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Estrogen Regulation of Growth Hormone Action

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GH plays a pivotal role in regulating body growth and development, which is modulated by sex steroids. A close interplay between estrogen and GH leads to attainment of gender-specific body composition during puberty. The physiological basis of the interaction is not well understood. Most previous studies have focused on the effects of estrogen on GH secretion. There is also strong evidence that estrogen modulates GH action independent of secretion. Oral but not transdermal administration of estrogen impairs the metabolic action of GH in the liver, causing a fall in IGF-I production and fat oxidation. This results in a loss of lean tissue and a gain of body fat in postmenopausal women and an impairment of GH effect in hypopituitary women on GH replacement. The negative metabolic sequelae are potentially important because of the widespread use of oral estrogen and estrogen-related compounds.

Estrogen affects GH action at the level of receptor expression and signaling. More recently, estrogen has been shown to inhibit Janus kinase/signal transducer and activator of transcription signaling by GH via the induction of suppressor of cytokine signaling-2, a protein inhibitor for cytokine signaling. This represents a novel paradigm of steroid regulation of cytokine receptors and is likely to have significance for a diverse range of cytokine function. (*Endocrine Reviews* 25: 693–721, 2004)

I. Introduction
II. Endogenous Estrogen and GH Action
  A. GH/IGF-I axis
  B. Growth and body composition
III. Exogenous Estrogen and GH Action
  A. Estrogen effects on GH/IGF-I axis
  B. Biological effects
  C. Physiological implications
  D. Therapeutic implications
IV. Cellular and Molecular Mechanisms
  A. GH receptor and binding protein
  B. GH receptor signaling
  C. Estrogen receptors
  D. Estrogen regulation of GH receptor
  E. Cytokine regulation by nuclear hormone receptors
V. Conclusion

I. Introduction

GH REGULATES GROWTH and body composition through a complex process that combines mitogenic and metabolic actions elaborated through mechanisms dependent and independent of IGF-I. The liver plays a pivotal role in the metabolic process. It is a target organ of GH action, the major source of circulating IGF-I, and a sex steroid-responsive organ.

Estrogens are responsible for the development of secondary sexual characteristics and play a major role in reproductive function in women. There is a close interplay between estrogens and GH in the regulation of growth and development as exemplified in puberty. The increases in GH and estrogen trigger a growth spurt, which is accompanied by dramatic changes in physical development resulting in the attainment of gender-specific body composition.

The regulatory interaction by estrogens on GH may occur at many levels: secretion, clearance, and action. Many previous studies have focused on estrogen effects on GH secretion at the hypothalamic and pituitary level. There is emerging evidence that estrogens modulate GH action independent of secretion through effects exerted on the liver. This has come from observations that orally but not transdermally administered estrogens impair the metabolic action of GH and that estrogen influences responsiveness to GH replacement therapy in adults. The metabolic sequelae of suppressed IGF-I production and lipid oxidation may have potential importance because of the widespread use of oral estrogens and estrogen-related compounds. The interplay between estrogens and circulating peptide growth factors also has relevance for cancer and cardiovascular disease.

There have been major advances in the understanding of how hormones act on cells. Specific signaling pathways and transcriptional mechanisms have been defined for hormones of different classes. Additional interactions between hormone classes can occur in the cell after receptor activation through signaling crosstalk. This represents another level at which the interactive effects between hormones may occur.

This review will cover the impact of estrogens on the

Abbreviations: ALS, Acid labile subunit; AP1, activator protein 1; CIS, cytokine-inducible SH2-containing protein; CRP, C-reactive protein; ECW, extracellular water; ER, estrogen receptor; ERE, estrogen response element; FFA, free fatty acid; GHBP, GH binding protein; GHR, GH receptor; GR, glucocorticoid receptor; HDL, high-density lipoprotein; HNF-4, hepatocyte nuclear factor-4; IGFBP, IGF binding protein; IFNγ, interferon γ; IL-6R, IL-6 receptor; IRS, insulin receptor substrate; JAK2, Janus kinase 2; LDL, low-density lipoprotein; LPL, lipoprotein lipase; OC, oral contraceptive; PI3K, phosphatidylinositol-3′-kinase; PIAS, protein inhibitor(s) of activated STAT; PLC, phospholipase C; PTP, protein tyrosine phosphatase; SH2, Src homology 2; SHP, SH2-containing PTP; SOCS, suppressor(s) of cytokine signaling; Sp1, specific protein 1; STAT, signal transducer and activator of transcription; TACE, TNF-α converting enzyme; UTR, untranslated region; VLDL, very low-density lipoprotein.

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metabolic action of GH in physiological (endogenous) and pharmacological (exogenous) circumstances and recent advances in the understanding of the molecular mechanisms by which estrogens regulate GH action.

II. Endogenous Estrogen and GH Action

Growth can be classified into pre- and postnatal periods. Prenatal growth is not dependent on GH or estrogen. Animals lacking GH or estrogen receptors (ERs) show no discernible growth phenotype at birth, and babies with GH deficiency or insensitivity syndrome (Laron dwarfism) are not small for gestational age (1–4). Postnatal growth comprises infancy, childhood, and pubertal components of growth before the final attainment of height and sexual maturity (5, 6). There are several lines of evidence that gonadal steroids govern the sexual dimorphism in body composition in adults (7). The major support for the hormonal influences on gender-related differences in body composition comes from observations made during puberty when gonadal steroid and GH secretion demonstrate marked sexual dimorphism. The individual action of these hormones and their interaction have a major role in the growth and development during puberty and early adult life. This section will review changes in the GH/IGF-I axis and body composition and the interrelationships with endogenous estrogen throughout a female's lifespan, and, in particular, at the biological milestones of puberty and menopause when major changes in gonadal status occur.

A. GH/IGF-I axis

1. Childhood and adolescence. GH secretion rate varies over a 125- to 150-fold range within healthy children and adults. The influence of age and gonadal steroids on GH secretion has been extensively reviewed in the *Endocrine Reviews* (8). GH secretion rates are stable in the decade before puberty. During puberty, a 1.5- to 3-fold increase in the pulsatile secretion of GH occurs, together with over a 3-fold increase in serum IGF-I concentration, which peaks at 14.5 yr in girls and 1 yr later in boys (9).

The parallel increases in GH and gonadal steroid concentrations suggest regulatory interactions in the secretion of these hormones. Estrogens in girls and androgens in boys were proposed as the major steroid hormones driving GH-mediated postnatal growth. However, more recent studies have suggested that estrogens also play a role in stimulating GH secretion in boys. Gonadal steroid priming enhances the GH response to pharmacological stimuli in both sexes (10, 11). GH and estrogen levels show positive correlations in prepubertal girls and boys (12, 13). Testosterone supplementation stimulates GH secretion and increases IGF-I levels in boys. However, the effect of testosterone is dependent on aromatization to estrogen because treatment of boys with nonaromatizable androgens (oxandrolone and dihydrotestosterone) fails to increase GH secretion (14–16). This is further supported by the studies in pubertal boys and adult men given tamoxifen, an antiestrogen, which abrogates the stimulatory effect of testosterone on GH (17, 18). In addition, 46XY patients with complete androgen insensitivity syndrome have plasma levels of estrogen and IGF-I that are highly correlated and fall in parallel after gonadectomy (19).

2. Adult life. There is ample evidence that GH secretion is regulated by estrogens in adult life. Frantz and Rabkin (20) first reported ambulatory GH levels to be higher in women than in men and noted levels to vary during the menstrual cycle. Stimulated and spontaneous GH secretion is higher in young women than in postmenopausal women or young men, with the difference strongly correlated with circulating estradiol levels (21–23). The difference in levels of GH and IGF-I between men and women is also lost after menopause (21). Careful studies of GH secretion during the menstrual cycle reveal spontaneous GH secretion and IGF-I concentrations to be higher during the periovulatory phase than the early follicular phase (24). In a 6-yr prospective study of perimenopausal women, there was a greater decline in IGF-I levels in those who achieved menopause than in the group who remained premenopausal, demonstrating the independent effects of estrogen on the GH/IGF-I axis from aging (25). Results from these observational studies provide strong support that estrogen or related ovarian factors that are cosecreted with estrogen play a role in the physiological regulation of GH secretion in women.

During pregnancy, there is a dramatic increase in circulating estrogen levels such that by term, the production rate of estradiol of 20 mg/d is approximately 200 times that of the nonpregnant state. In humans, two related genes with tissue-specific expression are responsible for production of distinct molecular isoforms of GH: the GH-N gene in the anterior pituitary, and the GH-V gene in the placenta (26–28). During human pregnancy, secretion of pituitary GH is progressively suppressed and replaced by secretion of placental GH into the maternal circulation (29–31). Placental GH is the major regulator of maternal serum IGF-I levels and has similar metabolic and somatogenic effects as pituitary GH, but no lactogenic activity (32–34). The factors regulating placental GH secretion are not known.

Besides gonadal steroids, GH secretion in adults is strongly influenced by nutrition, adiposity, and physical fitness. Because these factors vary greatly between individuals, they may confound the interpretation of data if their contributions are not recognized. GH secretion declines more rapidly with increasing age in men than in women between the third and fifth decades (35). The possibility that body fat contributes to the apparent age effect is supported by data from studies showing abdominal fat mass to be an independent predictor of integrated 24-h GH secretion (36, 37). These observations may explain why GH secretion declines more rapidly with age in men than in women and why there is a rapid decline in GH secretion during menopause in women, a period when there may be significant gains in visceral fat mass. The interrelation between GH secretion, body composition, and peripheral metabolism is complex and closely interrelated, and the causal relationship is obscure (37).

B. Growth and body composition

GH plays a critical role in childhood growth. Body growth results from a complex interplay of metabolic hormones,

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growth factors, and nutrition on energy and tissue metabolism, resulting in growth of soft tissue, organs, and skeleton, and expansion of body fluid compartments (38). These effects lead to significant changes in body composition (39). Although axial growth of the skeleton stops at the end of childhood, GH continues to stimulate the same metabolic processes and tissue mitogenic effects in the adult (40), which has been the subject of a number of reviews (40–42). In the adult, GH is a major regulator of substrate utilization and body composition, having major effects on fat and protein metabolism. GH stimulates lipolysis to enhance oxidative utilization of fat, which conserves the utilization of body protein for energy metabolism at times of nutrient deprivation. As a potent stimulator of protein synthesis and fat utilization, GH positively regulates body protein and negatively regulates body fat (40, 41). GH plays an important role in sodium homeostasis by stimulating renal tubular reabsorption of sodium that leads to an expansion of the extracellular water (ECW) compartment (43, 44). ECW, protein, and bone mass are the major components of fat-free mass in the body and are all positively regulated by GH. Because GH deficiency in children results in growth retardation, it also disrupts the metabolic process, causing major changes in body composition (45). Very few studies have investigated the impact of GH on the body composition of growing hypopituitary children. These few studies have observed reductions in body fat mass and increases in body cell mass, intracellular and extracellular water, with some of these changes reversing when GH treatment was stopped (46–51). Thus, GH exerts a change in body composition that accompanies the growth process.

1. Childhood and adolescence. There is little difference in the amount or proportion of fat and lean tissue mass between boys and girls during infancy and early childhood. However, a difference in soft tissue composition emerges at the start of puberty, with girls having 6% more body fat than boys by 10 yr of age (38). Leptin levels are similar in prepubertal boys and girls but rise to significantly higher levels by late puberty in females (52). During puberty, a gradual increase in fat mass occurs in girls, whereas the opposite occurs in boys. The reduction in fat mass in boys is accompanied by a reciprocal and almost equal increase in lean body mass, which continues into the early twenties (38, 39). The progressive increase in lean mass after puberty is GH-dependent because it does not occur in adolescents with severe GH deficiency (53). A gender difference in final adult height also emerges at the end of the pubertal growth spurt. That the increase in GH output is similar in both sexes during the pubertal growth spurt indicates that the sexual dimorphism in body composition and height cannot be explained by GH alone. It is likely that the gonadal steroids play some role in this process, acting either directly or indirectly by modifying the action of GH.

There is a close physiological interaction between the GH/IGF-I and gonadal system in the regulation of puberty. Puberty is delayed in boys and girls with GH deficiency or insensitivity (11, 54, 55). GH treatment reduces the delay in the onset of puberty in children with isolated GH deficiency (55). This interaction between GH and gonadal function may be due to GH induction of local production of IGF-I in the ovary and testis (55, 56). Thus, interactions between GH and gonadal function play a major direct role in the regulation of pubertal growth.

There is some evidence that gonadal steroids modulate the growth stimulatory effects of GH. Most of the evidence relates to the effects of androgens. Boys with constitutional delay of growth and development have reduced serum levels of GH, IGF-I, and sex steroids compared with age-matched controls (57). Administration of testosterone leads to significant increases in GH, IGF-I, and growth velocity (14, 58, 59). Adolescent boys with hypopituitarism, however, require full replacement doses of both testosterone and GH to normalize plasma IGF-I levels and achieve maximal growth potential (57, 60). Androgens therefore require the presence of GH to exert their full growth-promoting effects (61). None of these studies reported changes in body composition.

Virtually nothing has been reported on what effect estrogens may have on the growth-promoting effects of GH in hypopituitary girls (11). Examination of the interactions between estrogen and the GH/IGF-I axis have been largely confined to studies of short but otherwise normal girls and girls with Turner's syndrome. Low doses of estrogen accelerate short-term growth in girls with Turner's syndrome as well as in prepubertal boys (62, 63). In contrast, high doses of estrogen inhibit growth rates in adolescent girls and boys with tall stature (64). Children with precocious puberty have 1.5 times higher integrated concentrations of GH, which fall to half the pretreatment level after 6 months of GnRH agonist therapy (65, 66). In normally growing boys progressing through puberty, circulating estradiol levels correlate positively with growth velocity before the time of peak growth velocity and negatively after this time (67). These various findings are consistent with estrogen enhancing growth through augmentation of GH-dependent chondrocyte proliferation at the growth plate. However, because estrogen itself has major, and sometimes opposite, effects on epiphyseal chondrocyte growth, it renders the contribution from GH-mediated mechanisms difficult to determine (38, 64, 68, 69). The interactions between estrogen and GH at the growth plate remain unclear because no evidence exists clarifying the pivotal interdependent roles of these two hormones at the cellular level of the growth plate.

Gonadal steroids and GH are likely to interact during puberty to induce a dramatic increase in growth. However, striking differences in body composition and height emerge in the absence of significant differences in GH output between sexes. Because evidence that sex steroids directly stimulate growth is poor, interplay between the two systems is likely to occur through a modulatory effect, that is, with estrogen attenuating and/or androgens augmenting the action of GH.

2. Adult life. The difference in fat and lean tissue between men and women is maintained throughout adult life. Gender difference in leptin levels also persists throughout adult life (52). Within these broad differences, there are interesting differences in distribution of fat and muscle mass between sexes. Men harbor a larger proportion of fat in the trunk, whereas women deposit more fat in the lower body (70). The

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association between visceral fat mass and total body fat is greater in men for all ages (71, 72). Whereas lean tissue mass is greater in men, more is in the upper body compared with women after adjustments for weight and height (70, 73). Regardless of regional differences in the fat and muscle mass, the differences in body composition that exist between sexes are contrary to what may be predicted from a greater level of GH secretion in women. This observation again suggests a significant effect of gonadal steroids on body composition and possibly an action of estrogen opposite to that of GH.

The influence of estrogens on body composition has been studied by a number of investigators by observing changes in fat mass in women going through the menopause. The results are conflicting because increase (74, 75), reduction, and no change (76, 77) have been reported. Most cross-sectional comparisons report higher body fat and lower lean mass in postmenopausal women in comparison to premenopausal counterparts (75, 76, 78).

In a study of two groups of similar-aged women who differed by menopausal status followed longitudinally, lean body mass declined to a greater extent in the postmenopausal group, whereas body fat increased to a comparable extent in both groups (79). In a prospective longitudinal study of premenopausal women of perimenopausal years, Poehlman *et al.* (74) observed that the group who developed menopause gained more body fat and lost more fat-free mass, and also had greater concomitant reductions in resting energy expenditure and physical activity. Energy intake did not differ from the premenopausal group. This study demonstrates that natural menopause is associated with changes in lifestyle and behavior that significantly affect energy balance, making it impossible to deduce the contribution of estrogen to body compositional change. However, most studies agree that menopause is associated with an altered distribution of body fat toward the abdomen (74, 75, 80).

There is increasing evidence that estrogen directly influences adipose tissue function. Lipoprotein lipase (LPL) activity responsible for the hydrolysis of circulating triglycerides into free fatty acids (FFAs) for uptake and storage by adipose tissue is influenced by sex steroids (81). Men have less LPL activity and lower LPL mRNA levels in both gluteal and abdominal sc adipose tissue than women, and the LPL activity and mRNA levels are greater in abdominal sc than in gluteal fat in men, whereas the reverse is found in young lean women (82). Adipocytes from different regions respond differently to lipolytic agents (83). A recent study comparing *in vitro* abdominal and gluteal sc adipose tissue metabolism from peri- and postmenopausal women (84) found basal lipolysis and the adipose tissue LPL activity to be lower in both abdominal and gluteal sc adipose tissue in postmenopausal women. These collective findings provide a plausible mechanism explaining how estrogen status affects fat distribution in women.

Pregnancy is accompanied by marked changes in hormonal status, energy requirement, and body weight. It is likely that the marked changes in the maternal hormonal milieu produce a suitable environment for the fetus and for the mother throughout pregnancy. During pregnancy, there is an increase in energy need due to an increase in resting metabolic rate, increased deposition of fat, but no major changes in energy intake (85, 86). This is most likely met by changes in total energy expenditure, diet-induced thermogenesis, amount of energy used for physical activity, and energy intake (85). Longitudinal and cross-sectional studies have demonstrated that there is a considerable gain in total body fat mass occurring mainly in the first and second trimesters of pregnancy (85, 87). The change in fat mass is in concert with a parallel loss in fat-free mass that probably reduces the energy requirements of the mother, in turn contributing to acquisition of body fat (87). O'Sullivan *et al.* (88) have shown that a metabolic shift to reduce fat oxidation occurs during pregnancy, and this provides a mechanism for increasing fat stores without a need for significant increase in dietary intake. Serum estrogen levels correlated negatively with fat oxidation, suggesting that endogenous estrogen status may regulate total body fat using varying physiological states.

III. Exogenous Estrogen and GH Action

In the section above, we reviewed evidence for a tight relationship between estrogen and GH status. This included observations that estrogen and GH concentrations are strongly correlated during puberty (89) and that GH levels are higher in women than men (21, 90, 91), and highest in the periovulatory phase of the menstrual cycle when estrogen concentrations are maximal (24, 92). In men, the stimulatory effect of androgens on GH secretion is dependent on prior aromatization to estrogens (17, 93). The collective observations imply that estrogens play a major and positive role in regulating the GH/IGF-I axis in both sexes. In this section, the impact of exogenous estrogen administration on the GH/IGF-I axis and body composition will be discussed.

A. Estrogen effects on GH/IGF-I axis

Oral estrogen administration to Turner's girls and postmenopausal women increased circulating GH levels (94, 95). However, the treatment effects of estrogen on IGF-I were variable, with reports of no change (96) and even a fall in IGF-I levels (94, 95). An early study using a large estrogen dose also reported suppression of IGF-I activity in postmenopausal women (97). Thus, data reporting estrogen effects on GH/IGF-I in women seem confusing in that estrogen treatment augmented GH secretion but was accompanied by variable and even suppressive effects on circulating IGF-I. Many studies have since reported that hormone replacement therapy reduces IGF-I levels (98–100).

The liver is a sex steroid-responsive organ, as well as the major site of GH-regulated metabolism and the principle source of IGF-I (101). Many aspects of hepatic function are perturbed by supraphysiological concentrations of estrogen in the portal circulation after oral administration. Because estrogen is actively metabolized by the hepatic cytochrome system, a dose severalfold in excess of daily production rates must be administered orally to achieve an adequate systemic effect. The delivery of such a large dose into the portal system creates a pharmacological concentration not seen in the natural state. This so-called first-pass effect results in stimulation of the synthesis of angiotensinogen, clotting factors,

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lipoproteins, and the binding proteins for several steroid hormones. These effects are circumvented when estrogen is administered by a nonoral route (102, 103).

GH circulates in blood bound to a high-affinity binding protein (GHBP). The liver is a major source of GHBP, which is derived from proteolytic cleavage of the extracellular domain of the GH receptor (GHR) (see *Section IV.A.2*). GHBP alters the distribution and pharmacokinetics of GH and is likely to modulate GH action (104). Recent evidence has emerged that estrogens exert profound effects on this component of GH/IGF-I physiology in a route-dependent manner.

1. Route of administration. Weissberger *et al.* (105) tested the hypothesis that the route of estrogen administration had a major impact on the GH/IGF-I axis by comparing the effects of oral (ethinyl estradiol, 20 μg/d) and transdermal (17β-estradiol, 100 μg) administration in postmenopausal women. Both replacement regimens resulted in significant and comparable reductions in circulating levels of LH and FSH, indicating systemic bioequivalence of estrogen. Administration of oral ethinyl estradiol resulted in a 3-fold increase in mean 24-h GH concentrations to a level indistinguishable from weight-matched premenopausal women. In contrast, transdermal administration of 17β-estradiol did not affect mean 24-h GH concentrations. Oral estrogen administration resulted in a uniform and significant reduction in mean IGF-I levels, whereas transdermal estrogen delivery induced a small but significant increase in mean IGF-I levels. The mean GHBP activity was similar between pre- and postmenopausal women but increased significantly only with the oral route. The increase in binding activity arose from an increase in GHBP concentrations and not in affinity (106, 107). Thus, estrogen treatment in postmenopausal women induced a significant route-dependent effect on the GH/IGF-I axis. Bellantoni *et al.* (108, 109) have reported that oral but not transdermal estrogen augments GH secretory response to GHRH. The GH response to graded doses of GH-releasing peptide is augmented by oral estrogen, which reduced circulating IGF-I levels (110).

Several investigators have confirmed that an oral, but not a nonoral, route of estrogen administration lowers circulating IGF-I (111–113). Studies in the rat have shown that ip administration of estrogen inhibits hepatic IGF-I mRNA expression (114). Thus, it is likely that production of IGF-I from the liver was suppressed as a consequence of the pharmacological effect of oral ethinyl estradiol administration. A first-pass mechanism is also likely to explain the different effects of oral and transdermal estrogens on serum GHBP, because the liver is rich in GHRs from which GHBP is derived (105, 115). Because GHBP has been shown *in vitro* to blunt GH action (116, 117), complexing of GH could be another mechanism explaining the fall in IGF-I despite an increase in GH concentration. We have proposed that the increase in GH secretion is not a primary effect of estrogen, but stems secondarily from a loss of negative feedback inhibition by IGF-I (105) (Fig. 1).

The fall in IGF-I is opposed by progestogens with androgenic actions (118, 119). Neutral progestogens have no effect, whereas the more androgenic progestogens exert a greater effect. Conversely, in women administered transdermal estrogens, IGF-I increased only with the most androgenic progestogens (119). The relationship between the progestogen androgenicity and IGF-I may explain why increases in IGF-I during progestogen and transdermal estrogen administration are not reported by all studies (105, 108).

2. Different estrogen formulations. The estrogen types used in many of the above studies were not identical (105, 108, 111–113). Consequently, the data do not totally exclude the pos-

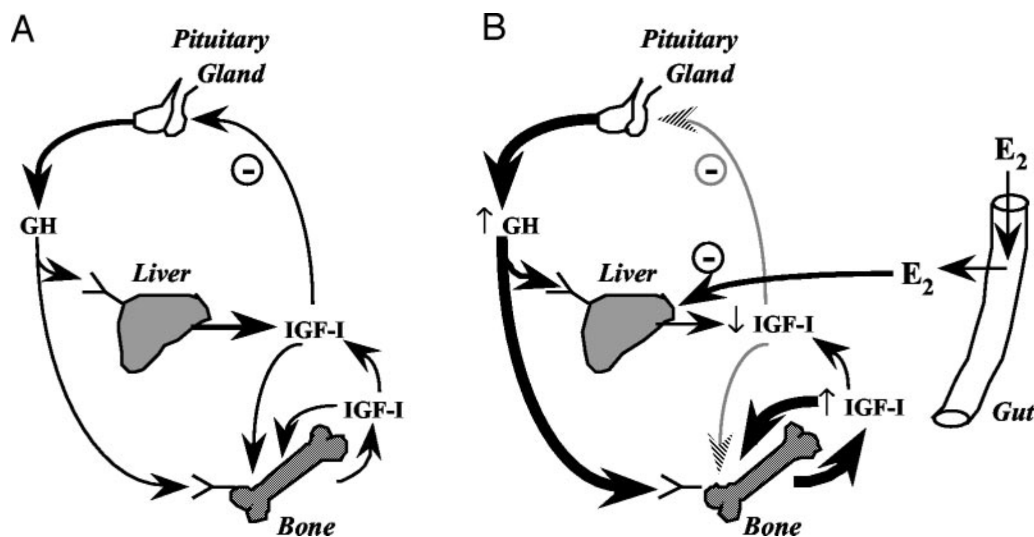

FIG. 1. GH and IGF-I action and the effect of oral estrogen. A, Current concepts surrounding IGF-I-mediated action of GH. Peripheral tissues such as bone respond to the combined effects of liver-derived IGF-I from the circulation (endocrine) and that derived from local generation (paracrine). Both sources contribute to feedback inhibition of GH release from the pituitary. B, Impact of oral estrogen administration, which inhibits hepatic IGF-I production to reduce circulating IGF-I. The loss of feedback inhibition results in a stimulation of GH secretion, which could stimulate peripheral tissues such as bone and muscle to enhance paracrine IGF-I action leading to higher circulating levels of procollagen or increased lean body mass. That this does not occur (see *Section III.B.1*) suggests that endocrine IGF-I is a more important regulator of peripheral tissue stimulation than local IGF-I. E_2, Estradiol.

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sibility that the contrasting effects of oral and transdermal preparations reflect intrinsic chemical differences rather than the dissimilar routes of administration. It has been reported that induction of hepatic protein synthesis by ethinyl estradiol appears to be greater than its ability to suppress gonadotropin secretion when compared with other estrogen types. These hepatic effects were not entirely eliminated when ethinyl estradiol was administered parenterally via the vaginal route (120, 121).

Kelly *et al.* (122) compared the effects of three different oral formulations (ethinyl estradiol 20 μg, conjugated equine estrogen 1.25 mg, and estradiol valerate 2 mg). All three estrogen formulations induced a significant fall in LH and FSH, in parallel with reciprocal elevations of SHBG and angiotensinogen. GHBP rose in parallel with these hepatic proteins. Each of the three estrogen formulations significantly reduced IGF-I levels and increased mean 24-h GH and GHBP concentrations (Fig. 2). The increase in mean 24-h GH concentrations during treatment was significantly and inversely related to the percentage fall in IGF-I levels. This inverse order of effect induced by the three estrogen types provides further support that the stimulation of GH secretion arises from reduced feedback inhibition by IGF-I. The increase in GHBP in response to oral but not transdermal estrogen indicates GHBP to be an estrogen-sensitive hepatic protein similar to SHBG and angiotensinogen (106).

The qualitatively similar responses displayed by all three estrogen formulations indicate that the reduction in IGF-I levels is an intrinsic effect of oral administration on the liver regardless of type (122). If this is true, elevation of estrogen in the systemic circulation to sufficiently high levels should induce a similar effect on hepatic function. Indeed, Friend *et al.* (123) observed that circulating IGF-I levels in postmenopausal women fell when transdermal patches were administered at seven times the therapeutic dose and that this was accompanied by an increase in GH concentrations, changes indistinguishable from those achieved by oral administration of 2 mg 17β-estradiol. Taken together, the results indicate that it is the high estrogen concentration that impairs hepatic IGF-I synthesis regardless of how this is achieved via the portal or systemic circulation.

3. Effects on IGF binding proteins (IGFBPs). Six members of the IGFBP family (IGFBP-1 to -6) have been identified. IGF-I circulates almost entirely as a ternary complex bound to IGFBP-3 and the acid labile subunit (ALS), both of which are strongly GH-regulated. The ternary complex alters the pharmacokinetics, distribution, and tissue availability of IGF-I (124). ALS is synthesized exclusively by the liver (125), whereas many peripheral tissues produce IGFBP-3. ALS is affected by estrogens in a manner similar to IGF-I (126) (Fig. 2). Thus, effects of estrogen on ALS are route- and dose-dependent and unaffected by estrogen formulation. Because ALS and IGF-I are colocalized in hepatocytes (127, 128), the route dependency of estrogen action thus suggests a first-pass hepatic effect similar to that on IGF-I.

The effects of oral estrogen on IGFBP-3 are less consistent. Some (118, 126) (Fig. 2), but not all, studies report a suppressive effect in postmenopausal women (98, 99, 109, 129). The reasons for the conflicting observations are not clear. A

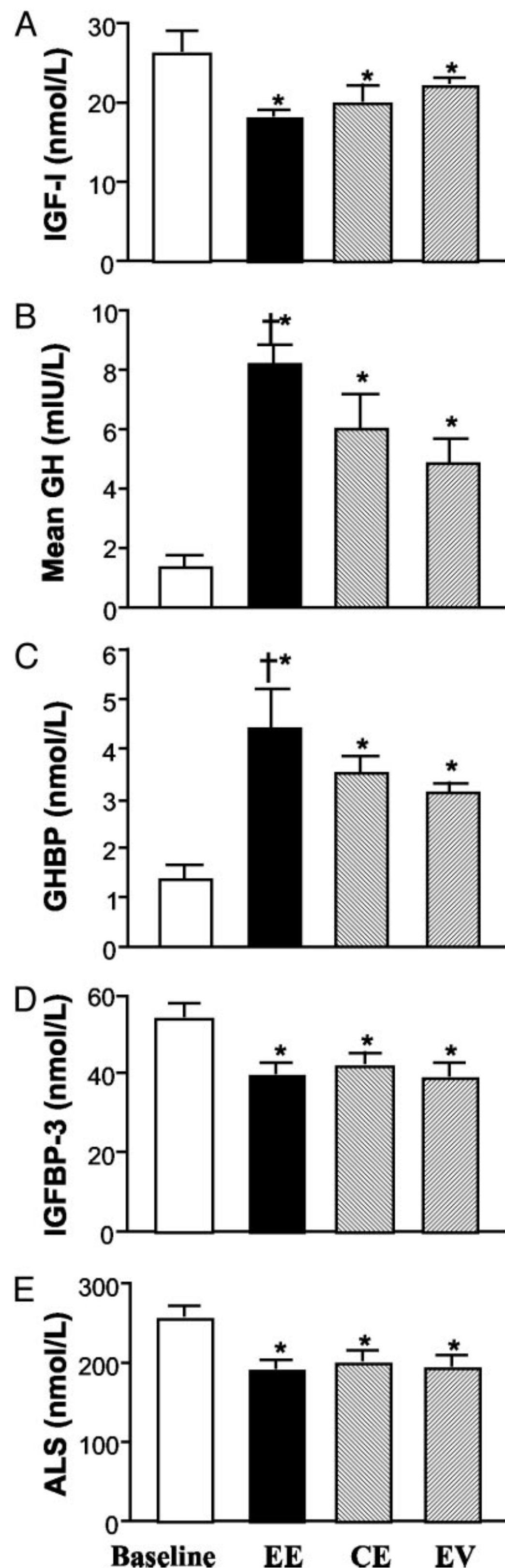

FIG. 2. Mean (± SE) concentrations of IGF-I (A), mean 24-h GH (B), GHBP (C), IGFBP-3 (D), and ALS (E) in six postmenopausal women before and during treatment with ethinyl estradiol (EE), conjugated equine estrogen (CE), and estradiol valerate (EV). *, $P < 0.05$ *vs.* baseline; †, $P < 0.05$ *vs.* EV.

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putative effect on hepatic IGFBP-3 may be diluted out by peripheral tissue sources of the binding protein. It has been shown that IGFBP-3 serves as a passenger protein in the ternary complex with any unbound IGFBP-3 rapidly cleared from the circulation (124). The reduction in IGFBP-3 level observed with oral estrogen could arise secondarily from a primary reduction in IGF-I and ALS levels. In contrast to IGF-I and ALS, IGFBP-3 is synthesized in Kupffer cells rather than in hepatocytes (125, 128) and could be regulated differently by estrogens.

In hypogonadal GH-deficient women, oral estrogen treatment also significantly reduced IGFBP-3 and ALS, indicating that the effects are independent of GH (126). The changes in IGF-I in each of the interventions paralleled the changes in both IGFBP-3 and ALS. Therefore, estrogens exert inhibitory effects on all three components of the IGF-I ternary complex in a route- and dose-dependent manner.

IGFBP-1 is also a liver-derived protein, acutely regulated by nutrition and insulin, both of which inhibit its synthesis. It binds the small fraction of free IGF-I and attenuates the hypoglycemic effect of the growth factor (130). Estrogens exert a route-dependent effect on circulating IGFBP-1 levels; in contrast to its suppressive effect on ALS, the oral route increases circulating IGFBP-1 (111–113, 118). The effect of increased IGFBP-1 can be predicted to reduce further the free fraction of IGF-I, which would be expected to reduce its activity. Thus, estrogens exert profound effects on liver-derived IGFBPs when administered by the oral route, although the effects are variable. Because IGFBPs alter the tissue availability and potency of IGF-I, estrogens are likely to modify the biological action of IGF-I in a route-dependent manner. The other members, IGFBP-2, -4, -5, and -6, are produced in extrahepatic tissues. IGFBP-5, which is derived from skeletal tissue, is GH-regulated, whereas the rest are not. Very little is known about the regulatory interactions between estrogens and these binding proteins (131).

B. Biological effects

The anabolic actions of GH are mediated through IGF-I, whereas other metabolic actions such as lipolysis and induction of insulin resistance do not involve IGF-I. Because three major components of the GH/IGF-I axis (GH, IGF-I, and GHBP) are markedly affected by oral but not transdermal estrogen administration, the question of whether estrogen exerts significant biological effects dependent on its route of administration has been the subject of recent studies.

1. Bone and connective tissue. GH promotes growth of a variety of body tissues, including those of connective and skeletal tissues. Increases in propeptides for type I collagen (the predominant protein matrix of bone) and type III collagen (the major structural protein in soft connective tissue) occur during GH treatment, indicating stimulation of bone and nonbone collagen synthesis (132–134). GH treatment also increases serum osteocalcin, a marker of bone formation (135). It is likely that these anabolic effects of GH are mediated by IGF-I because specific receptors for IGF-I are present in fibroblasts (136, 137) and osteoblasts (138), and IGF-I has been shown to directly stimulate collagen synthesis (139, 140) and replication of these cells (141, 142). Thus, increases in circulatory markers of connective tissue metabolism after GH treatment are likely to reflect the biological effects of IGF-I.

The markers of connective and bone tissue metabolism are influenced by the route of estrogen treatment (143). Levels of osteocalcin, procollagen I and III fell in parallel with IGF-I during oral estrogen, despite a 3-fold increase in circulating GH levels, and rose during transdermal treatment in concert with an increase in IGF-I. Both treatments suppressed gonadotropins to a similar degree, indicating that the systemic effects of estrogen arising from the two delivery routes were equivalent.

The dissociation of the GH/IGF-I axis induced by oral estrogen gives an interesting insight into the relative importance of endocrine and local IGF-I in the control of peripheral tissue growth. According to the somatomedin hypothesis, the fall in serum IGF-I levels induced by oral estrogen will be accompanied by a reduction in peripheral tissue metabolic activity as indicated by reduced levels of bone and connective tissue markers (Fig. 1B). This reduction was in fact observed, and so the finding provides indirect evidence that endocrine IGF-I is a more important determinant of peripheral tissue growth in humans than local IGF-I. These data stand in contrast to those from mice, with conditional knockout of liver IGF-I production displaying little attenuation in body growth (144, 145).

2. Glucose metabolism. The stimulation of GH secretion induced by oral estrogen therapy may impair carbohydrate metabolism because GH causes insulin resistance (146, 147). Studies have failed to find a difference between oral and parenteral estrogen replacement on glucose tolerance in nondiabetic postmenopausal women (112, 148). However, using the euglycemic clamp technique to assess insulin sensitivity, O'Sullivan and Ho (148) observed that the mean glucose infusion rate required to maintain euglycemia was slightly lower during oral than transdermal estrogen although the difference did not reach significance. During the transdermal estrogen phase, mean nonesterified FFA concentration was suppressed to a significantly lower level by insulin. The data suggest a route-dependent effect of estrogen on glucose metabolism with insulin sensitivity lowered during oral estrogen administration.

Studies in young women treated with contraceptive steroids reveal an unequivocal negative effect of more potent doses of estrogens (ethinyl estradiol 20–30 μg/d) on insulin sensitivity. Women taking oral contraceptive (OC) steroids exhibited up to a 30–40% lower insulin sensitivity (149–151). Perseghin *et al.* (149) observed that the estrogen-treated group had higher circulating levels of FFA, β-hydroxybutyrate, and triglycerides than the untreated control group. Because OC steroids reduce IGF-I and increase GH levels (152, 153), we propose that the development of insulin resistance is GH-mediated.

3. Substrate oxidation. Studies of GH replacement in GH-deficient adults show unequivocal evidence that GH plays a major role in regulating substrate oxidation and body composition (154–156). Stimulation of lipid oxidation and protein synthesis during GH treatment leads to a progressive fall

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in body fat and increase in lean body mass in GH-deficient adults. These metabolic effects of GH are imparted by a complex interplay of IGF-I-mediated and direct actions of GH.

GH stimulates resting energy expenditure and the oxidative metabolism of fat (154, 156). The effects of GH on energy balance and substrate utilization are two important mechanisms that lead to a reduction in body fat. The liver plays a pivotal role in fat metabolism and is a major site where fatty acids are oxidized after their release from peripheral fat stores. The effect of estrogen is an interesting situation because of the known stimulatory action of GH and possible first-pass effects of oral administration on hepatic fat oxidation. O'Sullivan *et al.* (157) compared the effects of oral and transdermal estrogen treatment on substrate oxidation in a randomized crossover study in 18 postmenopausal women. No significant difference in resting energy expenditure or basal lipid oxidation was observed between the two routes of estrogen therapy. Ingestion of a standardized mixed-meal acutely suppressed lipid oxidation during each treatment phase. However, when compared with the transdermal route, oral estrogen administration suppressed lipid oxidation to a greater degree (157). The suppression of lipid oxidation was accompanied by a matched reciprocal stimulation of carbohydrate oxidation. These changes likely represent hitherto unrecognized first-pass effect of estrogen on hepatic lipid metabolism.

The finding of a suppressive effect of oral estrogen on fat oxidation supports an earlier observation made in an 18-yr-old girl treated with high doses of ethinyl estradiol for tall stature (152). Oral ethinyl estradiol treatment with doses of 60, 100, and 200 μg/d produced a reversible, dose-dependent suppression of lipid oxidation associated with a reversible increase in carbohydrate oxidation. The degree of lipid oxidation suppression was far greater than that observed in postmenopausal women and is likely due to the greater potency of the synthetic estrogens.

How hepatic lipid oxidation is reduced by estrogen is not known. One possible mechanism may involve the increase in circulating GHBP, which could attenuate the stimulatory effects of endogenous GH on fat oxidation. The finding that insulin sensitivity was impaired during oral estrogen treatment argues against this possibility. Another mechanism may involve a direct effect of estrogen on the liver, the major site of fatty acid metabolism. *In vitro* studies have shown that pharmacological concentrations of estrogen reduce ketogenesis (a product of fatty acid oxidation) and increase fatty acid incorporation into triglycerides (158, 159). These *in vitro* findings are in accordance with clinical observations that oral but not transdermal estrogen therapy stimulates hepatic triglyceride synthesis and increases triglyceride levels (160). Because intrahepatic fatty acid metabolism is partitioned between oxidative and nonoxidative (incorporation into triglycerides) pathways, it is likely that estrogen regulates the metabolic fate of intrahepatic FFA by directing them away from oxidative into lipogenic pathways committed to very low-density lipoprotein (VLDL) synthesis and export.

4. Body composition. In the crossover study of postmenopausal women, no significant changes in body weight were observed between both routes of estrogen therapy after 6 months, nor did body weight change significantly with either treatment (157) (Fig. 3). Mean bone mineral density increased during oral and transdermal estrogen therapy, with the increases not being significantly different between the two routes. However, significantly different effects on fat mass and lean body mass were observed between the two routes of estrogen therapy. When the effects of both routes of estrogen administration were compared, oral therapy led to a significant increase in fat mass of 1.2 kg, equivalent to a 5% change in body fat (Fig. 3). No significant change occurred during the transdermal estrogen phase. Oral estrogen therapy also induced an equal loss in lean body mass equivalent

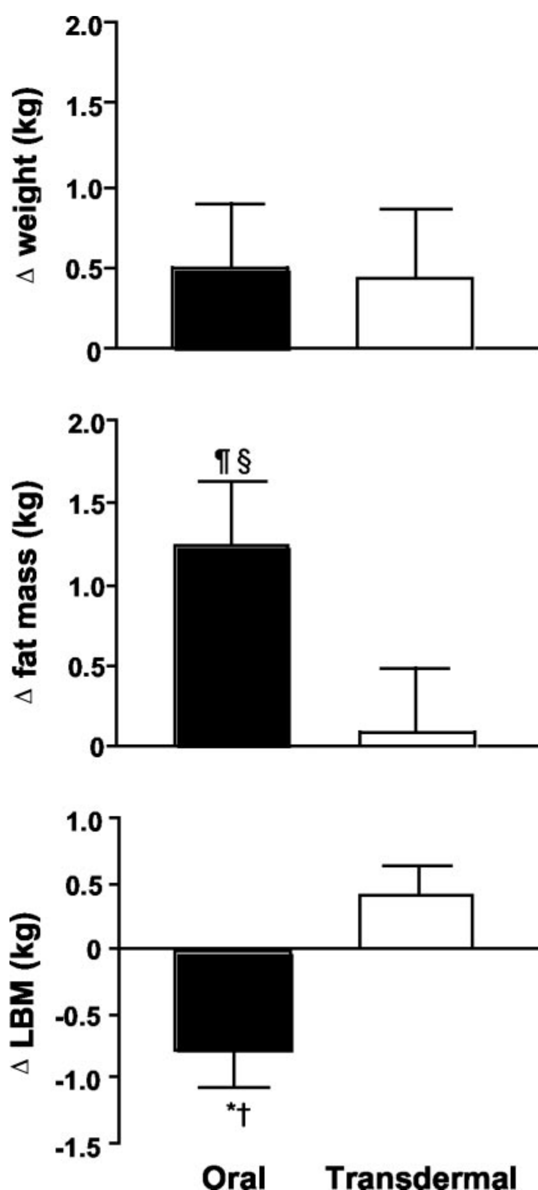

FIG. 3. Change (mean ± SE) in body weight (*top*), fat mass (*middle*), and lean body mass (*bottom*) in postmenopausal women after 24 wk of oral and transdermal estrogen treatment. ¶, $P < 0.05$ oral *vs.* transdermal; *, $P < 0.01$ oral *vs.* transdermal; †, $P < 0.02$ *vs.* before oral estrogen treatment; §, $P < 0.005$ *vs.* before oral estrogen treatment. [Adapted with permission from O'Sullivan *et al.* (157).]

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to a 3% change compared with that observed during transdermal estrogen therapy. This difference was accounted for by a significant decrease in lean body mass of 0.8 ± 0.3 kg with oral therapy and a small but nonsignificant increase in lean body mass during transdermal estrogen therapy (Fig. 3). Thus, when compared with the transdermal route, oral estrogen therapy was accompanied by a significant decrease in lean body mass and a significant increase in whole body fat mass. We speculate that long-term suppression by oral estrogen of hepatic IGF-I production leads to a loss of protein mass and suppression of lipid oxidation leading to accumulation of body fat. The different routes of estrogen therapy induced significant changes in body composition without a change in body weight. The change in lean body mass also supports the importance of circulating IGF-I in positively regulating anabolism (Fig. 1).

Studies on the effect of estrogen replacement on body weight and fat in menopause have given conflicting results. Some have reported an increase (161, 162) or no change (163–165) in weight, whereas others have observed a reduction (166, 167), no change (165, 168), or an increase (79) in body fat. Some of the confusing data may arise from selection bias inherent in cross-sectional comparisons between treated and untreated women because of the known behavioral and socio-demographic difference between users and nonusers (165). However, two prospective randomized studies have reported disparate findings in total body fat despite using similar methods to quantify body fat (79, 166). Most studies agree that estrogen replacement induces a redistribution of body fat from the truncal to lower body areas, which can be explained by regional differences in adipose tissue sensitivity to estrogen, possibly mediated through the action of LPL activity responsible for the hydrolysis of circulating triglycerides into FFA (166, 168–170).

The finding that the route of estrogen administration confers divergent effects on lipid metabolism provides some insights as to why there are conflicting reports on estrogen and body fat as cited above. Because there is strong *in vitro* evidence that estrogen enhances basal lipolytic activity (84), estrogen replacement may be expected to reduce body fat in postmenopausal women. However, this effect could be diminished or nullified when estrogen is administered orally because the suppression of hepatic fat oxidation by oral estrogen reduces global fat utilization. The oral route was the predominant mode of administration in all the above studies. The net effect, however, will be dependent on the balance between these opposing effects of oral estrogen on fat metabolism, the dose of estrogen, duration of therapy and lifestyle factors affecting energy balance. The differences in these factors and thus in the net effect would explain the varying results of these studies.

5. Lipoprotein metabolism and proinflammatory markers. The role of estrogens in cardiovascular function has been the subject of intensive research for many years. Although controversy persists as to whether estrogens provide cardiovascular protection, there is general agreement that the roles they play in lipoprotein metabolism and in the regulation of proinflammatory factors have relevance to vascular function. The liver plays a pivotal role in lipoprotein metabolism and is a source of a number of proinflammatory factors. Many clinical studies have reported that GH regulates lipoprotein metabolism and influences circulating proinflammatory markers, suggesting interactions between these two hormonal systems that influence vascular endothelial function. The effects of estrogen on lipoprotein metabolism and on circulatory inflammatory markers are route-dependent. The stimulation of GH secretion with the oral route raises the question as to whether some of the oral estrogen effects may be GH-mediated.

Oral estrogen administration reduces total and low-density lipoprotein (LDL)-cholesterol and increases high-density lipoprotein (HDL)-cholesterol and triglyceride levels. These changes occur as a consequence of increased hepatic expression of the LDL receptor, which leads to the LDL-cholesterol catabolism while stimulating the hepatic production of triglyceride-rich VLDL (171, 172). The effects of oral estrogen on LDL-cholesterol metabolism may be mediated through increased GH secretion. Studies in the rodent have shown that estrogen up-regulation of the hepatic LDL receptor does not occur in the absence of GH. *In vitro* studies of human liver show that GH stimulates the expression of this receptor (173, 174). GH increases the catabolism of LDL-cholesterol and of apolipoprotein B from the circulation through this mechanism (173, 174), which stimulates the hepatic synthesis and secretion of VLDL triglycerides (175). GH enhances the turnover of VLDL and LDL-cholesterol, which may in turn explain the increase in HDL-cholesterol concentration (176) and increase in LDL particle diameter in response to GH treatment (177).

Estrogens administered via a nonoral route exert only modest effects on total, LDL-, and HDL-cholesterol. In contrast to oral administration, serum triglyceride levels fall instead of rise during transdermal estrogen treatment (178). These observations are consistent with the absence of a GH-mediated effect occurring against a background of an estrogen-mediated action on lipid and lipoprotein metabolism (160, 179). Because oral estrogens stimulate GH secretion, it is conceivable that some of its effect on lipoprotein metabolism is mediated by GH induction of hepatic LDL receptor expression. Because oral estrogen suppresses hepatic fat oxidation, this is likely to enhance the shunting of FFA toward production of VLDL, thereby increasing blood levels of triglycerides. The current evidence, at least in rodents, suggests that GH plays a significant role in mediating some of the effects of estrogen on hepatic lipoprotein metabolism.

C-reactive protein (CRP) is an acute-phase reactant and a marker of underlying systemic inflammation. It is strongly associated with the risk of cardiovascular disease in both men and women (180, 181). There is evidence that many proinflammatory factors are estrogen-regulated. An increase in serum concentrations of CRP and proinflammatory cytokines such as IL-6 and TNF-α occurs during menopause (181, 182). *In vitro* studies have shown that estrogen directly inhibits IL-6 and TNF-α gene expression, both of which are secreted by vascular endothelial cells, vascular smooth muscle cells, and monocytes/macrophages (182).

However, these inhibitory effects of estrogen are in conflict with cross-sectional studies reporting serum CRP levels to be higher in postmenopausal women receiving estrogen treat-

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ment (183). This treatment effect of estrogen is route-dependent, occurring with oral but not transdermal administration (184). This is likely a first-pass effect causing increased hepatic synthesis of CRP and represents an undesirable effect of oral estrogen, which may have longer-term implications on vascular function.

There is strong evidence that GH negatively regulates many proinflammatory cytokines. Serum levels of CRP, IL-6, and TNF-α are increased in adults with GH deficiency and fall in response to GH replacement (185, 186). Monocyte production of IL-6 and TNF-α is increased in patients with GH deficiency and reduced by GH treatment (185). Levels of CRP are low in active acromegaly and increase with disease control (187). These data indicate that GH directly or indirectly reduces inflammation by modulating serum levels of cytokines and markers of inflammation. Thus, deficiency in both GH and estrogen is associated with increased serum concentrations of CRP, IL-6, and TNF-α.

The paradigm posed by oral estrogen administration because of its impact on the GH/IGF-I axis is interesting. It is conceivable that estrogen repletion along with enhanced GH secretion may reduce levels of proinflammatory cytokines. However, the opposite was observed; CRP levels rose with oral but did not change with transdermal estrogen administration in postmenopausal women, indicating powerful hepatic induction of this reactant, sufficient to overcome any putative effect of GH. The possibility that oral estrogen treatment abrogates the fall in proinflammatory factors during GH treatment of hypopituitary women has not been studied.

The longer-term implications of the stimulatory effect on proinflammatory markers are uncertain but likely to be nonfavorable. The regulation of proinflammatory cytokines by estrogens and their impact on vascular homeostasis function is a complex area and the subject of recent review (182). Understanding of the mechanisms and effects will provide much-needed insight into the controversies surrounding the value of estrogen therapy in cardiovascular health (164, 188).

C. Physiological implications

1. Normal women. The effects of oral estrogen on hepatic IGF-I production and fat oxidation are diametrically opposite to those of GH. These effects of estrogen are dose-related and may have physiological relevance if endogenous estrogens rise into a range sufficient to perturb GH action. Friend *et al.* (123) have demonstrated that elevating systemic estrogen levels to a mean of 750 pmol/liter by transdermal delivery significantly reduces circulating IGF-I in postmenopausal women. This is a level attained in the periovulatory phase.

How can this finding be reconciled with the observation in the menstrual cycle that GH and IGF-I levels are highest in the periovulatory phase of the menstrual cycle when estrogen levels peak (24)? It is possible that these changes could be mediated by ovarian substances with GH-releasing properties that are cosecreted with estrogens. Among possible candidates are androgens, the levels of which also peak during the periovulatory phase. The ovary also secretes peptides of the activin-inhibin family, which exert central neuroendocrine and pituitary actions unrelated to gonadototroph function. Activin is unlikely to be a candidate because it has not been shown to inhibit GH synthesis (189). A role for inhibin has not been explored, but it has been reported to stimulate GHRH secretion from the placenta (190). In summary, the correlations between endogenous estrogen and GH status may be explained by cosecretion of ovarian factors that stimulate GH release.

If estrogen played a significant role in stimulating GH secretion, GH status would be expected to fall significantly after the menopause. This has not been observed. Although spontaneous GH secretion falls progressively with advancing years, an abrupt reduction over the menopausal years does not occur, and most of the change is explained by age-associated increases in body or abdominal fat (36, 37).

Studies addressing whether fat oxidation varies during the menstrual cycle in relation to estrogen levels have not been undertaken. Evidence for a physiological effect of endogenous estrogens is provided by the observation that whole body fat oxidation is lower in women than in men (149), although this does not rule out a stimulatory effect of androgens. The modulatory effect of estrogen on fat metabolism may explain changes in body fat occurring at specific developmental milestones throughout a woman's life. Sexual dimorphism in body fat emerges during puberty in the absence of any major differences in GH concentrations between girls and boys. A critical mass of body fat is required for reproductive function. By the end of puberty, sc fat in girls is 50–90% higher, depending on the site of measurement. There is compelling evidence that rising levels of estrogen mediate metabolic adaptation in pregnancy. Early pregnancy is associated with an increase in fat mass, a teleologically desirable state that ensures adequate fetal and postpartum nutrition. At 24 wk gestation, women gained an average of 3.9 kg of fat without any significant changes in energy intake (85). O'Sullivan *et al.* (88) observed postprandial fat oxidation to be lowest in pregnancy and highest in postmenopausal women, revealing that fat oxidation was strongly and inversely related to estrogen status. The authors postulated that the high estrogen status of pregnancy facilitated efficient fat storage without the need for dietary change.

D. Therapeutic implications

1. Normal women. Estrogens are among the most widely used therapeutic substances. Nearly half of the young women aged 20–24 yr in the United Kingdom and one third of the single women in Denmark take estrogen in the form of OC steroids (191). In a large survey of breast cancer, between 10 and 20% of postmenopausal women in a control group were on hormone replacement treatment for more than 5 yr (192). The observation in postmenopausal women that oral estrogen causes detrimental changes in body composition has important implications for estrogen use in health and disease.

The perturbation of hepatic fat oxidation by oral estrogen may explain many observations associated with the use of OCs that remain poorly understood. Weight gain is a well-recognized effect of OC use, which induces insulin resistance and abnormal fatty acid metabolism (149). Nearly half of OC users reported a greater than 2 kg increase in an Australian survey (193). The cause has been attributed to fluid retention,

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although direct evidence of ECW expansion is lacking. Weight gain is often reported as a perceived problem because many studies have found little or no change (194). In the study by O'Sullivan *et al.* (157), oral estrogen changed body composition but not body weight because of reciprocal shifts in fat and lean body mass. Because fat is less dense than lean tissue, it is likely that the greater volume occupied by fat is perceived as an increase in weight. Studies of the effects of OC use on body fat are scarce. Two cross-sectional studies found no significant difference in body fat between OC users and the control group. However, these studies employed insensitive techniques such as bioelectrical impedance, anthropometry, and infrared interaction (195, 196). The impact of OC on body fat awaits careful prospective evaluation using modern sensitive techniques. Fatty liver or steatosis is a recognized consequence of OC use (197). We speculate that this arises from suppression of hepatic fatty acid oxidation by estrogens causing shunting to lipid synthesis.

The body compositional consequences of oral estrogen therapy in postmenopausal women are similar to those of aging, which is accompanied by a progressive increase in fat mass and a decline in lean body mass (157). These changes appear to be of biological significance when compared with the spontaneous increase in fat mass of 5–10% (1.5–2.5 kg) per decade and decrease in lean body mass of 2.5% (1–1.5 kg) per decade observed in normal aging women (198–200). When compared with the transdermal route, oral estrogen administration for only 6 months induced changes in body composition equivalent to those occurring spontaneously over a 5- to 10-yr period. The traditional route of estrogen administration may compound the undesirable changes in body composition that are already occurring with aging.

2. Hypopituitary women. The issue of estrogen replacement is especially relevant in the hypopituitary woman with GH deficiency because IGF-I levels are already low. IGF-I in the GH-deficient state is lowered further by oral estrogen but unaffected by transdermal therapy (126, 201). This observation may explain why some investigators have observed IGF-I levels to be lower in hypopituitary women than men, despite having a similar degree of impaired GH responses to insulin-induced hypoglycemia (202–204). However, none of these studies compared IGF-I levels between estrogen users and nonusers, so that it is not possible to ascertain whether the gender difference can be solely explained by the use of oral estrogen. Women with hypopituitarism may be more susceptible to the hepatic effects of oral estrogen administration because of the loss of feedback GH response. The level and degree of IGF-I suppression is greater in GH-deficient women than postmenopausal (GH-sufficient hypogonadal) women in response to oral estrogen treatment (205). Thus, estrogen replacement by the oral route may aggravate existing metabolic and body compositional abnormalities of the GH-deficient state. For the same reason, women with Laron syndrome (that is, with GH insensitivity) should not take estrogens by the oral route.

3. Acromegaly. Over 30 yr ago, before IGF assays were developed, high doses of estrogen were used empirically to control the symptoms of acromegaly. Wiedemann *et al.* reported that the bioactivity of sulfation factor, a measure of somatomedin activity, was suppressed by estrogens (97, 206). This treatment fell into disrepute because of the high incidence of side effects arising from high-dose estrogen treatment. The doses of ethinyl estradiol of 0.5–1.0 mg used are 30–50 times those currently used in OC preparations. With the development of the first RIAs for IGF-I (somatomedin C), Clemmons *et al*. (207) reported that oral estrogen reduced somatomedin C in blood. A preliminary study has reported that therapeutic doses of estrogen normalized IGF-I and reduced the metabolic and body compositional effects of GH excess in a group of women with mild acromegaly (208). These promising data suggest that oral estrogen may be an economic, safe, and effective adjuvant treatment for control of acromegaly. Because GH concentrations did not change during treatment, the data strongly support the importance of circulating IGF-I in mediating the biological consequences of GH excess in humans.

4. Selective ER modulators (SERMs). Advances in estrogen biology have revealed the existence of different classes of estrogen-related compounds (classical estrogens, SERMs, and phytoestrogens). SERMs are nonsteroidal compounds that exhibit estrogen agonistic and antagonistic actions in a tissue-specific manner. Little is known about the effects of SERMs and phytoestrogens on hepatic metabolic and endocrine function. Oral estrogens exert major regulatory effects on hepatic lipoprotein metabolism. There is evidence that SERMs exert actions on hepatic lipid metabolism that differ from classical estrogens. Raloxifene reduces LDL-cholesterol, but unlike oral estrogen, this SERM has no effects on circulating levels of HDL-cholesterol, plasminogen activator inhibitor, or triglycerides (209).

Several studies have reported that SERMs including tamoxifen, raloxifene, and droloxiefene reduce circulating IGF-I in women with breast cancer (210, 211). Raloxifene treatment reduces circulating IGF-I but not IGFBP-3 levels in postmenopausal women (212). In a cross-sectional study, postmenopausal women on raloxifene treatment had lower IGF-I/IGFBP-3 ratios than untreated controls (211). A preliminary report in postmenopausal women observed the IGF-I suppressive effects of 60 and 120 mg of raloxifene to be less than those of 1 and 2 mg of estradiol valerate, but effects on fat oxidation were comparable (205). Much more work is needed to delineate the effects of SERMs and the extent of their interactions on the GH/IGF-I axis and impact on substrate metabolism.

5. GH replacement in adults

a. Serum IGF-I. To address whether oral estrogen antagonizes the metabolic effects of exogenous GH, Wolthers *et al.* (201) compared changes in IGF-I, fat oxidation, and protein metabolism in hypopituitary women on GH treatment during oral and transdermal administration. GH treatment significantly increased IGF-I levels in a stepwise, dose-dependent manner during both routes of estrogen treatment. However, mean IGF-I levels were significantly lower during oral estrogen at each GH dose (Fig. 4). The rates of postprandial lipid oxidation and of protein synthesis were stimulated by GH but remained significantly lower during oral

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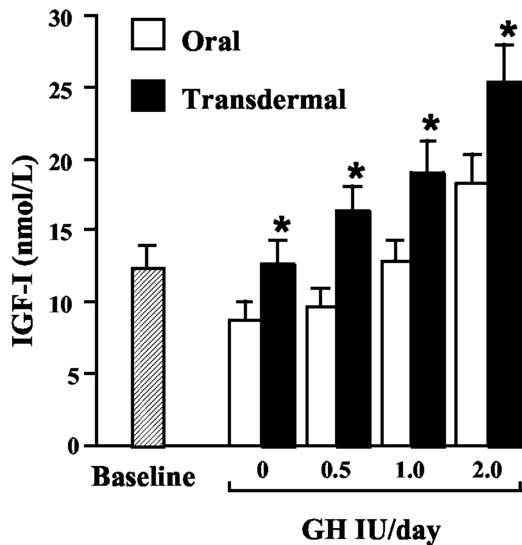

FIG. 4. Serum IGF-I concentrations (nmol/liter, mean ± SE) before and during incremental dosages of GH (0.5, 1.0, and 2.0 IU/d) during oral and transdermal estrogen therapy in GH-deficient women. Conversion factor of GH dose was 3 IU/mg. *, $P < 0.05$ by ANOVA, oral *vs.* transdermal. [Reproduced with permission from Wolthers *et al.* (201).]

estrogen treatment. Thus, estrogen exerts significant route-dependent attenuating effects on GH action in women with organic GH deficiency.

Several studies have reported that IGF-I levels are lower in GH-deficient women than men replaced with similar doses of GH (213) and that the dose of GH required to maintain a comparable level of IGF-I is higher in women than men (214–218). Although these studies did not provide subgroup analysis on gonadal status and estrogen treatment, it is likely that lower sensitivity to GH in women arose from concomitant oral estrogen treatment, the most widely used mode of replacement. Using IGF-I as an endpoint for dose determination, Cook *et al.* (219) observed that GH requirements in men were not different from those in women not taking estrogens, but that women taking oral estrogens required at least a 2-fold greater dosage of GH. Janssen *et al.* (220) reported that switching from oral to transdermal estrogen therapy increased serum IGF-I by 30% during GH replacement therapy.

b. Body composition. Gender-related changes in body composition in response to GH replacement treatment in hypopituitary adults have been consistently observed from investigators regardless of the body composition techniques used. Johannsson *et al.* (213, 221) observed that the increases in fat-free mass and total body water were more marked in men than women, as was the reduction in total body fat. In a placebo-controlled 9-month trial, Burman *et al.* (222) observed that GH treatment induced a greater reduction in the proportion of total body fat and in abdominal fat mass in men than women, as estimated by dual-energy x-ray absorptiometry. A large prospective study reported a progressive separation in fat mass, body nitrogen, and body potassium (indirect measures of body protein and cell mass, respectively) between men and women treated for up to 5 yr with GH (217, 223). In the latter studies, the mean daily GH dose was similar between sexes. However, because there was a significant weight difference, women actually received a larger weight-adjusted dose, indicating that they harbored an even greater degree of GH resistance than is apparent from the biochemical and body compositional change. The men also showed greater changes in bone composition. In the same 5-yr study, increases in lumbar spine bone mineral density were more marked in men than women (217). The greater effect on the skeleton in men has also been observed in other studies (218).

The observation that oral estrogen attenuates the protein anabolic and lipid utilization effects of GH (201) might explain in part the reduced efficacy of GH treatment in hypopituitary women. In the above studies, most of the hypopituitary women were replaced with estrogen, although the route of therapy was not specified. However, it can be assumed that the majority employed the oral route because this was the usual mode of administration. Most of the hypogonadal men were also replaced with androgens in these studies. The possibility that androgens may amplify the biological actions of GH has not been investigated. Studies in short children have reported that androgens increase the growth of GH-deficient children during GH treatment (11). Therefore, a positive regulatory effect of androgens should be considered as an additional mechanism explaining the clear sexual dimorphism in GH responsiveness.

c. Economic implications. The observation that estrogens taken orally attenuate the biological effects of GH has economic implications. A 2-fold higher dose of GH was required to achieve the same IGF-I level as that observed on transdermal therapy using standard estrogen replacement regimens. This approximates to an additional 1 IU (0.3 mg) of GH per day, which translates to a greater cost of approximately U.S. $4,400 per year per patient. The prevalence of hypopituitarism is estimated to be between 175 and 400 per million (224), giving an approximation of 50,000–120,000 adults with GH deficiency in the United States. Assuming that half are women and most are likely to be treated for coexisting hypogonadism, the potential cost saving to the community is approximately U.S. $110–250 million simply by switching to the transdermal route. Therefore, transdermal estrogen delivery will not only confer more cost-effective GH treatment when using IGF-I to monitor the response to therapy, as is recommended by the Growth Hormone Research Society (225); it might also be anticipated that effects on other important endpoints will be achieved with a lower daily dose of GH.

IV. Cellular and Molecular Mechanisms

The previous sections have provided strong evidence that estrogen exerts complex effects on the GH/IGF-I axis. The attenuation of GH-induced effects on the IGF system, fat oxidation, protein synthesis, and body composition in hypopituitary women strongly suggests that estrogen directly modulates GH action independent of the effect on secretion. Noteworthy is the increase in GHBP levels with oral estrogen administration, suggesting an effect on the GHR. This section

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will provide a review of pertinent information on GHR structure, signaling, and regulation; ER function; and recent work identifying the suppressor of cytokine signaling as a novel regulatory link between the two systems.

A. GH receptor and binding protein

The GHR belongs to the cytokine receptor family, which has more than 25 members, including the receptors for prolactin, leptin, erythropoietin, and a number of IL and interferons (3, 226). The highest level of GHR expression is found in the liver, followed by muscle, fat, kidney, and heart (227, 228). The receptor protein comprises 620 amino acids and consists of an extracellular ligand-binding domain (221–249 amino acids), a single transmembrane domain (24 amino acids), and a cytoplasmic domain (347–353 amino acids) for signal transduction (229).

1. GHR gene. The GHR transcript is approximately 4.7 kb in size and is encoded by 10 exons (230, 231). Exon 1 contains the 5′-untranslated region (UTR), and exon 2 encodes a signal peptide of 18–20 amino acids and the first five amino acid residues of the extracellular domain. Exons 3–7 encode most of the extracellular domain, whereas exon 8 encodes the transmembrane domain. Exons 9 and 10 together encode the entire cytoplasmic domain. Exon 10 also contains the 3′-UTR sequence.

There is a high degree of heterogeneity in the 5′-UTRs (exon 1) of the GHR gene in all species studied. Nine variants have been identified in humans (V1-V9) (232–234) and between two and nine variants in other species (229, 235–239). All variants splice to a common site 9–11 bp upstream of the translation start site (ATG) in exon 2, resulting in the generation of the same receptor protein. Of the nine exon 1 transcripts, the V1 and V2 variants are the predominant forms expressed in human liver and extrahepatic tissues, respectively (234). Other variants are expressed at low levels, and their contribution to the GHR pool is unknown. In animals, the V1 transcript appears to be most important because its abundance is closely correlated with the levels of hepatic GHR protein, serum GHBP, and circulating IGF-I (240, 241). Whether this is also the case in humans is not clear.

The 5′-flanking regions of V1 reveal interesting regulatory sequences, including the canonical TATA boxes, a half estrogen response element (ERE) site, a half glucocorticoid response element site, and binding sites for the signal transducer and activator of transcription 5 (STAT5), activator protein 1 (AP1), and AP3 (234, 242–246). A liver-specific transcription factor, hepatocyte nuclear factor-4 (HNF-4), also binds to the V1 promoter (242) and may provide for regulation of liver-specific expression of V1.

Unlike V1, the 5′-flanking region of V2 variant lacks a consensus TATA box, but has GC-rich sequences that bind the transcription factor, specific protein 1 (Sp1) (234, 247). Sp1 induces the expression of many genes with TATA-less promoters through direct interaction with components of the basal transcriptional machinery (248). The binding of Sp1 to the V2 promoter may account for ubiquitous expression of this variant in a wide range of tissues.

In summary, the GHR gene has a complex 5′-UTR structure, with multiple exon 1 sequences controlled by distinct promoters. This appears to be necessary for coordinating GHR expression in a tissue-dependent manner. Very little is known about how various promoters interact to regulate GHR transcription.

2. GHBP. As mentioned above, circulating GH binds to GHBP, which is derived from the GHR protein through proteolytic cleavage in humans (104). This process is catalyzed by the metalloprotease, TNF-α converting enzyme (TACE/ADAM-17) (249). In rodents, the binding protein is the product of a specific mRNA generated from alternate splicing of the GHR primary transcript. GHBP appears to have dual effects on GH action; it prolongs the circulating half-life of GH but competes with tissue GHRs for GH binding. The level of GHBP in the circulation has been proposed to reflect the status of tissue GHRs (104), although direct experimental evidence is lacking. The proteolytic cleavage site is located eight amino acid residues from the transmembrane domain (between Pro^{238} and Phe^{239}) for the rabbit GHR (250). This region is highly homologous among species in which GHBP is generated from proteolysis (Fig. 5). Replacement of the sequence Ser^{237}-Pro^{238}-Phe^{239} by Asn-Ile-Leu as found in the murine GHR markedly reduces the susceptibility to cleavage (251). There is recent evidence that GHBP is located intracellularly and enhances the transcriptional activity of GH, prolactin, and erythropoietin (252). The mechanism and physiological significance of this intracellular action of GHBP remains to be determined.

GHBP in rats and mice is encoded by a specific mRNA of

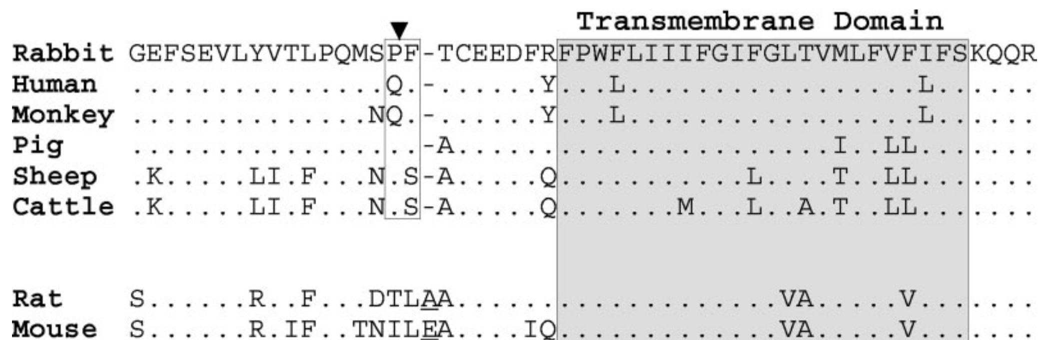

FIG. 5. Comparative analysis of the predicted cleavage sites for GHBP generation on the GHR extracellular domains of various species. The cleavage sites are denoted by the *unshaded box* and *arrowhead*. The transmembrane domains are *shaded*. Identical amino acid residues are indicated by *dots*. Gaps for maximal alignment are indicated by *dashes*, and the additional residues in the rodent GHRs are *underlined*.

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approximately 1.2 kb in size, generated by alternative splicing of exon 7 in the GHR primary transcript to a new exon designated 8A (253, 254). The rodent GHBP contains the extracellular domain and a short hydrophilic C terminus in place of the transmembrane and intracellular domains (229). Because of the differences in mechanisms for GHBP production, extrapolation of rodent findings to humans should be interpreted with caution.

3. Truncated GHR isoforms. Two transcripts formed from alternative splicing at exon 9 of the GHR gene have been identified in human liver (255, 256). The alternative splicing results in a frame shift, premature termination, and generation of truncated receptors of 279 amino acids (GHR1–279) and 277 amino acids in size (GHR1–277). Both of these isoforms bear intact extracellular and transmembrane domains but truncated cytoplasmic domains devoid of signaling function (256). The truncated isoforms are membrane-anchored and exhibit GH binding properties similar to those of the full-length GHR. By heterodimerizing with the full-length receptor, they regulate GHR signaling in a dominant negative manner (256–258).

GHR1–279 and GHR1–277 transcripts are detectable in a wide range of human tissues and cells, including the liver, fat, muscle, kidney, heart, prostate, lymphocytes, and fibroblasts (228). The relative abundance of the truncated isoform transcripts is approximately 10% and 0.5% of the full-length receptor, respectively, and varies between tissues. Because truncated GHR isoforms inhibit receptor signaling (256), their relative abundance may determine responsiveness to GH in different tissues. In extrahepatic tissues, such as muscle and fat, where the ratio of GHR1–279 to the full-length receptor is around 1:10, GH response may be inhibited by 30% as demonstrated by *in vitro* studies (256, 257).

The truncated GHR isoforms contribute significantly to GHBP production. Cells expressing GHR1–279 produce more GHBP than those with the full-length receptor (255, 256, 259). It has been proposed that the absence of an internalization motif located in the cytoplasmic domain renders the truncated receptors more susceptible to proteolytic cleavage (259, 260). Because the relative abundance of the truncated GHR isoform transcripts is proportionally higher in extrahepatic tissues, there may be more GHBP produced locally in these tissues.

What factors may regulate expression of the truncated GHR isoforms are unknown. Human clinical studies have demonstrated that estrogens inhibit GH action and increase circulating levels of GHBP (see *Section III*). Because these truncated isoforms inhibit receptor signaling and contribute to greater generation of GHBP, it is possible that estrogens may stimulate their expression.

B. GH receptor signaling

It has been proposed that GH binds at separate sites to two GHR molecules bringing about dimerization, which leads to activation of Janus kinase 2 (JAK2) (261). Recent work has revealed that a GH analog mutated to prevent receptor dimerization associates with two GHRs as the wild-type ligand does (262), and that full-length and truncated GHRs can be coprecipitated in the absence of ligand (263). These observations provide strong evidence that GHRs exist as a preformed dimer, as is the case for the erythropoietin receptor (264, 265) and the leptin receptor (266). It is likely that signal transduction is triggered by GH-induced conformational change of preformed GHR dimers (267, 268). The receptors for erythropoietin and leptin have been reported to undergo conformational changes that bring the receptor-associated JAK2 molecules into close proximity for transactivation (264–266).

1. JAK2. While the GHR lacks intrinsic protein kinase activity, the phosphorylation action required for signal transduction is mediated by JAK2, which binds to the receptor at a specific proline-rich motif (box 1) in the proximal cytoplasmic domain (226, 269). The central role of JAK2 in signal transduction is underscored by the finding that GHR mutants lacking the JAK2 binding site do not elicit signaling events (226). The JAK2 action is enhanced by a Src homology 2/α-collagen-related (SH2)-containing protein, SH2-Bβ (270, 271), but suppressed by Grb10 (272).

Activated JAK2 phosphorylates the GHR and a number of downstream signaling molecules, including: 1) the STATs; 2) SH-containing protein; 3) insulin receptor substrates (IRSs); 4) phospholipase C (PLC); and 5) p125 focal adhesion kinase (226, 273) (Fig. 6). SH-containing protein is involved in the Ras and MAPK pathway (*i.e.*, Raf/MEK/ERK), which mediates the mitogenic action of GH (274–276). IRSs activate the phosphatidylinositol-3′-kinase (PI3K) and Akt pathway responsible for the mitogenic and insulin-like actions (lipogenesis and antilipolysis) of GH (277). PLC activates protein kinase C, which is implicated in GH-induced lipolysis (278). p125 Focal adhesion kinase is associated with $p130^{Cas}$ and the pathway of MEKK, MKK, and JNK or p38, which may be involved in the cytoskeleton reorganizing effect of GH (279, 280). A comprehensive overview of all signaling cascades for GH action is beyond the scope of this review and has been discussed in detail elsewhere (226, 273). We will concentrate on STAT signaling, which is the best-characterized pathway for GHR signaling and relevant to estrogen regulation.

2. STAT. The STAT family comprises seven members (1, 2, 3, 4, 5a, 5b, and 6), all of which are cytosolic transcription factors essential for the action of cytokine receptors (281). After phosphorylation by JAKs, the STAT proteins form homo- or heterodimers that translocate to the nucleus and bind to specific motifs [TTC$(N)_3$GAA] in the promoter regions of targeted genes to induce transcription.

GH induces the phosphorylation of STAT5a and -5b and, to a lesser extent, of STAT1 and -3 (282). STAT5 proteins bind to specific phosphotyrosine residues on the GHR cytoplasmic domain and are phosphorylated by JAK2. They are involved in the expression of various GH-responsive genes, including β-casein, Spi2.1, IGF-I, ALS, insulin, HNF-6, and p450 CYP3A 6β-hydroxylase (282). Unlike STAT5, STAT1 and -3 directly associate with JAK2 rather than the GHR (282). These two STAT proteins mediate GH activation of the protooncogene c-*fos* through binding to the *sis*-inducible element in the oncogene promoter. In addition to tyrosine phosphorylation, GH stimulates serine phosphorylation of

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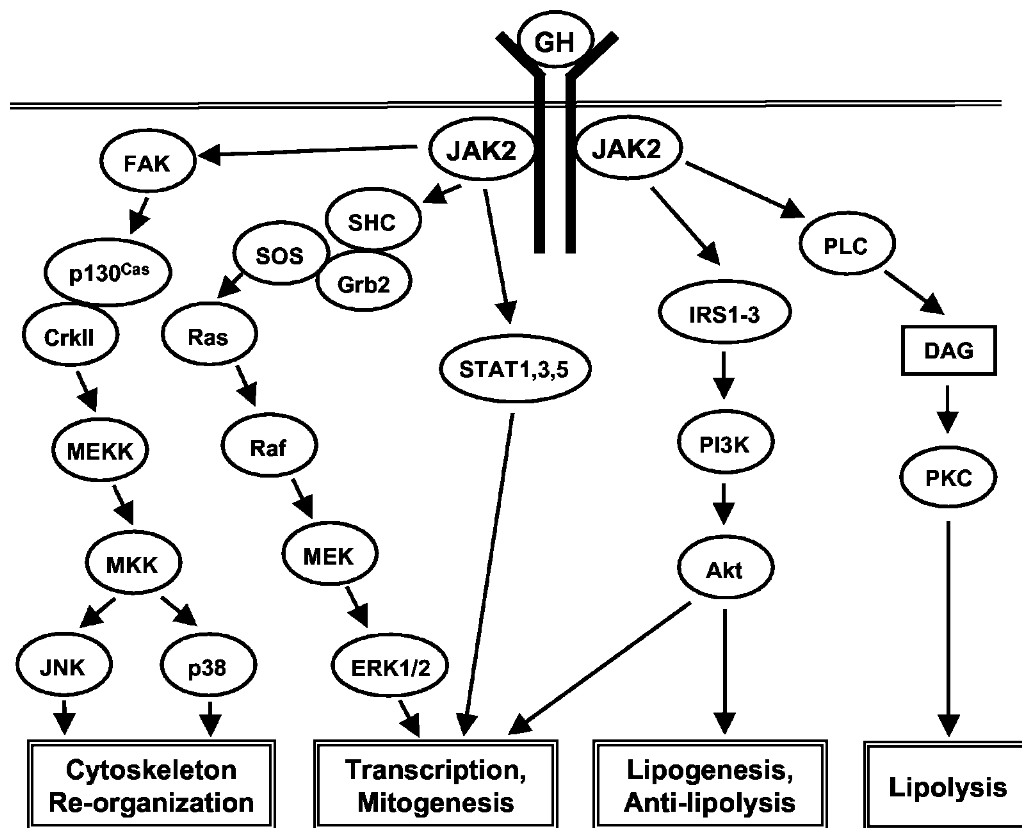

FIG. 6. Schematic representation of GHR signaling pathways.

STAT proteins, which augments DNA binding and transcriptional activation (282).

The transcriptional activity of STAT5 is modulated by other transcription factors. The glucocorticoid receptor (GR) acts as a coactivator of STAT5 in transcriptional regulation of β-casein by prolactin (283). STAT5 also forms a ternary complex with GR and a ubiquitous transcription factor, yin-yang 1, to activate the promoter of the Spi2.1 gene (284). In hepatic tissues, STAT5 acts synergistically with HNF-1α and HNF-4 to stimulate GH-induced promoter activity of the IGF-I and HNF-6 genes, respectively (285, 286).

STAT5b plays a more important role than other STAT proteins in mediating the growth-promoting and transcriptional actions of GH. Male STAT5b$^{-/-}$ mice have markedly impaired growth rate and reduced serum IGF-I levels (287, 288), whereas no changes in body growth and expression of GH-dependent genes have been detected in the STAT5a$^{-/-}$ mice (288, 289). STAT5b also plays a prominent role in activating hepatic expression of GH-dependent, sexually dimorphic genes, as exemplified by P450 enzymes in rodents (290). Pulsatile GH treatment simulating the male secretory pattern in rats markedly induces STAT5b phosphorylation and activates expression of the male-specific CYP2C11 gene. In contrast, continuous administration of GH mimicking the female pattern down-regulates STAT5b signaling and leads to expression of the female-specific CYP2C12 gene (290).

3. Negative regulators of GHR signaling. Termination of GHR signaling by negative regulators is an important mechanism for controlling GH action. Most data are derived from studies of the JAK2/STAT5 pathway, which have identified three groups of negative regulators: 1) the suppressors of cytokine signaling (SOCSs); 2) protein tyrosine phosphatases (PTPs); and 3) protein inhibitors of activated STATs (PIASs).

a. SOCS. SOCSs consist of a family of eight members, SOCS-1 to -7 and cytokine-inducible SH2-containing protein (CIS) (291–293). Each member contains a central SH2 domain and a highly conserved region of around 40 amino acids (designated SOCS box) at the C terminus (294). The SH2 domain mediates binding of the SOCS proteins to phosphotyrosine residues in specific cytokine receptors and/or JAKs, whereas the SOCS box is responsible for ubiquitin-linked proteosomal degradation of SOCS-associated proteins. SOCSs are expressed in response to a wide range of cytokines, hormones, and growth factors, resulting in feedback inhibition of receptor signaling (295).

GH stimulates the expression of CIS, SOCS-1, -2, and -3 in a number of cell lines and tissues including the liver, muscle, fat, and kidney (295–297). *In vitro*, JAK2/STAT5 signaling by GH is strongly suppressed by SOCS-1 and -3 and partially affected by CIS (298–300). The mechanisms by which these three SOCS proteins exert inhibitory effects are different (294). SOCS-1 binds to the catalytic loop of JAK2 and blocks its kinase activity (299). CIS has no direct interaction with JAK2 but competes with STAT5 for GHR binding (301). SOCS-3 binds to both the GHR and JAK2 and inhibits signaling probably by blocking the interaction between JAK2 and STAT5 (299, 301). CIS, but not SOCS-1 and -3, also

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mediates proteosomal degradation of the GHR/JAK2 complex to terminate GHR signaling (300).

Mice lacking SOCS-1 and CIS do not exhibit an altered growth phenotype, although SOCS-3 deficiency is prenatally lethal due to failure of erythrocytosis (294, 295). SOCS-$1^{-/-}$ mice die perinatally of excessive responses to interferon γ (IFNγ), which causes hematopoietic infiltration of multiple organs and fatty degeneration and necrosis of the liver (295). These animals have low body weight, probably secondary to immune disease. SOCS-$1^{-/-}$IFN$\gamma^{-/-}$ mice do not develop neonatal diseases but have chronic inflammatory diseases and polycystic kidneys at later stages of life (302). However, no growth abnormality has been recorded in these double knockout mice. CIS$^{-/-}$ mice do not have a distinct phenotype, whereas mice overexpressing CIS show growth retardation and defective development of mammary glands and T cells (294). These data suggest that there may be functional redundancy in CIS and other SOCS proteins, and that overexpression of CIS may provoke a nonphysiological effect on signaling of the GHR as well as other cytokine receptors.

Although a role of CIS and SOCS-3 in regulating GH action in physiological situations remains to be determined, they have been implicated in the development of GH resistance caused by catabolic agents and fasting. In catabolic states such as sepsis, bacterial infection, inflammation, and chronic renal failure, the production of cytokines including IL-1β, IL-6, and TNF-α reduces hepatic response to GH (303). Because these proinflammatory cytokines markedly induce the expression of SOCS-3 and CIS (304–308), it is conceivable that the two SOCS proteins suppress GHR signaling in a nonselective manner. Similarly, fasting augments GH-induced expression of SOCS-3 in rat liver, an effect contributing to GH resistance (309).

Unlike other SOCS proteins, SOCS-2 exhibits a biphasic effect on GH activation of the JAK2/STAT5 pathway, being inhibitory when expressed at low levels and stimulatory at high levels (298, 310). The stimulation appears to result from its ability to counteract the inhibitory action of endogenous SOCS-1 (298). Like CIS, SOCS-2 inhibits GHR signaling by blocking STAT5 binding to the receptor (301). However, this would not explain its antagonistic effect on SOCS-1 inhibition, and thus, there may be other mechanism(s) by which SOCS-2 regulates the JAK2/STAT5 pathway.

Evidence that SOCS-2 is the major physiological negative regulator of GH action has been convincingly demonstrated in mice lacking the SOCS-2 gene (294). SOCS-$2^{-/-}$ mice exhibit a gigantism phenotype indistinguishable from the GH-transgenic mice (311). Similarly, the high-growth mice, a strain genetically selected for postnatal overgrowth, have a disrupted SOCS-2 locus (312). However, whereas SOCS-$2^{-/-}$ mice have normal serum GH and IGF-I levels (311, 313), the high-growth mice have reduced GH and increased IGF-I levels (312), suggesting that factors other than deregulation of GH and IGF-I secretion may contribute to the overgrowth phenotype. Greenhalgh *et al.* (313) have demonstrated that STAT5 activation by GH was prolonged in hepatocytes from SOCS-$2^{-/-}$ mice. They further showed that SOCS-$2^{-/-}$STAT5b$^{-/-}$ mice had a normal growth rate. The collective data thus suggest that SOCS-2 regulation of the JAK2/STAT5 pathway is critical for controlling the growth-promoting action of GH.

Paradoxically, overexpression of SOCS-2 in transgenic mice results in enhancement rather than impairment in growth as might be predicted from the findings in SOCS-$2^{-/-}$ mice (310). This observation may be explained by the *in vitro* findings that SOCS-2 has dual effects on GHR signaling. Unlike the growth response, neuron production from progenitor cells is increased in SOCS-2-transgenic mice but decreased in SOCS-$2^{-/-}$ mice (314). Because GH inhibits neuron differentiation, it is conceivable that overexpression of SOCS-2 may attenuate whereas SOCS-2 deficiency may enhance the suppressive effect of GH on neurogenesis (314). These findings also suggest that SOCS-2 may exert different dose-effects on GHR signaling in the central nervous system and peripheral tissues.

b. PTP. PTPs are a large family of over 100 enzymes that modulate cellular function by removing phosphate from phosphotyrosine residues in signaling molecules (315). Three of the phosphatases, the SH2-containing PTP (SHP)-1, SHP-2, and PTP-1B, negatively regulate GHR signaling (273, 316). The mechanisms for their actions appear to be different.

GH induction of JAK2 phosphorylation and STAT5 activation is prolonged in the absence of SHP-1 (317). Abrogation of SHP-2 binding to the GHR prolongs phosphorylation of the receptor, JAK2 and STAT5b, and significantly enhances GH-induced STAT5b transcriptional activity (318). Interestingly, SHP-2 enhances GH activation of the *c-fos* promoter (319), possibly through its ability to recruit the adapter protein Grb2 (320, 321), thus linking the GHR to the MAPK pathway and hence *c-fos* promoter activation.

PTP-1B suppresses the phosphorylation of JAK2, leading to attenuation of transcription by STAT3 and STAT5 (316). PTP-1B regulation may contribute to the development of GH resistance induced by fasting as demonstrated in PTP-1B$^{-/-}$ mice. In wild-type mice, the ability of GH to stimulate JAK2 phosphorylation is markedly reduced during fasting compared with feeding (316). In contrast, the levels of JAK2 phosphorylation are similar in the fasted and fed PTP-1B$^{-/-}$ littermates. The hepatic expression of SOCS-2 in response to GH, but not of SOCS-3 and CIS, is augmented in the fasted PTP-1B$^{-/-}$ mice, suggesting that the negative regulators may work in a complementary manner to control GHR signaling. The factors that regulate the expression and activity of these phosphatases have not been investigated in detail.

c. PIAS. The PIAS family contains five cytosolic proteins, PIAS1, -3, -xα, -xβ, and -y (322), which play an important role in negative regulation of STAT transcription (322, 323). The actions of PIAS proteins appear to be highly specific, with PIAS1 and PIAS3 inhibiting DNA binding of STAT1 and STAT3, respectively. A corresponding PIAS homolog for STAT5 has not been identified (322). PIAS1 and -3 affect the receptor signaling of IL-6, ciliary neurotrophic factor, oncostatin M, and IFNγ (322, 323), but their role in GHR signaling remains to be demonstrated.

In summary, the identification of negative regulators for the JAK/STAT pathway has greatly advanced the understanding of the molecular mechanisms for regulation of GHR

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signaling. On the basis of this knowledge, future studies can be conducted to delineate how other signaling pathways of the GHR are controlled.

C. Estrogen receptors

Estrogen exerts its biological action through specific receptors (ERs), which are ligand-activated transcription factors belonging to the nuclear hormone receptor family (324–326). There are two ER subtypes, designated as ERα and ERβ, which are widely expressed in reproductive and nonreproductive tissues including the liver and skeletal tissues (327, 328). ERα and ERβ exhibit distinct but overlapping patterns of tissue expression, ligand binding, and transcriptional function, which are likely to explain tissue-specific response to estrogen (327, 329).

Upon ligand binding, the two ER subtypes form homo- or heterodimers that bind to a specific palindromic motif, the ERE, on the 5′-regulatory region of target genes (328). On the DNA template, ERs interact with several classes of coactivators and the components of the basal transcriptional machinery to initiate transcription. ERs can also modulate gene expression via physical interaction with other transcription factors. Both ERα and ERβ associate with the Jun/Fos family proteins and stimulate transcription via AP1 sites (330). ERα enhances the transcriptional activity of Sp1 by forming an Sp1-ER complex that binds to a motif containing the GC-rich Sp1 site and an ERE half-site (331). In contrast, ERα inhibits nuclear factor κB-induced IL-6 expression by forming an inactive complex with the transcription factor (332, 333).

In addition to this nuclear site of action, estrogen can activate cytosolic signaling effectors, such as MAPK, PI3K, PLC, cAMP, and intracellular Ca^{2+} (334). These nongenomic actions occur within minutes of treatment, in contrast to the hours required for genomic effects, and involve ERs or other estrogen binding factors in the plasma membrane and cytosol (334, 335). Estrogen also stimulates receptor signaling of growth factors, including IGF-I and epidermal growth factor. Upon ligand binding, ERα, but not ERβ, physically associates with the IGF-I receptor, and induces autophosphorylation and activation of the receptor, resulting in phosphorylation of the MAPKs, ERK1/2 (336). Estrogen also potentiates IGF-I-induced phosphorylation and/or activation of IRS-1, PI3K, and ERK1/2 and stimulation of cell proliferation (337).

Estrogen can activate STAT signaling in a JAK-independent manner. In porcine aortic endothelial cells, estrogen acts through cytosolic and/or membrane-associated ERα and ERβ to rapidly (*i.e.*, within minutes) induce phosphorylation of STAT3 and STAT5 on both tyrosine and serine residues, leading to nuclear translocation and transcriptional activity of the STAT proteins (338). Induction of STAT phosphorylation is a nongenomic action of estrogen that requires the ligand-binding domain, but not the DNA-binding domain or coactivator binding regions, of ERα. This action is mediated by c-Src, MAPK, and PI3K, probably via a direct interaction of ERs with these proteins (339).

D. Estrogen regulation of GH receptor

Recent studies have provided strong evidence that regulatory interactions at the level of GHR expression and signaling may offer the basis for some of the physiological interplay between estrogen and the GH systems (Fig. 7).

1. GHR expression

a. Rodents. In rodents, GH binding activity in the liver and serum GHBP levels are higher in females than in males (227, 340–342) and are strongly estrogen-regulated. GHR content in the liver and circulating GHBP concentrations increase during pregnancy in mice, with the levels falling rapidly postpartum (342). Ovariectomy or treatment with an estrogen antagonist reduces GHR protein and GHBP in female rats (343). Estrogen treatment increases hepatic GHR contents in a dose- and time-dependent manner in both male and female rats, whereas testosterone treatment has no effect (343).

In contrast to the binding studies, investigations quantifying total mRNA abundance of the GHR and GHBP in rat liver have failed to detect a gender difference or a significant response to estrogen treatment (240, 344). However, the levels of GHR/GHBP transcripts derived from the liver-specific exon 1 (V1) variant are higher in female than male rats (240, 344, 345). The abundance of the V1 transcripts after estrogen treatment and gonadectomy parallel the GH binding activities. Similarly, only the levels of the liver-specific GHR/GHBP transcripts increase during pregnancy in mice (346). These observations suggest that the liver-specific transcript is likely to be the major contributor to the sexual dimorphism of hepatic GHR and GHBP in rodents.

How estrogen up-regulates expression of the liver-specific GHR/GHBP transcripts is not understood. The presence of an ERE-like motif and an adjacent AP1 site in the promoter region of the liver-specific exon 1 variant provides a potential mechanism (347). The possibility that ER and AP1 proteins may interact cooperatively to regulate hepatic expression of this GHR transcript has not been investigated.

Estrogen-induced expression of the liver-specific GHR/GHBP transcripts in rodents is GH-dependent. Estrogen fails to affect GHR/GHBP mRNA abundance in GH-deficient rats (344) and increases the mRNA levels in cultured mouse hepatocytes only with GH cotreatment (348). The GH dependency may be explained by the observation that GH induces hepatic ER expression (348, 349).

GHR expression in extrahepatic tissues is also estrogen-regulated but appears to be tissue-dependent. Estrogen increases the GHR mRNA level in osteoblasts (350) but not in uterus (351). Moreover, estrogen decreases the GHR mRNA level in rat brain, whereas ovariectomy has the opposite effect (352). The mechanism for tissue-dependent regulation may arise from the differential expression of ER subtypes. ERα, but not ERβ, associates with Sp1 to activate Sp1-responsive genes (331). As mentioned above, Sp1 plays a prominent role in regulating expression of V2-containing GHR transcripts, which are the predominant variants in extrahepatic tissues (247, 353, 354). Estrogen may interact with Sp1 to induce GHR expression in a tissue-specific manner related to the ER subtype status in extrahepatic tissues.

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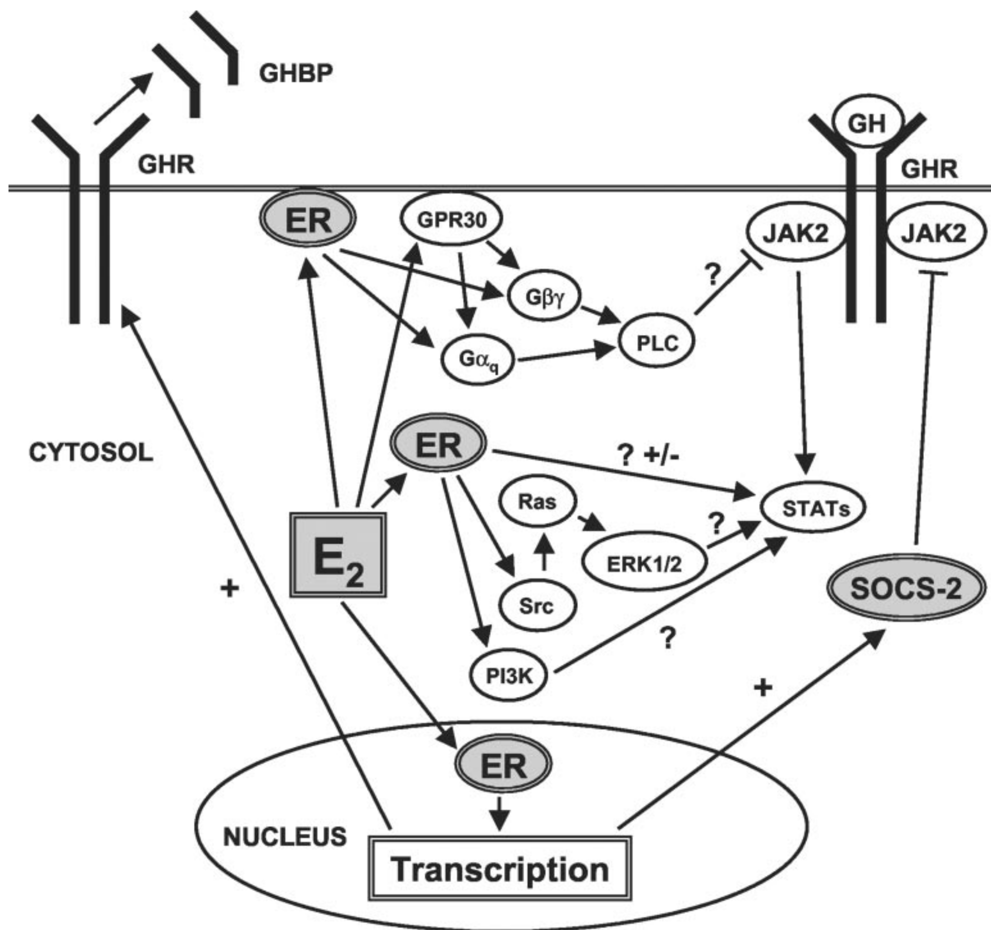

FIG. 7. Mechanisms for estrogen regulation of GHR expression and signaling through the JAK/STAT pathway. Stimulatory and inhibitory actions are indicated by + and ⊤ signs, respectively. Regulatory actions yet to be defined are denoted with *question marks*. E_2, Estradiol; GPR30, G protein-coupled receptor 30.

b. Human. Serum GHBP concentrations are significantly higher in women than in men (107). However, the binding protein level does not differ between pre- and postmenopausal women (106), nor does it vary with stages of the menstrual cycle (355). As described above, oral but not transdermal estrogen treatment significantly increases GHBP levels in blood (106). This effect is similar to those of other estrogen-sensitive hepatic proteins such as SHBG, T_4 binding globulin, and clotting factors (102, 120). These data suggest that estrogen is a regulator of GHRs in human liver, which is a major source of circulating GHBP.

There are very few data on mechanisms by which estrogen regulates GHR expression in human tissues. GH binding activity is increased in cultured osteoblasts by estrogen in a biphasic mode, with a maximal response at 10^{-12} M (350). GHR mRNA abundance shows a similar trend, although not reaching statistical significance. No studies on estrogen regulation of GHR expression in other human tissues have been reported.

How estrogen treatment increases the generation of GHBP in humans is not known. Potential mechanisms include 1) global stimulation of GHR expression, 2) selective induction of truncated GHR isoform expression, or 3) stimulated activation or expression of the metalloprotease, TACE/ADAM-17, which increases cleavage of the GHR. Studies are being undertaken in our laboratory to investigate the importance of these potential mechanisms.

2. GHR signaling. Our laboratory has recently reported that estrogen inhibits GH activation of the JAK/STAT pathway (296). The inhibition is dose-dependent and results from suppression of GH-induced JAK2 phosphorylation, leading to reduction in transcriptional activity of STAT3 and STAT5 (296). Estrogen does not affect PTP activity but stimulates expression of SOCS-2, which in turn inhibits JAK2 action (Fig. 7). These findings are supported by transcript profiling analysis of estrogen-responsive genes in mouse kidney, which shows that SOCS-2 expression is up-regulated by estrogen in an ERα-dependent manner (356). Given the central role of JAK2 in activating multiple signaling cascades of the GHR, estrogen may also affect other downstream pathways to exert a broader impact on GH action. The role of ER subtypes has also been investigated; ERα is more potent than ERβ in mediating this action of estrogen (T. H. Low, K.-C. Leung, and K. K. Y. Ho, unpublished observations). The role of SOCS-2 in mediating the inhibitory effects of estrogen is a novel mechanism of regulatory control of GH action by steroid hormones.

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Although the above mechanism reveals the inhibitory effect of estrogen to be indirect, there is some evidence supporting direct interactive crosstalk between signaling components of cytokines and steroid hormones, such as those between STAT5 and GR. However, evidence for direct interaction between ERs and STAT proteins is conflicting. Under conditions of ER and/or STAT overexpression, ERα and ERβ have been shown to physically associate with STAT3 and STAT5 after IL-6 and prolactin activation, respectively (357–359). However, this observation is not confirmed by experiments involving coimmunoprecipitation of ERα with endogenous STAT5 after GH activation (296). It is also not clear how ER association may affect STAT function because the above observations were made under conditions in which estrogen either inhibits (357, 358) or enhances the transcriptional activity of STATs (359).

Estrogen may interact with the JAK/STAT pathway through nongenomic mechanisms (Fig. 7). Estrogen rapidly activates c-Src, MAPK, and PI3K to induce phosphorylation and nuclear translocation of STAT3 and STAT5. This may conceivably cause desensitization of JAK/STAT signaling in response to GH due to reduced availability of STAT proteins in the cytosol. Estrogen may also suppress GHR signaling by activating PLC. Through membrane-associated ERα or the G protein-coupled receptor GPR30, estrogen activates the G protein subunits, Gα_q and G$\beta\gamma$, which in turn induce PLC activation (335, 360). PLC has been implicated in down-regulation of JAK2/STAT5 signaling by GH (361), although the mechanism has not been defined. In summary, current evidence indicates the existence of genomic and nongenomic avenues for estrogen regulation of GH action.

E. Cytokine regulation by nuclear hormone receptors

The many levels through which estrogen modulates GHR signaling are not unique. There is a wealth of evidence that steroid hormones influence the cellular action of many members of the cytokine receptor family by modulating their expression and signaling. Some notable examples are summarized in Table 1. Glucocorticoids stimulate mRNA expression of the IL-6 receptor (IL-6R), IL-2Rα, and IL-7Rα (344, 362, 363). However, it suppresses the expression of GHR (344, 362, 363), IL-3R (364), IL-11R, and oncostatin M receptor (365) and antagonizes IL-4Rα expression induced by IL-4 and phorbol myristate acetate (366). Similarly, estrogen increases the mRNA level of the prolactin receptor (367) and decreases those of IL-6R and gp130 (368). On the other hand, androgen decreases expression of the prolactin receptor (367). The mechanism by which these steroid hormones regulate cytokine receptor expression is not fully understood.

Nuclear hormone receptors also exert diverse effects on JAK/STAT signaling by cytokine receptors (Table 1). Glucocorticoids potentiate STAT5 transcriptional activity stimulated by GH and prolactin (283, 296, 369, 370) and enhance the STAT3 activity induced by IL-6 (371, 372). Similarly, mineralocorticoids and progesterone enhance STAT5 activation by prolactin (369), and 1α,25-dihydroxyvitamin D_3 potentiates GH stimulation of the JAK2/STAT5 pathway (373). Androgen does not affect prolactin-induced STAT5 transcription, but suppresses glucocorticoid enhancement of the prolactin action (369). Estrogen inhibits the signaling of IL-6 and leukemia inhibitory factor (296, 357, 358, 374). It has also been reported that estrogen either suppresses or augments STAT5 activation by prolactin (359, 369), although the reason for this discrepancy is unknown.

The GR does not affect JAK-induced STAT phosphorylation (372, 374) but acts as a coactivator to enhance nuclear translocation of the STAT proteins and prolong their DNA-binding capacity by preventing dephosphorylation (283, 374–376). Unlike glucocorticoids, 1α,25-dihydroxyvitamin D_3 suppresses GH-induced expression of SOCS-3 and CIS to enhance JAK2/STAT5 signaling (373).

The inhibitory action of estrogen on JAK2-mediated signal transduction may have broader implications beyond GH action, given that the JAK/STAT/SOCS systems are integral components of the cytokine receptor signaling. There is evidence that estrogen inhibits signaling of several members of the cytokine receptor family, including prolactin, IL-6, and leptin (296). Estrogen inhibition of lactation and sexual dimorphism in red cell mass and immune diseases is likely to be underpinned by regulatory control by estrogen of prolactin, erythropoietin, and proinflammatory cytokines. The role of androgens in the modulation of GH and cytokine actions is likely to be equally significant. There is a plethora of possible regulatory interactions between steroid hormones and cytokines, and much work is needed to understand their significance in health and disease.

TABLE 1. Cytokine receptor regulation by nuclear hormone receptors (NHRs)

NHR ligands	Effect	Cytokine receptors	Ref. no.
Receptor expression			
Glucocorticoid	↑	IL-2Rα, IL-6R, IL-7Rα	344, 362, 363
	↓	GHR, IL-3R, IL-4Rα, IL-11R, OSMR	364–366
Estrogen	↑	PRLR	367
	↓	IL-6R, gp130	368
Androgen	↓	PRLR	367
JAK/STAT signaling			
Glucocorticoid	↑	GHR, PRLR, IL-6R	283, 296, 369–372
Estrogen	↓	GHR, PRLR, IL-6R, LIFR	296, 357, 358, 369, 374
	↑	PRLR	359
Progesterone	↑	PRLR	369
Androgen	↓	PRLR	369
Mineralocorticoid	↑	PRLR	369
Vitamin D	↑	GHR	373

↑, Increase; ↓, decrease; OSMR, oncostatin M receptor; LIFR, leukemia inhibitory factor receptor; PRLR, prolactin receptor.

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V. Conclusion

Estrogen regulates the metabolic effects of GH. This effect is imparted not just at the level of secretion, but also at the level of GH action. The liver is a major site of regulatory interaction at which estrogen inhibits GH action in a dose-dependent manner. The detrimental but avoidable metabolic effects of oral estrogen on GH-regulated hepatic metabolic function add a new dimension of health consideration for all estrogen users from the young on OC steroids to the post-menopausal on estrogen replacement. Oral estrogen use in hypopituitary women may worsen an already abnormal body compositional state. In these patients, there are major economic implications because a larger dose of GH is required for an equivalent benefit. Conversely, estrogen could be an economic and convenient adjuvant treatment for women with active acromegaly. New classes of estrogen-related compounds such as SERMs and phytoestrogens are gaining widespread use. Their effects on the metabolic function of the liver are largely unknown but deserve further studies because of their long-term use and potential impact on body composition.

Estrogen attenuates GH action by suppressing GHR function. The GHR signals through the JAK/STAT pathway, which is negatively regulated by SOCS proteins. We propose that inhibition by estrogen of GHR signaling via up-regulation of SOCS-2 represents a novel paradigm of steroid hormone regulation of cytokine receptor function. Estrogen has already been shown to inhibit prolactin and IL-6 receptor signaling. There are many physiological observations, which may be explained by estrogen regulation of cytokine action. Thus, the inhibitory regulation of GHR signaling by estrogen mediated through the SOCS proteins is likely to have significance for the regulation of a diverse range of hormone and cytokine functions.

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