MECHANISMS IN ENDOCRINOLOGY

Paracrine and endocrine control of the growth hormone axis by estrogen

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Abstract

There is a strong biological link between the growth hormone (GH) and gonadal systems in growth, development and metabolism; however, regulatory interactions are poorly understood. Advances in estrogen biology and endocrine physiology have provided insights into mechanistic links between the two systems. Estrogens are synthesized from androgens by aromatase which is widely distributed in extragonadal tissues. Local generation of estrogens raise the possibility of paracrine control as an additional level to classical endocrine regulation of the GH system. To explore the mechanistic links, we review the pharmacology of estrogen, the effects of estrogen replacement, antagonism, and the impact of aromatase inhibition on the GH system as well as the metabolic sequelae. In men, estrogens derived from androgens drive the central secretion of GH, independent of the androgen receptor. In hypogonadal women, physiological replacement via a parenteral route evokes no effect while estrogen receptor antagonism and estrogen deprivation induce disparate effects, providing no consistent evidence that estrogens regulate the central secretion of GH via paracrine or endocrine mechanisms. However, delivery of estrogen by the oral route inhibits hepatic IGF-1 production, in turn increasing GH secretion via reduced feedback inhibition. This endocrine route-dependent effect of oral estrogen compounds on hepatic function induces detrimental metabolic effects on hypogonadal women. In conclusion, estrogens regulate the secretion and action of GH via complex paracrine and endocrine interactions and impart metabolic effects in a route- and gender-dependent manner. The metabolic sequelae of compounds mimicking, antagonizing, or depleting estrogens, should be considered in tailoring and optimizing their use.

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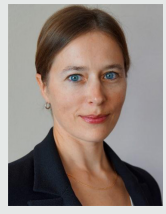

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Introduction

The strong relationship between sexual development and growth reflects a close interaction between the gonadal and GH systems. Observations that sex steroid priming in children enhanced GH response to provocative stimulation (1) provide early evidence that gonadal steroids enhanced GH secretion, triggering substantial efforts to elucidate the mechanisms by which gonadal steroids activate the GH system.

There have been major conceptual advances in understanding the regulation and function of the endocrine system. The endocrine system is defined as a system where the function of an organ is controlled by chemical messengers produced by another organ. Secreted hormones also act locally, affecting the function of neighboring cells. This local mode of action, known as paracrine regulation, is an integral part of the endocrine system. Further insight into the complexities of control has been provided by the discovery of another level of paracrine regulation in which a hormone transported in blood functions as a 'prohormone' that can either be converted to an 'active hormone' or deactivated by specific enzymes expressed locally in the target tissues. An example is type II and type III deiodinases and the activation of T4 to T3 or deactivation to reverse T3, respectively (2). In addition, the 'prohormone' can be transformed to distinct end products with disparate physiological actions, for example, the conversion of testosterone by aromatase to estradiol or by 5α-reductase to DHT (3, 4). These discoveries have provided major insights into the molecular pathophysiology of sex steroids and their interactions within the endocrine system. The information has provided strong evidence for estrogen being a key regulator of the GH system, mediating actions previously attributable to androgens. This review covers the biological links between the estrogen and GH, the biochemistry and pharmacology of estrogen, effects of estrogen agonism, antagonism and depletion, as well as clinical implications arising from modulating the GH system.

Biological links between estrogen and GH

There is a distinct gender difference in adult GH secretion. Random and mean circulating GH levels are higher in women than in men (5, 6, 7, 8). Support for endocrine-mediated regulation of GH secretion by estrogen comes from observations in women during puberty, menstrual cycle, and menopause, where changes in blood estrogen levels are associated with corresponding changes in GH concentrations. GH secretion increases with increasing stages of puberty in girls, especially in late puberty, when circulating estrogen is tightly linked to increased GH secretion (9, 10). The observation that estrogen priming in girls amplify the peak GH response to provocative stimulation is consistent with a stimulatory effect on GH secretion (1). In studies of pre-menopausal women, mean GH levels are higher during the luteal than the early follicular phase and peak in the late follicular phase when circulating estradiol levels are highest (11, 12, 13). GH levels are higher in pre-menopausal than post-menopausal women (5) and GH response to stimulation is substantially reduced after ovariectomy in pre-menopausal women (14). The collective observations of a strong association between estrogen and GH status at various stages of development and life suggest that estrogen positively regulates GH secretion.

Androgens and GH

There are also strong links between androgens and GH. Martha *et al*. observed that GH secretion increases during sexual development peaking in late puberty (15). Androgen priming amplifies the GH response to provocative stimulation in boys, mirroring estrogen effects in GH in girls possibly suggesting gender-distinct partitioning of androgens and estrogen action on the GH axis. Until then, it had been known that androgens such as testosterone can be converted to estradiol by the enzyme aromatase whereas some synthetic androgens cannot. Using this knowledge to probe sex steroid effects, two studies addressed the question as to whether androgens stimulated GH secretion directly. The first study observed that oxandrolone, a synthetic androgen that cannot be aromatized, failed to stimulate GH secretion in peripubertal boys (16). The second study demonstrated in hypogonadal men that testosterone enhanced GH secretion but that this was prevented by prior treatment with tamoxifen, an estrogen receptor antagonist (17). Thus, in males, androgens may not activate GH secretion directly but rather their actions depend on conversion to estradiol to activate the GH axis.

Estrogen biology

Estrogens produced from the gonads are derived from androgens by the action of the aromatase enzyme.

The cloning of the aromatase gene led to the discovery that aromatase is widely expressed in extragonadal tissues including the CNS, bone, adipose tissue, breast and vasculature. Estrogen synthesized within these compartments acts predominantly at the local tissue level in a paracrine or intracrine fashion (18). Thus, the total amount of estrogen synthesized within extragonadal sites may be small but the local tissue concentrations achieved are probably high exerting biological influence locally. In menopause, tissue concentrations of estradiol in the breast are up to ten-fold higher than that in blood (19, 20). There is growing evidence supporting the importance of extragonadal biosynthesis of estrogens in health and disease (18).

In humans, aromatase is highly expressed in the hypothalamus and the pituitary gland in females and males (21). Within the human pituitary, aromatase is localized to gonadotrophs, lactotrophs, somatotrophs and thyrotrophs (22). The importance of aromatase in GH biology has only been recently appreciated from the extra gonadal manifestations of aromatase deficiency. Somatotrophs of female mice rendered aromatase inactive by knockout technology are hypoplastic, exhibiting markedly reduced GH expression (23). The observation that men lacking the aromatase gene are also marked deficient in GH (24) adds further evidence supporting a pivotal role for estrogens in the development and function of the somatotrophs in humans.

Within the human pituitary gland, estrogen receptors (ERs) are expressed in somatotrophs with expression highest in lactotrophs (25). ERs are also widely distributed in the human hypothalamus including the paraventricular nucleus, ventromedial nucleus and lateral hypothalamus with some differences in intensity and distribution between the sexes (26).

Animal studies have localized ERs to both GHRH (27) and somatostatin neurons (28). These observations provide anatomical evidence for the machinery necessary for the local formation and action of estradiol on the pituitary and hypothalamus.

GH axis and estrogen pharmacology

The key elements of the GH system are the secretion of GH from the hypothalamus and its feedback inhibition by IGF-1 produced from the liver by the action of GH. The control of GH secretion from the hypothalamus is mediated principally by GHRH, ghrelin and somatostatin along with a host of metabolic, nutritional and stressor factors (29, 30), a review of which is beyond the scope of this paper. Sex steroids regulate a range of hepatic functions, such as lipoprotein metabolism, fat oxidation, and they also modulate the action of GH on the production of IGF-1. Estrogen inhibits the action of GH on the liver including the production of IGF-1, while androgens exert an opposite effect (31, 32, 33, 34). Thus, the feedback of IGF-1 on GH secretion is modulated by sex steroids arising from their hepatic effects of GH action.

Estrogens and related compounds are among the most widely used therapeutic agents. They are available as oral and parenteral formulations. Because estrogen is actively metabolized by the hepatic cytochrome system, a dose several fold in excess of daily production rates must be administered orally to achieve an adequate systemic effect (30, 35). The necessity to deliver such a large dose into the portal system creates a pharmacological concentration not usually seen in the physiological environment. This so-called first-pass effect results in stimulation of the synthesis of clotting factors and the binding proteins for several hormones (SHBG, TBG and CBG), but the inhibition of IGF-1 synthesis (36, 37, 38). The first-pass hepatic effects are circumvented when estrogen is administered in physiological doses by a non-oral route (35). The route-dependent impact arises from concentration-dependent effect of estradiol on the liver such that hepatic function is perturbed when non-physiological elevations are achieved by parenteral delivery (39). The pharmaco-kinetic and -dynamics of estradiol are key considerations necessary to interpret the results from regulatory studies of the GH system.

Estrogen and the GH system

We will review the studies investigating estrogen effects on GH secretion, covering *in-vitro* and clinical studies with a perspective on clinical relevance.

In rat pituitary cell cultures, estradiol at physiological concentrations increases spontaneous GH secretion while enhancing secretion stimulated by GHRH (40, 41). Studies employing tissue-specific knockout demonstrate unequivocally that the effects of estradiol are mediated via Ers (42). Thus, pre-clinical evidence supports direct stimulation of GH secretion by estrogen in the pituitary.

Studies in men

As indicated above, the first evidence that suggested that the GH stimulatory effect of androgens was indirect

came from studies using non-aromatizable androgens and ER blockade. Link and colleagues reported that 24 h GH secretion did not change in a group of peripubertal boys treated with oxandrolone, a non-aromatizable androgen, in contrast to a group treated with testosterone propionate (16). Malhotra *et al.* confirmed in boys with constitutional delay that oxandrolone failed to enhance GH secretion while exerting clear-cut androgenic effects (43). Along with another study showing that DHT failed to stimulate GH secretion in contrast to testosterone (44), the collective evidence do not support a direct effect of androgens in stimulating GH secretion.

The report by Weissberger *et al.* that the stimulation of GH secretion by testosterone in hypogonadal men was inhibited by tamoxifen, an ER antagonist, provided the first evidence that aromatization to estradiol mediated this effect of testosterone (17). Equally important was the observation that blood IGF-1 rose with testosterone and fell with ER blockade, indicating that aromatized estradiol drives GH secretion centrally. Birzniece and co-authors have confirmed in healthy adult men that tamoxifen inhibits arginine-evoked GH secretion and reduces blood IGF-1 levels (45, 46).

A mechanistic role for estradiol in stimulating GH secretion has been reinforced in studies investigating the effects of aromatase inhibition. Birzniece *et al.* observed that arginine-evoked GH secretion and corresponding IGF-1 levels were reduced by letrozole, a potent inhibitor of aromatase activity in normal men (46). The studies demonstrate that aromatase plays a critical role in mediating the central effects of testosterone on GH secretion.

Testosterone can also be converted to DHT in local tissues by 5α-reductase. Veldhuis *et al.* investigated whether androgens acting through the androgen receptor via the generation of DHT has any effect on GH (47). They compared the effects between 5α-reductase and aromatase inhibition after testosterone treatment in normal men. The authors observed that the enhancement of GH secretion by testosterone was unaffected by 5α-reductase inhibition which reduced DHT. In contrast, this effect was abrogated by aromatase inhibition which markedly reduced corresponding blood estradiol levels, indicating that AR activation does not affect GH secretion.

Further evidence that estradiol is the mediator of male hormone action has been provided by elegant estradiol add-back studies in hypogonadal men. Roelfsema and colleagues observed in a group of normal men rendered hypogonadal by a GnRH agonist and in whom aromatase activity was also inhibited that estradiol but not testosterone add-back restored GH secretion (48). These collective results provide good evidence that estradiol derived from androgens drives the central GH secretion in men.

Studies in women

The approach in women has been similar to intervention studies using estrogens, ER antagonists and aromatase inhibitors in post-menopausal women. However, the interpretation of GH changes is confounded by the clinical pharmacology of estrogen compounds as described above.

Route-dependency

An invariant finding from studies employing estrogen treatment by the oral route is the stimulation of GH secretion mirrored by a fall in circulating IGF-1, with IGF-1 suppression dependent on the dose and potency of estrogen formulations (35, 49, 50). The inhibition by estrogen of GH action arises from the inhibition of hepatic GH receptor signaling (38). The fall in IGF-1 also occurs in parallel with a rise in estrogen-sensitive hepatic proteins such as SHBG and of GHBP. Therefore, the question of whether estrogens stimulate GH secretion centrally cannot be addressed by studies using estrogen delivered by the oral route because of inescapable perturbation of hepatic function.

Estrogen can be replaced physiologically by a parenteral route avoiding first-pass metabolism. Employing a transdermal route, Weissberger *et al.* observed that estradiol replacement did not affect mean GH secretion over 24 h compared to a three- to four-fold increase by the oral route in post-menopausal women (Fig. 1) (35). The physiological nature of transdermal estradiol replacement of 100 µg/d was confirmed by the finding that SHBG level was unaffected. Bellantoni *et al.* investigated the effects of transdermal delivery of three different replacement doses (50, 100 and 150 µg) of estradiol to post-menopausal women (51). They observed a dose-dependent increase in circulating estradiol levels in the range of the follicular phase of young women. There was no significant change in mean GH and IGF-1 levels but there was a fall in the GH response to GHRH. In a later study, Bellantoni and colleagues confirmed that the route-dependent effect of estradiol on the GH-IGF-1 system while noting that transdermal delivery of 100 µg estradiol did not affect GH and IGF-1 levels in contrast to the effects of oral delivery (52).

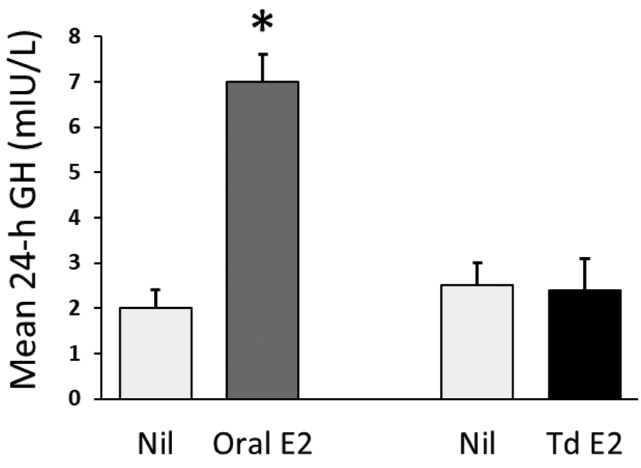

Figure 1

Mean 24-h serum GH concentrations in seven post-menopausal women studied before (nil) and during oral ethynyl estradiol (20 µg daily) or transdermal estradiol (100 µg patches twice weekly) therapy for 2 months. Oral estradiol significantly (*$P < 0.001$) increased serum GH in the face of reduction in IGF-1 by about 30%, which was not observed with transdermal estradiol treatment. Data are presented as mean ± S.E.M.. E2, estradiol; Td, transdermal. Adapted from (35).

The route-related effects likely arise from a concentration effect on the liver induced by a higher concentration of estradiol in the portal circulation after oral delivery. If this is true, it should be possible to reduce IGF-1 by parenteral delivery by increasing systemic estradiol concentration to a level sufficient to inhibit hepatic IGF-1 production. Friend *et al.* studied the transdermal effects of administering a supraphysiologic dose of estradiol on the GH system in post-menopausal women (53). This resulted in an increase in GH secretion and a reciprocal fall in IGF-1, along with an increase in SHBG, changes similar to the effects of oral delivery of 1 mg estradiol in this cross-over study. A mean estradiol concentration of 928 pmol/L was achieved with transdermal delivery, four- to five-fold higher than that observed in the mid-follicular phase. Thus, supraphysiological levels of estradiol in portal blood from oral dosing affects the function of the liver reducing IGF-1 production that in turn lessens the negative feedback on GH secretion and GH levels increase.

Concentration effect

What estradiol dose and concentration in blood are appropriate for assessing the effects without affecting hepatic function? Clues are revealed in two separate studies employing slightly different doses. Transdermal delivery of 50–100 µg estradiol to achieve blood concentration of at least 367 pmol/L did not affect GH and IGF-1 concentrations in post-menopausal and younger women with premature ovarian failure (54). In comparison, transdermal delivery of 200 µg/d which attained a mean peak estradiol level of 560 pmol/L was associated with a slight increase in mean GH concentration (55). In this latter study, the investigators observed no significant change in IGF-1 concentration but noted a significant fall in IGFBP-3 and an increase in SHBG levels, indicative of perturbation of hepatic function. Thus, evidence required to address whether estradiol stimulates GH secretion centrally must be selected from replacement studies employing a parenteral route that does not perturb hepatic function. When appropriately selected, these studies (35, 51, 52, 54) do not provide evidence supporting an endocrine role for estradiol in the central stimulation of GH secretion in adult hypogonadal women.

Estrogen receptor blockade

With poor evidence supporting an endocrine role of estrogen, the question of whether local estrogen regulates GH secretion has been addressed by using ER antagonists. Tamoxifen, as a selective estrogen receptor modulator (SERM) possesses tissue-specific antagonistic and agonist effects. While it exerts ER antagonist centrally, tamoxifen possesses estrogen agonist effects on the liver (56, 57). Birzniece *et al.* compared the effects of orally administered tamoxifen and estradiol valerate on GH stimulation by arginine in post-menopausal women (33). Tamoxifen reduced and estradiol valerate enhanced the GH response to arginine (Fig. 2). IGF-1 fell and SHBG levels rose with both treatments. The blunted GH response observed with tamoxifen in the face of reduced IGF-1 feedback inhibition indicates that GH secretion was suppressed by estrogen receptor antagonism. Because circulating estradiol was unaffected by tamoxifen treatment, these data suggest a persuasive role of local estrogen in the central control of GH secretion in hypogonadal women.

Aromatase inhibition

To extend investigations exploring possible paracrine control of GH secretion, Birzniece *et al.* studied whether aromatase inhibition replicated the effects of tamoxifen in post-menopausal women (Fig. 3) (46). Tamoxifen reduced GH secretion, however, letrozole, a potent aromatase inhibitor, imparted no significant effect. Estradiol concentration was unmeasurably low throughout the

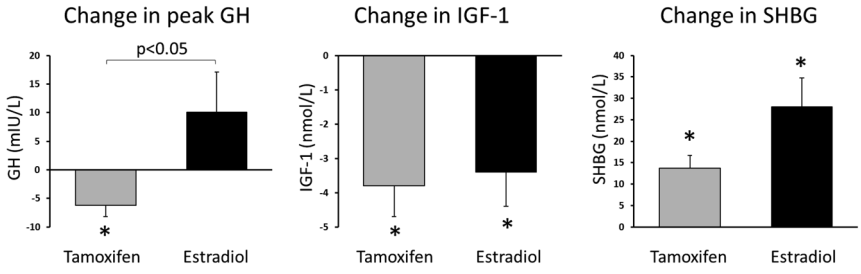

Figure 2
Change from baseline in peak GH response to stimulation, serum IGF-I and SHBG during oral treatment with tamoxifen (20 mg/d) and estradiol valerate (2 mg/d) in post-menopausal women. GH data are presented as maximum increment in serum GH over pre-stimulation GH levels during arginine infusion. Conversion factor: 1 mIU/L = 0.33 g/L. Data are expressed as mean ± S.E.M. *$P < 0.05$ compared with baseline. Adapted from (33).

study in the post-menopausal women, in whom estrone levels fell by more than 95%, providing evidence of letrozole action. In men, whom letrozole administration reduced GH secretion and IGF-1 levels, letrozole markedly reduced both estradiol and estrone levels (46). Thus, inhibiting aromatase did not affect GH secretion in post-menopausal women, an unexpected finding suggesting that estrogens may not drive GH secretion in women with low estrogen milieu.

How can a fall in GH secretion from ER blockade with tamoxifen be explained? Evidence from the oncology field shows that tamoxifen exerts direct effects on signaling pathways inhibiting tumor cell proliferation that are not estrogen receptor-mediated (58). It has also been observed that tamoxifen exerts a direct dose-dependent inhibitory effect on GH secretion from pituitary somatotrophs *in-vitro* in the absence of added estradiol (59). Thus, it is conceivable that the blunting of GH secretion may be mediated through mechanisms not involving the estrogen receptor.

Androgens

The effect of androgens on the GH-IGF system in women has been investigated in the setting of HIV wasting. Schurgin *et al.* observed that transdermal administration of 150 µg of testosterone increased IGF-1 but not mean GH concentration (60). In a study in men with HIV wasting, the same group reported that testosterone treatment did not change IGF-1 but significantly reduced mean GH levels, findings different from those in normal men (61). These observations suggest that patients with HIV wasting are not an appropriate model for investigating the interactions of sex steroids and the GH system. To our knowledge, there have been no studies in women of reproductive age exploring whether androgens amplify GH secretion and if so whether this requires aromatization.

In summary, studies in men show that estrogens mediate the central stimulation of GH secretion in men. In hypogonadal women, physiological estrogen replacement has no effect while ER antagonism and estrogen deprivation induce disparate effects, providing no consistent evidence that estrogens regulate the central secretion of GH.

Clinical implications

In adults, GH regulates energy and substrate metabolism and body composition optimizing functional and structural integrity of the body. Estrogen compounds and derivates are used for a range of conditions including growth disorder, hypogonadism, contraception, osteoporosis, and breast cancer. Their use is likely to carry secondary effects on metabolic health arising from how the GH system is affected.

The route-dependent effects of estrogen carry important metabolic consequences for the use in hypogonadism and for contraception for women. For hypopituitary women who are GH deficient (GHD), oral estradiol replacement not only worsens the consequences of GHD but also attenuates the therapeutic benefits of GH replacement therapy (39). During GH replacement in hypopituitary women, concurrent estrogen treatment by the oral route attenuates protein anabolism and fat oxidation (62). These effects are exacerbated by prescribing contraceptive steroids or SERMs rather than estradiol in therapeutic doses (63, 64). The route-dependent effects are equally relevant in menopause. In comparison to transdermal treatment of post-menopausal women, oral

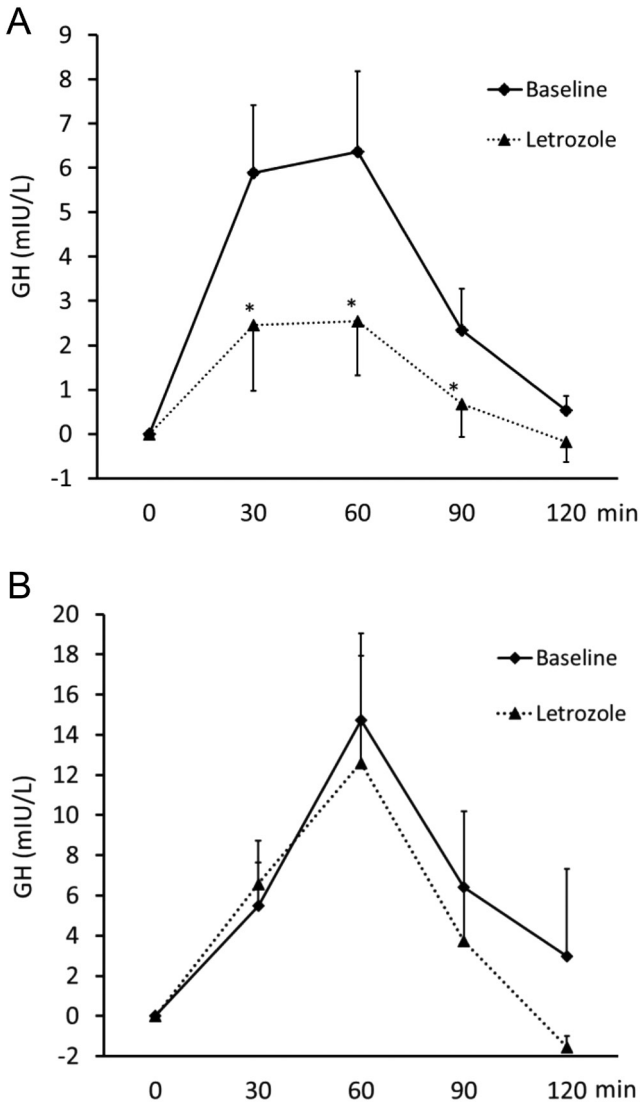

Figure 3

GH response to arginine stimulation at baseline (diamond) and during aromatase inhibitor letrozole (triangle) treatment in eight healthy men (A), and in seven healthy post-menopausal women (B). Data are presented as mean increment in serum GH after arginine infusion over 120 min. *$P < 0.05$ compared to baseline. Conversion factor: 1 mIU/L= 0.33 µg/L. Adapted from (46).

estrogen replacement increased fat mass by 1.2 kg over 6 months and a reduced lean body mass by an equivalent amount (65), as well as reducing insulin sensitivity (66).

The endocrine-mediated effects of estrogens and SERMs on hepatic GH action have been exploited for adjuvant treatment of acromegaly (30, 67). Tamoxifen treatment normalized IGF-1 in almost 50% patients with acromegaly (68). Clomiphene citrate, another SERM, reduced IGF-1 to normal in a similar proportion of men with acromegaly (69). Thus, oral estrogens and SERMs can be offered as inexpensive adjuvant treatment of active acromegaly in a gender-appropriate manner.

SERMs and aromatase inhibitors are established adjuvant treatments in breast cancer. While used as effective estrogen-inactivating drugs, they exert disparate effects on the GH system. Tamoxifen inhibits GH secretion and hepatic IGF-1 production in contrast to aromatase inhibitors which does not affect the GH system in women (46). Steatosis develops as a consequence of tamoxifen but not aromatase inhibitor therapy for breast cancer and fatty liver is a common finding in GHD (70, 71, 72). As GH stimulates hepatic triglyceride export by stimulating VLDL secretion (73), the development of steatosis may arise secondarily from GH insufficiency induced by tamoxifen (74). Thus, aromatase inhibitors possess metabolic advantages over SERMs in the adjuvant therapy of breast cancer.

Conclusion

Complex regulatory inter-relationships involving paracrine and endocrine mechanisms underlie the interaction between estrogen and the GH system. There are gender-dependent effects of estrogen on both the secretion and action of GH. In men, GH secretion by estrogen is activated via a paracrine manner from aromatization of androgens. In women, the evidence supporting a role for estrogen in the central regulation of GH secretion is sparse. The inhibitory effects of estrogen on hepatic GH function are mediated by an endocrine mechanism. There is a need for future studies to assess central mechanisms regulating GH secretion in pre-menopausal women. Estrogens, estrogen antagonists and estrogen depleting medications can exert significant gender-related effects on metabolic health and in turn, may impart detrimental or beneficial effects depending on the clinical context.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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