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# Symposium: Diet, nutrition and exercise in reproduction

## Metabolic effects of obesity on reproduction

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## Abstract

Obese women are characterized by similar comorbidities to men, particularly type 2 diabetes mellitus and cardiovascular diseases. Moreover, they also develop some specific problems, including fertility-related disorders and some hormone-dependent forms of cancer. The relationship between excess body fat and reproductive disturbances appears to be stronger for early-onset obesity. Early onset of obesity, particularly during adolescence, favours the development of menses irregularities, chronic oligo-anovulation and infertility in adulthood. Moreover, obesity in women can increase the risk of miscarriage and impair the outcome of assisted reproductive technologies. The main factor implicated in the association between obesity and fertility-related disorders is insulin excess, which accompanies insulin resistance. Hyperinsulinaemia may be directly responsible for the development of androgen excess, through its effects in reducing sex hormone-binding globulin synthesis and circulating concentrations, and in stimulating ovarian androgen production rates. Androgen excess, in turn, represents one of the major factors leading to altered ovarian physiology and associated ovulatory disturbances. Obesity-associated hyperleptinaemia may represent an additional factor involved in anovulation, not only through the induction of insulin resistance, but also through a direct impairment of ovarian function.

***Keywords:*** *metabolism, obesity, reproduction*

### Association between worldwide epidemic of obesity and fertility disorders in women

The world is facing an emergency in public health, due to the increasing epidemic of obesity and related disorders (Haslan and James, 2005). The price of obesity is represented by a long list of comorbidities and social, psychological and demographic problems. Obese women are characterized by similar comorbidities to men, particularly type 2 diabetes mellitus (T2DM) and cardiovascular diseases (Ford, 2004). On the other hand, they also have some specific problems, including fertility-related disorders and some hormone-dependent forms of cancer (Pasquali *et al.*, 2003; Linnè, 2004). There is increasing evidence that infertility in women is more deeply counteracted by the presence of overweight and obesity, particularly when they develop early in life, such as during infancy or pubertal age. In addition, in the last 2 decades particular emphasis has been placed on the relationship with polycystic ovarian syndrome (PCOS), one of the more common causes of infertility in women, and obesity, which is believed to play an important role in its pathophysiological processes and complications, both during fertile life and even after menopause (Gambineri *et al.*, 2002).

This article will focus attention on the impact of obesity on prevalent infertility disorders in women, including PCOS. Specific paragraphs will discuss: (i) the epidemiological and clinical evidence of the association between obesity and reproductive disorders; (ii) the pathophysiological aspects by which obesity may impair fertility, with particular emphasis on androgen disorders; (iii) major factors explaining the strong association between obesity and PCOS and potential pathophysiological mechanisms of obesity on PCOS. For this

purpose, the article will mostly focus on relevant reviews rather than original papers published in the last 10–15 years. The effects of obesity on pregnancy rates and complications, together with the effects on delivery and fetal morbidities and mortality will not be included in this article, and readers should therefore refer to recent reviews on these issues (Linnè, 2004; Mitchell *et al.*, 2005).

## Obesity and reproductive disorders: epidemiological aspects

The association between obesity and alterations of the reproductive functions in women was recognized a long time ago. More than 50 years ago, Rogers and Mitchell (1952) found that 43% of women affected by various menstrual disorders, infertility and recurrent miscarriages were overweight or obese. Much later, Hartz and colleagues (1979) showed that the presence of anovulatory cycles, oligoamenorrhoea, hirsutism and infertility, separately or in association, were significantly higher in obese than in normal-weight women. Similar findings have been reported by others (Norman and Clark, 1998).

The relationship between excess body fat and reproductive disturbances appears to be stronger for early-onset obesity, particularly during the adolescent age, although this still represents a controversial issue, largely due to the heterogeneity of the overweight or obese preadolescent or adolescent populations investigated (Azziz, 1989). There are several epidemiological studies suggesting that changes in body weight or body composition are critical factors regulating pubertal development in young women. The discovery of leptin provided a unique explanation in this complex circuit, leptin being a main product of body fat (Considine *et al.*, 1996) and, at the same time, regulating the gonadotrophin surge which initiates the development of pubertal stages (Farooqi *et al.*, 1999). Indirect confirmation of this association derives from evidence that in leptin deficient ob/ob mouse the reproductive system remains pre-pubertal (O'Rahilly, 1998). Other studies have repeatedly reported that the age of menarche generally occurs at a younger age in obese girls than in normal-weight counterparts (Pelusi and Pasquali, 2003). Just as the onset of menarche is earlier in obese women, so data also suggest that the onset of ovarian failure and increased production of FSH at menopause occurs several years earlier in obese than in normal-weight women (Norman and Clark, 1998). In adolescent and young women the age of onset of obesity and that of menstrual irregularities are significantly related (Pasquali and Casimirri, 1993). In addition, there are data indicating that the association with menstrual disorders may be more frequent in girls with onset of excess body weight during puberty than in those who were obese during infancy. These findings have been confirmed in a large study performed in approximately 6000 women by Lake and co-workers (Lake *et al.*, 1997), who found that obesity in childhood and the early 20s increased the risk of menstrual problems. It is therefore likely that overweight and obesity do contribute to a significant proportion of menstrual disorders in young women. Early onset of obesity, i.e. before adult age, favours the development of menses irregularities and chronic oligo-anovulation, and may therefore play a role in the development of PCOS (Pelusi and Pasquali, 2003).

Although many multiparous women are obese, there is nonetheless evidence that obesity may also affect fertility rates in women within the fertile age. In the Nurses' Health Study (Rich-Edwards *et al.*, 1994) it was reported that the risk of ovulatory infertility increased in women with increasing body mass index (BMI) values. Several other cross-sectional and prospective studies have produced similar findings (Norman and Clark, 1998; Linnè, 2004). In a recent observational study performed in the United Kingdom, the authors found that lifestyle (smoking, alcohol and coffee consumption) and BMI had a significant and cumulative dose-dependent and weight-dependent negative impact on fecundity (Hassan and Killick, 2004). In addition, there are consistent data indicating that obesity is also associated with an increased risk of miscarriage (Norman and Clark, 1998). On the other hand, while examining a large group of nulliparous healthy women who presented for artificial insemination due to infertility of their partners, Zaadstra and co-workers (1993) found that body fat distribution as well as fat amount were associated with a decreasing chance of conception.

Obesity can also impair the outcome of assisted reproductive technologies. The lower probability of a healthy liveborn described in obese women seems to be the result of a combination of lower implantation and pregnancy rates, higher preclinical and clinical miscarriage rate, and increased complication during pregnancy for both mother and fetus (Hall and Neubert, 2005). Studies performed in infertile women undergoing assisted reproduction indicate that the ovary plays a leading but not exclusive role in the fertility prognosis of these patients (Fedocksák *et al.*, 2004). The endocrine and metabolic environment may in fact affect oocyte quality and, therefore, embryo development, implantation and pregnancy outcome. The endometrium too seems to play a subtle role in the more negative reproductive outcome of obese women, according to the recent studies based on the ovum donation model. Numerous reviews have been published on this topic in the last few years (Moschos *et al.*, 2002; Hall and Neubert 2005).

Due to the growing world epidemic of obesity in the last decade, there is thus a need for much more updated investigations in this area in order to evaluate whether this is associated with a parallel increase of related adverse effects on fertility in women.

## Abdominal obesity as a hyperandrogenic state in women

The role of adipose tissue is crucial in controlling the balance of sex hormone availability in non-adipose target tissues. In fact, adipose tissue is able to store various lipid-soluble steroids, including androgens. Most sex hormones appear to be preferentially concentrated within the adipose tissue rather than in the blood. As a consequence, since the amount of fat in obesity is larger than the intravascular space, and the steroid concentration in adipose tissue is much higher than in plasma, the steroid pool in obese individuals is greater than that found in normal-weight individuals (Gambineri *et al.*, 2002).

It is well known that an increase in body weight and fat tissue is associated with several abnormalities of sex steroid

balance, particularly in women of reproductive age (Pasquali *et al.*, 2003). Such alterations involve both androgens and oestrogens and, overall, their carrier protein, sex hormone-binding globulin (SHBG). Changes in SHBG concentrations lead to an alteration of androgen and oestrogen delivery to target tissues. SHBG concentrations are regulated by a complex of factors, including oestrogens, iodothyronines and growth hormone as stimulating agents, and androgens and insulin as inhibiting factors (Von Shoultz and Carlstrom, 1989). The net balance of this regulation is probably responsible for the decrease in SHBG concentration observed in obesity.

In addition, body fat distribution has also been demonstrated to substantially affect SHBG concentrations. In fact, women with central obesity usually have lower serum SHBG concentrations in comparison to their age- and weight-matched counterparts with peripheral obesity (Pasquali *et al.*, 1993). This seems to be mostly dependent on higher circulating insulin, due to the inhibitory capacity of insulin on SHBG synthesis by the liver (Plymate *et al.*, 1988). Reduction of circulating SHBG results in an increase in the metabolic clearance rate of circulating SHBG-bound steroids, specifically testosterone, dihydrotestosterone and androstenediol, the principal active metabolite of dihydrotestosterone (Samojlik *et al.*, 1984; Kirshner *et al.*, 1990). However, this effect is compensated by consequent increases in their respective production rates. In fact, women with central obesity have higher testosterone production rates than those with peripheral obesity (Kirshner *et al.*, 1990). The maintenance of normal circulating concentrations of these hormones in obesity predicts the presence of a sophisticated regulatory system that can adjust both the production rate and the metabolic clearance rate of these hormones according to body size (Kurtz *et al.*, 1987). Due to the greater reduction of SHBG concentrations, the free testosterone fraction tends to be higher in women with central obesity than in those with peripheral obesity (Evans *et al.*, 1990). An inverse correlation exists between waist to hip ratio (WHR), or other indices of body fat distribution, and testosterone and SHBG concentrations, regardless of BMI values (Evans *et al.*, 1990). Obesity also affects the metabolism of androgens not bound to SHBG. In fact, both production rates and metabolic clearance rates of dehydroepiandrostenedione and androstenedione are equally increased in obesity (Kurtz *et al.*, 1987).

Therefore, a condition of 'relative functional hyperandrogenism' appears to be associated with the central obesity phenotype in women. Whether this condition may be related to the complex pathophysiological series of events leading to chronic oligo-anovulation in obesity has not been adequately investigated. On the other hand, it is well known that hyperandrogenic states such as PCOS are typically associated with menstrual disturbances and chronic infertility, and, in addition, that androgen excess plays a dominant role in determining these disorders. In addition, there is evidence that the association between obesity and PCOS tends to synergistically increase reproductive abnormalities. This issue will be discussed in a subsequent paragraph. In addition to these alterations, other metabolic derangements are suggested to play a crucial role in the association between obesity, particularly the abdominal phenotype, and infertility problems in women.

# Insulin resistance, hyperinsulinaemia and leptin in simple obesity: pathophysiological insights

## Insulin resistance and hyperinsulinaemia

Together with the well-known actions at the level of classic target organs such as liver, adipose tissue and muscles, insulin plays a role in the regulation of other tissues/organs, particularly the ovary, besides the pituitary and the adrenal gland. At the ovarian level, insulin acts by interacting with its own receptor and by the insulin-like growth factor-I (IGF-I) receptor, which have been detected in human models throughout all ovarian compartments, such as granulosa, thecal and stromal tissues (Poretsky *et al.*, 1999). It has also been definitively proved that insulin is able to stimulate ovarian steroidogenesis both in granulosa and thecal cells. In fact, insulin increases 17α-hydroxylase and 17–20 lyase (both components of the P450c17 enzyme system) activity (Nestler and Jakubowicz, 1996) and stimulates the expression of 3β-hydroxysteroid dehydrogenase in human luteinized granulosa cells. Conversely, the role of insulin on aromatase activity is rather discordant, in-vitro studies having demonstrated either a stimulatory or a lack of effect (Pasquali *et al.*, 2003). In addition, insulin appears to increase the sensitivity of pituitary gonadotropes to gonadotrophin-releasing hormone (GnRH) action (Poretsky *et al.*, 1999) and to reinforce the ovarian steroidogenic response to gonadotrophins, by mechanisms probably related to an increase of the LH receptor number (Poretsky *et al.*, 1999). Moreover, together with its effects on SHBG synthesis (Plymate *et al.*, 1988), insulin has a negative effect on both hepatic and ovarian IGF binding protein-1 (IGFBP-1) synthesis, which bind sex steroids and IGFs respectively, to regulate ovarian growth and cyst formation and to modulate adrenal steroidogenesis (Poretsky *et al.*, 1999). In-vitro studies have shown that insulin may also increase 17α-hydroxylase and 17–20 lyase activity in the adrenals either directly (L'Allemand *et al.*, 1996) or by strengthening the responsiveness of the enzyme to adrenocorticotrophic hormone (ACTH) stimulation (Moghetti *et al.*, 1996).

Insulin resistance can be defined as a reduced biological effect of insulin for any given concentration of insulin. The definition and accurate quantification of insulin resistance are important and a number of methods are available for its measurement, including the hyperinsulinaemic euglycaemic clamp technique, and the minimal model (Beck-Nielsen, 2000). Because of the complexity of these approaches, which are usually reserved for clinical research, great efforts have been made in the search for simpler tests. These include fasting insulin alone, the homeostasis assessment method [(HOMA): (fasting glucose, mmol/l × fasting insulin, mIU/ml)/25] (Matthews *et al.*, 1985) and the quantitative insulin-sensitivity check index [QUICKI: 1/(log insulin fasting + log glucose fasting)], which have been extensively reviewed (Katz *et al.*, 2000). These tests are simple to perform, being based on fasting insulin concentrations and glucose concentrations alone, and can be used for investigational and clinical purposes. However, even these simple procedures need to be carried out in carefully standardized conditions. Moreover, it should also

be emphasized that once a significant defect of insulin secretion exists, the value of these measurements may be poor and can be misleading (Beck-Nielsen *et al.*, 2000).

The definition of insulin resistance in obesity and associated clinical disorders, such as, for example, PCOS, largely depends on the methodology used. Some aspects of insulin action in obesity resemble those seen in PCOS (Dunaif, 1997; Poretsky *et al.*, 1999). Whatever the method used, however, it is evident that most patients with obesity are insulin resistant and hyperinsulinaemic, particularly when the abdominal phenotype is present. In addition, hyperinsulinaemia often develops in these patients, as a compensatory mechanism to force reduced insulin sensitivity in peripheral tissues.

As reported above, excess insulin may play a role in determining altered androgen secretion and metabolism in abdominal obesity. In fact, insulin acts as a true gonadotrophic hormone; therefore insulin excess may be responsible for excess androgen production from the ovaries (Dunaif, 1997; Poretsky *et al.*, 1999). The mechanisms by which obesity may induce an insulin-resistant state have been extensively summarized elsewhere (Matthaei *et al.*, 2000; Kahn *et al.*, 2005). Briefly, enlargement of adipose tissue mass, in particular of visceral fat depot, increases the availability of several metabolites [such as free fatty acids (FFA) and lactate] which are able to affect the secretion and the metabolism of insulin as well as its peripheral action. Insulin resistance in obesity can also be related to tumour necrosis factor-α (TNF-α) and to leptin, both products of adipose tissue. TNF-α mediates serine phosphorylation of insulin receptor substrate-1 (Hotamisligil *et al.*, 1996), which has been shown to interfere with the action of both insulin and IGF-I, by inhibiting insulin receptor and type I IGF receptor tyrosine kinases respectively, and by stimulating IGFBP production (Poretsky *et al.*, 1999). TNF-α can also inhibit signalling through PPAR-γ (Poretsky *et al.*, 1999). Leptin may contribute to the insulin resistance of obesity via mechanisms similar to TNF-α (Poretsky *et al.*, 1999).

Metabolic disorders such as the development of insulin resistance and compensatory hyperinsulinaemia, which are associated with obesity, may have some implication on infertility disorders and hyperandrogenic states. A significant proportion of the infertile or sub-fertile population are obese or overweight (Mitchell *et al.*, 2005), with a plethora of reproductive complications (Hartz *et al.*, 1979; Lake *et al.*, 1997). The development of obesity and insulin resistance commonly go hand-in-hand with the development of fertility problems but the link between this metabolic state and infertility still needs to be defined (Mitchell *et al.*, 2005).

Leptin, a product of the OB gene, is not only an adipose-derived messenger of the amount of energy stores to the brain, but also a crucial hormone for a number of diverse physiological processes, including gonadal function and reproduction (Moschos *et al.*, 2002). In peripheral tissues, leptin generally has a fat metabolizing role with limited direct effect on glucose metabolism (Mitchell *et al.*, 2005). However, leptin antagonizes insulin action and decreases its production by pancreatic β-cells (Seufert *et al.*, 2004), and it indirectly affects glucose metabolism, for example glucose transport in skeletal muscle via the hypothalamus and central nervous system (Minokoshi *et al.*, 1999). Evidence suggests that leptin increases lipolysis in adipose tissue, cells, and in skeletal muscle, but it appears less critical to liver function (Cohen *et al.*, 2001). It has therefore been suggested that increased leptin may have a role in development of insulin resistance in humans. Obese individuals are characterized by increased rather than reduced leptin concentrations, and leptin mRNA expression in fat cells is significantly related to excess body weight and fat (Considine *et al.*, 1996). Hyperleptinaemia is thought to be indicative of leptin resistance at central levels, thereby explaining the lack of reduced feeding in the presence of excess leptin concentrations (O'Rahilly, 1998). Notably, in addition to its metabolic actions, there is also strong evidence for a close physiological interplay between leptin and the hypothalamic–pituitary–gonadal (HPG) axis in humans. A link with insulin resistance and fertility was described early on, with leptin-deficient mice having increased adiposity, displaying severe insulin resistance and diminished fertility, both of which were restored with leptin administration, but not by calorie restriction or weight loss (Chehab *et al.*, 1996). Leptin participates in the regulation of the HPG axis at both central and gonadal levels. Leptin in fact regulates gonadotrophin secretion, this effect being dependent on the high expression of leptin receptors in the hypothalamus and the pituitary (O'Rahilly, 1998; Moschos *et al.*, 2002). Moreover, leptin receptors (i.e. long and short isoforms) are also widely expressed in the human ovaries and testes (Moschos *et al.*, 2002), and this provides the basis for a potential regulation by leptin of the HPG function. Extensive studies in animal models and humans have produced evidence that leptin is essential for the development of mature HPG dynamics and that, in this condition, obesity and hypogonadism are strongly related (Moschos *et al.*, 2002). In fact, ob/ob mice are infertile and hypogonadic (Chebab *et al.*, 1996) and both hormone disturbances and infertility can be restored by treatment with recombinant leptin (Chebab *et al.*, 1996). The presence of adequate circulating concentrations of leptin is essential for the activation of the HPG axis at puberty in humans, because patients with congenital leptin deficiency (Faroqui *et al.*, 2002) and leptin receptor mutations (Clement *et al.*, 1998) fail to undergo pubertal development, which can be restored by leptin administration (Faroqui *et al.*, 2002).

Whether leptin is involved in the regulation of controlling HPG axis in simple obesity and whether this occurs differently in obese men and obese women has been poorly investigated. Leptin and sex hormone blood concentrations are strongly related in humans (Pasquali, in press) and leptin appears to decrease testosterone production and blood concentrations in proportion with increasing body weight in men, as reviewed recently (Pasquali, in press). The interaction between sex steroids and leptin is emphasized by their coordinated relationship during a menstrual cycle in normal cycling women (Geisthovel *et al.*, 2004). In human ovaries, leptin may also exert a direct inhibitory effect on both granulosa and thecal cell steroidogenesis (Agarwal *et al.*, 1999), and in female rats high leptin concentrations in the ovary may interfere with the development of dominant follicles and oocyte maturation (Duggal *et al.*, 2000), therefore impairing ovulation rate (Duggal *et al.*, 2000). Convincing studies on the effect of excess leptin on gonadotrophin or ovarian sex hormone release in obese women, whether normally cycling or with ovulation impairment, are however still lacking. In the last years, interest has been focused on leptin concentrations in women with PCOS, particularly in those with obesity. To date, contradictory

results have been reported on leptin concentrations in women with PCOS, and higher concentrations than those expected for their BMI or normal concentrations have been detected (Gambineri *et al.*, 2002). Whether leptin may play a role in the pathophysiology of ovarian dysfunction in PCOS therefore remains to be demonstrated.

In summary, there is evidence from many studies performed in experimental animals and humans that the presence of insulin resistance, as occurs in obesity, particularly the abdominal phenotype, may have a profound direct or indirect impact on ovarian function, and thus on fertility. This involves different sites of action, including the ovarian tissues, the neuroendocrine functions, and several other factors. Insulin excess, which accompanies insulin resistance, may be directly responsible for the development of androgen excess which, in turn, is associated with ovulatory disturbances. Obesity-associated hyperleptinaemia may represent an additional factor involved not only in the development of insulin resistance, but also in the impairment of ovarian function. Other adipocytokines potentially involved in the regulation of insulin action and regulation of the HPG axis in women have been suggested to play a role in reproduction, but their role still remains partially unknown. On the other hand, several recent reviews on the potential role of ghrelin (Pasquali, in press), adiponectin and resistin (Mitchell *et al.*, 2005) are available for further reading.

## Obesity and PCOS: pathophysiological aspects

PCOS, one of the most common causes of infertility due to anovulation, affects 4–7% of women (Ehrmann, 2005). Although it was considered that PCOS may have some genetic component (Urbanek and Spielman, 2002; Fratantonio *et al.*, 2005) and that the clinical features of this disorder may change throughout the lifespan, starting from adolescence to postmenopausal age, no effort has been made to define differences in phenotype and clinical presentation according to age. Indeed, in the last decade, it has been widely recognized that several features of the metabolic syndrome, particularly insulin resistance and hyperinsulinaemia, are inconsistently present in the majority of women with PCOS. In addition, obesity and the metabolic syndrome may affect more than half of these women. The association between PCOS and excess fat leads to marked worsening, with particular reference to altered fertility state. Later in life, it becomes clear that the association between obesity (particularly the abdominal phenotype), insulin resistance, and PCOS renders affected women more susceptible to develop T2DM, with some differences in the prevalence rates between countries, suggesting that environmental factors are important in determining individual susceptibility (Gambineri *et al.*, 2002; Legro, 2002; Legro *et al.*, 2005). This represents an important factor in the evaluation of PCOS throughout life, and implies that PCOS by itself may not be a hyperandrogenic disorder exclusively restricted and relevant to young and fertile aged women, but may also have some health implications later in life. In young women with PCOS, hyperandrogenism, menstrual irregularities, overweight and insulin resistance may occur together, emphasizing the pathophysiological role of excess androgen and insulin on PCOS (Ehrmann, 2005). Symptoms related to androgen excess, such as oligo- or amenorrhoea and infertility, other than obesity, conversely represent the major complaints of adult PCOS women of reproductive age.

Mechanisms by which obesity influences the pathophysiology and clinical expression of PCOS are complex and not completely understood (Gambineri *et al.*, 2002). However, obesity is believed to play a distinct pathophysiological role in the development of hyperandrogenism in women with PCOS. In an obese PCOS woman, the presence of obesity in her mother during pregnancy appears to influence the susceptibility to develop hyperandrogenism and the PCOS phenotype of the daughter later in time, although pathophysiological mechanisms have not been defined (Cresswell *et al.*, 1997). On the other hand, it has been recently postulated that in-utero androgen excess may be an important factor programming subsequent PCOS development during puberty (Abbott *et al.*, 2002). This theory appears to be substantiated by several studies performed in non-human primates and sheep (Gambineri *et al.*, 2002). By this mechanism, it has been suggested that a primary ovarian disorder may occur early in the woman's life, leading to the development of a hyperandrogenized ovary later in life. The dangerous effects of early (pre-adolescence) onset androgen excess is further emphasized by recent studies reporting abnormal metabolic and endocrine profiles consistent with the metabolic syndrome or pre-PCOS signs in girls with precocious adrenarche and precocious pubarche, and possible metabolic syndrome and PCOS development in childhood or adolescent girls with a history of these precocious disorders (Ibanez *et al.*, 2000).

Several factors are relevant to understanding the complex pathophysiological network relating obesity with PCOS. They include insulin, the insulin-growth-factor system, the opioid system, oestrogens and several cytokines, particularly leptin, which have been extensively reviewed in recent articles (Poretsky *et al.*, 1999; Gambineri *et al.*, 2002) and are briefly discussed here. It is well known that obesity, particularly the abdominal phenotype, is a condition of insulin resistance and compensatory hyperinsulinaemia. The following paragraph will extensively summarize the concept of insulin resistance in PCOS and its role in the development of metabolic sequelae, with particular emphasis on glucose homeostasis and the metabolic syndrome. Contrary to what occurs in the classic target tissues (i.e. muscle, liver, adipose tissue) of insulin action, that became resistant to insulin, the ovaries remain responsive to insulin throughout the interaction with its own receptor. In the last two decades, a large number of in-vitro and in-vivo studies have demonstrated that in the ovaries of PCOS women, excess insulin is capable of stimulating steroidogenesis and excessive androgen production by the theca cell system (Dunaif, 1997; Poretsky *et al.*, 1999; Gambineri *et al.*, 2002). The excess in local ovarian androgen production induced by excess circulating insulin may also cause premature follicular atresia and thus favour anovulation (Poretsky *et al.*, 1999). It can therefore be speculated that insulin resistance and hyperinsulinaemia, which develop together with the obese state, play a dominant role in favouring hyperandrogenism and infertility in women susceptible to the development of PCOS.

The influence of obesity on hyperandrogenism can also be mediated by other factors and mechanisms. As in simple obesity, a hyperoestrogenic state is present also in obese PCOS women. Excess oestrogens may exert positive feed-back regulation on gonadotrophin release, triggering in turn a

rise in ovarian androgen production, according to a still valid theory proposed many years ago by Yen (1980). An additional factor involved in the dysregulation of this complex circuit may be an increased tone of the opioid system, which has been demonstrated to be present in obesity, as well as in women with PCOS (Gambineri *et al.*, 2002). Several studies have shown that β-endorphin is able to stimulate insulin secretion (Gambineri *et al.*, 2002). The possibility that increased opioid activity may favour the development of hyperinsulinaemia and, in turn, of hyperandrogenaemia, is further supported by the finding that acute and chronic administration of opioid antagonists, such as naloxone and naltrexone, suppresses both basal and glucose-stimulated insulin blood concentrations (Lanzone *et al.*, 1991; Sir-Peterman *et al.*, 1998). Whether alterations of the opioidergic system play a causative role in the infertility of some women is, however, still undefined. On the other hand, there are studies showing that opioid antagonists given to obese PCOS women may improve menses (Pasquali and Casimirri, 1993).

Several peptides, particularly leptin, are currently emerging as potential candidates involved in the pathogenesis of hyperandrogenism and infertility in PCOS women. The previous paragraph summarized the potential mechanisms by which leptin may be involved in obesity-associated ovulatory dysfunction. This may also be relevant for the pathophysiology of PCOS, although the exact role of leptin in this disorder needs to be further clarified, as reported above.

Women with PCOS and obesity are characterized by several distinct features with respect to those presenting with normal weight and various studies have uniformly demonstrated that obese PCOS women are characterized by significantly lower SHBG plasma concentrations, and worsened hyperandrogenism in comparison with their normal-weight counterparts (Gambineri *et al.*, 2002). In addition, a negative correlation between body fat mass and circulating androgens has been reported (Gambineri *et al.*, 2002). It has also been repeatedly reported that a higher proportion of obese PCOS women complain of hirsutism and menstrual disturbances than do normal-weight women (Gambineri *et al.*, 2002). Therefore, there is consistent evidence that the increase in body weight may favour a worsened hyperandrogenic state in women with PCOS.

As reported above, it is well documented that women with PCOS have a high prevalence of abdominal distribution of body fat, even if they are normal-weight (Gambineri *et al.*, 2002). The impact of abdominal obesity on PCOS may be greater than expected, since this phenotype is associated with more pronounced hyperandrogenism and insulin resistance than the peripheral body fat phenotype. As in women with simple obesity (see previous paragraph), increased free fatty acid (FFA) efflux from the highly lipolytic abdominal fat to the liver and muscles may represent the most important link between abdominal obesity and the insulin resistant state in PCOS (Holte *et al.*, 1995). In addition, PCOS women with the abdominal phenotype present with a higher prevalence of menstrual abnormalities and acanthosis nigricans (a cutaneous marker of insulin resistance) and a tendency towards more severe hirsutism compared with PCOS women with the peripheral obesity phenotype (Gambineri *et al.*, 2002).

Androgens in turn may favour the development of insulin resistance and associated hyperinsulinaemia. In fact, at the level of visceral fat depots, testosterone stimulates lipolysis and, in the muscle, testosterone modifies the histological structure by increasing type II, less insulin-sensitive fibres (Gambineri *et al.*, 2002). These androgen-dependent mechanisms may have a further important impact on the insulin resistant state in women with PCOS, particularly in the presence of abdominal fatness.

## Obesity and PCOS: insulin resistance and the metabolic syndrome

Insulin resistance in women with PCOS appears more common than in the general population (Ehrmann, 2005). In one study examining the characteristics of more than 1000 consecutive women with androgen excess, Azziz and co-workers (2004) found that 716 of them had PCOS and were characterized, as a group, by hyperinsulinaemia and insulin resistance. Interestingly, 60% of them were obese, which emphasizes the concept that obesity *per se* may be an amplifier of a cause of this metabolic derangement On the other hand, insulin resistance may be present even in PCOS women of normal weight (Gambineri *et al.*, 2002). Reports on the prevalence of insulin resistance in women with PCOS are not homogeneous, depending on the sensitivity and specificity of the test employed. Due to the lack of epidemiological studies, available data refer to clinical studies performed in different centres worldwide and only include PCOS patients attending each institution for medical problems or personal complaints, particularly hirsutism, menstrual abnormalities, infertility or obesity.

Both fasting and glucose-stimulated insulin concentrations are usually significantly higher in PCOS than in non-PCOS controls (Dunaif, 1997; Poretsky *et al.*, 1999; Gambineri *et al.*, 2002). Studies examining insulin sensitivity by using different methods have demonstrated that PCOS women have significantly lower insulin sensitivity compared with age- and weight-matched controls. In addition, they demonstrated that almost all obese PCOS women have some degree of insulin resistance, whereas this abnormality is present in more than half their non-obese PCOS counterparts (Dunaif, 1997; Poretsky *et al.*, 1999; Gambineri *et al.*, 2002). Some studies, however, found some degree of deficiency in the first phase of insulin secretion in selected groups of PCOS women with obesity investigated in the US (Ehrmann *et al.*, 1995; Dunaif *et al.*, 1997), which were not confirmed by other studies performed in Europe (Holte *et al.*, 1995; Gambineri *et al.*, 2004). Interestingly, a more recent study using the i.v. glucose tolerance test technique showed that in women with normal weight, insulin sensitivity and β-cell function is preserved, whereas glucose effectiveness (which is the insulin-independent glucose uptake) was conversely decreased (Gennarelli *et al.*, 2005). At variance, other relevant papers did not confirm decreased insulin sensitivity in PCOS. Holte and co-workers (Holte *et al.*, 1994) reported that some differences in the insulin sensitivity index (ISI), defined as the ratio of the glucose disposal rate ($S_i$) to the insulin concentrations at the end of a euglycaemic hyperinsulinaemic clamp, were present only in subjects with high BMI levels. Interestingly, while examining insulin sensitivity (measured by the i.v. glucose tolerance test) in relation to the presence of a heredity

risk for T2DM, Ehrmann and colleagues (1995) were not able to find any difference in ISI between obese PCOS and controls in affected women with a negative family history, whereas the difference in ISI was present in those affected women with a positive history. Morin-Papunen and co-workers (2004) found that, compared with adequate control groups, only obese PCOS women were more insulin resistant, without any difference between normal-weight PCOS and controls.

There are several conceptual reasons to indicate that insulin resistance and the metabolic syndrome should be considered as separate entities (Kahn *et al.*, 2005). On the other hand, it has been shown that the greater the number of factors defining the metabolic syndrome, the higher the probability that affected subjects are insulin resistant (Cheal *et al.*, 2004). This indicates the need to carefully collect the data for each parameter, and if necessary to include some other parameters in the definition of the metabolic syndrome.

There is evidence that worsening insulin resistance in the long term may represent an important factor in the development of glucose intolerant states in PCOS (Pasquali *et al.*, 1999; Wang and Norman, 2004; Legro *et al.*, 2005). Clinical studies have in fact shown that glucose intolerance is present, at the first clinical examination, in as many as 30–40% of obese PCOS women in the US (Dunaif, 1997) and, probably, to a lesser extent in those living in Europe (Gambineri *et al.*, 2004), whilst it is uncommon in their normal-weight counterparts (Gambineri *et al.*, 2004). These findings indicate that obesity may contribute to determine the insulin resistant state and may impair glucose tolerance in PCOS. Although insulin resistance seems to play a determining role in the development of diabetes, the presence of insulin resistance does not immediately imply a concomitant alteration of glucose tolerance. In fact, most obese insulin-resistant PCOS women still have a normal glucose tolerant state. On the other hand, it has recently been found that PCOS women with impaired glucose tolerance or T2DM are significantly more insulin-resistant and hyperinsulinaemic than those with normal glucose tolerance, regardless of the presence of obesity (Gambineri *et al.*, 2004). Prospective studies in PCOS women also found that insulin resistance tends to worsen over time together with an increment of insulin and C-peptide response to an oral glucose challenge, and that, in several cases, glucose intolerance appears (Pasquali *et al.*, 1999; Legro *et al.*, 2005). Taken together, these findings strongly support the role of insulin resistance in the development of altered glucose tolerance states in PCOS women.

Some recent studies have used the NECP/ATPIII criteria to assess the prevalence of the metabolic syndrome in PCOS women. Glueck and co-workers (Glueck *et al.*, 2003) studied 138 PCOS patients and found a prevalence rate of 46%, whereas, more recently, Apridonidze and co-workers (2005) found a prevalence of 43% by retrospectively reviewing the medical charts of 106 PCOS women attending the Endocrine Clinic of Richmond, Virginia. Both these studies, therefore, described a prevalence of the metabolic syndrome in PCOS nearly 2-fold higher than that reported in the general population investigated in the cited NHANES III report, matched for age and body weight. Apridonidze and co-workers (2005) also described higher free testosterone and lower SHBG concentrations in those women with the metabolic syndrome compared with those without it, as well as a higher prevalence of acanthosis nigricans and a tendency towards a greater family history for PCOS. These results were in accordance with a cross-sectional population-based study conducted by Korhonen and co-workers (2003) and Pasquali *et al.* (2005). Studies in American (Ehrmann *et al.*, 1999), Asian (Weekariet *et al.*, 2001) and Italian (Gambineri *et al.*, 2004) subjects have also shown that women with PCOS have a tendency to early development of impaired glucose tolerance and T2DM when compared with the general population.

Taken together, these findings demonstrate that the prevalence of the metabolic syndrome and T2DM in women with PCOS is higher than that of the general population, regardless of ethnicity and geographical area. They also indicate a strong association between the metabolic syndrome, the hyperandrogenic state, and obesity, particularly the abdominal phenotype. Whether factors associated with the metabolic syndrome may affect fertility in PCOS has been partly discussed in a previous paragraph. Notably, being obese, particularly the abdominal phenotype, a constitutive component of the metabolic syndrome, it is likely that the cause of worsened fertility, particularly in PCOS women, may be related to mechanisms which reflect, at least in part, the hormonal and metabolic derangements which are strictly related to its presence. However, this possibility deserves more convincing studies.

## Summary and conclusions

Obesity, particularly the abdominal phenotype, is associated with several abnormalities of sex steroid balance in women, including an increased androgen production rate and decreased serum concentrations of SHBG, with a condition of insulin resistance and compensatory hyperinsulinaemia, and with excess leptin concentrations and a leptin resistant state. All these factors may directly or indirectly impair ovarian function, thus explaining the well-defined association between obesity and infertility in women. A diagram illustrating the complex nature of the relationship between obesity and reproductive abnormalities in simple obesity and obesity-related PCOS is shown in **Figure 1**.

It has been postulated that the increasing epidemic of obesity worldwide may also favour the high prevalence of obesity among women with PCOS. Obesity has a profound impact on the PCOS phenotype, being associated with more severe hyperandrogenism and insulin resistant state and fertility disorders. Insulin resistance and hyperinsulinaemia are key features of women with PCOS, particularly in the presence of obesity. This has important effects on the pathophysiology of this disorder and largely contributes to fertility problems in PCOS women (**Figure 1**). Insulin excess does in fact have a direct responsibility in favouring androgen excess and oligo-anovulation in PCOS. On the other hand, insulin resistance represents the main pathophysiological event leading to the development of the metabolic syndrome, which affects almost 50% of women with PCOS. Recognition of these abnormalities in PCOS women may be relevant for both treatment and preventive strategies. It is in fact well defined that PCOS by itself, and even more so in the presence of obesity and a positive family history, may increase individual susceptibility to an early development of T2DM.

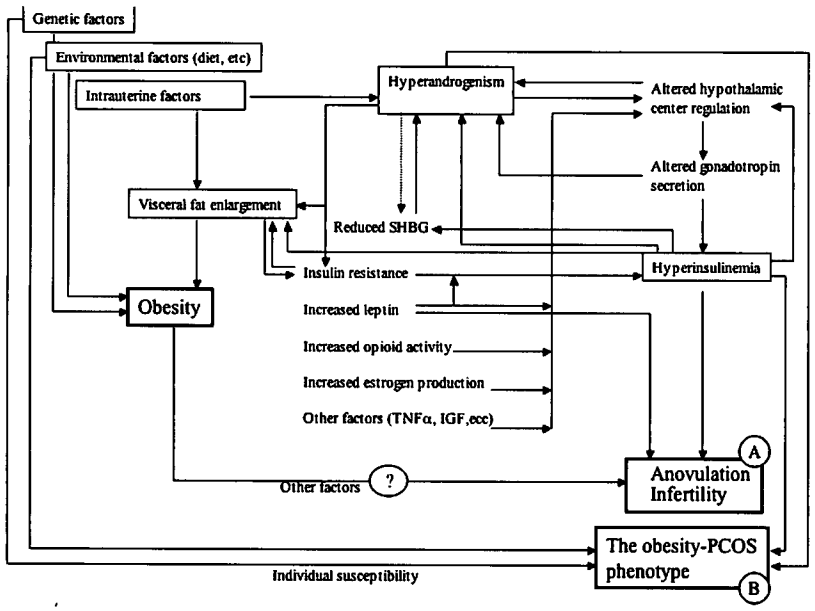


**Figure 1.** Diagram illustrating the complex nature of the relationship between obesity and reproductive abnormalities in simple obesity and obesity-related polycystic ovarian syndrome (PCOS). The diagram illustrates how factors related to obesity may participate in the complex interaction between androgens, neuroendocrine centres, insulin and insulin resistance, leptin and other factors and gonadal and extragonadal mechanisms leading to reproductive disorders in simple obesity (the abdominal phenotype) (box A), and particularly when it is associated with PCOS (box B). SHBG = sex hormone-binding globulin; TNF α = tumour necrosis factor α; IGF = insulin-like growth factor.

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# Fetal cells identified in cervix at 5 weeks' gestation

Several approaches have been designed to collect fetal cells early in pregnancy in order to genetically type the fetus. The most widely used methods include chorionic villous sampling at ~10 weeks and amniocentesis at the second trimester. Each of these involves interference with the developing embryo, and the late termination of pregnancies where the fetus carries a genetic disorder. Reports over recent years have indicated that fetal cells colonize the mother, and can be collected for prenatal diagnosis in the earlier stages of pregnancy. A report from an Italian group of investigators has now revealed that fetal cells can be collected from the cervix uteri at 5 weeks' gestation, i.e. 2 weeks earlier than the 7 weeks previously believed to be the earliest stage of pregnancy for collection.

Intrauterine lavage was used to collect the fetal cells. This approach can be achieved in various ways including sampling the external os, lower cervical canal, lower uterine pole and uterine cavity. In this case, transcervical cells were collected just prior to pregnancy termination. For lavage, a 2.5 mm inner diameter needle attached to a flexible catheter was used. It was attached to a syringe and inserted through the cervix just past the internal os under general anaesthesia. A total of 10 ml of saline was injected, and 10 s later 2.5 ml were withdrawn for cell analysis. For comparisons, a sample of maternal blood was also collected immediately after the lavage, and a sample of placenta was collected after termination. Screening of the uterine samples revealed no spermatozoa, and the trophoblast cells could be identified and collected since they were short and scarcely branched and displayed more limited arborescence than those collected in later stages of gestation. They could be sorted microscopically from the aspirated tissues.

DNA sequences were measured by quantitative PCR in samples of maternal blood, placental extracts and intrauterine samples. Short tandem repeats were used for the analysis of chromosomes X, Y, 13, 18 and 21. Fetal sex was classified through the non-polymorphic sequence of the amelogenic region, which corresponds to specific items for the X and the Y chromosomes. Measuring the peaks of expression helped to confirm the presence of fetal paternally-derived cells in maternal blood and placenta that trophoblast cells had been collected from the samples. The mother had a peak of amelogenin corresponding to an X chromosome, and was heterozygous for markers specific to chromosomes 13, 18 and 21. The application of FISH identified the presence of XY cells among maternal contaminants in the uterine sample. The fetus was male with X and Y chromosome markers, and carried heterozygous autosomal markers' which were identical in the uterine and placental samples. The authors stress that more studies are essential to confirm the safety of their procedures and why trophoblast cells can be found in the uterine lumen. Their study may open new avenues for extremely early prenatal diagnosis.

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