MECHANISMS IN ENDOCRINOLOGY

Estradiol as a male hormone

Nicholas Russell[1,2] and **Mathis Grossmann**[1,2]

[1]Department of Medicine Austin Health, University of Melbourne, Heidelberg, Victoria, Australia and [2]Department of Endocrinology, Austin Health, Heidelberg, Victoria, Australia

Correspondence should be addressed to M Grossmann
Email
mathisg@unimelb.edu.au

Abstract

Evidence has been accumulating that, in men, some of the biological actions traditionally attributed to testosterone acting via the androgen receptor may in fact be dependent on its aromatization to estradiol (E2). In men, E2 circulates at concentrations exceeding those of postmenopausal women, and estrogen receptors are expressed in many male reproductive and somatic tissues. Human studies contributing evidence for the role of E2 in men comprise rare case reports of men lacking aromatase or a functional estrogen receptor alpha, short-term experiments manipulating sex steroid milieu in healthy men, men with organic hypogonadism or men with prostate cancer treated with androgen deprivation therapy (ADT) and from observational studies in community-dwelling men. The collective evidence suggests that, in men, E2 is an important hormone for hypothalamic–pituitary–testicular axis regulation, reproductive function, growth hormone insulin-like growth factor-1 axis regulation, bone growth and maintenance of skeletal health, body composition and glucose metabolism and vasomotor stability. In other tissues, particularly brain, elucidation of the clinical relevance of E2 actions requires further research. From a clinical perspective, the current evidence supports the use of testosterone as the treatment of choice in male hypogonadism, rather than aromatase inhibitors (which raise testosterone and lower E2), selective androgen receptor modulators and selective estrogen receptor modulators (with insufficiently understood tissue-specific estrogenic effects). Finally, E2 treatment, either as add-back to conventional ADT or as sole mode of ADT could be a useful strategy for men with prostate cancer.

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Introduction

Estrogens were demonstrated in the urine of men in the 1920s (1) and in the testis in 1952 (2). In 1937 Steinach and Kun demonstrated that administration of large doses of testosterone to men increased the estrogenic activity of their urine and inferred *in vivo* conversion of testosterone to estrogens (3). Subsequent advances included the identification, isolation, sequencing and regulatory characterization of the aromatase cytochrome P450 enzyme, product of the *CYP19A1* gene, the enzyme responsible for aromatization of testosterone to 17β

Invited Author's profile

Mathis Grossmann, MD, PhD, FRACP, is a physician-scientist trained in both basic biology and in clinical endocrinology. He is a professor of medicine at the Department of Medicine (Austin Health), University of Melbourne and consultant endocrinologist and head of men's health at Austin Hospital, Melbourne, Australia. His research focuses on the regulation of musculoskeletal and cardiometabolic health by androgens and estrogens, both at the clinical and the molecular levels.

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estradiol (E2) and androstenedione to estrone (E1), the major endogenous estrogens (4). Many other non-aromatized endogenous steroids, estrogen metabolites and environmental and pharmaceutical compounds with diverse structures have minor estrogenic activity (5, 6).

For this review the PubMed database was searched using the MeSH terms 'estradiol' and 'men', from inception to November 1, 2018. Reference lists from relevant retrieved articles were used to identify further relevant papers. Although pre-clinical models have been instrumental in elucidating relevant biological mechanisms, this review will predominantly discuss human studies. Table 1 provides a summary of conclusions drawn from these human studies.

Physiology and metabolism of estrogens

Circulating E2 in men

One quarter to one half of circulating E2 is estimated to originate from direct testicular secretion, with the rest resulting from peripheral aromatization of testosterone, particularly in adipose tissue, muscle, bone and brain (7, 8, 9). Median serum E2 concentrations are around 150 pmol/L in healthy young men and 90 pmol/L in healthy older men, compared to 400 pmol/L in premenopausal women, while healthy male circulating testosterone concentrations are substantially higher, ranging from about 10 to 30 nmol/L, although these concentrations vary across studies performed in different populations and using different assay methodologies (10, 11).

Metabolism of estrogens

The predominant metabolic pathway for E2, the most potent endogenous estrogen, is reversible oxidation to E1 by the widely distributed 17β hydroxysteroid dehydrogenase (12). For infusions of labeled steroid, oxidation of E2 to E1 is more rapid than the reductive reaction (13). E2 and E1, the parent estrogens, undergo irreversible hydroxylation at the 2-, 4- or 16-carbon positions by cytochrome P450 enzymes, particularly CYP1A2 and CYP3A4, located in the liver and other tissues (14). The 4-hydroxy-estrogens are similar in potency to the parent estrogens, while the 2-hydroxy-estrogens are less potent and therefore, because they retain ER-binding affinity, may be relatively anti-estrogenic (15). The 2- and 4-hydroxylated metabolites undergo methylation to less active forms (16). The 16α hydroxyestrogens, including estriol (E3), retain minor estrogenic activity (17).

In a parallel metabolic pathway, the parent estrogens, and their metabolites, are reversibly inactivated through conjugation with sulfate, glucuronide or glutathione, by widely distributed conjugation enzymes (16, 18). Estrogen conjugates can not only be excreted in urine or bile but also may circulate in blood and reach target tissues where deconjugation may occur to release active estrogens. Despite their abundance, estrogen metabolites are not commonly measured, although this is likely to change with increasing clinical availability of mass spectrometry (16).

Estrogen receptors in men

ERα and ERβ, encoded by the *ESR1* and *ESR2* genes respectively, are members of the nuclear receptor superfamily. Multiple isoforms of each receptor type exist, created by differential splicing of exons (19). More recently, the transmembrane G-protein-coupled estrogen receptor 1 (GPER) was identified (20). ERs are expressed throughout the human male reproductive tract, and also in male brain, cardiovascular system, liver, bone, adipose tissue, pancreatic islets and skeletal muscle (21).

ER signaling occurs via a number of distinct pathways (22). The classic ligand-dependent pathway involves ligand-activated ER dimerization with direct DNA binding to estrogen response elements (EREs) in gene promoters leading to the regulation of transcription. ERα and ERβ bind to the same ERE. Other ligand-dependent pathways involve interaction of the ER with coregulators and additional transcription factors, allowing interaction with gene promoters that do not contain ERE. The ER can also be activated to modulate gene transcription in a ligand-independent fashion, by second messenger kinases involved in signal transduction by receptors of growth factors. There are also non-genomic pathways by which ER ligand binding induces rapid physiological effects (23).

Intracrinology of estrogens

Most clinical studies infer biological actions from serum E2 concentrations, based on the classical endocrine concept that gonadal-produced sex steroids circulate to target tissues to exert their effects. These studies do not take into account cellular uptake, either after dissociation from SHBG or albumin or perhaps as an SHBG-bound complex (24), nor local production and metabolism of sex steroids in target tissues themselves, with potential for autocrine and paracrine actions. As reviewed elsewhere (25), some extragonadal tissues possess the

Table 1 Biological roles of estradiol in men.

System	Process	Comment	Clinical reference
HPT axis	Negative feedback	• E2 produces negative feedback on GnRH pulse frequency, probably indirectly via KNDy neurons. • E2 produces negative feedback on LH pulse amplitude by reducing pituitary gonadotrope sensitivity to GnRH.	26, 27, 28, 29, 30, 31
Reproduction	Testicular descent and spermatogenesis	• ER signaling appears to play a role in testicular descent and spermatogenesis	35, 36, 38
	Maintenance of libido and erectile function	• E2 appears to have a beneficial but non-essential role	47, 50, 53, 54
	Prostate development, hypertrophy, carcinogenesis	• *In vitro* and animal models suggest a role for ER signaling in *in utero* prostate gland development. • There is no definitive human data to define any role of E2 in BPH or carcinogenesis. • In men with prostate cancer, clinical evidence is consistent with a net antineoplastic effect of estrogens if used at supraphysiological doses to reduce circulating T to castrate concentrations.	64, 65
GH-IGF-1 axis	Pituitary GH secretion	• E2 appears to be the principal determinant of GH secretion with effects at both hypothalamic and pituitary level	68, 69
	Hepatic IGF-1 secretion	• E2 inhibits hepatic IGF-1 response to GH	71
Bone	Growth	• E2 stimulates the pubertal growth spurt in boys likely via GH–IGF-1 axis stimulation • E2 directly causes late pubertal growth plate closure	82, 89
	Attainment and maintenance of bone mass	• E2, acting via ERα appears to be the predominant sex steroid for attainment and maintenance of normal bone mineral density and microstructure	32, 82, 89, 96, 97, 102, 103, 104, 105
Body composition	Muscle mass and strength	• Minimal role if any	54
	Fat mass	• E2 appears to prevent gain of fat mass. Data are mixed on whether this is a depot specific effect	54, 121
Metabolism	Glucose metabolism	• E2 appears to improve insulin sensitivity. Data are mixed on whether this is hepatic, peripheral or both	121, 129, 133
	Lipid metabolism	• E2 may not have any direct effect on lipid metabolism	68
Brain	Masculinization of brain anatomy and physiology	• No role for E2 in brain development in men has been defined. Where reported, men with congenital ERα deficiency and aromatase deficiency have had cisgender identity	35, 47
	Cognition	• No role for E2 in cognition has been proven and small trials of E2 add-back in men with castrate levels of T have not demonstrated any cognitive benefit	152, 153
Temperature regulation	Vasomotor stability	• E2 is the predominant sex steroid responsible for prevention of hot flushes	53, 104, 157

BPH, benign prostate hypertrophy; E2, estradiol; ER, estrogen receptor; GH, growth hormone; HPT, hypothalamic–pituitary–testicular; IGF-1, insulin-like growth factor-1; KNDy, kisspeptin, neurokinin B and dynorphin; T, testosterone.

capacity for *de novo* sex steroid synthesis and/or for metabolism of circulating precursors, such as adrenal produced dehydroepiandrosterone sulfate, and express steroidogenic enzymes not reported to be active in gonadal tissues. The net biologic effects of such locally acting estrogenic compounds in individual target tissues are not known but, in combination with the use of inaccurate immunoassays, may contribute to the relatively modest associations of circulating E2 concentrations with biologic phenotypes reported in many clinical studies.

Hypothalamic–pituitary–testicular axis regulation

Human gonadotropin-releasing hormone (GnRH) neurons express ERβ but not ERα (26). It is likely that E2 acts on ERα in kisspeptin, neurokinin B, and dynorphin-expressing (so-called KNDy) neurons in the infundibular nucleus of the hypothalamus to regulate GnRH neurons indirectly (27). E2 also has direct effects on pituitary gonadotropin production (28, 29).

In parallel experiments in healthy and GnRH-deficient men (with idiopathic hypogonadotropic hypogonadism; IHH) receiving fixed GnRH treatment (generating a *de facto* hypothalamic clamp), the effect of an aromatase inhibitor (AI) on luteinizing hormone (LH) secretion was examined (30). By comparing LH responses in healthy men to LH responses in those undergoing the hypothalamic clamp, pituitary-dependent and hypothalamic-dependent effects of E2 withdrawal could be distinguished. In both groups, AI treatment suppressed serum E2 and elevated serum testosterone into the supraphysiological range. Despite the testosterone rise, LH increased in both groups, suggesting that aromatization of testosterone to E2 is required for negative feedback on LH secretion. For men undergoing the hypothalamic clamp, the site of this E2 effect must have been pituitary, and the authors inferred that, in normal physiology, E2 reduces pituitary sensitivity to GnRH, thereby reducing LH pulse amplitude. LH increments in healthy men were approximately twice those recorded in IHH men undergoing the hypothalamic clamp. This differential suggested an additional hypothalamic site of E2 action, likely to reduce GnRH pulse frequency. A subsequent study subjecting healthy and IHH men to experimental castration and simultaneous inhibition of aromatase by high-dose ketoconazole, and testosterone, E2 or placebo add-back concluded that, while E2 has dual feedback, its predominant negative effect occurs at the hypothalamus (31).

Rochira *et al.* examined gonadotropin secretion pre and post physiological E2 add-back in two men with congenital aromatase deficiency (29). Such men have normal to high serum testosterone. In these men, E2 add-back reduced LH pulse frequency, suggesting a role for circulating E2 in reducing hypothalamic GnRH action. When these men were given GnRH to stimulate gonadotropin secretion, E2 add-back reduced LH and follicle-stimulating hormone (FSH) secretory peaks and area under the curve, suggesting that circulating E2 also directly acts on the pituitary to reduce sensitivity to GnRH.

Direct androgen receptor (AR)-mediated negative hypothalamic-pituitary feedback also may occur in men. In a 2-year randomized controlled trial (RCT) of pharmacological doses of the non-aromatizable androgen dihydrotestosterone (DHT) designed to assess androgen effects on prostate growth in healthy older men, DHT suppressed testosterone, E2, LH and FSH to castrate concentrations (32). However, supraphysiological doses of pure androgen agonists, while providing evidence of the potential for pharmacological AR-mediated effects, do not prove that these effects are important in physiology. Experimental parallel design studies referred to above (31) using medical castration with ketoconazole and testosterone add-back have also concluded that testosterone exerts a direct negative hypothalamic feedback not requiring its aromatization to E2.

Additional indirect evidence comes from 46,XY women with complete androgen insensitivity syndrome (CAIS) due to inactivating AR mutations. These women have, despite circulating E2 sufficient to cause a female external phenotype, high concentrations of testosterone and LH. This suggests that E2 is not sufficient to maintain a normal LH concentration, but that AR-mediated effects are also required to reduce LH production (33). Whether these inferred effects are organizational during *in utero* hypothalamic–pituitary programming or activational AR-dependent effects in adulthood is not resolved by these case observations.

Overall, human studies suggest that E2-mediated negative feedback on gonadotropin activation involves both hypothalamic and pituitary actions. Moreover, while E2 is important for this negative feedback, it is likely that AR-mediated effects also play a role.

Reproduction

Spermatogenesis

A comprehensive discussion of the role of E2 in testicular function and spermatogenesis is beyond the scope of this manuscript and recent reviews exist (21). Briefly, ERα and ERβ are expressed throughout the human male reproductive tract, including in germ cells, although there are conflicting data in certain cell types. Aromatase is found in human immature germ cells, spermatozoa, efferent ductules of the testis and epididymis (21). While studies in mice have demonstrated important roles for estrogen signaling in male fertility, studies in men have been less dramatic and less conclusive.

Male ERα-knockout mice are infertile due to disruption of fluid reabsorption in the efferent ductules of the epididymis (34). Testicular function has been reported in two men with homozygous missense mutations in *ESR1* causing non-functional ERα. The first man, at age 28 years, had descended testes of normal volume and normal sperm count with a minor reduction in sperm viability (35). The second man, at age 18 years, had a cryptorchid right testis, hypoplastic left testis and primary hypogonadism, but no semen analysis was reported (36).

Aromatase-knockout mice exhibit progressively impaired spermatogenesis with aging (37), and abnormalities have been reported in men with congenital aromatase deficiency. In most cases, testicular size was normal (38, 39, 40, 41, 42, 43, 44, 45), although a few men had low testicular volume (46, 47, 48, 49), and cryptorchidism has been commonly reported (43, 45, 47, 48). Where reported, sperm counts were normal (42, 44) or reduced (39, 46), commonly with altered sperm motility (44, 46). A few men with aromatase deficiency have undergone testicular biopsy, showing varying degrees of impaired spermatogenesis including germ cell arrest (46, 48, 49). These observations suggest a role for E2 in testicular descent and spermatogenesis. The fact that some men appear to have normal testicular function despite congenital lack of E2 has been postulated to be related to dietary phytoestrogen intake (37), although there is no direct evidence for this in humans. An alternative explanation is that men with aromatase deficiency would be expected to retain ligand-independent ERα signaling in the reproductive tract (50).

Libido and erectile function

The evidence from men with congenital non-functional ERα or aromatase deficiency suggests that E2 is not essential for male libido and erectile function (39, 46), but various lines of evidence support some physiological role. In non-blinded studies, two men with aromatase deficiency had increments in libido and sexual activity during E2 treatment, either alone (51) or with testosterone (47). Non-definitive evidence from men with prostate cancer suggests that androgen deprivation therapy (ADT) using estrogens may better maintain sexual function than surgical castration (52) and GnRH analogs (53).

In a physiological experiment, Finkelstein *et al.* gave a GnRH analog with or without AI to 400 healthy young men for 16 weeks (54). Participants were further randomized to add-back with one of five transdermal testosterone gel doses that produced serum testosterone concentrations ranging from castrate to mildly supraphysiologic, either with E2 clamped to very low concentrations (AI cohort) or with the expected corresponding E2 increments for each testosterone dose increment (non-AI cohort). This design allowed for discrimination of testosterone from E2-dependent effects. Testosterone-related effects were assessed directly by comparing changes between groups of men in whom E2 was clamped. E2-related effects were inferred indirectly, by comparing mean change for each outcome in the AI cohort with the outcome in the non-AI cohort, and also by linear regression modeling of the cohort–testosterone dose interaction. In this analysis, E2 clearly exerted a positive effect on sexual interest and erectile function, independent of testosterone. Assignment to the AI cohort was not randomized and there was no placebo, so the possibility of systematic differences between cohorts that were not due to differences in serum E2 is not completely excluded. Moreover, although not reported, it cannot be excluded that potential adverse effects of AI (e.g. hot flushes and associated sleep disturbance), in addition to their E2-lowering actions, might have contributed to reductions in libido.

Prostate

Estrogens are synthesized by aromatase in the prostate stroma (55) and have autocrine and paracrine actions. ERα appears to be primarily located in prostate stromal cells while ERβ is found primarily in the epithelium (56). GPER has been demonstrated in stromal cells and epithelial progenitor cells (57). Estrogens appear to have a role during normal prostate development *in utero*, a time of high systemic estrogen exposure (56, 57). However, unphysiologically timed or dosed estrogen exposure during prostate gland development influences later prostate cancer risk in animal models (58).

Aromatization to E2 is hypothesized to be important in testosterone-stimulated prostate growth, although there are clearly AR-mediated effects illustrated by the fact that 5α-reductase inhibitors are effective in treating symptoms of benign prostatic hypertrophy (BPH) (63). A recent small trial ($n=31$) was conducted to differentiate aromatization from non-aromatization-dependent effects of testosterone on BPH in men receiving testosterone replacement for age-associated mildly low testosterone (59). Men were randomized to placebo, transdermal testosterone or transdermal testosterone plus AI. The primary outcome was change in prostate volume after 12 months. Men receiving testosterone alone had a small significant increase in prostate volume compared with placebo-treated and testosterone/AI-treated men, leading the authors to infer that the tropic effects of testosterone on the prostate are aromatization dependent. Adding support to this hypothesis, in a 2-year trial of DHT treatment of older men; gonadotropins, E2 and T were suppressed, with maintenance of AR-signaling (32). In this trial, there was no difference in prostate growth between DHT and placebo arms. However, these results are difficult to interpret because the study was designed to test the hypothesis that DHT would actually inhibit prostate growth via ERβ-mediated effects of its metabolite, 3βAdiol.

Aberrant aromatase expression has been demonstrated in human prostate adenocarcinoma epithelial cells suggesting the potential for autocrine signaling via ERα (55). ERα activation has proliferative effects in the prostate, but ERβ- and GPER-mediated effects appear to be antiproliferative and anticarcinogenic (60, 61). As a result, the net effect of estrogenic signaling in normal prostate or prostate cancer cells has been difficult to predict, and biologic effects may differ between healthy and diseased prostate. Evidence from rat models suggests important roles for genotoxicity of hydroxylated estrogen metabolites in inducing prostate carcinogenesis (62).

The clinical evidence is largely consistent with a net antineoplastic effect of estrogen-based treatment if used at supraphysiologic doses to reduce circulating testosterone to castrate concentrations in men with prostate cancer. Conversely, in men with castrate resistant prostate cancer, trials of estrogen signaling ablation through the use of AI in addition to surgical or medical castration did not show any clinical benefit (63). While benefits in early studies using oral estrogens were negated by cardiovascular toxicity due to the prothrombotic hepatic first-pass effect (64), more recent smaller studies using high-dose transdermal E2 as a sole mode of ADT have not reported increased cardiovascular risks (65). Overall, these clinical trials suggest that potential prostate cancer-promoting effects of estrogenic signaling appear outweighed if pro-carcinogenic testosterone signaling is abolished.

In summary, the collective evidence from rare case studies (including ER and aromatase-deficient men) and experimental studies in healthy younger and older men is consistent with the fact that ER signaling plays a role in various aspects of male reproductive health, including testicular descent, spermatogenesis and sexual function. ER signaling also has complex effects on the prostate gland, but the consequences for prostate health and disease are poorly understood.

Growth hormone insulin-like growth factor-1 axis

Men with congenital aromatase deficiency have impaired stimulated growth hormone (GH) secretion and low insulin-like growth factor-1 (IGF-1) (66). Exogenous E2 in these men was not able to normalize GH secretion (66), possibly because of abnormal development of the GH–IGF-1 axis in the setting of congenital E2 deficiency or because restoration of normal circulating E2 concentration is insufficient if local E2 production remains impaired (67). Additional lines of evidence from preclinical models, women, and indirect evidence from men, suggest that E2 is important in regulation of GH secretion by both direct and indirect mechanisms (67, 68).

Using a T/E2 clamp in healthy older men (GnRH analog plus intramuscular (IM) testosterone add-back plus AI plus transdermal E2 or placebo controls), Roelfsema *et al.* isolated the effect of E2 on GH secretion (68). The primary endpoint, overnight GH secretion on day 22 of the intervention, was 50% higher in the E2 arm than the transdermal placebo arm. Mean E2 concentration 301 ± 66 pmol/L was triple the basal E2 concentration (91 ± 5 pmol/L). Pulsatile GH secretion was strongly positively correlated with E2 concentration. The authors concluded that E2 is the principal determinant of GH secretion in men. In pituitary stimulation tests, there was no difference in GH secretion between groups, raising the possibility that the E2 effect may be at the level of the hypothalamus. However, Erα and ERβ are expressed in pituitary somatotrophs, and *in vitro* data show that, although the GH promoter lacks ERE, E2 acts via ERα and ERβ to stimulate pituitary-specific positive transcription factor 1, a potent GH transcription factor, which in turn upregulates somatotroph GH synthesis and release (69).

E2-stimulated GH in Roelfsema's study did not produce an increase in IGF-1 concentration (68). Despite stimulating GH release, E2 inhibits hepatic IGF-1 response to GH so that the net effect of E2 on IGF-1 depends on relative exposure of hypothalamus/pituitary and liver (70, 71). E2 inhibits the hepatic IGF-1 response to GH by stimulating suppressor of cytokine signaling (SOCS)-2, which inhibits the Janus kinase 2 (JAK)2 phosphorylation downstream of the GH receptor, and thus, the transcription signal for IGF-1 (70).

In summary, E2 rather than testosterone appears the main sex steroid regulator of the GH-IGF-1 axis in men.

Bone

Osteoblasts, osteocytes, osteoclasts and marrow stromal cells contain ER and AR (72, 73). The weight of evidence points to E2, acting via ERα, as the predominant sex steroid in the development and maintenance of the male skeleton (74). Androgens have a smaller but important role, particularly in promoting periosteal apposition to increase bone size in men.

E2 has multiple direct and indirect actions, the net effect of which is to maintain remodeling balance by reducing bone resorption and increasing bone formation.

Based largely on preclinical evidence, and some studies in women, the predominant actions of E2 are to inhibit differentiation and promote apoptosis of osteoclasts and promote differentiation and inhibit apoptosis of osteoblasts. E2 alters cytokine production from bone and immune cells in ways that limit bone resorption, in particular by reducing T-cell tumor necrosis factor-α production, and the secretion ratio of nuclear factor kappa B ligand to osteoprotegerin by osteoblast lineage cells (73). In preclinical studies, ligand-independent effects of ERα and ERβ in osteoblast lineage cells are important for mechanotransduction, the homeostatic mechanism by which bone strength of load-bearing bones is regulated according to the power of the muscles acting upon them (75).

E2, linear bone growth, cessation of growth and achievement of peak bone mass

E2 has concentration-dependent effects on skeletal growth. Pre-puberty, serum and tissue E2 is extremely low, and skeletal growth, more appendicular than axial, is mediated by the GH–IGF-1 axis (76). Skeletal sexual dimorphism does not arise until puberty (77). Men are taller than women, predominantly because of a longer period of prepubertal growth, leading to longer leg length (78).

In early puberty, in boys and girls, increased serum and tissue E2 concentrations stimulate the pubertal growth spurt, with acceleration of truncal and deceleration of appendicular growth (79, 80, 81). The E2 effect on growth in boys was demonstrated by the growth charts pre and post initiation of E2 treatment in a young man with congenital aromatase deficiency. At age 17 years, before treatment, bone age was 12 years, Tanner stage V, testosterone concentrations were supranormal and E2 was undetectable. In this man, growth continued after initiation of E2 treatment, with an increase in relative height from the 50th centile at age 17 to the 90th centile at age 20 (40). In contrast, in an older (age=27 years) man with congenital aromatase deficiency, E2 treatment initiation resulted in rapid epiphyseal closure and termination of linear growth within 12 months (82). These observations do not resolve the mechanism of the early pubertal effect of E2 on increasing linear growth in men. Pre-clinical studies suggest that this is likely to be an indirect action via E2-mediated stimulation of the GH–IGF-1 axis. This has been recently reviewed elsewhere (83).

In late human puberty, higher E2 availability at the growth plate in both sexes causes growth plate closure and cessation of growth (79). This is a direct effect of E2 in inhibiting proliferation and promoting apoptosis of chondrocytes (84). Higher E2 in girls stimulates an earlier cessation of growth so that boys have a longer period of intra-pubertal growth and greater adulthood truncal length (78). Men with prepubertal hypogonadism do not experience a pubertal growth spurt, but develop tall stature with eunuchoid proportions, characterized by greater leg and lesser truncal length, because of failure of E2-mediated growth plate closure. However, the tall stature in Klinefelter syndrome has a pre-pubertal onset and is likely to be partly due to an increased dose of growth-promoting genes on the additional X chromosome (such as sex chromosome-related short stature homeobox-containing gene, *SHOX*, which influences chondrocyte differentiation) (85).

ESR1 (86) and *CYP19* (87) polymorphisms are associated with adult height but large genome wide association studies (GWAS) on adult height have identified hundreds of other loci across the genome. In a recent study of over 250,000 individuals of European ancestry, 697 variants, clustered in 423 loci explained 20% of the heritability in height (88). The genes in these loci are particularly highly expressed in tissues related to chondrocytes and osteoblasts and included many newly associated genes involved in skeletal and cartilage development.

The two reported men with non-functional ERα had delayed bone maturation. In the first case, in which testicular size and serum testosterone were normal (35, 89), there was absence of the pubertal growth spurt and delayed epiphyseal closure resulting in continued linear growth into adulthood. This was accompanied by reduced bone mass, even adjusted for low bone age. Cortical thickness and trabecular volume were reduced. The second man had an undescended right and hypoplastic left testis, low serum testosterone and delayed bone age, but bone mineral density (BMD) data were not reported (36). Interestingly the best characterized man with non-functional ERα (89) had a low bone remodeling state, which is in contrast to the high bone remodeling seen in men with congenital aromatase deficiency (40) and hypogonadism (90, 91). This may indicate a non-ligand-dependent role for ERα in promoting bone remodeling or roles for ERβ or GPER in suppressing remodeling (36, 74). These non-ERα-mediated effects of E2 are potentially over-activated in men with non-functional ERα-induced E2 resistance, which results in high serum E2 and could be invoked to explain other features of the skeletal phenotype. However, the phenotype is similar in men with congenital aromatase deficiency: lack of pubertal growth

spurt; continued linear growth after sexual maturation, leading to tall stature; genu valgum and low bone mass. This phenotype is remediable with E2 therapy (40, 82). Together, these observations suggest that E2 signaling via ERα is critical in normal male bone development.

Peak bone mass at the completion of growth is an important determinant of lifetime fracture risk (77). It is a function of bone mineral content and bone size. During puberty, men have greater periosteal bone expansion than females, resulting in greater cortical width and peak bone mass in adulthood. Women have less periosteal bone formation and predominantly increase cortical width by endocortical apposition (77). Evidence from rodents suggests that sex differences in radial bone growth depend on an intact GH–IGF-1 axis (92), but the neonatal testosterone surge imprints the adult male GH secretory pattern (93). In rodents, there appears to be direct AR-mediated effects in late puberty (92). This also appears to be the case in humans because women with 46,XY CAIS have deficits in spine BMD despite never being estrogen deplete (94). Additionally, the Gothenburg Osteoporosis and Obesity Determinants (GOOD) study of a large homogenous cohort of 18- to 200year-old men reported that periosteal circumference at the time of peak bone mass was positively associated with cross-sectional serum-free testosterone and negatively associated with free E2, noting that sex steroids were measured by immunoassay rather than more accurate methods, and that free hormone fractions were calculated rather than measured directly (95). However, at least some of the testosterone effect on periosteal expansion in men is aromatase dependent because E2 treatment of a 17 year-old man (bone age 12 years) with congenital aromatase deficiency produced a dramatic increase in bone size and cortical thickness (40).

SNPs in *CYP19* are associated with serum E1 and E2 concentrations in European and Chinese adult men in GWAS (96, 97). Mendelian randomization analysis using summary results from the European GWAS (97) and BMD data from various cohorts contributing to the Genetic Factors in Osteoporosis Consortium reported a positive relationship between serum E2 and BMD at the lumbar spine (LS) and femoral neck to the extent that a 3.7 pmol/L genetically increased serum E2 was associated with a 0.048 standard deviation increase in LS BMD measured in adulthood (97). The mean age of participants in the various cohorts ranged from 18 to 76 years. Within general caveats of Mendelian analyses, this provides strong evidence of a causal link between E2 and BMD. In contrast, testosterone concentrations were not associated with these SNPs. Whether genetically determined E2 is most important in the achievement of peak bone mass or in the attenuation of bone loss in older men is not resolved by Mendelian randomization.

In the GOOD study, peak areal BMD in a large homogenous cohort of 18- to 20-year-old men was shown to be associated with aromatase gene polymorphisms, with the association mediated by differences in cortical bone size (a function of cross-sectional area and thickness) (98). The polymorphisms associated with greater cortical bone size were associated with higher cross-sectional testosterone, suggesting lower aromatase activity, although serum E2, measured by immunoassay, was no different between genotypes, and statistically, testosterone concentrations did not account for the effect of polymorphisms on bone mass. Thus, the mechanism of this association remains unclear. The authors speculated that differences in earlier, pubertal, testosterone concentrations, not measured in this cross-sectional study, may be responsible for differences in periosteal apposition leading to the differences in cortical bone thickness.

E2 and bone loss in older men and in models of hypogonadism

Severe hypogonadism in older men induces accelerated bone remodeling and deterioration in bone density and microarchitecture (91). Observational and experimental evidence suggests that these effects are mostly E2 dependent.

Among older men, epidemiological studies have generally found closer correlations between serum E2, particularly non-SHBG-bound E2, and BMD, microstructure and fractures than with serum testosterone and these parameters (99, 100, 101). AIs, which lower E2 and increase testosterone (102), and treatment with DHT, a non-aromatizable androgen which lowers E2 concentrations (32), both reduce BMD in men.

Short-term experiments of GnRH analog-induced hypogonadism in healthy men combined with AI treatment and add-back of testosterone or E2 have assessed the relative importance of each hormone in maintenance of BMD and suppression of abnormal bone remodeling. In one such study, Falahati-Nini *et al.* used a GnRH analog plus AI in 59 healthy older men with transdermal add-back of physiological doses of testosterone and E2. Following baseline assessment of bone remodeling markers at week 3, either T, E2, both or neither were withdrawn, and repeat measurements were made at week 6 (90). Sex steroid withdrawal leads to an increase

in urinary deoxypyridinoline and N-telopeptide of type 1 collagen, indicating resorption of mineralized matrix. Significant increases were prevented by E2 add-back, even without testosterone, and in an analysis of variance model, testosterone had no independent effect. In this study, the osteoblast lineage marker, pro collagen type 1 amino-terminal propeptide (P1NP), fell with testosterone and E2 withdrawal, and this effect was prevented by E2 but not testosterone.

In Finkelstein's study (discussed above) (54), 16 weeks of induced hypogonadism in healthy younger men caused elevations in serum beta carboxyl-terminal type 1 collagen telopeptide (CTX) and declines in BMD by quantitative computed tomography which were greater in men who received AI at each active testosterone replacement dose (103). In another 4-week RCT enrolling men rendered chronically hypogonadal with GnRH analogs for prostate cancer, transdermal E2 add-back reduced serum CTX and increased P1NP, consistent with short-term anti-resorptive and pro-osteoblastic effects (104). These studies suggest that the high remodeling rate and bone loss that occurs in male hypogonadism is primarily due to E2 deficiency. However, short-term studies revealing differential changes in biomarkers of resorption and formation cannot be used to infer long-term effects of sex steroid withdrawal or treatment, because of steady state coupling of these processes.

Longer-term interventional data are available from a bone sub-study of an ongoing trial of ADT with transdermal E2 (high E2, castrate testosterone) versus GnRH analogs (castrate testosterone and E2) in men with advanced prostate cancer (105). This sub-study enrolled 74 men who underwent dual energy x-ray absorptiometry (DXA) scans at baseline and annually for 2 years. The primary endpoint, 1-year change in spine BMD, was analyzable for 60 participants, with an estimated difference between arms was 6.7% (95% CI 3.7–9.7) in favor of E2.

Serum E2 and fractures in men

Many observational studies have observed an association between serum E2, particularly free E2, and fracture risk in older men (100, 106, 107). In general, attributable risks are modest, and the association is not a universal finding (108, 109, 110). A high-quality longitudinal study measuring sex steroids by mass spectrometry in 1700 men (mean age 76.9 ± 5.5 years) showed no association of E2 with change in BMD or incident fractures over 6 years, although free E2 was not reported and only 10% of participants had an incident fracture (110). A large Mendelian randomization study of E2-associated and testosterone-associated SNPs on fracture risk was recently reported (111). This study used data from 175,583 men of European origin, captured as part of the UK Biobank. The men enrolled in the study were aged 56.9 ± 8.1 years and self-reported 17,650 fractures. A genetically reduced serum E2 by 1 SD (35.2 pmol/L) was robustly associated with an increased risk of any self-reported fracture (OR 1.35; 95% CI 1.18–1.55). There was no causal effect of genetic alterations in serum testosterone on fracture risk.

Although further research is required in populations in whom hypogonadism has been rigorously excluded, reported mass spectrometry reference ranges for serum E2 range from 38 to 196 pmol/L in men aged <65 years, 23 to 154 pmol/L (65–75 years), 22 to 166 pmol/L (75–85 years) and 22 to 174 (>85 years) (112). Thresholds of 37–92 pmol/L have been suggested to be necessary for skeletal health in men, based on observational and experimental data (103, 113).

In summary, the available data suggest that, while testosterone has direct effects on bone remodeling and bone microstructure, and indirect effects though anabolic effects on skeletal muscle, E2 appears to be the sex steroid predominantly important for skeletal health in men.

Body composition and glucose and lipid metabolism

Body composition: E2, muscle and fat

In men, aromatase activity is reported in skeletal muscle (114) and adipose tissue (9). Aromatase is strongly expressed in the stroma of human adipose tissue, although nearly all of these data are from women (115). ERs are also expressed in human adipose tissue (116) and there is low level expression of ERα in human skeletal muscle (117).

E2 does not appear to be important for muscle size or strength. In Finkelstein's study (discussed above) (54), healthy younger men rendered hypogonadal with GnRH analogs lost muscle mass, size and strength over 16 weeks. These losses were testosterone dependent, decreasing below a threshold of approximately 6.9 mmol/L, with no independent effect of E2.

In the same study, experimental profound hypogonadism caused gains in subcutaneous and intra-abdominal fat (54). These were predominantly due to E2 deficiency as they occurred in AI-treated men, irrespective of testosterone replacement dose. In the AI-treated men, percentage body fat and subcutaneous fat area gains were attenuated in men receiving supraphysiological

testosterone replacement, suggestive of pharmacological AR-mediated effects. This is in agreement with RCTs reporting reductions of fat mass using pharmacological doses of non-aromatizable androgens (32, 118). Such AR-mediated effects on fat mass might be secondary to increased muscle mass, and lipolytic myokine production (119) or diversion of pluripotent stem cells from adipogenesis and toward myogenesis (120).

In an RCT, Juang *et al.* treated young obese men without diabetes with a GnRH antagonist plus supraphysiological transdermal testosterone add-back with either dutasteride to inhibit reduction to DHT (dutasteride group), AI to inhibit aromatization to E2 (AI group), or placebo (testosterone-only group) (121). A separate group received placebos for all four treatments (placebo group). Body composition by DXA was assessed at baseline and 14 weeks. Compared with the placebo and AI groups, serum E2 increased, proportion fat-free mass increased and proportion fat mass decreased in the dutasteride and testosterone-only groups suggesting an E2-dependent effect. As others have pointed out, systemic E2 withdrawal in the context of testosterone add-back in Finkelstein's and Juang's studies would be expected to lower GH and IGF-1 which might have contributed to the body composition effects attributed to E2 withdrawal (122).

Other human data support a role for E2 in limiting fat gain. Men with congenital aromatase deficiency are generally overweight or obese with excess abdominal adiposity (38, 39, 40, 42, 44, 45, 46, 48, 49). In one 9-month RCT randomizing abdominally obese men older than 40 years (n=31) to transdermal DHT, transdermal testosterone or placebo, DHT treatment increased visceral adipose tissue (VAT) with no change in subcutaneous adipose tissue (SAT) or lean mass compared to testosterone-treated men (123). In another 24-month RCT of DHT treatment to inhibit prostate growth in 114 healthy men 50 years of age or older, DHT increased lean mass by 1.0–1.5 kg together with a small increase in grip strength as expected (32). However, there was a decrease in fat mass by 1.0–1.5 kg in the DHT group with a small increase in the placebo group, although the between-group difference was non-significant. VAT and SAT were not distinguished in this study. Because DHT is non-aromatizable, and profoundly suppresses serum E2 and testosterone, a specific effect of E2 in limiting fat mass, predominantly VAT, mass could be inferred from these studies. However, as discussed above, another theoretical mechanism of effect is via the DHT metabolite 3βAdiol, an agonist at Erβ. Erβ is expressed in human adipose tissue (116) and in pre-clinical studies appears to be a negative regulator of peroxisome proliferator-activated receptor-gamma-induced adipogenesis (124). The hypothesis of depot-specific effects of E2 are supported by differential expression of ERβ1 isoforms, which are reduced in VAT compared to SAT (116).

Effects of obesity on E2

Observational studies have consistently found a negative linear correlation between serum testosterone and body mass index in men (125). Insulin resistance lowers SHBG and therefore total testosterone, but as obesity increases, free testosterone also falls, and this is associated with low or inappropriately normal LH. One hypothesized mechanism is that in obesity, excess adipose tissue aromatase activity elevates E2 concentrations, which increases negative feedback on LH production. In the 1970s higher E1 and E2 serum concentrations and urinary production rates were reported in obese men compared with lean controls (126). However, there are little subsequent data to support the excess aromatization hypothesis. For example, a study by Dhindsa *et al.* employed mass spectrometry and equilibrium dialysis to accurately measure total and free E2 in obese men with type 2 diabetes with either subnormal or normal serum free T (127). Men with subnormal free testosterone had lower E2 concentrations than those with normal serum free testosterone. In Dhindsa's study, and also in the European Male Aging Study in which men mostly did not have diabetes (128), testosterone and E2 concentrations were positively correlated.

Glucose metabolism

Subnormal serum testosterone is prevalent in men with type 2 diabetes. Contrary to the hypothesis that this low testosterone is due to negative feedback from elevated serum E2 produced by excessive adipose tissue aromatase, Dhindsa *et al.* showed that E2 in men with type 2 diabetes is low and remains directly correlated with serum testosterone (127).

E2 may have beneficial effects on glucose metabolism. Men with congenital aromatase deficiency are reported to have insulin resistance, which improves with E2 treatment (129). In male mice, E2 appears to have direct beneficial effects, acting via hepatic ERα in the liver to improve insulin sensitivity (130). Rodent studies also suggest beneficial effects of E2 signaling on insulin sensitivity in other tissues, such as the brain, skeletal muscle, adipose tissue and pancreatic islet cells (131). E2 may also

have intracrine actions; in male mice, overexpression of aromatase targeted to white adipose tissue (WAT) increased WAT E2 concentrations and expression of *Glut4* and *Irs1*, associated with improved insulin sensitivity, without measurable changes in circulating sex steroid concentrations (132). In a hyperinsulinemic–euglycemic clamp study in healthy young men randomized to AI or placebo, AI treatment reduced insulin-stimulated peripheral glucose disposal but had no effect on insulin-mediated suppression of endogenous glucose production, letting the authors infer a skeletal muscle effect (133). However, as in other studies of AI alone (without GnRH analog to suppress gonadotropins), treatment results in an increase in testosterone which confounds interpretation.

In the study by Juang *et al.* (described above) (121), insulin sensitivity was measured by euglycemic–hyperinsulinemic glucose clamp at baseline and 14 weeks. There were significant between-group differences in change in insulin sensitivity, with the dutasteride group (GnRH analog plus testosterone plus dutasteride) showing increases in insulin sensitivity which were not seen in the testosterone-only (GnRH analog plus testosterone) or AI group (GnRH analog plus testosterone plus AI). The authors infer an effect of E2 in improving insulin sensitivity which is inhibited by DHT. Both global and peripheral insulin sensitivity improved.

Lipid metabolism

In men with prostate cancer, ADT with GnRH analogs, which reduces both testosterone and E2 to castrate concentrations, appears to alter lipids in a pattern distinct from the dyslipidemia of metabolic syndrome. Studies have been non-randomized by necessity and have made heterogeneous observations about changes in lipid parameters. Additionally, many of these studies were uncontrolled, and likely confounded by ADT-induced changes in body composition, insulin resistance and physical activity. Most (134, 135, 136) but not all (137) controlled studies report that ADT is associated with a modest increase in total cholesterol (TC) (7–10%) and triglycerides (TG) (25%). These studies observed either an increase (134, 135) (up to 7%) or no change (136) in low-density lipoprotein cholesterol (LDL-C) and an increase (134) (up to 20%) or no change (135, 136) in high-density lipoprotein cholesterol (HDL-C). In a 6-month placebo-controlled trial of GnRH analog therapy in 50 men with benign prostatic hyperplasia, TC, HDL-C and TG increased compared to placebo, with no change in LDL-C (138). Lipids returned to baseline after the intervention. In another RCT enrolling 15 healthy men, induction of hypogonadism using a GnRH analog increased TC and HDL-C compared to placebo with no change in LDL-C or TG (139). Testosterone replacement prevented these changes, but the study was not designed to differentiate aromatization-dependent effects.

Roelfsema *et al.* performed a short-term clamp study to isolate the effect of physiological E2 concentration on lipids and inflammatory markers in healthy older men (140). This design avoided confounding effects of changes in body composition and physical activity. Over 3 weeks, 74 men were rendered acutely hypogonadal by a GnRH antagonist followed by randomization to IM testosterone add-back or placebo, AI or placebo, and transdermal E2 patch or no patch. Four groups were analyzed: IM placebo (T−E2−); IM T plus oral placebo (T+E2+); IM T plus oral AI (T+E2−) and IM T plus AI plus E2 patch (T+E2++). Mean serum testosterone concentrations were 14.9–17.3 nmol/L at baseline. The intervention produced testosterone concentrations of 5.7 nmol/L in the testosterone-E2 group, different from the 25.9 to 29.3 nmol/L in the three groups receiving testosterone add-back. Mean serum E2 concentrations were 77–99 pmol/L at baseline, then 4 pmol/L in the testosterone+E2− group, 35 pmol/L in testosterone−E2− group, 115 pmol/L in the T+E2+ group and 301 pmol/L in the T+/E2++ group. Despite an 80-fold range of serum E2 concentrations encompassing the physiological range, linear regression analysis showed that TC, LDL-C, HDL-C, TG, lipoprotein (a) and apolipoprotein B were unrelated to serum E2 or testosterone concentrations. While larger studies are needed to confirm these findings, they suggest that the lipid changes observed in other studies might be due to body composition or activity changes, pharmacological rather than physiological actions or other confounders.

Vascular reactivity and atherosclerotic plaque

Experimental evidence in men rendered hypogonadal for treatment of prostate cancer suggests that low dose oral E2 add-back beneficially reduces responses to vasoconstrictors and enhances basal endothelial nitric oxide production (141). Observational data are inconclusive as to whether E2 has effects on atherosclerosis progression in men (142, 143).

In summary, the data suggest that the metabolically beneficial effects of testosterone on body composition are mediated via aromatization of E2 to limit adiposity, including VAT, but that the anabolic effects of testosterone on muscle mass are largely a direct effect via AR signaling.

With respect to sex steroid-mediated beneficial effects on insulin sensitivity and glucose metabolism, experiments in male rodents and men indicate that these are, at least in part, mediated via E2 signaling in multiple metabolically active tissues. Inferences on effects on lipids and atherosclerosis are limited due to the lack of appropriately designed studies.

Brain

Antenatal effects on brain structure and gender-related behavior

Brain structure and physiology, and resultant cognition and behavior, are sexually dimorphic. The greatest distinctions pertain to structures and behaviors relevant to reproduction. These differences are attributable to differences in gonadal hormone secretion, and the effects of sex chromosome-encoded genes (144, 145, 146).

In mammalian brain development, the default phenotype is considered to be female, with masculinization dependent on time-critical exposure (prenatal in primates, early postnatal in rodents) to sex steroids from the fetal testis (147). In rodents (148), and several other animal species (146), perinatal, gonadal testosterone-induced masculinization of the brain, defined by subsequent male-typical sexual behavior in adulthood, is aromatization dependent. The male fetus has higher brain E2 concentrations than the female because uniquely in the male, significant gonadal steroid production occurs prenatally (147). Brain E2, locally formed from gonadal testosterone, is responsible for un-methylating epigenetically repressed masculinizing genes (149) leading to brain masculinization.

Observations in humans suggest that testosterone, acting via the AR, is important in male gender development while any aromatization-dependent effects are less important (146). Unlike in rams, in which AI treatment during gestation disrupted normal male copulatory behavior (150), men with congenital aromatase deficiency, where reported, have male gender identity and heterosexual orientation (47, 51). Conversely, XY women with CAIS have a female gender identity despite elevated serum testosterone and E2 concentrations, demonstrating that AR-mediated actions are important in human brain masculinization (151). Furthermore, the single reported male lacking a functional ERα had a male gender identity and heterosexual orientation suggesting that ERα lacks an important role in human brain masculinization (35). However, biological mechanisms contributing to gender identity and sexual orientation in humans are obscured by psychological and social factors.

Cognition

Few relevant, appropriately controlled intervention studies exist in men in which the cognitive effects of testosterone and E2 can be delineated. In two small (n=25–27) short-term randomized trials of oral E2 versus placebo in older men receiving GnRH analogs for prostate cancer, E2 did not improve cognition in the specific domains tested (152, 153). Testosterone treatment of older men with mildly low testosterone and mild memory impairment was ineffective at improving cognition over 12 months (154).

In summary, while E2, acting locally, is critical for brain masculinization during development in rodents, in men, brain masculinization appears to track more closely with testosterone signaling via the AR, although data are limited. Whether sex steroids have effects on cognition is not clear.

Vasomotor stability

The profound sex steroid deficiency induced by ADT with GnRH analogs produces vasomotor symptoms (VMS) in the majority of men (155). In men, as in perimenopausal women, E2 withdrawal is the mediator of this effect. In women E2 withdrawal has been shown to cause release of hypothalamic neurokinin B, a paracrine regulator of heat dissipation effectors (156). In Finkelstein's paradigm of experimental testosterone and E2 depletion in young men with differential add-back of testosterone with or without AI, men receiving AI had a greater incidence of VMS, and even supraphysiologic serum testosterone concentrations were unable to prevent hot flushes if serum E2 remained <37 pmol/L (157). In a preliminary report of an ongoing RCT comparing two modes of ADT for prostate cancer, high-dose transdermal E2 versus standard GnRH analog therapy, men in the E2 arm had a lower incidence of hot flushes at 6 months (8 vs 46%) (53). Finally, in a 4-week RCT, low-dose transdermal E2 add-back in men receiving GnRH analog therapy, reduced hot flush frequency-severity scores (104).

Effects of excess E2 in men

Excess exposure to estrogens in men can cause gynecomastia, hypogonadotropic hypogonadism, and, if the exposure is pre-pubertal, premature epiphyseal closure

leading to short stature. This phenotype occurs in the rare, autosomal dominant, aromatase excess syndrome that results from subchromosomal rearrangements that enhance aromatase transcription. (158). The first rearrangements were described in 2003 (159) but cases of familial gynecomastia, likely due to this syndrome have been noted since antiquity (160).

Gynecomastia is the most consistent effect of excess exposure to estrogens in boys and men. In addition to the aromatase excess syndrome, gynecomastia has been described in cases of estrogen-secreting testicular tumors (161) and Sertoli cell proliferation in Peutz Jegher Syndrome (162), excess aromatase expression by hepatocellular carcinoma (163), occupational exposures, abuse of aromatizable androgenic steroids and intentional pharmacological use of estrogens including in transgender women and in men with prostate cancer (164). Gynecomastia is also seen without absolute estrogen excess. Male breast tissue expresses both ER and AR. In females, estrogens stimulate breast tissue, whereas androgens inhibit it (165). This understanding has been extrapolated to men (166). Gynecomastia can occur in circumstances where there is absolute androgen deficiency or where the ratio of circulating free testosterone to free E2 is reduced (164). Examples of the latter include conditions in which SHBG is increased such as thyrotoxicosis or aging (because SHBG binds testosterone more avidly than E2, SHBG elevation reduces free testosterone more than it does free E2) (167).

Klinefelter syndrome commonly causes gynecomastia (168). It has traditionally been considered a condition of absolute excess circulating E2. However, a recent meta-analysis has reported that this association was driven by studies using inaccurate assay technologies and is not present if only studies using high-quality E2 assays with sufficient accuracy in the low male range are considered (169). In this analysis, men with Klinefelter syndrome did have an increased circulating E2/T ratio, suggesting an increase in aromatization of available testosterone, the mechanism of which is not established.

Since the 1940s, high doses of synthetic oral estrogens and parenteral E2 have been used to induce profound hypogonadotrophic hypogonadism as a way of controlling locally advanced and metastatic prostate cancer. While the use of oral estrogens has stopped because of the high risk of thrombosis caused by upregulation of hepatic clotting factors by the first-pass metabolism or these estrogens, there is ongoing interest in high-dose parenteral E2 which does not have this effect (170). Other than gynecomastia, this treatment appears to be well tolerated and safe, and may prevent skeletal fracture and vasomotor symptoms associated with castration using GnRH agonists or surgery.

Endocrine-disrupting chemicals

The Endocrine Society defines an endocrine-disrupting chemical (EDC) as an 'exogenous chemical or mixture of chemicals, that interferes with any aspect of hormone action' (171). Many EDCs, such as bisphenol A, p,p′-dichlorodiphenyltrichloroethane (DDT, now banned), perfluorooctanoic acid (PFOA), and polychlorinated biphenyls (PCBs, now banned) bind to and activate ERs. Pre- and perinatal exposure to some of these EDCs, and others, have obesogenic, diabetogenic and reproductive effects in rodent studies and there is epidemiological evidence to support similar effects in humans (6). However, causation in humans has not been proven and EDCs can act through diverse mechanisms, including inhibition of androgen production and action, and direct toxic effects on endocrine and reproductive tissues. Therefore, it is impossible at present to isolate particular estrogenic effects of particular EDCs that are responsible for any particular clinical effect in men.

Tissues in which androgen action is independent of aromatization

In certain tissues, AR-mediated actions are clearly predominant. The *in utero* development of male external genitalia is AR mediated. Micropenis and hypospadias can occur in XY male infants with partial androgen insensitivity syndrome, and topical DHT, which is not aromatized, can produce penile growth (172). The higher male reference range for hemoglobin reflects the impact of androgen action on erythropoiesis. The mechanisms appear to be via AR-mediated reduction in hepatic hepcidin production, thus increasing iron availability for red cell production (173). Male-pattern body hair growth is also AR mediated, illustrated by the efficacy of 5α-reductase inhibitors for treating androgenic alopecia (174).

Clinical implications of E2 physiology in men

Knowledge that E2 has important physiological roles in men has clinical applications. Firstly, standard therapy in hypogonadal men should be replacement with T (175), and not with non-aromatizable androgens, selective androgen

receptor modulators or AIs to enhance endogenous testosterone production. These latter approaches do not correct the deficiency of estrogen action. Moreover, AIs require hypothalamic–pituitary responsiveness and are not effective in secondary organic hypogonadism or indeed in primary organic hypogonadism where circulating gonadotrophins are already high. In a 12-month RCT of AI therapy in older symptomatic men with low serum testosterone, BMD declined in those treated with AI despite increases in serum testosterone (102). Similar negative effects on BMD have been shown with DHT (32). AIs do have off-label clinical roles in boys with aromatase excess syndrome, precocious puberty due to testotoxicosis, and possibly idiopathic short stature, in which cases they appear to improve adult height (176). AIs and antiestrogens are also clinically useful in boys and men with gynecomastia due to E2 excess (177). However, concerns over skeletal safety of AIs in boys and men remain (176).

The clinical utility of selective estrogen receptor modulators (SERMs) in middle aged and older men with low testosterone is also under investigation (reviewed in (178)). Like AIs, SERMs can enhance endogenous testosterone production in men with preserved hypothalamic–pituitary responsiveness, but, in contrast to AIs, have some ER agonistic activity in somatic tissues such as the skeleton. Clinical trials to date however have been relatively small, short term and have not been designed to provide definitive evidence for clinical use (178). Furthermore, limited data suggest that the SERM, raloxifene, is an inadequate substitute for E2 when skeletal maturity has not yet been attained. Unlike subsequent transdermal E2 treatment, 12 months of raloxifene proved ineffective in fusing epiphyses in a young man with congenital aromatase deficiency (179). Forearm BMD improved in association with raloxifene treatment, but bone age did not advance.

Secondly, there are potential roles for E2 treatment in men requiring ADT for prostate cancer currently under investigation, either in the form of high-dose transdermal E2 as the method of achieving medical castration or as low-dose transdermal add-back to mitigate vasomotor symptoms and bone and metabolic side effects of GnRH analog therapy (170). Some clinical evidence already suggests E2 therapy is effective for vasomotor symptoms in men undergoing such therapy (180).

Thirdly, measurement of serum E2 concentration in the clinic, ideally with mass spectrometry, is useful in the evaluation of possible E2 excess (177, 181) and in differentiating the rare causes of deficient E2 action leading to persistent linear growth, delayed bone age and osteoporosis. This differential includes estrogen resistance, aromatase deficiency and rare combined defects of steroid synthesis (182).

At present, serum E2 measurement should not be part of the routine evaluation of clinical conditions such as hypogonadism or osteoporosis because there is no evidence that E2 measurements provide clinically actionable information beyond that of circulating testosterone. Access to validated gold standard mass spectrometry E2 measurement techniques for routine clinical use is still limited for many clinicians, and measurement by immunoassay is highly inaccurate and tends to overestimate E2 at the low serum concentrations present in men (183). Even if circulating E2 can be accurately measured, because E2 in men is produced locally and diffusely in aromatase-containing tissues, and acts in a paracrine fashion, it is unclear to what extent serum E2 concentrations reflect sufficiency of estrogenic effects in any particular tissue. Serum E2 thresholds establishing sufficiency of estrogen action in various tissues have been proposed (170). Clearly however, given the importance of E2 in male health, further studies, using increasingly available mass spectrometry assays, are needed to define the utility of serum E2 measurements in clinical practice.

Conclusion

Recent evidence has demonstrated that many biological actions historically attributed to testosterone are instead, at least in part, mediated by its aromatization product E2. The data are strongest for effects on bone, fat mass, insulin resistance and VMS. The relevance of these data is that clinically efficacious treatment of male hypogonadism is best achieved with testosterone, which provides ‘three hormones in one’ – testosterone, DHT, E2. Conversely, this evidence raises caution regarding the use of selective androgen receptor modulators, non-aromatizable androgens and AIs for male hypogonadism, and emphasizes the need for better understanding of the tissue-specific effects of SERMs, which are also used off label by some practitioners for this purpose. They also suggest that E2, either as sole ADT or as add-back to conventional GnRH analog-based ADT, may be a promising treatment to mitigate some of the adverse effects of ADT given to men with prostate cancer. Most current studies in men are relatively small, short term, and the design of experimental studies does not always recapitulate physiology. More research is needed to better

understand the relative roles of testosterone versus E2 in somatic and reproductive studies and to dissect the relative biologic roles of circulating versus locally produced E2.

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