0163-769X/87/0802-0132$02.00/0
Endocrine Reviews

Vol. 8, No. 2
Printed in U.S.A.

The Gonadotropic Function of Insulin*

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I. Introduction

DURING the last decade, it has become apparent that the pituitary gonadotropins, FSH and LH, are not the exclusive regulators of ovarian function. Several other hormones and various growth factors also play a role in ovarian physiology. Among these hormones and growth factors are insulin and insulin-like growth factors (IGFs).

It is conceivable that a deficiency or an excess of insulin or IGFs could significantly alter ovarian function. In fact, insulin-dependent diabetes mellitus (IDDM), a disorder characterized by insulinopenia, is associated with clinical manifestations of ovarian hypofunction: primary amenorrhea, late menarche, anovulation, low pregnancy rate, and early menopause. Furthermore, insulin resistance, the cardinal feature of which is hyperinsulinemia, is associated with clinical manifestations of ovarian hyperstimulation, primarily hyperandrogenism.

It is becoming increasingly clear that insulin plays an important role in ovarian physiology; however, the mechanisms of insulin action on the ovary remain largely hypothetical. We will review clinical observations and experimental studies that shed light on the role of insulin in ovarian function.

II. Clinical Observations and *in Vivo* Studies

The idea that insulin influences ovarian physiology stems from clinical observations among patients with IDDM and patients with various syndromes of insulin resistance.

A. IDDM

Early observations among patients with IDDM demonstrated that insulin deficiency was associated with ovarian hypofunction. In the preinsulin era, amenorrhea was common among young women with diabetes. In a 1925 review of their experience regarding growth, development, and prognosis of children with diabetes, Joslin *et al.* (1) wrote, "Unaided by insulin, no girl in our series developed menstruation after the onset of diabetes." After 2 months to 1 yr of insulin therapy, menstruation commenced in four girls ranging in age from 13 to 17 yr.

As insulin therapy became widely available, this picture changed; menarche developed in most girls with diabetes, although at a somewhat later age than in normal controls. In 1954, Bergqvist (2) reported that among nondiabetic girls (n = 114), the average age of menarche was 13.9 yr, whereas among diabetic girls (n = 33), the average age of menarche was 15.0 yr; (the difference was statistically significant).

In 1982, Djursing and co-workers (3) reported that menarche still occurred about 1 yr later in diabetic women than it did in nondiabetic women. The average age of menarche among nondiabetic, anovulatory controls (n = 45) was 13 yr, whereas among diabetic women, either ovulatory (n = 9) or anovulatory (n = 22), the average age of menarche was 14 yr (the difference was not statistically significant). The disappearance, from 1954 to 1982, of a significant difference in age of menarche between diabetic women and nondiabetic women could reflect either the later study's smaller number of patients or improved management of diabetes in recent years.

In 1954, Bergqvist (2) examined also the number of pregnancies and the age of onset of menopause among diabetic women. Among 169 diabetic women (ranging in age from 18–45 yr), the number of pregnancies with a duration of 28 weeks or more was 48, instead of a predicted 77. Regarding the age of onset of menopause, the author concluded, "On the whole it seems that the menopause occurs at a slightly lower age in diabetic women than in the comparative group of nondiabetic women" (the difference was not statistically significant) (2).

Sex hormone patterns among women with diabetes have been the subject of surprisingly few studies.

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* Supported in part by a grant from the United Parcel Service.

† Recipient of a First Independent Research Support and Transition (FIRST) Award from the National Institutes of Health (HD-22738).

Walton and co-workers (4), in a study of endocrine abnormalities associated with hemochromatosis, reported that estradiol levels were lower among women with diabetes than among women with hemochromatosis or cirrhosis (122 *vs.* 146 or 130 pmol/liter). Normal controls were not studied, unfortunately, rendering interpretation of the study's results somewhat difficult. The low estradiol levels among women with diabetes, however, lent support to the idea that ovarian hypofunction was associated with the insulin-deficient state.

Zumoff and co-workers (Zumoff B. Z., L. Miller, L. Poretsky, C. D. Levit, E. H. Miller, U. Heinz, M. Kalin, and R. S. Rosenfeld, personal communication) found that follicular phase progesterone levels were significantly lower among women with IDDM than among normal controls. Progesterone levels correlated negatively with glycosylated hemoglobin, suggesting that some factor involved in glucose metabolism (such as insulin) contributed to the low progesterone levels.

Djursing and co-workers (3) found that median levels of FSH, LH, estradiol, and testosterone were lower among anovulatory diabetic women than among ovulatory diabetic women, ovulatory controls, or anovulatory controls. The authors concluded that the deficiency in ovarian hormone production among the anovulatory diabetic patients was associated with "either a derangement in gonadal-pituitary feedback mechanism or a depression of pituitary function at or above the pituitary level."

To evaluate the relationship between insulin and the pituitary-gonadal axis, Rueda and Urdinola (5) measured insulin levels and LH levels among patients with polycystic ovarian syndrome (PCO); there was no correlation between the two hormones.

Distiller and co-workers (6) examined pituitary responsiveness to LHRH among diabetic women (n = 12), diabetic men (n = 7), and normal controls. Both the men with diabetes and the women with diabetes had significantly blunted responses of LH and FSH to LHRH. Maximum changes in LH correlated inversely with fasting plasma glucose levels.

Several investigators have examined the effects of insulin deficiency on the ovary in animals. Liu and coworkers (7) demonstrated that ovarian weights and ovary/body weight ratios were significantly lower among rats with Alloxan-induced diabetes than among nondiabetic animals. Response of ovarian weight to FSH also was impaired in diabetic animals. Insulin reversed these abnormalities, producing a dose-related increase in the ovary/body weight ratio, which increased until it normalized. Circulating levels of LH and of FSH were similar among diabetic animals and controls. The authors concluded that "... the decreased ovarian weight in the diabetic immature rats was probably not due to a lack of pituitary gonadotrophic stimulation, but mainly due to an insulin deficiency." They hypothesized that insulin deficiency in some way decreased responsiveness of the ovaries to gonadotropins.

Bestetti and co-workers (8) evaluated gonadotropic function in female rats with streptozotocin-induced diabetes. Median eminence content of LHRH was low and plasma LH response to LHRH was inadequate, suggesting that gonadotropin deficiency was the cause of ovarian hypofunction. The arcuate nucleus and the median eminence contained numerous degenerate axons, and LH gonadotroph cells were altered (these changes were evident to an even greater degree among diabetic male rats). The authors hypothesized that the abnormalities were caused by insulin deficiency.

Another hypothetical cause of ovarian hypofunction in IDDM is a concomitant deficiency of IGF-1, a substance shown to have gonadotropic activity in *in vitro* studies (9). Maes and co-workers (10) recently showed that rats with experimental IDDM do indeed have low levels of IGF-1. Tan and Baxter (11) reported that the mean serum IGF-1 level was significantly lower among patients with IDDM than among normal controls (0.41 $\pm$ 0.17 U/ml *vs.* 0.84 $\pm$ 0.26 U/ml, $P < 0.001$).

The association between functional ovarian abnormalities and IDDM suggests that insulin plays a role in the regulation of ovarian function. It has been extremely difficult to determine, however, which of the many metabolic derangements of uncontrolled diabetes underlies the abnormalities in ovarian function. Hyperglycemia, insufficient body weight, insulin deficiency, gonadotropin deficiency, IGF-1 deficiency, or any combination of these factors could conceivably affect the ovary. Contributing further confusion to the issue is the fact that some patients with IDDM are hyperinsulinemic, due either to overzealous treatment or to appropriate treatment in response to insulin resistance (12). Thus, patients with IDDM today actually constitute a heterogeneous group in terms of serum insulin levels, which may be low, normal, or high. This heterogeneity may account for the fact that the overall ovarian function in treated patients with IDDM differs only marginally from that of normal controls.

B. Insulin resistance

Further evidence supporting the idea that insulin affects ovarian function has arisen from clinical observations among patients with various syndromes of insulin resistance and acanthosis nigricans. These patients exhibit features of ovarian hyperstimulation, primarily hyperandrogenism. Patients with syndromes of insulin resistance and acanthosis nigricans are heterogeneous in terms of etiology of insulin resistance and clinical pres-

entation but homogeneous in terms of serum insulin levels: all are hyperinsulinemic. Several different entities can be distinguished.

Patients with the type A syndrome are young, thin women with PCO or hyperthecosis and a genetic deficiency in the number of insulin receptors (13). Patients with the type B syndrome are usually somewhat older than those with the type A syndrome. Type B patients have features of autoimmune disease, and their insulin resistance is caused by insulin-receptor autoantibodies (14–16). Another group comprises several patients whose clinical picture resembles that of the type A syndrome, but their number of insulin receptors is normal (17–23). It is thought that the patients in this latter group have postbinding abnormalities in the insulin action pathway; indeed, in some cases, investigators already have demonstrated abnormalities in the tyrosine-kinase activity of the insulin receptor β-subunit (19, 20, 24). Other unusual syndromes of hyperinsulinemia and hyperandrogenism include lipoatrophic diabetes (25, 26), leprechaunism (27, 28), and the Rabson-Mendenhall syndrome (29, 30).

The association between hyperinsulinemia and hyperandrogenism is not limited to unusual syndromes. Acanthosis nigricans, insulin resistance, and hyperandrogenism occur also in approximately 5% of obese women; these women constitute probably the largest subset of patients with the syndrome (31). Their insulin receptor number is down-regulated by the hyperinsulinemia of obesity and normalizes with caloric restriction.

Another common disorder, PCO, also is characterized by varying degrees of hyperandrogenism and insulin resistance (32). Burghen and co-workers (33) found insulin resistance among both obese patients and nonobese patients with PCO. Plasma insulin levels correlated with plasma testosterone levels and androstenedione levels. Dunaif and co-workers (34) similarly demonstrated that insulin resistance, even in the absence of obesity, was common among women with PCO.

It has been of considerable interest that in spite of heterogeneity in clinical picture and in insulin receptor status, various degrees of hyperandrogenism accompany each of the hyperinsulinemic states (25). The source of the excessive androgens initially was unclear. Taylor and co-workers (35) found that among women with the type B syndrome, only those who were premenopausal exhibited hyperandrogenism. The difference between premenopausal women and postmenopausal women pointed to the ovary as the source of excessive androgen formation, a hypothesis confirmed by subsequent studies.

McNatty and co-workers (36) catheterized the ovarian and the adrenal veins in a patient with hyperandrogenism and insulin resistance and demonstrated that the ovary indeed was the source of the patient's excessive testosterone and androstenedione. Similar findings were observed in a patient with autoantibodies to the insulin receptor (35).

These clinical observations have demonstrated that insulin resistance, regardless of its cause, is closely associated with hyperandrogenism. Two distinct points of view concerning the basis of this association have evolved.

One hypothesis asserts that hyperandrogenism produces insulin resistance (33). This hypothesis has been supported by at least two observations. The first observation, reported by Cole and Kitabchi (37), was that administration of estrogen and progesterone to a patient with PCO caused remission of insulin resistance. The second observation was reported by Shoupe and Lobo (38). They found that administering the androgen antagonist spironolactone to patients with PCO significantly reduced not only testosterone levels, but also fasting insulin levels. These two studies thus showed that reducing the degree of hyperandrogenism reduced also the degree of insulin resistance.

The hypothesis that hyperandrogenism causes insulin resistance does not explain, however, either the cause of hyperandrogenism or the mechanism by which hyperandrogenism could produce insulin resistance. The latter is particularly problematic in cases in which the cause of insulin resistance is apparent (*e.g.* insulin receptor autoantibodies or a genetic deficiency in the number of insulin receptors).

Two recent studies argue against the hypothesis that hyperandrogenism causes insulin resistance. Stuart and co-workers (39) matched patients having acanthosis nigricans with controls according to weight and androgen levels. The hyperandrogenized, obese controls exhibited significantly less hyperinsulinemia than did the acanthotic subjects, suggesting that hyperandrogenism or obesity was not the cause of insulin resistance in the acanthotic group. In the second study, Geffner and co-workers (40) administered a long acting LHRH agonist to three patients with PCO and hyperinsulinemia. Despite suppression of ovarian androgen production, hyperinsulinemia persisted. These two studies thus contradict the hypothesis that hyperandrogenism causes hyperinsulinemia.

The second hypothesis regarding the association between hyperandrogenism and insulin resistance proposes that hyperinsulinemia produces ovarian hyperstimulation, manifested principally as hyperandrogenism (35, 41). A recent study designed by Nestler and co-workers (42) to test this hypothesis failed to find any supporting evidence. These investigators administered insulin via the hyperinsulinemic-euglycemic clamp technique for 12 to 16 hr to four normal women and one woman with hyperandrogenism, insulin resistance, and acanthosis ni-

gricans. Although each woman became hyperinsulinemic (the average serum insulin level was 1832 ± 292 μU/ml), none demonstrated a rise in serum testosterone. The meaning of these findings, as noted by the authors, is unclear, because the length of time during which hyperinsulinemia was maintained was extremely brief. Perhaps months or years of hyperinsulinemia are necessary to produce hyperandrogenism.

The hypothesis that hyperinsulinemia produces hyperandrogenism remains attractive, even in view of these findings, because the idea provides a unifying explanation for hyperandrogenism in all of the above mentioned syndromes. The hypothesis raises two questions, however. The first question is: Why would hyperandrogenism be the principal manifestation of ovarian hyperstimulation? There are several proposed mechanisms explaining this phenomenon, and they are discussed below (see *Insulin or IGFs and Ovarian Steroidogenesis*). The second question is: How could the ovary remain sensitive to insulin in insulin-resistant states? To explain this paradox, it was postulated that insulin acts upon the ovary by binding not only to ovarian insulin receptors, but also to ovarian IGF-1 receptors (35). This hypothesis incorporates the knowledge that insulin, in high concentrations, interacts with IGF-1 receptors (the specificity spillover phenomenon) (43) and that IGF-1 stimulates steroidogenesis (9). Thus, one of the first steps in *in vitro* studies examining the effects of insulin on ovarian function was identification of insulin receptors and IGF-1 receptors in the ovary.

III. *In Vitro* Studies

Insulin initiates its action by binding to an insulin receptor. The insulin-receptor complex is rapidly brought from the plasma membrane into the cell (internalization), and the insulin molecule is degraded. The receptor may then either undergo degradation or be recycled to the cell membrane (44).

It is thought that the insulin signal is transmitted via a recently discovered phenomenon involving phosphorylation of the insulin receptor β-subunit. Kasuga and coworkers (45) reported that insulin-stimulated insulin receptor phosphorylation occurred in a dose-dependent fashion and reached maximum intensity the first minute after insulin binding (45). Found to be a tyrosine-specific protein kinase, the receptor apparently can acutely initiate phosphorylation of various intracellular proteins. It still is unclear as to which postbinding event initiates insulin's metabolic effects; at this time, however, phosphorylation of the insulin receptor β-subunit appears to be the most likely candidate.

Insulin binds also to the IGF-1 receptor, although with an affinity lower than that with which it binds to the insulin receptor (43, 46). Insulin and IGF-1 are structurally similar: positions 1 to 29 of the IGF-1 molecule are homologous to the insulin β-chain; positions 42 to 62 are homologous to the α-chain; and the three-dimensional structures of the two peptides are virtually identical (47). Also sharing many features are the peptides' receptors (48). Both insulin receptors and IGF-1 receptors are heterotetramers, consisting of two α-subunits (mol wt ~ 130,000) and two β-subunits (mol wt ~ 90,000). The α-subunits serve as hormone-binding sites, whereas the β-subunits undergo autophosphorylation (of their tyrosine residues) and serve as tyrosine kinases (45, 49). Although insulin and IGF-2 also are structurally similar, their receptors differ, and insulin does not bind to the IGF-2 receptor (44).

A. Insulin receptors and IGF-1 receptors in the ovary

Because insulin initiates its action by binding to either insulin or IGF receptors, tissues serving as target organs for insulin are expected to contain either one or both receptors.

1. Insulin receptors and IGF-1 receptors in animal ovarian tissue. Both insulin receptors and IGF-1 receptors have been identified in animal and in human ovarian cells. Rein and Schomberg (50) identified insulin receptors in porcine granulosa cells. The [^{125}I]insulin binding was saturable and dependent on pH, temperature, and incubation time (optimal conditions were: 2 h, 25 C, pH 7.5). The level of insulin-receptor binding remained constant during the course of follicular development.

Veldhuis and co-workers (51) found IGF-1 receptors (using [^{125}I]somatomedin-C) in swine granulosa cells. Low affinities for insulin and for multiplication-stimulating activity and absent affinity for desoctapeptide insulin demonstrated specificity of the binding sites. Davoren and co-workers (52) found specific high affinity, low capacity IGF-1 binding sites in granulosa cells from immature hypophysectomized estrogen-treated rats.

Insulin receptors and IGF-1 receptors thus have been clearly demonstrated in animal granulosa cells.

2. Insulin receptors and IGF-1 receptors in human ovarian tissue. We previously have reported finding specific insulin-binding sites in human ovarian tissue: in stromal tissue from normal women, in stromal tissue from women with PCO, in follicular tissue from normal women, and in purified human granulosa cells (53, 54). Receptors were abundant in all types of ovarian cells and were characterized by the following: high affinity to insulin (ED_{50} ~ 10 ng/ml); low affinity to the insulin analog proinsulin (ED_{50} ~ 100 ng/ml); and recognition by antiinsulin receptor antibodies (serum *B*-2), but not by monoclonal anti-IGF-1 receptor antibodies (α-IR-3) (53).

Jarrett and co-workers (55) found insulin-binding sites in cell membrane fractions from normal human ovaries. Maximum binding occurred by 4 h at 15 C and was similar to that of other traditional insulin target tissues. Insulin binding with luteal phase ovarian cell membranes resembled that with follicular phase ovarian cell membranes.

Initial attempts to demonstrate IGF-1 receptors in human ovarian tissue showed only minimal [^{125}I]IGF-1 binding in granulosa cells from two women undergoing *in vitro* fertilization procedures (53). Later, however, IGF-1 binding sites were found in ovarian stroma from a patient with hyperthecosis and insulin resistance (Pepper, G. M., L. Poretsky, J. L. Gabrilove, and M. M. Ariton, submitted for publication). Subsequent experiments clearly identified high affinity IGF-1 receptors in purified human granulosa cells (Gates, G. S., S. Bayer, M. Seibel, L. Poretsky, J. S. Flier, and A. C. Moses, submitted for publication). Insulin receptors and IGF-1 receptors thus have been clearly demonstrated in the human ovary.

B. Insulin receptor phosphorylation

The earliest known postbinding event in the insulin action pathway is phosphorylation of the insulin receptor β-subunit (45), a process thought to serve as the signal for insulin action. Among some insulin-resistant patients, insulin receptor binding characteristics are normal, but insulin receptor phosphorylation or tyrosine kinase activity is defective (19–21); these findings suggest that defective insulin receptor phosphorylation or defective tyrosine kinase activity is the cause of insulin resistance in some cases. Insulin receptor phosphorylation or tyrosine kinase activity has been demonstrated in mononuclear cells (56), lymphocytes (56), erythrocytes (57), or fibroblasts (58). Two recent studies have demonstrated insulin receptor phosphorylation in swine ovarian cells or human ovarian cells.

Veldhuis and co-workers (59) showed that insulin stimulated phosphorylation of the insulin receptor β-subunit in cultured swine granulosa cells. Neither somatomedin-C (IGF-1) nor desoctapeptide insulin produced the effect, demonstrating specificity of the reaction. We reported that insulin stimulated phosphorylation of the insulin receptor β-subunit in purified human stromal ovarian membranes (53). These studies thus have demonstrated insulin receptor phosphorylation both in human and in animal ovarian tissues; in regard to phosphorylation, therefore, insulin interaction with its ovarian receptor resembles that with receptors in other target tissues.

C. Insulin or IGFs and ovarian steroidogenesis

The idea suggested by *in vivo* observations, that insulin affects ovarian hormone production, logically led to *in vitro* studies of the effects of insulin on ovarian cell steroidogenesis. It is known that ovarian granulosa, theca, or medullary tissue from premenopausal women, postmenopausal women, or women with PCO can secrete steroids in culture (60–63). Studies involving short term cultures of animal or human ovarian tissue have examined the effects of insulin or IGFs on ovarian cell steroidogenesis.

1. Effects of insulin or IGFs on steroidogenesis in animal ovarian tissue. Insulin-stimulated progesterone secretion has been demonstrated in porcine granulosa cells, in bovine granulosa cells, and in porcine thecal cells. Channing and co-workers (64) reported that insulin produced a 7- to 8-fold increase in progesterone secretion in cultured porcine granulosa cells. Veldhuis and co-workers (65) also studied the effects of insulin on cultured porcine granulosa cells; they found augmentation not only of progesterone secretion but also of progesterone synthesis (65). The peptide's effects were saturable and dose- and time-dependent. The process required synthesis of protein and RNA, augmentation of mitochondrial cytochrome P-450 content, and utilization of cholesterol in the side chain cleavage reaction.

Savion and co-workers (66) reported that insulin increased progesterone secretion by cultured bovine granulosa cells, and Barbieri and co-workers (67) reported that insulin increased progesterone secretion by cultured porcine thecal cells.

Insulin's stimulatory effects on ovarian steroidogenesis are not limited to progesterone production. Davoren and co-workers (52) showed that in cultured rat granulosa cells, insulin increased estrogen production, and Barbieri and co-workers (67) showed that in cultured porcine thecal cells, insulin increased androstenedione production.

These studies thus have demonstrated insulin-stimulated steroidogenesis in cultures of animal ovarian cells.

The regulation of granulosa cell function by IGFs has been the subject of many studies and an excellent review (9). IGFs stimulate steroidogenesis in porcine, bovine, or rat granulosa cells *in vitro* and act synergistically with FSH or LH (68–70).

2. Effects of insulin or IGFs on steroidogenesis in human ovarian tissue. Few studies have examined the effects of insulin or IGFs on steroidogenesis in human ovarian tissue. Barbieri and co-workers (71) studied the effects of insulin on incubated ovarian thecal or stromal fragments from a patient with hyperandrogenism, insulin resistance, and acanthosis nigricans. Insulin increased

accumulation of androstenedione, testosterone, progesterone, and estradiol in the thecal cell incubations, and increased accumulation of androstenedione, testosterone, and dihydrotestosterone in the stromal cell incubations. These investigators also incubated stromal fragments from four patients with hyperandrogenism and found that insulin increased accumulation of testosterone and androstenedione (72).

We reported that low concentrations (10 ng/ml) of insulin or IGF-1 stimulated progesterone production by cultured human granulosa cells. Monoclonal antibody to the IGF-1 receptor blocked the steroidogenic effect of IGF-1, but not of insulin, suggesting that each hormone induced progesterone synthesis through its own receptor (73).

These studies have established clearly that insulin or IGFs can stimulate ovarian steroidogenesis in various *in vitro* cell systems. The mechanisms by which insulin could possibly stimulate ovarian steroidogenesis are reviewed below.

3. Possible mechanisms by which insulin or IGFs stimulate ovarian steroidogenesis. The mechanisms by which insulin or IGFs stimulate ovarian steroidogenesis remain largely unknown. Possible mechanisms can be broadly classified into nonspecific or specific effects of insulin or IGFs on steroidogenesis. The nonspecific effects comprise the classic actions of insulin on glucose transport, on amino acid uptake, and on DNA synthesis; these effects would improve cell viability and consequently enhance steroidogenesis. The specific effects include a direct effect of insulin on steroidogenic enzymes (*e.g.* aromatase activity), synergism between insulin and LH or FSH, or modulation of LH receptor induction by insulin.

Nonspecific effects of insulin on ovarian cells. The first two possible nonspecific effects whereby insulin could enhance steroidogenesis are through enhanced glucose or amino acid metabolism. Allen and co-workers (74) found that insulin stimulated transport of 2-deoxyglucose and α-amino-isobutyric acid in cultured granulosa cells from unbred heifers. Weber and co-workers (75) showed that IGF-1 (somatomedin-C) or insulin increased glucose oxidation in cultured porcine granulosa cells. These studies thus demonstrated the presence of insulin's classic effects in ovarian cells.

Several studies have assessed the effects of insulin or IGFs on the viability and morphology of cultured ovarian cells.

Channing and co-workers (64) demonstrated that insulin changed the morphology and the viability of cultured porcine granulosa cells. Insulin caused the cells to grow in an epitheloid manner and increased the number of intracellular lipid granules. After 2, 4, or 6 days in culture, insulin produced a dose-dependent increase in cell number; the effect was attributed to improved plating efficiency and enhanced cell viability.

Savion and co-workers (66) demonstrated that insulin enhanced proliferation of cultured bovine granulosa cells. An insulin concentration of 0.03 μg/ml significantly stimulated cell proliferation, whereas a concentration of 1.0 μg/ml saturated the effect. Insulin increased cell density 6-fold and shortened the average population doubling time from 41 to 28 h. Somatomedin-C (IGF-1) in concentration as low as 1.0 ng/ml also was mitogenic, and the increase in cell number was dose dependent. Saturation of this effect was not achieved at the highest concentration tested (60 ng/ml). Somatomedin-C appeared to be more potent than insulin in stimulating cell proliferation. Insulin or somatomedin-C acted synergistically with fibroblast growth factor, epidermal growth factor, or high density lipoprotein; any of the latter three factors alone had no stimulatory effect on cell number.

May and Schomberg (76) demonstrated that insulin dramatically influenced monolayer growth and maintenance in porcine granulosa cell cultures; the effect was observed only during 24–146 h of incubation. Insulin also induced cultured swine granulosa cells to adopt an epitheloid appearance.

In summary, these studies showed that insulin or IGF-1 significantly affected the morphology and the viability of cultured porcine or bovine granulosa cells. Under the influence of insulin or of IGF-1, the cells acquired an epitheloid appearance; the number of lipid droplets and vacuoles increased; and cell plating efficiency, cell maintenance, and cell proliferation were stimulated. The mechanisms of these phenomena are the subject of debate (77).

Specific effects of insulin or IGF-1 on steroidogenesis: Effects of insulin or IGF-1 on aromatase activity. Insulin possibly stimulates ovarian steroidogenesis by influencing aromatase activity. Garzo and Dorrington (78) demonstrated that in cultured human granulosa cells, insulin alone or with FSH stimulated aromatase activity. Studies by Veldhuis and co-workers (65), in contrast, showed that in swine granulosa cells, insulin suppressed aromatization of testosterone to estradiol.

Adashi and co-workers (79) examined the effects of somatomedin C (IGF-1) on aromatase activity in cultured rat granulosa cells and found that the peptide potentiated FSH-induced aromatase activity. As these experiments varied in cell type studied and dose and type of peptide employed, it is difficult to compare their results or draw conclusions about the direction in which insulin or IGF-1 affects aromatase activity. That insulin or IGF-1 did affect aromatase activity in each of these studies, however, suggests that such an effect is involved in the regulation of ovarian cell steroidogenesis.

Synergism between insulin or IGF-1 and LH or FSH. Several studies have demonstrated that insulin and the gonadotropins act synergistically to augment ovarian cell steroidogenesis. Incubation of porcine granulosa cells (64, 65, 80) or rat granulosa cells (52) with insulin and the gonadotropins synergistically augmented progesterone production. Incubation of rat granulosa cells (52) or human granulosa cells (78) with insulin and the gonadotropins synergistically augmented estrogen production. Studies of thecal cells from swine (67) or stromal cells from normally cycling women (71, 72) showed synergistically augmented androstenedione production.

Adashi and co-workers (69) demonstrated synergism between IGF-1 and FSH on induction of progesterone synthesis in cultured rat granulosa cells; progesterone synthesis was stimulated to a level several times higher than the maximal level achieved with FSH alone.

The mechanism for this synergism is largely unknown. Adashi and co-workers (81) showed that IGF-1 enhanced FSH-induced intracellular accumulation of cAMP. This finding is very interesting, as neither insulin nor IGF-1 alone affects intracellular cAMP levels (44), whereas the gonadotropins do (82). Possibly the synergism between the gonadotropins and IGF-1 or insulin (the latter acting through the IGF-1 receptor) is mediated through increased intracellular cAMP production.

It is of interest that IGF-1 acts synergistically not only with the pituitary gonadotropins but also with estradiol [in granulosa cells (83)] and with TSH [in cultured thyroid cells (84)]. These findings suggest the exciting possibility that IGF-1 acts as a universal amplifier of various hormonal stimuli.

Insulin or IGF-1 modulation of LH-receptor induction. A third possible specific mechanism whereby insulin or IGF-1 could stimulate ovarian steroidogenesis is through modulation of LH receptor induction. An increased number of LH receptors would enhance steroidogenic cell responsiveness to LH, resulting in stimulation of steroidogenesis. Support for this hypothesis has come from several studies. Davoren and co-workers (52) found that insulin enhanced induction of LH receptors in rat granulosa cells (52). Adashi and co-workers (85) showed that IGF-1 enhanced FSH induction of LH receptors in cultured rat granulosa cells. Mondschein and Schomberg (86, 87) found enhanced induction of LH receptors with other growth factors, suggesting that this mechanism of action may be common for a variety of peptides.

4. Why does insulin-induced ovarian hyperstimulation manifest primarily as hyperandrogenism? It is not quite clear why insulin-induced ovarian hyperstimulation manifests primarily as hyperandrogenism. One possible explanation involves the idea that insulin inhibits aromatase and could thus produce a relative preponderance of androgens. Several studies investigating this hypothesis have yielded differing results (see *Effects of insulin or IGF-1 on aromatase activity*).

A second possible explanation is that insulin affects exclusively the ovary's androgen-producing cells (stromal or thecal cells). Multiple *in vitro* studies with animal granulosa cells and with human granulosa cells have demonstrated that insulin also stimulates progesterone and estrogen production (see *Insulin or IGFs and ovarian steroidogenesis*), suggesting that the peptide's gonadotropic effects are not limited to the ovary's androgen-producing cells.

A third potential explanation is based on the fact that the mass of androgen-producing cells in the ovary exceeds that of estrogen- or progesterone-producing cells. Thus, even if the gonadotropic action of insulin were directed equally at all ovarian cells, hyperandrogenism would be a predominant feature. The merits of this simple explanation have not been tested.

We propose yet another explanation. It is known that LH induces differentiation of ovarian interstitial cells into androgen-producing cells and stimulates androgen production by these cells (88). As insulin acts synergistically with LH *in vitro* (see *Synergism between insulin or IGF-1 and LH or FSH*), perhaps hyperinsulinemia enhances LH-induced differentiation of ovarian interstitial cells and LH-induced androgen production, resulting in androgen excess. The androgen excess would induce follicular atresia, with loss of estrogen-producing cells. Androgen-producing cells would replace the atretic follicles (88) and could perpetuate the cycle by producing more androgens in response to LH and insulin. Hyperinsulinemia thus would produce an excess of androgens, but not of the other ovarian hormones.

D. Insulin and IGFs in ovarian fluid

The hypothesis that insulin or IGFs affect ovarian steroidogenesis could attain physiological significance only if it were shown that the peptides had access to ovarian steroidogenic cells *in vivo.*

Because some ovarian cells have limited access to the blood supply, it is important to determine whether insulin or IGFs are present in follicular fluid. Diamond and co-workers (89) recently reported that insulin was present in human follicular fluid and that follicular fluid insulin concentrations correlated positively with follicular fluid progesterone levels. Hammond and co-workers (90) showed that *in vitro* porcine granulosa cells produced IGFs and IGF binding-proteins, raising the possibility that the ovary produces IGF-1 *in vivo.* IGF-levels in preovulatory follicular fluid were significantly greater than those in either serum or immature follicles, further supporting this possibility.

These studies provide evidence that insulin and IGFs have access to ovarian steroidogenic cells *in vivo*; the correlation between follicular fluid insulin levels and progesterone levels lends further support to the idea that insulin affects ovarian steroidogenesis *in vivo*.

IV. Summary

We have reviewed the role of insulin in ovarian physiology. Clinical observations and experimental data strongly support the hypothesis that insulin possesses gonadotropic activity, when acting alone or with FSH or LH. This idea is further supported by the recent discovery of insulin in follicular fluid.

The idea that insulin has gonadotropic function can explain a variety of clinical observations, which otherwise are difficult to understand. For example, manifestations of ovarian hypofunction (primary amenorrhea, late menarche, anovulation, low pregnancy rate, and early menopause) in IDDM can be understood if it is accepted that insulin is necessary for the ovary to reach its full steroidogenic potential. The idea that insulin affects ovarian steroidogenesis also helps to understand the observation that hyperandrogenism frequently accompanies each of the various insulin-resistant states, regardless of the latter's etiology (*e.g.* genetic deficiency in the number of insulin receptors, antiinsulin receptor antibodies, obesity, *etc.*). The explanation for this association (Fig. 1) is based on the idea that hyperinsulinemia intensifies ovarian steroidogenesis, which manifests clinically as hyperandrogenism. Continuous stimulation of the ovary by insulin over a long period of time possibly produces morphological ovarian changes, such as hyperthecosis or polycystic changes; these changes commonly are observed among women with insulin resistance.

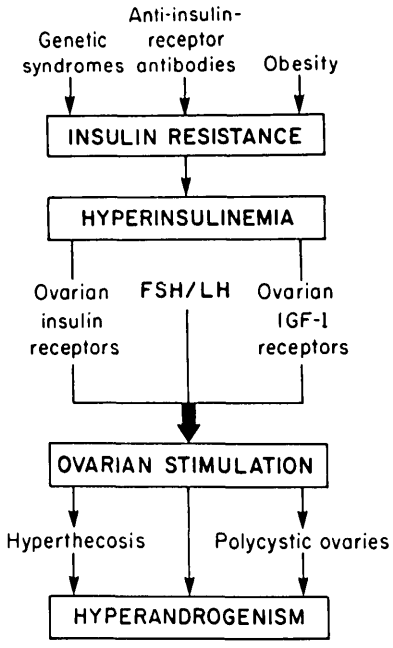

FIG. 1. The proposed pathogenesis of hyperandrogenism in insulin-resistant states is illustrated above. Insulin resistance of any etiology (*e.g.* genetic abnormalities of the insulin receptor, antiinsulin receptor antibodies, obesity, *etc.*) produces hyperinsulinemia. The massive quantity of insulin binds to ovarian insulin receptors and ovarian IGF-1 receptors and acts synergistically with FSH and LH to produce ovarian hyperstimulation. The hyperstimulation may produce hyperthecosis or polycystic changes in the ovary. Hyperandrogenism results either through these changes or directly from ovarian hyperstimulation.

The effects of insulin on ovarian cells are mediated possibly through binding of the peptide to its own receptor or to the IGF-1 receptor (the specificity spillover phenomenon). The latter could be an important mechanism in cases of insulin resistance.

Potential mechanisms underlying the gonadotropic activity of insulin include direct effects on steroidogenic enzymes, modulation of FSH or LH receptor number, synergism with FSH or LH, or nonspecific enhancement of cell viability.

The gonadotropic function of insulin adds yet another note to what has been termed a symphony of insulin action. Further investigation into this new area may yield greater insights not only into normal ovarian physiology, but also into the pathogeneses of such diverse entities as PCO, obesity, diabetes mellitus, and the syndromes of insulin resistance and acanthosis nigricans.

Acknowledgments

We wish to thank Dr. Barnett Zumoff of The Mount Sinai School of Medicine and Dr. Jeffrey S. Flier and Dr. Alan C. Moses of Harvard Medical School for their helpful comments.

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