Chapter 17
Impact of Obesity on Female Reproductive Health

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Introduction

In the USA, more than 70% of the adult population is either overweight (BMI >25) or obese (BMI >30). An estimated 73% of the adult population were overweight or obese in 2008, reflecting the continued and steady increase in body weight over time. Indeed, nearly 50% of women in the USA aged 15–49 are overweight or obese [1]. Several well known medical complications are associated with obesity, including the risk of atherosclerosis, cardiovascular disease, hypertension, diabetes, and cancer. Obesity is caused by an imbalance between energy intake and energy expenditure. The proximate cause of obesity is the excessive accumulation of white adipose tissue; the major form of energy reserve in the body.

Obesity poses unique reproductive health risks in women. Obesity and excessive weight gain in pregnancy are associated with maternal and fetal complications of pregnancy, including preterm labor, gestational diabetes, preeclampsia, operative delivery, fetal macrosomia, and birth defects [2–7]. Additionally, childhood obesity in girls is associated with early onset of puberty, menstrual irregularities during adolescence and polycystic ovary syndrome (PCOS) [8, 9]. Obesity can increase the risk of anovulation and may also decrease ovulatory response to fertility treatment [10]. Moreover, the efficacy and safety of hormonal contraceptives can be severely compromised by increased body weight [11]. Conversely, weight reduction enhances reproductive outcomes and diminishes obesity-related maternal, perinatal, cardiovascular, and cancer risks [12–15]. The fact that most of the obesity-associated deleterious effect on female fertility is ameliorated by bariatric surgery strongly suggests a causative role for obesity in the development of these adverse effects on reproduction. Although several published reports have shown that maternal obesity is associated with increased morbidity and mortality for both mother and offspring, the mechanisms underlying the

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increased reproductive health risk associated with maternal obesity are not well understood. In this chapter, we aim to discuss the impact of obesity on female reproduction with a focus on the effect of leptin on fertility problems in obese women. Finally, we comment on the possible cross talk in the molecular and cellular pathways utilized by leptin and insulin to alter reproductive processes in obese women with PCOS.

Energy Homeostasis and Reproduction

In humans, reproductive function declines at both extremes of energy balance. The possibility of a factor secreted by white adipose tissue that reflects the level of total body energy stores and possibly regulates hypothalamic control of feeding was first proposed by Kennedy [16, 17] and is supported by classic cross-circulation (parabiosis) experiments in rodents [18, 19]. This was later confirmed by the discovery of leptin by positional cloning in 1994 [20]. In humans, the amount of body fat is the principal determinant of circulating levels of leptin [21]. Leptin is secreted in pulses that are positively correlated to gonadotropins, estradiol, and thyrotropin [22]. Leptin secretion in women has a diurnal pattern characterized by a nocturnal rise that synchronizes with a similar rise in gonadotropins and estradiol [23], suggesting a functional link between leptin and the hypothalamic–pituitary–ovarian axis.

Leptin and reproduction. Leptin is a major regulator of food intake and energy homeostasis. In addition, leptin relays information to the brain about the adequacy of peripheral energy stores to sustain reproduction. When energy stores are adequate, leptin suppresses feeding behavior and permits neuroendocrine functions that facilitate energy expenditure [24–26]. Conversely, a lower level of serum leptin reflecting inadequate energy stores elicits adaptive responses by diverting energy resources away from energy demanding processes, such as reproduction to those functions that are essential for the survival of the organism [27, 28]. This is reflected in clinical conditions associated with hypoleptinemia. For instance, a congenital absence of leptin as seen in individuals with homozygous mutations in the gene coding for leptin is associated with infertility in humans and in mice [29–32]. Infertility also characterizes acquired conditions associated with low levels of circulating leptin such as exercise-induced amenorrhea, functional hypothalamic amenorrhea, and anorexia nervosa [33, 34]. Leptin treatment has been shown to restore fertility in adult humans with congenital or acquired leptin deficiency [33, 34]. However, the precise mechanisms by which leptin acts to normalize reproductive function have not been fully defined.

Leptin and the hypothalamic–pituitary–gonadal axis. Previous studies suggest that leptin acts at the hypothalamic level to stimulate the release of gonadotropin releasing hormone (GnRH) and consequently the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the pituitary [35, 36]. More recently, studies from our laboratory [37, 38] and others [39–42] have suggested that leptin has distinct local effects on the ovary independent of its action on the hypothalamic-pituitary axis. These effects appear to be bimodal in nature. At physiological

concentrations, leptin increased estrogen production from human luteinized granulosa cells [42], and both estradiol and progesterone production from in vitro cultures of preantral mouse follicles [40]. Leptin has also been shown to enhance nuclear and cytoplasmic maturation of the oocyte and to induce ovulation via an LH-independent pathway in the mouse [39]. In contrast to supraphysiological concentrations, leptin inhibited ovarian steroidogenesis. For example, leptin at a concentration of 10 ng/ml increased the in vitro production of progesterone from porcine granulosa cells in vitro, but inhibited progesterone production at 1,000 ng/ml [43]. Further evidence that leptin may have a direct effect on the ovary was provided by a recent clinical study in which recombinant human leptin was used to treat hypoleptinemic lipodystrophic patients for a period of 10 months. Regular menses were restored in eight of ten women with primary amenorrhea or irregular menses [33], but this treatment was without any significant effect on serum levels of LH or FSH. This suggests that the therapeutic effect of leptin in women with lipodystrophic atrophy may be mediated directly at the level of the ovary or at least via a mechanism that does not alter circulating levels of gonadotropins. We have previously shown that the impaired folliculogenesis in leptin-deficient mice is associated with a subnormal number of follicles, a higher level of granulosa cell apoptosis, and an increased number of atretic follicles [37]. Subsequently, we compared the effect of gonadotropin treatment on ovarian follicle development in prepubertal leptin-deficient mice to age-matched controls [38]. The ovarian responsiveness to gonadotropin administration appeared to be subnormal in the leptin-deficient mice (fewer follicles developed to the late antral stage, and animals failed to ovulate), suggesting that leptin may directly alter the process of folliculogenesis independent of its action in hypothalamic-pituitary axis.

These data and others further suggest that leptin may be necessary to maintain a normal ovarian responsiveness to gonadotropins. Perhaps leptin signaling in the ovary may be required to maintain a normal expression levels of FSH and LH receptors, or in the absence of leptin signaling, the affinity of these receptors for their ligands may be reduced. One of the commonest reproductive disorders associated with obesity in women is PCOS. PCOS is characterized by excessive ovarian androgen production, anovulation, and polycystic ovaries (PCO). Obesity may exacerbate PCOS and conversely, PCOS may worsen the degree of obesity. In the next few sections of this chapter, we will use PCOS as our disease entity to explore the complex relationship between obesity and reproductive health in women.

Pathogenesis of Polycystic Ovary Syndrome

A large proportion, 60–80%, of women with PCOS have high concentrations of circulating testosterone, and about 25% have high concentrations of dehydroepiandrosterone sulfate (DHEAS), leading some investigators to surmise that uncontrolled steroidogenesis might be the primary abnormality in this disorder [44, 45]. PCO have a thickened thecal layer, and thecal cells derived from these ovaries

secrete excessive androgens in vitro under basal conditions or in response to LH stimulation [44, 45]. The excessive androgen production that characterizes the polycystic ovary may result from the very high ratio of LH to FSH levels or it may be due to an intrinsic abnormality of the ovary in PCOS.

LH and FSH are synthesized and secreted by gonadotropes in the anterior pituitary gland. Each of these hormones is comprised of a heterodimer of a common α-subunit non-covalently bound to a unique β-subunit. The α-subunit is encoded by a single gene, whereas the unique β-subunits arise from separate genes and confer biological specificity. The pulsatile secretion of GnRH from the hypothalamus is essential for determining the relative proportion of LH and FSH synthesized within the gonadotrope. Consequently, an increased pulse frequency of hypothalamic GnRH favors transcription of the β-subunit of LH over the β-subunit of FSH; conversely, decreased pulse frequency of GnRH favors transcription of the β-subunit of FSH, which decreases the ratio of LH to FSH. Several previous studies have shown that women with PCOS display unusual patterns of gonadotropin secretion characterized by excessive secretion of LH but normal secretion of FSH. This pattern of secretion gives rise to an abnormally high ratio of circulating LH to FSH in the serum of some patients with PCOS [46–49]. Whereas LH regulates the androgenic synthesis of theca cells, FSH is responsible for regulating the aromatase activity of granulosa cells, thereby determining how much estrogen is synthesized from androgenic precursors. When the concentration of LH increases relative to that of FSH, the ovaries preferentially synthesize androgens.

Because women with PCOS appear to have an increased LH pulse frequency, it has been inferred that the pulse frequency of GnRH must be accelerated in the syndrome. It is not clear whether this accelerated pulse frequency is due to an intrinsic abnormality in the GnRH pulse generator or caused by the relatively low levels of progesterone resulting from infrequent ovulatory events. Since progestins decreases the GnRH pulse frequency, low circulating progestin levels in women with PCOS may lead to acceleration in the pulsatility of GnRH, increased levels of LH, and overproduction of ovarian androgens. The relative increase in pituitary secretion of LH leads to an increase in androgen production by ovarian theca cells. Increased efficiency in the conversion of androgenic precursors in theca cells leads to enhanced production of androstenedione, which is then converted by 17β-hydroxysteroid dehydrogenase (17β-HSD) to form testosterone or aromatized by the aromatase enzyme to form estrone. Within the granulosa cell, estrone is then converted into estradiol by 17β-HSD. The adipose tissue is a major endocrine organ that secretes adipocytokines with autocrine, paracrine, and endocrine actions that may modulate the effects of both LH and insulin on ovarian androgen production.

PCOS and obesity. PCOS affects 5–10% of women in their reproductive years and a substantial proportion of these patients are obese, have profound peripheral insulin resistance with consequent hyperinsulinemia from enhanced basal insulin secretion and decreased hepatic insulin extraction [50, 51]. Although anovulatory infertility is common among women with PCOS, the cause of ovulatory dysfunction in these women is poorly understood. Insulin resistance and pancreatic β-cell dysfunction, both independent of obesity and unrelated to the actions of androgens

on insulin dynamics, are strongly associated with PCOS [52–57]. Insulin interacts synergistically with LH within the theca cells of PCO (in which theca cell hyperplasia is usually present) to cause activation of the enzyme, P450c17α, the key enzyme in the biosynthesis of ovarian androgens such as testosterone. Hyperinsulinemia may also have adverse effects in women with PCOS through its action at non-ovarian sites. These include the pituitary through enhancement of pituitary LH pulse amplitude, the liver through suppression of hepatic synthesis of steroid hormone binding-globulin (SHBG) and the adrenal gland through stimulation of P450c17α activity, thereby increasing adrenal androgen production.

Metformin, an insulin-sensitizing agent, has been used for the treatment of PCOS in adult women. Metformin acts by decreasing hepatic glucose production and increasing peripheral insulin sensitivity. Studies in lean and overweight adult women have demonstrated beneficial effects of Metformin, particularly in the prevention of type 2 diabetes. Metformin lowers androgen levels, improves lipid profiles, and increases insulin sensitivity. It may therefore delay or prevent metabolic syndrome and cardiovascular morbidity in the long-term. Metformin appears to be safe in the short term and is generally well tolerated. In addition, several small, short term studies have demonstrated safety and beneficial effects of metformin in girls with PCOS [58, 59]. Although metformin is effective in treating the insulin resistance and the consequent metabolic complications of PCOS, it is less effective in the treatment of anovulation in women with PCOS [58]. This suggests that other factors probably in conjunction with insulin may be involved in the pathogenesis of the ovulatory defect in PCOS.

Currently, the mechanism by which hyperinsulinemia is related to the altered ovarian steroidogenesis in the presence of peripheral insulin resistance that is characteristic of PCOS is unknown. However, the insulin resistance associated with PCOS appears to be tissue selective and has been reported in muscle, adipose tissue and the liver, but not in the ovary [52, 53]. Perhaps, in women with PCOS, the ovary maintains normal sensitivity to insulin raising the possibility that the hyperinsulinemia associated with this disorder directly augments ovarian response to the gonadotropins. Previous studies have shown that insulin acting through its cognate receptors promotes hyperandrogenemia and exacerbates the severity of PCOS by facilitating ovarian and adrenal steroidogenesis and decreasing hepatic production of SHBG thereby increasing free androgen levels [47, 50]. Insulin also inhibits hepatic synthesis of SHBG and thereby increases free or bio-available testosterone levels [60]. Furthermore, insulin decreases hepatic and ovarian synthesis of insulin-like binding protein-1(IGFBP-1), which promotes insulin-like growth factor 1 (IGF-1) actions, such as stimulation of ovarian androgen production [60]. Therefore, hyper-insulinemia contributes to hyperandrogenism and ovarian dysfunction in women with PCOS. A further adverse effect of hyperinsulinemia on the ovary in women with PCOS includes the arrest of ovarian follicle development at 5–10 mm, well before a mature follicle would be expected to ovulate [60] thereby contributing towards anovulation. The arrest of follicular maturation in these women is reminiscent of the follicular response that we recently described in female leptin-deficient mice following gonadotropin stimulation [38]. These findings suggest a functional link

between insulin and leptin-signaling pathways that may be operational in the ovaries of women with PCOS.

Cross talk between leptin and insulin-signaling pathways in PCOS. The JAK-2/STAT-3 pathway is the major signaling mechanism activated by leptin in the hypothalamus to regulate energy and metabolic homeostasis. This pathway is not essential for regulating reproductive function [61–64]. This notion is supported by the findings from a recent study showing that although leptin activation of the STAT-3 signaling pathway is critical in the regulation of body weight and some neuroendocrine functions (thyroid, adrenal, and lactation), hypothalamic STAT-3 signaling is not essential for maintaining reproductive function [65]. This suggests that leptin may regulate reproduction through a STAT-3-independent signaling pathway. A possible alternative to STAT-3 system for leptin signaling is the mitogen-activated protein kinase (MAPK) signaling cascade. The extracellular regulated kinase (ERK) members of the MAPK family are serine/threonine kinases that are activated by a wide range of stimuli and are components of the well-defined Ras/MAPK signaling cascade [66].

Previous studies have shown that when insulin levels are reduced by hypocaloric diet or treatment with insulin sensitizers, the reproductive and metabolic outcomes in obese women with PCOS remarkably improves even before significant weight loss is observed [12]. Although the mechanism underlying this improvement in reproductive profile is unclear, it may involve the MAPK signaling pathway [67]. The MAPKs are mediators of signal transduction from the cytosol to the nucleus. Previous studies have shown that alternative signaling pathways, including the MAPK and protein kinase B (Akt) pathways are associated with LH-induced changes in steroid biosynthesis. This has been corroborated by a more recent study that compared androgen biosynthesis in propagated normal and PCOS theca cells via MAPK kinase (MEK1/2) and ERK1/2 phosphorylation. That study revealed that MEK1/2 phosphorylation was decreased more than 70%, and ERK1/2 phosphorylation was reduced 50% in PCOS cells as compared with normal cells. This suggests a causative role for alterations in the MAPK pathway in the pathogenesis of excessive ovarian androgen production in PCOS. This pathway may be particularly relevant to the reproductive consequences of obesity because an alteration in the MAPK pathway has also been implicated in excessive ovarian androgen production associated with leptin in oocytes [67].

The MAPK signaling pathway is a possible point of cross-talk between leptin and insulin signaling. MAPK signaling can be activated via the long or the short leptin receptor isoforms. The short form of the leptin receptor which is the dominant form expressed in the oocyte is capable of activating MAPK signaling in white adipose tissue, monocytes, and osteoblast precursor cells [68–70]. In a recent study, leptin treatment increased phosphorylated MAPK content and enhanced oocyte maturation at all stages of follicular development [40]. Significantly, this leptin-stimulated oocyte maturation was inhibited by MAPK inhibitor, suggesting that leptin enhances oocyte maturation via activation of the MAPK pathway.

Another potential point of cross talk between leptin and insulin involves insulin signaling. Leptin has been reported to activate some components of insulin signaling, including insulin receptor substrate-1 (IRS-1) and insulin receptor substrate-2 (IRS-2) [71]. One of the major downstream pathways activated by IRS proteins involves recruitment of the regulatory subunit of PI3K to tyrosine-phosphorylated IRS proteins, leading to subsequent activation of the lipid kinase and the phosphorylation of phosphatidylinositol 4,5-bisphosphate to phosphatidylinositol 3,4,5-trisphosphate (PIP_3) [72–76]. Compelling evidence suggests that members of PI3K family control cell cycle progression, differentiation, and survival [77]. Several biological effects of PI3K are mediated through the activation of the downstream target Akt [78]. Akt, a serine/threonine protein kinase, also known as protein kinase B (PKB), is the mammalian homolog of the transforming viral oncogene v-Akt. Akt is recruited to the plasma membrane by the products of PI3K in response to a variety of stimuli (hormones, growth factors, and cytokines). Then, Akt is phosphorylated at threonine 308 and at serine 473 by a still undefined kinase [79, 80]. This double phosphorylation activates Akt. The significant role of this system in granulosa cell differentiation was recently demonstrated by Zeleznik et al. [81]. They found that when adenovirus vectors were employed to modulate specific intracellular signaling systems in undifferentiated granulosa cells, the dominant active PKB vector amplified FSH-induced aromatase and LH receptor mRNA levels. Conversely, the dominant negative PKB vector completely abolished the actions of FSH suggesting that PKB is an essential component of the FSH-mediated granulosa cell differentiation.

Conclusions

Clinical and experimental data support the concept that leptin plays a major role in regulating reproductive function. Although previous studies suggest that one target site for leptin's action on reproductive processes is the hypothalamus (via the regulation of gonadotropin secretion) studies from our laboratory and others suggest that leptin may also act directly on the ovary as well. Based on these findings, we hypothesize that leptin is essential for maintaining the normal response of ovarian follicles to FSH and LH signaling. In the absence of leptin, folliculogenesis is compromised. Obese patients have elevated serum leptin levels but may exhibit leptin resistance so that some biological effects of leptin are compromised. These individuals are predisposed to PCOS and metabolic syndrome. A systematic study of the molecular pathways involved in leptin signaling in the ovary is essential to define the role of leptin as a causative factor in the altered sex hormone profiles and amenorrhea seen in obese women. Specifically, studies designed to unravel the complex interactions between hyperleptinemia, hyperinsulinemia, and hyperandrogenemia in PCOS not only elucidates the intricate pathophysiology, but may also potentially lead to the development of novel therapeutic approaches for the treatment of obesity-related reproductive disorders.

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