History of Estrogen: Its Purification, Structure, Synthesis, Biologic Actions, and Clinical Implications

Richard J. Santen[1] and Evan Simpson[2,3]

[1]Division of Endocrinology and Metabolism, University of Virginia, Charlottesville, Virginia, 22908; [2]Hudson Institute of Medical Research, Clayton, Victoria 3168, Australia; and [3]Centre for Reproductive Health, Queen's Medical Research Institute, University of Edinburgh Medical School, Edinburgh EH16 4TJ, United Kingdom

ORCiD numbers: 0000-0002-6715-5192 (R. J. Santen).

This mini-review summarizes key points from the Clark Sawin Memorial Lecture on the History of Estrogen delivered at Endo 2018 and focuses on the rationales and motivation leading to various discoveries and their clinical applications. During the classical period of antiquity, incisive clinical observations uncovered important findings; however, extensive anatomical dissections to solidify proof were generally lacking. Initiation of the experimental approach followed later, influenced by Claude Bernard's treatise "An Introduction to the Study of Experimental Medicine." With this approach, investigators began to explore the function of the ovaries and their "internal secretions" and, after intensive investigations for several years, purified various estrogens. Clinical therapies for hot flashes, osteoporosis, and dysmenorrhea were quickly developed and, later, methods of hormonal contraception. Sophisticated biochemical methods revealed the mechanisms of estrogen synthesis through the enzyme aromatase and, after discovery of the estrogen receptors, their specific biologic actions. Molecular techniques facilitated understanding of the specific transcriptional and translational events requiring estrogen. This body of knowledge led to methods to prevent and treat hormone-dependent neoplasms as well as a variety of other estrogen-related conditions. More recently, the role of estrogen in men was uncovered by prismatic examples of estrogen deficiency in male patients and by knockout of the estrogen receptor and aromatase in animals. As studies became more extensive, the effects of estrogen on nearly every organ were described. We conclude that the history of estrogen illustrates the role of intellectual reasoning, motivation, and serendipity in advancing knowledge about this important sex steroid. (***Endocrinology*** **160: 605–625, 2019)**

This mini-review covers the salient points of the Clark Sawin Memorial Lecture delivered by the authors at the 2018 Endocrine Society annual meeting. Our knowledge about estrogen has been driven by astute observations of multiple investigators. The research findings have been rapidly translated into development of clinically applicable estrogen preparations. The discoveries have led to the birth control pill, prevention and treatment of breast cancer, menopausal hormone therapy, induction of puberty in hypogonadal girls, prevention and treatment of osteoporosis, and vaginal estrogen therapy, among others. Recent clinical and experimental data demonstrate important effects of estrogen on bone and reproductive function in men. With the involvement of a myriad of investigators in estrogen research, multiple controversies have arisen, some of which are still unresolved.

The Sawin presentation concentrated on the rationale for the various experiments, the data obtained, the controversies, and identification of the three types of investigators contributing to estrogen research (as pointed out by Fuller Albright). Albright, a giant in the field of endocrinology, quoted the US Supreme Court justice Oliver Wendell Holmes to define three categories of investigators as paraphrased succinctly: "One story intellects—fact

ISSN Online 1945-7170

Received 31 May 2018. Accepted 20 July 2018.
First Published Online 18 December 2018

Abbreviations: DMBA, dimethylbenzanthracene; ER, estrogen receptor.

collectors who have no aims beyond their facts; two story intellects who compare, reason and generalize using the labors of fact collectors as well as their own; three story intellects who idealize, imagine, predict—their best illumination comes from above through the skylight" (a phrase that the authors define as inspiration) (1).

Role of Historical Observations

In antiquity, the role of estrogens could only be inferred by astute observations both of external appearances and, later on, by anatomic dissection. The depiction of "King" Tutankhamun (1341 to 1323 BC) from a statue found in his tomb strongly suggests gynecomastia (Fig. 1) and represents an historical observation of estrogen excess in a male (2). Although some have suggested that this is merely artistic license, the similar representation of gynecomastia in his brother Smenkhkare, as well as his relatives Amenophis III and Akhenaten, leads us and others to think that this was indeed gynecomastia, suggesting to many the possibility of familial aromatase excess. Availability of Tutankhamun's DNA should allow this to be diagnosed. Alternatively as suggested by Bernadine Paulshock, androgen insensitivity may have been responsible.

Figure 1. Photo of Tutankhamun taken by one of the authors (R.J.S.) at the Cairo Museum. Tutankhamun ruled from 1332 to 1323 BC. The statue depicts the appearance of gynecomastia, although some observers have speculated that this was only artistic license (2).

Knowledge at the Height of the Roman Civilization

Physicians practicing in Rome often referred to the thoughts of Greek writers from their experience several centuries before. They knew that Aristotle (384–322 BC), for example, thought that only male semen was incorporated into the fetus and that the female played no role in the generative material (3). Soranus of Ephesus (AD 98–138), the most noted gynecologist at the time, contradicted Aristotle, in writing that both the male and female produce "seeds" necessary for conception (4–6). Galen (AD 129–200), the most accomplished of all medical researchers in antiquity and physician to the philosopher emperor Marcus Aurelius (author of *The Meditations of Marcus Aurelius*), appeared to agree with Soranus but not Aristotle (3, 7, 8). He observed female "testes" and concluded from these observations that the structures he saw corresponded to male testes and served the same purpose, namely production of semen (3).

Interestingly, Galen thought that menstruation represented a form of auto-phlebotomy and represented a means to eliminate unfavorable circulating humors, a concept not contradicted until several centuries later (3). Soranus commented on several aspects of female development. He noted a clear temporal association between menses and breast development and noted that these two events occur at approximately age 14 (6). This is of interest because it is generally agreed that the age of menarche has decreased from age 16 to 17 to age 13 during the past two centuries, a finding postulated to be due to increased fat deposition and leptin levels (9–12). This raises the question whether nutrition during the Roman civilization was better than two centuries ago in Western countries. The change in age of menstruation from AD 1800 to 1980 is thought to have resulted from the improvement in nutrition during that time period (9). As further evidence of the power of observation, Soranus also noted that masculine-appearing females and those exercising excessively failed to menstruate. He also commented on contraception, noting that blockade of the cervical os as an effective means of preventing conception was possible (6).

Post-Renaissance Period

Thomas Wharton (AD 1614–1673) was interested in ductless vs ductal glands. He was famous for naming

Wharton's duct, which connects the submandibular gland with the mouth. He thought, based on appearance, that the ovaries and testes are similar and that damage to either results in infertility (13). He reasoned on this basis, as did Soranus and Galen, that the ovary must produce sperm. Later, Augustin Nicolas Gendrin (AD 1796–1890) postulated that menstruation was related to ovulation, thus contradicting Galen's theory of auto-phlebotomy (3, 7, 8, 14, 15).

Dawn of Experimental Era

In 1855, Claude Bernard, the father of experimental medicine, spearheaded the transition from clinical observation to experimentation (16). He developed the concept of internal secretion when observing that glucose released by the liver is transported by blood into many distant tissues. This observation led to a plethora of studies of internal secretion, basically involving three experimental steps: (i) removal of the gland with documentation of ensuing signs and symptoms, (ii) transplantation of the gland back into the body with demonstration of the reversal of signs and symptoms, and (iii) administration of the extract of the gland (organ therapy) in patients lacking that gland with the finding of reversal of signs and symptoms. With respect to the ovary, these steps were taken during a period of years.

Step 1

In the 1880s, Robert Battey developed the ability to perform oophorectomy safely in women (14, 17). This operation, called the Battey operation, became popular and was performed for multiple reasons, including dysmenorrhea and bleeding from myomata. After removal of the ovaries, patients developed hot flashes and vaginal atrophy, leading to the hypothesis that the ovary makes some type of substance that in its absence causes various symptoms (14, 17).

Step 2

In 1896, Emil Knauer from Vienna removed the ovaries from rabbits and observed uterine atrophy, which he could prevent by transplanting the ovary at a distant site, confirming the postulate of internal secretion by the ovaries (18–20).

Step 3

In 1897, Hubert Fosbery successfully used ovarian extracts to treat a patient with severe hot flashes (21). In his published report, he stated, "when at last the periods ceased, the patient was much troubled with frequent and violent flushings which at night in winter would wake her up, the face being in burning heat"; "I ordered 5-grain palatinoids of ovarian gland [a form of medicine capsule, as ground-up ovary tastes terrible] three times a day"; and "The flushings rapidly became less frequent and intense and were nearly cured by the time three dozen palatinoids were taken." This description seemed rather convincing, although later it might have been attributed to a placebo effect (21).

History of Organ Therapy

The use of extracts of glands to treat patients became common in the later 19th and early 20th centuries. In the 1880s, Brown-Séquard (AD 1847–1894), thought to be the father of organ therapy, administered extracts of monkey testicles to patients with the concept that this would relieve symptoms due to specific hormone deficiencies (22). He administered these extracts to himself and reported increased strength and muscle mass, less fatigue, and an increase in mental faculties (23). He also suggested organ therapy for women whose ovaries had been removed. In response to Brown-Séquard's wide influence, organ therapy then began to be used for many maladies, including mental disorders (24).

A "deep dive" into history revealed that Brown-Séquard was not the first to use organ therapy widely. In actuality, San Si Miao (AD 623–682) from China first administered the organ extracts of deer and sheep thyroid to patients with goiter and observed beneficial effects (Supplemental Fig. 1) (25, 26). He went from city to city expounding this therapy and demonstrating efficacy. From this and his publication of more than 40 volumes of medical information, he was then known as the "King of Medicine." Later, in AD 1025, the Chinese prepared extracts of male and female urine, purportedly using powdered components to treat hypogonadism in men and dysmenorrhea in women, and other clinical disorders. The detailed methods describing preparation of these urine extracts is published in detail in the book *The Genius of China* and is summarized in Table 1 (27). What was actually contained in these extracts will remain unknown until the methods are replicated and analyzed with modern methods.

Therapeutic Innovation Based on Evolving Concepts

As the concepts regarding the function of the ovaries evolved, therapies were designed based on the current knowledge. In 1896, Sir George Beatson, a surgeon, described the first effective hormonal ablative therapy for

Table 1. Methods of Preparing Urine Extracts to Treat Hypogonadism in Men, Dysmenorrhea in Women, and Other Clinical Disorders

Steps	Description
1	Obtain 150 gallons of urine
2	Heat to facilitate evaporation
3	Seal and sublimate and obtain residue
4	Grind to powder and make pill
5	Administer five to seven pills with wine or warm soup
6	Male urine used for hypogonadism, impotence, and beard growth
7	Female urine used for dysmenorrhea

Data from Temple R. The Genius of China: 3,000 Years of Science, Discovery, and Invention. Prion, 1983.

treatment of breast cancer (28–30). He based his rationale on an experience "moonlighting" on a farm where he was the physician for the owner. There he learned several facts about lactation and began to study this phenomenon. Based on his knowledge of histology, he appreciated similarities between the tissue appearance of lactational changes, characterized by increasing breast proliferation and the same phenomenon in histologic sections of breast cancer. Also, he knew that oophorectomy prolonged the time of lactation in cows, a practice common in Australia at that time. From these observations, he postulated a regulatory role of the ovary on benign and malignant breast and sought to apply this concept clinically. With seemingly great courage, he decided to remove the ovaries surgically in premenopausal women with breast cancer. Notably, he demonstrated both partial and complete, temporary remissions. This work initiated the field of surgical ablation of endocrine glands as treatment of hormone-dependent breast cancer. To the authors of this manuscript, this represented " three story thinking," primarily with inspiration as to the rationale.

Purification of Estrogen

A major accomplishment at the time was the purification of estrogen, a feat primarily involving three key investigators, Edgar Allen, Edward Doisy, and Adolph Butenandt. In 1923, Edgar Allen, a reproductive physiologist, was studying the role of follicular fluids obtained from the ovary of sows on uterine weight, vaginal maturation, and sexual receptivity (31, 32). A serendipitous circumstance leading to a key collaboration came from the fact that Allen had no car. Taking advantage of a friendship developed while playing on a faculty baseball team, he rode to work in St. Louis in the Model T Ford of Edward Doisy, an organic biochemist and fellow baseball enthusiast (33). This allowed extensive conversations leading to the appreciation of the potential for a synergistic, scientific relationship and commencement of work together—Allen provided follicular fluid from sows and Doisy purified the estrogenic activity. The biologic endpoint, uterine weight, was later called the Allen-Doisy test.

Progress was slow until Selmar Ascheim and Bernard Zondek, working in Germany, demonstrated large amounts of estrogenic activity in the urine of pregnant women (34). With this biological material as a source, Doisy crystallized estrone and presented this finding at the 13th International Physiologic Congress in Boston in 1929 (35, 36). Fuller Albright observing the presentation commented, " The potency of these crystals is so great that one gram could restore the sex cycle in more than nine million rats" (1). Likely, Albright began to think about the implications, an approach characterizing him as a "three story investigator." After discovering the crystals, Doisy went home and told his wife that they were going to be rich. On second thought, he gave the two resulting patents (both awarded on 24 July 1934) to St. Louis University, which has used the monies in an established foundation to this day to support the biochemistry department in the medical school.

Groundbreaking research findings often occur simultaneously in two completely independent laboratories. The German investigator Adolph Butenandt had simultaneously purified and crystalized estrone when he heard about Doisy's talk from a colleague who had attended the Boston meeting. This led Butenandt to rush into print several months before Doisy's manuscript was published (37). Ironically, Butenandt (along with Leopold Ruzincka) won the Noble Prize for this work (as well as for the crystallization of testosterone), whereas Doisy had to wait a decade to win his Nobel Prize for the purification of vitamin K. Somewhat later, estriol and estradiol were isolated and purified (38, 39). As expected, the problem of fully characterizing the structure of estrogens required major feats of organic chemical investigation, leading to controversies among investigators. A major one was whether estrogens contained three or four cyclohexane rings, a controversy illustrated by a photograph of Butenandt displaying four fingers, which aptly conveyed his opinion [see Fig. 3 in Simpson and Santen (40)].

Early Clinical Applications

Within 5 years, several estrogen preparations were commercially available from multiple pharmaceutical companies that recognized the clinical and commercial benefits of estrogen. The various products included theelin, progynon, emmenin, oestroform, folliculin, and amniotin. The ready availability of these agents facilitated rapid clinical advances. In 2 years, Albright demonstrated that estrogen blocked hot flashes; in 5 years, bone loss; and in 6 years, ovulation (41–45). Of interest was that only one patient needed to be studied with cyclic on–off therapy with estrin (preparation of estrogen based on the Doisy formula) to convincingly demonstrate inhibition of hot flashes (43) (Fig. 2). Estrin also provided relief of dysmenorrhea (45). The observation that estrogen markedly increased the amount of bone in male pigeons (46) (Fig. 3) stimulated Albright to show that estrogens prevent bone loss in postmenopausal women (44, 47, 48) (Fig. 4). This emergence of these new findings led Albright to establish a clinic specifically related to reproduction. In this clinic, Albright demonstrated

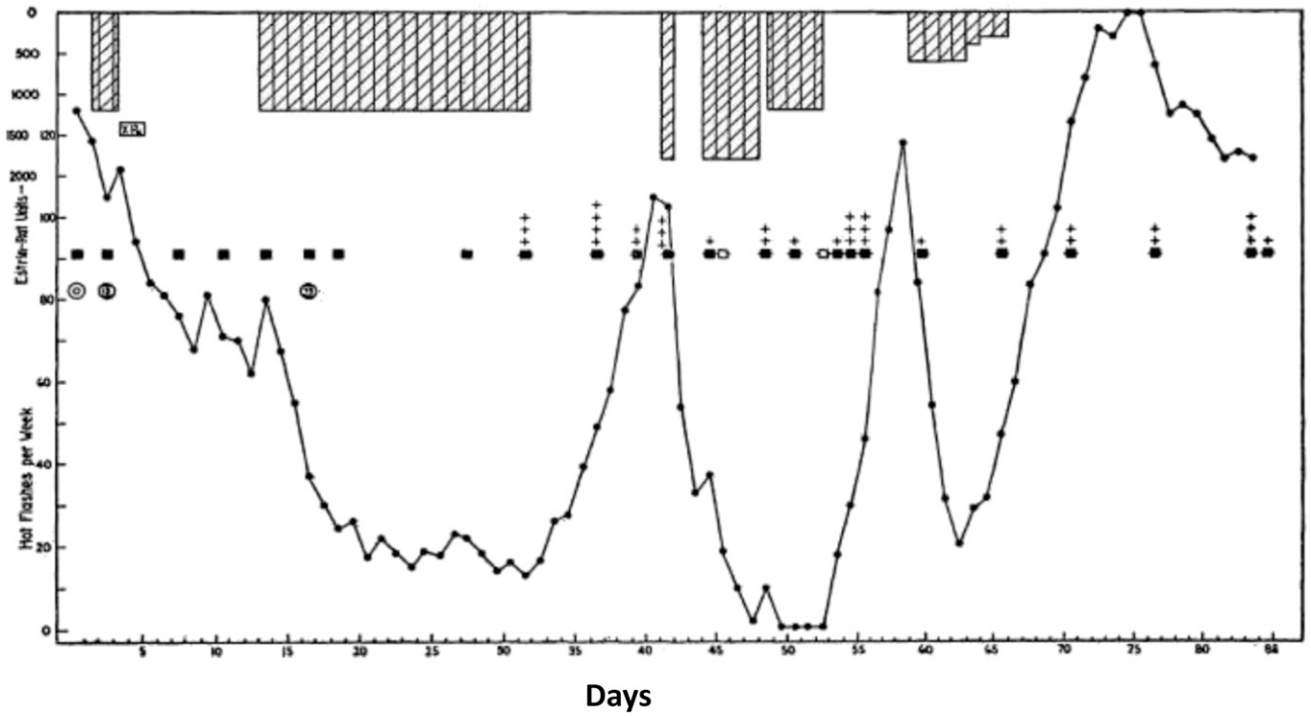

Figure 2. Treatment of a patient with estrin as shown by the hatched bars. The number of hot flashes per week are shown on the vertical axis. [Reproduced with permission from Albright F. Studies on ovarian dysfunction III: the menopause. Endocrinology 1936;20:24–39.]

blockade of ovulation with administration of estrogen during the early follicular phase of the menstrual cycle (42, 43).

Physiology of Breast Cancer

From George Beatson's work, the role of estrogen on breast cancer growth was known, but an animal model was needed to study the precise physiologic mechanisms.

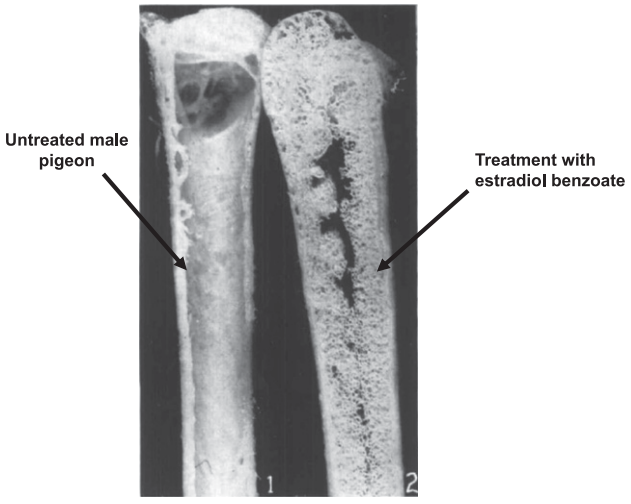

Figure 3. The femoral bone from an untreated male pigeon is shown on the left and after 36 days of estrogen on the right (46). [Reproduced with permission from Pfeiffer CA, Gardner WU. Skeletal changes and blood calcium level in pigeons receiving estrogens. Endocrinology 1938;23:485–491.]

Charles Huggins had won the Nobel Prize for demonstrating that castration in men could cause prostate tumor regression (49). Surprisingly, late in his career he developed the first animal model of hormone-dependent breast cancer. Motivation is often an interesting component of research progress (50–54). One of the coauthors (R.J.S.) had the opportunity to ask Huggins why he embarked upon the new area of research late in his career. He responded "The Ben May laboratory had no operating room and I had to take the dogs to Northwestern for the orchiectomies in my wife's station wagon. One day she said, "Charlie, your dogs are no longer going to befoul my station wagon and you had better find something else to work on." He chose to develop the dimethylbenzathracene (DMBA) breast cancer model, which allowed much information to be obtained about the hormone responsiveness of this common neoplasm in women.

Development of the Birth Control Pill

Several key steps led up to this monumental achievement (55, 56). In 1949, Russell Marker found a method to markedly reduce the cost of synthesizing progesterone by identifying precursors in a specific Mexican yam. He spent several months testing more than 100 yams, leading to the identification of the one with the highest level of diosgenin tested (55, 57, 58). He used this as a starting material for the synthesis of progesterone, which resulted

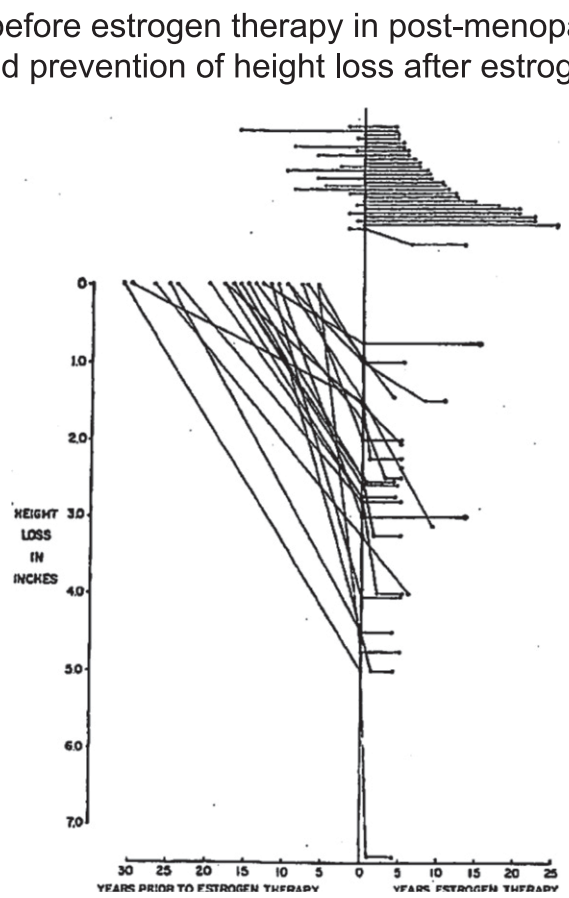

Figure 4. The vertical axis shows the height of patients before starting estrogen therapy (left of the vertical line) and after starting hormone therapy (right of the vertical line). This clearly shows that estrogen prevents loss of height in postmenopausal women (48). [Reproduced with permission for electronic publication only from Wallach S, Henneman PH. Prolonged estrogen therapy in postmenopausal women. JAMA 1959; 171:1637–1642.]

in a lowering of the price from US$200 per gram to US$5 (55). Gregory Pincus and Min-Chueh Chang then demonstrated blockade of ovulation in animals with progestins. Clinical trials in Puerto Rico, involving several individuals, including John Rock, demonstrated the efficacy of contraception in women (55, 56). The background for these studies was that Margaret Sanger, founder of the "National Birth Control League," later named Planned Parenthood, was well ahead of her time and strongly encouraged contraceptive research in the early 1920s. A surprisingly current quote from her at that time stated that "No woman can call herself free who does not control her own body." The extensive research required a substantial amount of funding. Katharine Dexter McCormick, inheritor of the McCormick fortune from the McCormick Reaper, contributed nearly one-third of the money required but notably also imparted intensive intellectual input into birth control pill research (55).

John Rock, a staunch Roman Catholic obstetrician/gynecologist from Boston, thought that his church would approve the birth control pill, but this did not happen. A little known fact is the history of the Catholic Church's deliberations on the morality of the birth control pill. A large and distinguished panel was convened by Pope John XXIII to consider whether "artificial birth control" was moral. The majority report of more than 60 experts considered it moral and their report was leaked to *The New York Times* ["An analysis of the majority report 'Responsible Parenthood' and its recommendations of abortion, sterilization, and contraception" by Richard J. Fehring (59)]. John XXIII died and his successor, Pope Paul VI, accepted the minority opinion of no more than 15 panel members and declared oral contraceptives to be morally unacceptable. Interestingly, most Catholic women in the United States approve of use of oral contraceptives despite church teaching.

Several outcomes ensued after approval of Enovid, the first oral contraceptive in 1960. Laws were changed to legalize contraceptives. This represented the first practical ability of families to determine the number of children desired and superseded the use of condoms and the "rhythm method," derided by some as a form of Russian roulette. Availability of birth control pills led to substantial changes in the role of women in society and the workplace and putatively was one of the most

powerful influences on several social aspects, including sexual behavior, gender roles in society, and family economics (60).

Discovery of the Estrogen Receptor

Novel techniques led to initial studies involving radioisotopes of high specific activity, namely tritium and carbon-14, which were used to label steroids such as estradiol.

Elwood Jensen, another "three story investigator," first identified estrogen receptors (ERs) utilizing this methodology. He administered labeled estradiol to castrate rats and demonstrated specific uptake into the uterus and vagina (61) (Fig. 5). He then demonstrated that ERs in women could be used to predict which breast tumors were hormone-dependent and would respond to hormone therapy (62). As a mentor, he stimulated his mentees to purify the ER and study its detailed molecular mechanisms.

Aromatase

Discovery

The aromatization of androstenedione to estrone and testosterone to estradiol was first suggested by Bernard Zondek in 1934 (34, 63). Later, Steinach and Kun "injected large quantities of male hormone (testosterone propionate) into men and quantitated the excretion of estrogenic substances in the morning urine" (64). The finding that rat units of excretion increased from 0 to 36 to a maximum of 1200 confirmed that androgens served as precursors for the product estrogens, a relationship suggesting the presence of aromatization. As often occurs in science, a controversy arose as to the naming of the enzyme. Some investigators called the enzyme "estrogen synthase" and others "aromatase," but in the long run, aromatase won out (65).

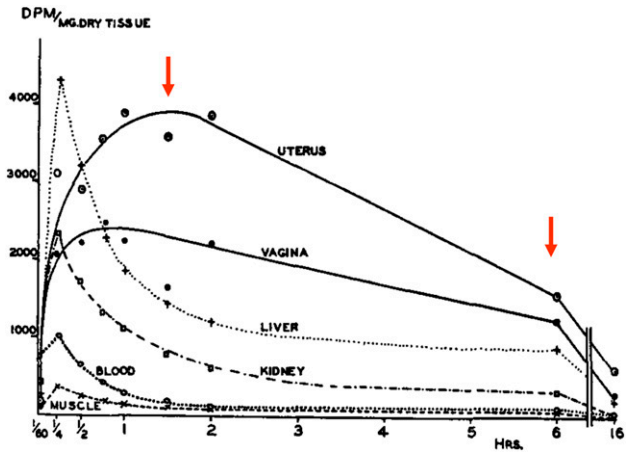

Figure 5. Administration of tritiated estradiol to oophorectomized rats. The disintegrations per minute (DPM) of isotope per milligram of dry tissue are shown on the vertical axis and time is shown on the horizontal axis (61). The red arrow on the left points out the peak concentration of isotope in the uterine tissue at 1.5 h, and the red arrow on the right the defection point in the decline at the 6-h time point. [Reproduced with permission for electronic publication only from Jensen EV. On the mechanism of estrogen action. Perspectives in Biology and Medicine 1962;6:47–59.]

Major biochemical studies were initiated in the 1960s. Ken Ryan studied and characterized placental aromatase and Harry Brodie and others the enzymatic steps involved (66–73). The initial two hydroxylation steps involved, steps 1 and 2, are generally agreed to involve sequential hydroxylations on the C19 methyl group. The third hydroxylation step remained elusive for more than two decades. Notably, recent studies from Guengerich's group have shed additional light on this issue (74). As shown in Supplemental Fig. 2 (26, 74), he suggested two possibilities for step 3 and outlined the specific interactions necessary. This synthesis pathway highlights how one enzyme can remarkably catalyze three separate steps.

Purification

The tools necessary for protein purification in the 1980s involved multiple steps using sequential chromatographic columns and serial eluants with measurement of enzyme activity in the various fractions. The tritiated water assay, which used placental microsomal aromatase, developed earlier by Siiteri and Thompson (75–78), facilitated these studies by considerably simplifying the enzyme activity assay and reducing the need for thin layer chromatography. The overall process of purification was technically difficult, time-consuming, and costly. Nonetheless, Yoshio Osawa, Peter Hall, Larry Vickery, Frank Bellino, Norio Muto, Marrku Pasanen, Olavi Pelkonen, Evan Simpson, and Carole Mendelson purified the human aromatase cytochrome P450 protein from human placental microsomes (79–84). Final demonstration of activity required the confirmation of the tritiated water assay with thin layer chromatographic demonstration of the conversion of androstenedione to estrogen, crystallization, and then recrystallization. When aromatase was originally studied in the early 1950s, it was logical to think that this reaction required three separate enzymes. Conceptually, it was difficult to imagine that one enzyme could cause hydroxylation of three separate sites and the double bond structure in the A ring. Nonetheless, the purified enzyme could clearly catalyze these disparate steps, a finding considered by all investigators to be definitive.

During this period, the techniques of recombinant DNA revolutionized the ease of determination of exact protein structures, both by considerably simplifying the process and by the sensitivity and specificity of the

methods. Availability of purified protein preparations of aromatase led to the generation of both polyclonal and monoclonal antibodies that could then be used for analyzing clones. The laboratories of Toda and Shizuta and Evans, Simpson, and Mendelson obtained clones complementary to aromatase transcripts. Expression libraries, and particularly one using the λgt11 phage, enabled isolation of a partial cDNA clone lacking the 5′ end of the cDNA but containing the heme binding region. Simpson and Mendelson carried out these studies and extended their findings using the 5′ rapid amplification of cDNA end technique (85) to identify the entire cDNA and 503 amino acid sequences. As biologic studies had identified aromatase in a number of species (75), a series of investigators used similar molecular biological methodology to identify aromatase sequences from numerous species and to compare and contrast the unique structural components of each (85–92).

Three-dimensional structure

As X-ray analysis of crystal structure provided a powerful tool to determine specific mechanisms for enzymatic activity, investigators in the aromatase field worked very hard for at least two decades to obtain crystals of this protein. They found this task to be problematic owing to the fact that the protein was membrane bound, relatively low in amount, and contained elements such as the heme configuration that were unstable during purification. As a means to overcome these obstacles, a number of groups sought to model the three-dimensional structure of the aromatase protein based on the known structures of other cytochrome P450 species (93–95). Promising candidates, known at that time, were soluble prokaryotic, cytochrome P450 species. Modeling attempts used these structures, which, however, had low sequence homology to human aromatase (96, 97). As the field evolved, the molecular biological tools enabled study of a wide variety of structures of mammalian microsomal P450 species that served as templates for model building (96–98). This approach, and particularly that used by Shiuan Chen and his group (98), proved to be remarkably predictive of later studies using human aromatase when it was finally crystalized (99).

In some instances in science, older, time-proven, but quite difficult techniques prove superior to newer, elegant molecular methodologic methods. This principle characterized the final crystallization of human aromatase. Starting with the original methodology of Yoshio Osawa (81), the group of Debashis Ghosh in Buffalo, New York, used protein chemistry methods to purify aromatase from human placenta in quantity and quality allowing crystallization. This process overall encompassed more than 20 years.

They then used this to obtain crystals that allowed characterization of the actual three-dimensional structure of human aromatase by X-ray spectroscopy (99) (Fig. 6). When all is taken into consideration, this would appear to be a remarkable achievement.

Genomic Structure

Exons I through X

The next step was to determine the sequence of the human aromatase gene. This was achieved using the aromatase cDNA sequences as probes for human genomic libraries. In this way, the exonic sequence together with the flanking sequences of the human gene were obtained (84, 92–96). The sequence contained nine coding exons, which were named by the Dallas group II to X, with the heme-binding region present in exon X. This region was ~30 kb in length. The first exon turned out to be upstream of the translational start site and moreover was different for each tissue site of expression. Namely, the placenta, ovary, and adipose tissue each

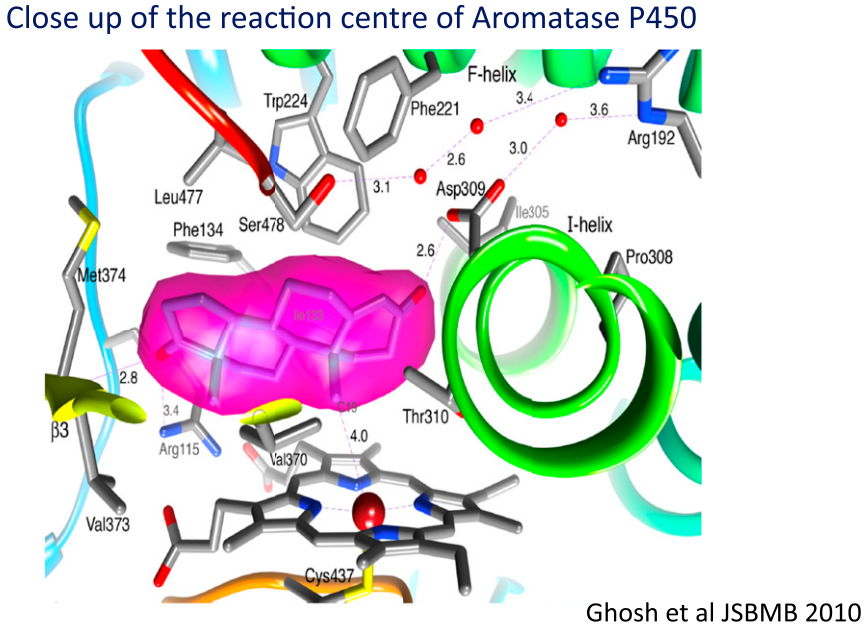

Figure 6. Close-up of the docking site for androstenedione in the structure of the aromatase enzyme obtained from analysis of aromatase crystals. [Reproduced with permission for electronic publication only from Ghosh D, Griswold J, Erman M, Pangborn W. X-ray structure of human aromatase reveals an androgen-specific active site. J Steroid Biochem Mol Biol 2010;118(4–5):197–202.]

exhibits a unique exon I (100–102). These are spliced into a common 3′ splice junction upstream of the start of translation in a tissue-specific fashion such that the sequence of the translated protein is always identical (Supplemental Fig. 3) (26). This was quite unexpected and important because the use of these untranslated first exons is directed by a number of tissue-specific promoters in a tissue-specific fashion. Because each of these promoters is uniquely regulated by a different cohort of *trans*-acting elements, this determines the tissue-specific regulation of aromatase expression and hence of estrogen biosynthesis.

When the sequence of the aromatase gene was matched to the published sequence of the human genome, there was a missing region between the most distant promoter, namely that of the placenta, and the remaining sequence. This alignment with genomic sequences enabled the gap to be closed by Bulun *et al.* (103) to reveal that the complete structure encompasses a span of ~123 kb. Within the P450 superfamily, aromatase is designated as family 19, with the human gene as *CYP19A*, and the mouse gene as *Cyp 19A*.

Promoter regulation

At the time, this type of regulation was poorly appreciated. As indicated above, tissue specificity was enabled by the presence of tissue-specific promoters upstream of the various first exons, which then stimulated the transcription of the aromatase message contained in exons II through X. Thus, for example, upstream of the placenta-specific first exon is a promoter region (promoter I.1) that is regulated by multiple factors as shown in Supplemental Fig. 4 (26). Notably, however, this first exon is located some 90 kb upstream of the translational start site and the entire intervening sequence is spliced out during (100) processing of the placental aromatase transcript. The specific processes differ in various tissues. For example, in the ovarian tissue (101, 102), the first exon (exon II) is proximal to the translational start site and its promoter.

In the ovary, promoter II contains two cAMP response elements and hence is regulated by cAMP through actions of FSH. Aromatase expression in adipose tissue is recognized to be present largely in the preadipocytes or stromal cells rather than the lipid-laden adipocytes. In these cells, aromatase is regulated primarily by another distal promoter (promoter I.4) that is regulated by class 1 cytokines such as IL-6 and also by TNFα. However, transcripts from the ovarian promoter II are also present in adipose stromal cells, and these become dominant in cancer-associated fibroblasts surrounding a breast tumor due to the action of prostaglandin E_2, probably produced largely by the tumorous epithelium (Supplemental Fig. 5) (75). The production of estrogen within cells and its binding to the estrogen in the same cells is an example of intracrinology (Supplemental Fig. 5) (26).

[The reader should note that two separate terminologies exist for alternative exons I as proposed by Mahendroo *et al.* (101) and Means *et al.* (102) (*i.e.*, exons I.1, I.2, I.3, I.4, 2a) and by Harada *et al.* (100) (*i.e.*, exons 1a, 1b, 1c, 1d, 1f, 2a).]

ER-Mediated Functions

ERα and ERβ

Both receptors are members of the nuclear receptor superfamily and contain six functional domains: amino terminal (A/B domain), DNA-binding C domain, hinge region (D domain), ligand-binding (E) domain, and carboxyl-terminal F domain (104–106). The A/B domain contains transcription activation function 1, and the ligand-binding domain contains transcription activation function 2 (104, 107). Binding of estradiol to both ERs changes the conformation of a critical portion of these receptors, helix 12, which exposes protein surfaces where both coactivators and corepressors can bind. These proteins can amplify or reduce the rate of transcription induced by binding to the estrogen ligand. Both ERα and ERβ contain a high degree of homology in the C and E domains but are divergent in the A/B, D, and F domains (104, 105). Alternative splicing and utilization of divergent start sites results in receptors of varying size. Additionally, breast cancers treated with hormone therapy develop ERα mutations that act in a ligand-independent fashion (108).

ERα levels are highest in the uterus and pituitary and in lesser concentrations in the liver, hypothalamus, bone, mammary gland, cervix, and vagina. ERβ levels are highest in the ovary (exclusively in the granulosa cells), lung, and prostate (109). Extensive knockout and knock-in experiments in mice have elucidated the specific physiologic roles of each receptor. As examples, ERβ is necessary for the proper differentiation of ovarian granulosa cells and efficient ovulation, whereas ERα is important for the function of the uterus (104, 110) and development of the mammary gland.

Sites of initiation of receptor-mediated events

The ER resides in the perimembranous region and can initiate events at that location. After synthesis, the ERs primarily localize to the nucleus due to a strong specific nuclear localization site originating in the D domain. ERα also is reported to be present in the mitochondria where it is involved in regulation of reactive oxygen species and apoptosis (111–113). Current concepts hold that events initiated at or near the cell membrane are

integrated with mechanisms involving nuclear initiation (112, 114–127).

Nuclear-initiated events

Both ERα and ERβ can act in the nucleus via three separate mechanisms: (i) direct binding to estrogen response elements, which classically consist of 5GGTC-AnnnTGACC palindromes or minor variants of this DNA sequence; (ii) tethering to AP-1 and SP-1, which in turn have their own response elements, or to activated MAPK; and (iii) ligand-independent receptor activation, which is thought to be possible when specific serines in the receptor structure are phosphorylated. Dimerization of two ER monomers is necessary for functionality. Chromatin immunoprecipitation sequencing studies have shown that the ER binds to 5000 DNA sites in the absence of ligand and 17,000 sites when ligand is present (128). Activation of transcription involves RNA polymerase II, which is only activated when ligand is present. This process involves the pioneering factor FOXA1, which causes chromatin remodeling and opens up sites for ER binding to the estrogen response elements (129). Coactivators and corepressors are important modulators of this process, as are complex enhancer elements. A simplified diagram of these events is illustrated in Supplemental Fig. 6 (26).

A myriad of investigators contributed to identifying each component in this complex fabric of functional activity. The details of each of these steps is beyond the scope of this minireview. However, to name just a few studies and investigators, Geoff Green, in conjunction with Pierre Chambon, and others cloned ERα and later crystalized this protein (Supplemental Fig. 7) (26, 130); Kuiper *et al.* (127) cloned ERβ; and multiple investigators studied genomic actions [see key manuscripts and reviews by Ken Korach, Donald McDonnell, John and Benita Katzenellenbogan, Serdar Bulun, Jack Gorski, Bert O'Malley, and many others (omitted because of space constraints)] (103, 104, 109, 115, 121, 125, 130–161).

Membrane-initiated actions

Pietras and Szego (162) first demonstrated membrane effects of estrogen and published these data in *Nature* in 1977. These studies were originally considered controversial and did not stimulate further investigations in this area. Two decades later, Ellis Levin and others began to explore this area intensively (112, 116–118). Studies during the past decade have clearly demonstrated a role for ERs residing near or in the plasma membrane and a role for ERα in the mitochondria (111, 113, 121, 136, 145, 162–174). Another estradiol-binding protein, GP 30, now called GPER1, also mediates multiple functions in various tissues (123, 173).

As a key issue related to the mechanism whereby ERα was directed to the plasma membrane, Razandi *et al.* (174) demonstrated that cytosolic ER is palmitoylated, which allows localization in or near the plasma membrane. Palmitoylation at cysteine 447 provides the mechanism for transport to the membrane where it binds to caveolin. Relevant studies then began examining the downstream signaling initiated by membrane ERα, which was found to be quite complex. Steps included rapid activation of several different components: IGF-1, epidermal growth factor, p21, Raf, MAPK, AKT, protein kinase C, release of nitric oxide, stimulation of prolactin secretion, and alteration of calcium and maxi-K channels (164). A marker of membrane signaling used by most investigators initially was MAPK, which is activated within 5 minutes of exposure to estradiol (164, 175, 176). Use of estradiol-linked dendrimer conjugates that could not enter the nucleus to activate MAPK (115, 153, 177) confirmed that this action was not nuclear. An important finding from these studies was that membrane-initiated events could ultimately result in regulation of transcription in the nucleus. Activation of MAPK, an event occurring at or near the membrane, resulted in ERα/MAPK complexes that entered the nucleus and bound to specific DNA response elements. There, activated MAPK can phosphorylate SRC3, RIP140, p300, and CREB1 (115, 177–179). The demonstration of ERα/MAPK complexes on DNA as shown by chromatin immunoprecipitation assays provided conclusive proof of this membrane to nuclear DNA pathway (115, 177). The signaling pathways that transduce the rapid effects of estradiol are complex and differ according to cell type.

Mitochondrial function

Mitochondria contain both ERα and ERβ. Estradiol can augment several mitochondrial DNA-encoded RNAs (113). Through ERβ, estrogen simulates manganese superoxide dismutase and reduces damage from reactive oxygen species. These actions lead to a reduction of apoptosis. An extensive literature has now been developed that identifies multiple effects of both ERα and ERβ on mitochondrial function (112, 113).

Estrogen acts on both sexes

Two prismatic cases clearly demonstrated the role of estrogen in men. Smith *et al.* (147) reported a male patient with a nonfunctional ERα, and Maffei *et al.* (180) reported a man with nonfunctioning aromatase. Both had osteopenia with genu valgum and continued to grow into their late 20s, lacking closure of their long bone epiphyses (Fig. 7). These findings indicated the role of

estrogen in epiphyseal closure and bone maintenance in men. Treatment in the aromatase-deficient male demonstrated that estrogen regulates lipids, enhances insulin sensitivity, lowers glucose, and normalizes liver function, all important metabolic and hepatic effects (Supplemental Figs. 8 and 9) (26). Further information was gained using genetically altered mice with ERα/β and aromatase knockout or knock-in conducted by Ken Korach, Evan Simpson, Matti Poutanen, and Raj Tekmal and their respective groups (75, 125, 181–186). These studies demonstrated a role of estrogen in spermatogenesis, sexual intromission, ejaculation, and maintenance of bone density. Many of these effects were also demonstrated by Finkelstein *et al.* (187) who conducted add-back experiments in men given GnRH agonists to suppress androgen and estrogen levels.

Clinical Studies

Early metabolic studies

A number of investigators began to extensively study the metabolism of various steroids using radioisotope kinetic studies in the late 1950s and 1960s. This provided the ideal methodology to examine the levels of aromatase activity under various clinical circumstances. Using these techniques, Pentti Siiteri and Paul MacDonald (188) studied the clinical expression of aromatase in patients as a function of both weight and age (Fig. 8). They demonstrated nearly a 10-fold increase in aromatase when comparing 90-pound subjects with 250- to 300-pound men and women. These results clearly showed that most of this estrogen was coming from the adipose tissue rather than the gonads and also demonstrated that extragonadal aromatization increased substantially as a function of increasing age (188).

Clinical implications of estrogen physiology

Animal models of breast cancer and prior observations with surgical oophorectomy and adrenalectomy in breast cancer patients suggested that an antiestrogen might also be effective treatment (49, 189–191). This suggested the use of tamoxifen in patients with breast cancer. The first clinical trial was reported by Cole *et al.* (192) in 1971 at the Christie Hospital in Manchester, UK, demonstrating a 22% response rate in 46 patients receiving 10 mg daily of tamoxifen. A study in 1973 reported a 40% response in 35 women receiving 20 mg daily (193). These results compared favorably with non–head-to-head studies in women treated with ablative surgery (oophorectomy or adrenalectomy) that were routine at that time and strongly suggested the efficacy of medical, antiestrogen therapy (194). Later extensive studies by Craig Jordan and associates identified a unique property of tamoxifen, that is, that it exerts antiestrogenic effects on breast but estrogenic effects on uterus and bone. These findings led to the coining of the term SERM (selective ER modulator) (195).

Several investigators postulated that inhibition of adrenal steroid synthesis would be as effective as tamoxifen as treatment of breast cancer in postmenopausal women. Accordingly, the nonsteroidal inhibitor aminoglutethimide (then thought to be a cholesterol side chain-inhibitor) was studied by Robert Cash, Kenneth Gale, "Dickie" Newsome, and Richard Santen with demonstration of antitumor efficacy (75, 196–201). Later, stimulated by the advice of Pentti Siiteri, biochemical studies were undertaken that demonstrated that aminoglutethimide was an effective aromatase inhibitor (75–78, 188). The use of isotopic kinetic technology allowed demonstration in 1978 of a 95% reduction of aromatase with aminoglutethimide (Supplemental Fig. 10) (26), which represented its major mechanism of action (200).

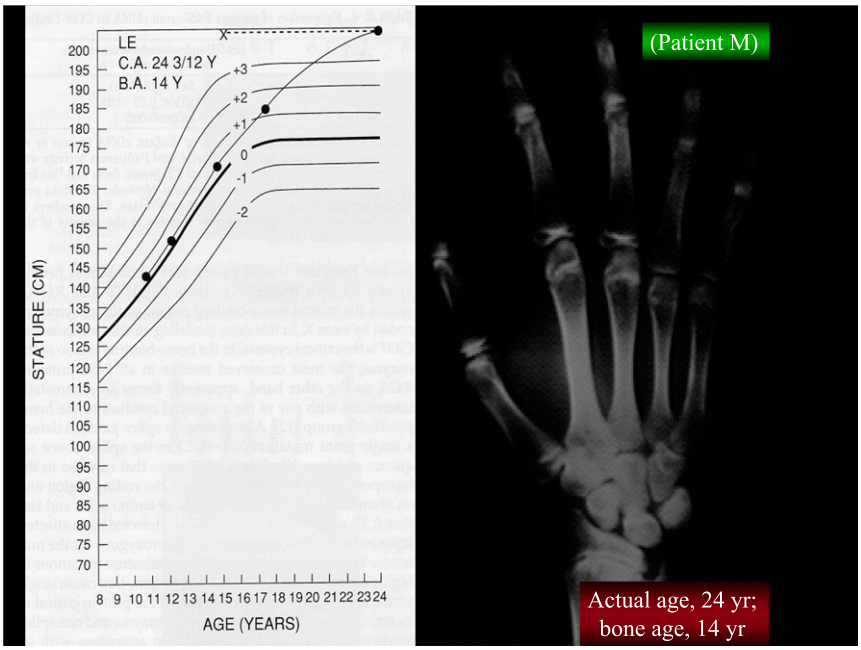

Figure 7. The growth curve over time in a patient with aromatase deficiency (left panel) and an X-ray of the hand (right panel) in the same patient at age 24 to demonstrate the lack of closure of the epiphyses. The lack of closed epiphyses is characteristic of a 14 year old and thus the term, bone age of 14 is used (180). [Reproduced with permission from Smith EP, Boyd J, Frank GR, *et al*. Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. N Engl J Med 1994;331(16):1057.]

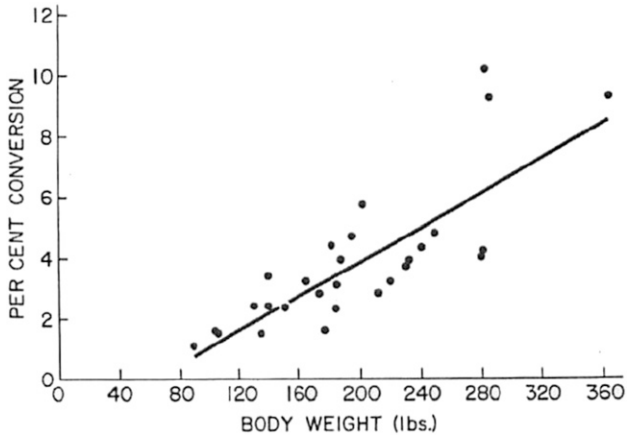

Figure 8. The relationship of the percentage conversion of testosterone to estradiol under steady-state conditions (vertical axis) to body weight in pounds (lbs) in postmenopausal women. Correlation coefficient, 0.74 (188). [Reproduced with permission from Siiteri PK, MacDonald PC. Role of extraglandular estrogen in human endocrinology. In: Greep RO, Astwood EB, eds. Handbook of Physiology. Washington, DC: American Physiologic Society, 1973: 615–629.]

Angela Brodie and her husband, Harry Brodie, focused on development of steroidal aromatase inhibitors. After preclinical studies were completed, Angela Brodie and her collaborators demonstrated the clinical efficacy of the steroidal aromatase inhibitor 4-OH-androstenedione (75, 202, 203) (Supplemental Fig. 11) (26). Full details of the history of development of aromatase inhibitors are detailed in another publication by the coauthors (40, 75). As blockade of estrogen synthesis with aromatase inhibitors or action with antiestrogens evolved, it became apparent that prevention of breast cancer with these agents might become possible. Trevor Powles pioneered the use of tamoxifen for breast cancer prevention (204). Many groups reported that high estradiol levels in postmenopausal women were associated with an increased risk of breast cancer (205, 206).

As responses to hormonal therapy were not uniform, a means was needed to predict which patients would respond to the antiestrogens and aromatase inhibitors. William McGuire (1937–1992), along with Elwood Jensen (1920–2012), had the vision to extensively correlate the presence of ERα with responsiveness of hormone-dependent breast cancer and to develop multiple concepts about breast cancer biology (62, 207–209). Use of selection for the presence of ERα allowed an enhancement of the percentage of responders to these agents. Interestingly, these studies demonstrated immediate and quite obvious efficacy of antiestrogens and aromatase inhibitors in a proportion of ER-positive patients (Supplemental Fig. 12) (26). The clear-cut responses observed support Charles Huggins' statement that "if your therapy works, you do not need statistics to prove it" (the quotation is from Festschrift for Olaf Pearson, Case Western School of Medicine, 1987, as related personally to R.J.S.).

Recent attention has focused on resistance to antiestrogens and aromatase inhibitors in women with breast cancer. An early focus was on estrogen receptor mutations by Fuqua *et al.* (210–212) who described a K303R mutation that induced hypersensitivity to estradiol. An interesting mechanism is the emergence of clones of cells with mutations of the ER that render them capable of ligand-independent actions (108). These mutations are generally not present in tumors when initially discovered but are observed in tumors exposed long term to hormonal therapies (108), presumably through a process of selection. These observations have led to the more common use of the selective receptor downregulator fulvestrant and the development of new, more potent selective receptor downregulators (213, 214). The rationale is to eliminate these receptors as a means of abrogating their function.

Other syndromes of estrogen excess and deficiency

Benign tumors and malignant tumors containing high levels of estrogen can cause gynecomastia as in the Peutz-Jeghers syndrome and hepatocellular carcinoma (215–217). Duplication, deletion, and inversion resulting from subchromosomal recombinations result in syndromes of aromatase overexpression (218, 219). Inactivity of aromatase in women can result in pseudohermaphroditism, hirsutism, and other abnormalities (220–222).

Controversy

Many controversies arose with respect to the use of estrogen for treatment of the menopause, and both proponents and opponents expressed strong opinions (57, 87, 223–232). One controversy is whether estrogens cause or prevent breast cancer. The Women's Health Initiative Trial suggests that conjugated equine estrogen, the estrogen used in that trial, reduced the risk of breast cancer by 23% (hazard ratio, 0.77; CI, 0.62 to 0.95) (233, 234) (Supplemental Fig. 13) (26). The mechanism for this is not clearly established but may reflect the proapoptotic effects of estrogens on occult, preexisting tumors (235–238). Interestingly, recent preclinical data suggest that the effects of conjugated equine estrogen may differ from those of estradiol (239). Another controversy is whether menopausal hormone therapy causes more benefit than harm in postmenopausal women. The recent Endocrine Society guideline concludes that benefits exceed harm in symptomatic women 50 to 59 years of age or $<$10 years postmenopausal when at low risk of breast cancer and coronary heart disease (231).

Complexity of estradiol effects

Recent reviews detail in considerable depth the actions of estrogen in women and in men and on brain (240), heart (114, 241), vasculature (114, 121), ovaries (125), bones (242), skeletal muscle (187), breast (205, 206, 243), adipose tissue (244), and reproductive tissue (104, 125). Studies of these actions in depth are found in the reviews cited above but were beyond the scope of the Clark Sawin Lecture.

Research into the various actions of estrogen has advanced rapidly in the past 5 years. To illustrate this point, the estrogen-related publications in the journal *Endocrinology* from 2013 until the present have been identified and categorized here. A major focus has been on the effects of estrogen on various aspects of brain function, including gonadotropin regulation, behavior, synaptic function, metabolism, and morphogenesis (245–280). Three other areas of emphasis have been bone (98, 281, 283), adrenal (284–287), and ovary (288–291). Other foci of study include pain mediation (292–294), uterus (295–297), glucose/insulin actions (298–302), and reproduction (303).

Topics and investigators left out

Any history must prioritize the issues raised, and this lecture illustrates this point well. We left out important effects on the brain, including behavior, gonadotropin regulation, and obsessive compulsive disorder, among others; cardiovascular actions; effects on the immune system; pubertal development; and management of menopause and actions on skeletal muscle. Finally, we apologize to the key investigators that we have failed to mention because of the time allotted to the lecture, but we refer readers to two reviews written by the coauthors (40, 75).

Acknowledgments

Access to audio and video of Sawin lecture: https://figshare.com/articles/Sawinlecturemp4/7605488.

Correspondence: Richard J. Santen, MD, Division of Endocrinology and Metabolism, University of Virginia, P.O. Box 801416, Charlottesville, Virginia 22908. E-mail: rjs5y@virginia.edu.

Disclosure Summary: The authors have nothing to disclose.

References

1. Albright F, Ellsberg R. *Uncharted Seas*. Portland, OR: Kalmia Press; 1990:3–95.
2. Paulshock BZ. Tutankhamun and his brothers. Familial gynecomastia in the Eighteenth Dynasty. *JAMA*. 1980;**244**(2):160–164.
3. Connell SM. Aristotle and Galen on sex difference and reproduction: a new approach to an ancient rivalry. *Stud Hist Philos Sci*. 2000;**31**(3):405–427.
4. Galanakis E. Apgar score and Soranus of Ephesus. *Lancet*. 1998;**352**(9145):2012–2013.
5. Karamanou M, Tsoucalas G, Creatsas G, Androutsos G. The effect of Soranus of Ephesus (98–138) on the work of midwives. *Women Birth*. 2013;**26**(4):226–228.
6. Temkin O. *Soranus Gynecology*. Baltimore, MD: Johns Hopkins University Press; 1956.
7. Boylan M. Galen. *J Hist Biol*. 1986;**19**(1):47–77.
8. Hankinson RJ. *The Cambridge Companion to Galen*. Cambridge, UK: Cambridge University Press; 2009.
9. Kaplowitz PB. Link between body fat and the timing of puberty. *Pediatrics*. 2008;**121**(Suppl 3):S208–S217.
10. Frisch RE, Revelle R. Height and weight at menarche and a hypothesis of menarche. *Arch Dis Child*. 1971;**46**(249):695–701.
11. Frisch RE, Revelle R. The height and weight of girls and boys at the time of initiation of the adolescent growth spurt in height and weight and the relationship to menarche. *Hum Biol*. 1971;**43**(1):140–159.
12. Frisch RE. Body fat, menarche, fitness and fertility. *Hum Reprod*. 1987;**2**(6):521–533.
13. Lorriaux DL. Thomas Wharton (1614–1673): codifying the glands. In: A Biographical History of Endocrinology. Oxford, UK: John Wiley & Sons; 2016:49–54.
14. Mettler CC, Mettler FA. Gynecology in the nineteenth century: ovariotomy. In: History of Medicine. Philadelphia, PA: The Blakiston Company; 1947.
15. Gendrin AN. Chapter 13. In: *Traité Philosophique de Médecine Pratique*. Paris, France: Baillière; 1838:987–1003.
16. Bernard C. An Introduction to the Study of Experimental Medicine. Reprinted in Classics of Medicine series from the original published in 1864 by Gazette Médicale de Paris. New York, NY: Macmillan Company; 1927.
17. Battey R. Normal ovariotomectomy. *Atlanta Medical and Surgical Journal*. 1873;**10**:321–339.
18. Knauer E. Ovarian transplantation in rabbits: a preliminary report. Contralb F Gyn., 1898.
19. Knauer E. On ovarian transplantation: labour and normal end of pregnancy after transplantation of ovaries in rabbits. Contralb F Gyn., no. 8, 1898.
20. Knauer E. Transplantation of the ovaries: an experimental study. Archiv F Gyn., 1898.
21. Fosbery WH. Severe climacteric flushings successfully treated with ovarian extract. *BMJ*. 1897;**1**:1039.
22. Brown-Séquard C. Note on the effects produced on man by subcutaneous injections of a liquid obtained from the testicles of animals. *Lancet*. 1889;**134**(3438):105–107.
23. Nieschlag E, Nieschlag S. Testosterone deficiency: a historical perspective. *Asian J Androl*. 2014;**16**(2):161–168.
24. Easterbrook CC. Organo-therapeutics in mental diseases. *BMJ*. 1900;**2**:813–823.
25. Miao Sun Si. Essential Formulas for Emergencies [Worth] a Thousand Pieces of Gold [in Chinese]; 652.
26. Santen RJ, Simpson E. Data from: History of estrogen: its purification, structure, synthesis, biologic actions, and clinical implications. figshare 2019. Deposited 26 January 2019. https://figshare.com/articles/Supplemental figures History of Estrogen/7635968.
27. Temple R. The science of endocrinology. In: The Genius of China: 3000 Years of Science, Discovery, and Invention. London, UK: Prion; 1998:127–130.
28. Beatson GT. On the treatment of inoperable cases of carcinoma of the mamma: suggestions for a new method of treatment, with illustrative cases. *Trans Med Chir Soc Edinb*. 1896;**15**:153–179.
29. Stockwell S. Classics in oncology. George Thomas Beatson, M.D. (1848–1933). *CA Cancer J Clin*. 1983;**33**(2):105–121.

30. Clarke MJ. Ovarian ablation in breast cancer, 1896 to 1998: milestones along hierarchy of evidence from case report to Cochrane review. *BMJ*. 1998;**317**(7167):1246–1248.
31. Allen E, Francis BF, Robertson LL. The hormone of the ovarian follicle: its localization and action in test animals and additional points bearing upon the internal secretion of the ovary. *Am J Anat*. 1924;**34**(1):133–181.
32. Allen E, Doisy EA. An ovarian hormone: preliminary report on its localization, extraction and partial purification and action in test animal. *JAMA*. 1923;**81**(10):819–821.
33. Doisy EA. An autobiography. *Annu Rev Biochem*. 1976;**45**(1): 1–9.
34. Rohde W. The contributions of Aschheim and Zondek to endocrinology. *Pediatr Endocrinol Rev*. 2010;7(4):323–327.
35. Doisy EA, Thayer S, Veler CD. The crystals of thefollicular ovarian hormone. *Proc Soc Exp Biol Med*. 1930;**27**(5):417–419.
36. Veler CD, Thayer S, Doisy EA. The preparation of the crystalline follicular ovarian hormone: theelin. *J Biol Chem*. 1930;**87**: 357–371.
37. Butenandt A. Über "Progynonein" kystallisiertes weibliches Exualhormon. *Naturwissenschaften*. 1929;**17**(45):879.
38. Huffman MN, Thayer SA, Doisy EA. The isolation of alpha-dihydrotheelin from human placenta. *J Biol Chem*. 1940;**133**: 567–571.
39. Thayer SA, Levin L, Doisy EA. Characterization of theelol. *J Biol Chem*. 1931;**91**:655–665.
40. Simpson E, Santen RJ. Celebrating 75 years of oestradiol. *J Mol Endocrinol*. 2015;**55**(3):T1–T20.
41. Albright F. The effect of hormones on osteogenesis in man. *Recent Prog Horm Res*. 1947;**1**:293–353.
42. Albright F, Halsted JA, Cloney E. Studies on ovarian dysfunction. *N Engl J Med*. 1935;**212**(5):192–195.
43. Albright F. Studies on ovarian dysfunction. III. The menopause. *Endocrinology*. 1936;**20**:24–39.
44. Forbes AP. Fuller Albright. His concept of postmenopausal osteoporosis and what came of it. *Clin Orthop Relat Res*. 1991; (269):128–141.
45. Sturgis SH, Albright F. The mechanism of estrin therapy in the relief of dysmenorrhea. *Endocrinology*. 1940;**25**:68–72.
46. Pfeiffer CA, Gardner WU. Skeletal changes and blood calcium level in pigeons receiving estrogens. *Endocrinology*. 1938;**23**:485–491.
47. Reifenstein EC Jr, Albright F. The metabolic effects of steroid hormones in osteoporosis. *J Clin Invest*. 1947;**26**(1):24–56.
48. Wallach S, Henneman PH. Prolonged estrogen therapy in postmenopausal women. *J Am Med Assoc*. 1959;**171**(12):1637–1642.
49. Huggins C. How Charles Huggins made his Nobel prize winning discovery—in his own words: an historic audio recording. Interviewed by Willard Goodwin and Elmer Bell. *Prostate*. 2012; **72**(16):1718.
50. Huggins C, Grand LC, Brillantes FP. Critical significance of breast structure in the induction of mammary cancer in the rat. *Proc Natl Acad Sci USA*. 1959;**45**(8):1294–1300.
51. Huggins C, Briziarelli G, Sutton H Jr. Rapid induction of mammary carcinoma in the rat and the influence of hormones on the tumors. *J Exp Med*. 1959;**109**(1):25–42.
52. Huggins C, Grand LC, Brillantes FP. Mammary cancer induced by a single feeding of polynuclear hydrocarbons, and its suppression. *Nature*. 1961;**189**(4760):204–207.
53. Huggins C, Grand L, Fukunishi R. Aromatic influences on the yields of mammary cancers following administration of 7,12-dimethylbenz(*a*)anthracene. *Proc Natl Acad Sci USA*. 1964;**51**:737–742.
54. Welsch CW. Host factors affecting the growth of carcinogen-induced rat mammary carcinomas: a review and tribute to Charles Brenton Huggins. *Cancer Res*. 1985;**45**(8):3415–3443.
55. Speroff L. *A Good Man: The Man, His Story, the Birth Control Pill*. Portland, OR: Arnica Publishing; 2009.
56. Goldzieher JW. The history of steroidal contraceptive development: the estrogens. *Perspect Biol Med*. 1993;**36**(3):363–368.
57. Watkins ED. *The Estrogen Elixir: A History of Hormone Replacement in America*. Baltimore, MD: Johns Hopkins University Press; 2007.
58. Colton FB. Steroids and "the pill": early steroid research at Searle. *Steroids*. 1992;**57**(12):624–630.
59. Fehring RJ. An analysis of the majority report "Responsible Parenthood" and its recommendations on abortion, sterilization, and contraception. In: Koterski JW, ed. *Life and Learning XIII: Proceedings of the Thirteenth University Faculty for Life Conference: 2003 at Georgetown University*. Washington, DC: University Faculty for Life; 2004: 120–152.
60. Goldin C, Katz LF. The power of the pill: oral contraceptives and women's career and marriage decisions. *J Polit Econ*. 2002; **110**(4):730–770.
61. Jensen EV. On the mechanism of estrogen action. *Perspect Biol Med*. 1962;**6**(1):47–59.
62. Block GE, Ellis RS, DeSombre E, Jensen E. Correlation of estrophilin content of primary mammary cancer to eventual endocrine treatment. *Ann Surg*. 1978;**188**(3):372–376.
63. Zondek B. Oesrogenic hormone in the urine of the stallion. *Nature*. 1934;**133**(3361):494.
64. Steinach E, Kun H. Transformation of male sex hormones into a substance with the action of a female hormone. *Lancet*. 1937; **230**(5954):845.
65. Balthazart J, Ball GF. New insights into the regulation and function of brain estrogen synthase (aromatase). *Trends Neurosci*. 1998;**21**(6):243–249.
66. Brodie HJ, Pillai AK, Hay CE. Studies on the mechanisms of estrogen biosynthesis. VII. 2β-Hydroxylation of estr-4-ene-3,17-dione in human placenta, in vitro. *Biochim Biophys Acta*. 1969; **187**(2):275–277.
67. Brodie HJ, Raab K, Possanza G, Seto N, Gut M. Further stereochemical studies of the catalytic reduction of delta-1,4-3-keto steroids with tritium. *J Org Chem*. 1969;**34**(9):2697–2702.
68. Brodie HJ, Kripalani KJ, Possanza G. Studies on the mechanism of estrogen biosynthesis. VI. The stereochemistry of hydrogen elimination at C-2 during aromatization. *J Am Chem Soc*. 1969; **91**(5):1241–1242.
69. Brodie HJ, Possanza G, Townsley JD. Studies on the mechanism of estrogen biosynthesis. V. Stereochemical comparison of aromatization in placental and microbiological systems. *Biochim Biophys Acta*. 1968;**152**(4):770–777.
70. Meyer AS. 19-Hydroxylation of Δ^4-androstene-3,17-dione and dehydroepiandrosterone by bovine adrenals. *Experientia*. 1955; **11**(3):99–102.
71. Meyer AS, Hayano M, Lindberg MC, Gut M, Rodgers OG. The conversion of Δ^4-androstene-3,17-dione-4-C^{14} and dehydroepiandrosterone by bovine adrenal homogenate preparations. *Acta Endocrinol (Copenh)*. 1955;**18**(2):148–168.
72. Meyer AS. Conversion of 19-hydroxy-Δ^4-androstene-3,17-dione to estrone by endocrine tissue. *Biochim Biophys Acta*. 1955;**17**(3): 441–442.
73. Morato T, Hayano M, Dorfman RI, Axelrod LR. The intermediate steps in the biosynthesis of estrogens from androgens. *Biochem Biophys Res Commun*. 1961;**6**(5):334–338.
74. Yoshimoto FK, Guengerich FP. Mechanism of the third oxidative step in the conversion of androgens to estrogens by cytochrome P450 19A1 steroid aromatase. *J Am Chem Soc*. 2014;**136**(42): 15016–15025.
75. Santen RJ, Brodie H, Simpson ER, Siiteri PK, Brodie A. History of aromatase: saga of an important biological mediator and therapeutic target. *Endocr Rev*. 2009;**30**(4):343–375.
76. Thompson EA Jr, Siiteri PK. The involvement of human placental microsomal cytochrome P-450 in aromatization. *J Biol Chem*. 1974;**249**(17):5373–5378.
77. Thompson EA Jr, Siiteri PK. Utilization of oxygen and reduced nicotinamide adenine dinucleotide phosphate by human placental microsomes during aromatization of androstenedione. *J Biol Chem*. 1974;**249**(17):5364–5372.

78. Thompson EA Jr, Siiteri PK. Subcellular distribution of aromatase in human placenta and ovary. *Horm Res*. 1979;**11**(4):179–185.
79. Mendelson CR, Wright EE, Evans CT, Porter JC, Simpson ER. Preparation and characterization of polyclonal and monoclonal antibodies against human aromatase cytochrome P-450 (P-450AROM), and their use in its purification. *Arch Biochem Biophys*. 1985;**243**(2):480–491.
80. Pasanen M, Pelkonen O. Solubilization and partial purification of human placental cytochromes P-450. *Biochem Biophys Res Commun*. 1981;**103**(4):1310–1317.
81. Osawa Y, Yoshida N, Fronckowiak M, Kitawaki J. Immunoaffinity purification of aromatase cytochrome P-450 from human placental microsomes, metabolic switching from aromatization to 1β and 2β-monohydroxylation, and recognition of aromatase isozymes. *Steroids*. 1987;**50**(1–3):11–28.
82. Hall PF, Chen S, Nakajin S, Shinoda M, Shively JE. Purification and characterization of aromatase from human placenta. *Steroids*. 1987;**50**(1–3):37–50.
83. Muto N, Tan L. Purification of oestrogen synthetase by high-performance liquid chromatography. Two membrane-bound enzymes from the human placenta. *J Chromatogr A*. 1985;**326**: 137–146.
84. Kellis JT Jr, Vickery LE. Purification and characterization of human placental aromatase cytochrome P-450. *J Biol Chem*. 1987;**262**(9):4413–4420.
85. Corbin CJ, Graham-Lorence S, McPhaul M, Mason JI, Mendelson CR, Simpson ER. Isolation of a full-length cDNA insert encoding human aromatase system cytochrome P-450 and its expression in nonsteroidogenic cells. *Proc Natl Acad Sci USA*. 1988;**85**(23):8948–8952.
86. Harada N. Cloning of a complete cDNA encoding human aromatase: immunochemical identification and sequence analysis. *Biochem Biophys Res Commun*. 1988;**156**(2):725–732.
87. McPhaul MJ, Noble JF, Matsumine H, Wilson JD. Cloning and expression of the chicken ovary aromatase P-450: expression of mRNA in tissues of the Sebright and Leghorn chicken. *Trans Assoc Am Physicians*. 1988;**101**:219–225.
88. McPhaul MJ, Noble JF, Simpson ER, Mendelson CR, Wilson JD. The expression of a functional cDNA encoding the chicken cytochrome P-450$_{arom}$ (aromatase) that catalyzes the formation of estrogen from androgen. *J Biol Chem*. 1988;**263**(31):16358–16363.
89. Toda K, Terashima M, Mitsuuchi Y, Yamasaki Y, Yokoyama Y, Nojima S, Ushiro H, Maeda T, Yamamoto Y, Sagara Y, Shizuta Y. Alternative usage of different poly(A) addition signals for two major species of mRNA encoding human aromatase P-450. *FEBS Lett*. 1989;**247**(2):371–376.
90. Hickey GJ, Krasnow JS, Beattie WG, Richards JS. Aromatase cytochrome P450 in rat ovarian granulosa cells before and after luteinization: adenosine 3′,5′-monophosphate-dependent and independent regulation. Cloning and sequencing of rat aromatase cDNA and 5′ genomic DNA. *Mol Endocrinol*. 1990;**4**(1):3–12.
91. Terashima M, Toda K, Kawamoto T, Kuribayashi I, Ogawa Y, Maeda T, Shizuta Y. Isolation of a full-length cDNA encoding mouse aromatase P450. *Arch Biochem Biophys*. 1991;**285**(2): 231–237.
92. Tanaka M, Telecky TM, Fukada S, Adachi S, Chen S, Nagahama Y. Cloning and sequence analysis of the cDNA encoding P-450 aromatase (P450arom) from a rainbow trout (*Oncorhynchus mykiss*) ovary; relationship between the amount of P450arom mRNA and the production of oestradiol-17β in the ovary. *J Mol Endocrinol*. 1992;**8**(1):53–61.
93. Laughton CA, Zvelebil MJ, Neidle S. A detailed molecular model for human aromatase. *J Steroid Biochem Mol Biol*. 1993;**44**(4–6): 399–407.
94. Graham-Lorence S, Amarneh B, White RE, Peterson JA, Simpson ER. A three-dimensional model of aromatase cytochrome P450. *Protein Sci*. 1995;**4**(6):1065–1080.
95. Williams PA, Cosme J, Sridhar V, Johnson EF, McRee DE. Mammalian microsomal cytochrome P450 monooxygenase: structural adaptations for membrane binding and functional diversity. *Mol Cell*. 2000;**5**(1):121–131.
96. Favia AD, Cavalli A, Masetti M, Carotti A, Recanatini M. Three-dimensional model of the human aromatase enzyme and density functional parameterization of the iron-containing protoporphyrin IX for a molecular dynamics study of heme-cysteinato cytochromes. *Proteins*. 2006;**62**(4):1074–1087.
97. Koymans LM, Moereels H, Vanden Bossche H. A molecular model for the interaction between vorozole and other non-steroidal inhibitors and human cytochrome *P*450 19 (*P*450 aromatase). *J Steroid Biochem Mol Biol*. 1995;**53**(1–6):191–197.
98. Chen S, Zhang F, Sherman MA, Kijima I, Cho M, Yuan YC, Toma Y, Osawa Y, Zhou D, Eng ET. Structure–function studies of aromatase and its inhibitors: a progress report. *J Steroid Biochem Mol Biol*. 2003;**86**(3–5):231–237.
99. Ghosh D, Griswold J, Erman M, Pangborn W. Structural basis for androgen specificity and oestrogen synthesis in human aromatase. *Nature*. 2009;**457**(7226):219–223.
100. Harada N, Utsumi T, Takagi Y. Tissue-specific expression of the human aromatase cytochrome P-450 gene by alternative use of multiple exons 1 and promoters, and switching of tissue-specific exons 1 in carcinogenesis. *Proc Natl Acad Sci USA*. 1993;**90**(23): 11312–11316.
101. Mahendroo MS, Means GD, Mendelson CR, Simpson ER. Tissue-specific expression of human P-450$_{AROM}$. The promoter responsible for expression in adipose tissue is different from that utilized in placenta. *J Biol Chem*. 1991;**266**(17):11276–11281.
102. Means GD, Kilgore MW, Mahendroo MS, Mendelson CR, Simpson ER. Tissue-specific promoters regulate aromatase cytochrome P450 gene expression in human ovary and fetal tissues. *Mol Endocrinol*. 1991;**5**(12):2005–2013.
103. Bulun SE, Takayama K, Suzuki T, Sasano H, Yilmaz B, Sebastian S. Organization of the human aromatase p450 (*CYP19*) gene. *Semin Reprod Med*. 2004;**22**(1):5–9.
104. Hamilton KJ, Hewitt SC, Arao Y, Korach KS. Estrogen hormone biology. *Curr Top Dev Biol*. 2017;**125**:109–146.
105. Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schütz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P, Evans RM. The nuclear receptor superfamily: the second decade. *Cell*. 1995;**83**(6):835–839.
106. Hewitt SC, Korach KS. Estrogen receptors: new directions in the new millennium. *Endocr Rev*. 2018;**39**(5):664–675.
107. Aagaard MM, Siersbæk R, Mandrup S. Molecular basis for gene-specific transactivation by nuclear receptors. *Biochim Biophys Acta*. 2011;**1812**(8):824–835.
108. Jeselsohn R, Bergholz JS, Pun M, Cornwell M, Liu W, Nardone A, Xiao T, Li W, Qiu X, Buchwalter G, Feiglin A, Abell-Hart K, Fei T, Rao P, Long H, Kwiatkowski N, Zhang T, Gray N, Melchers D, Houtman R, Liu XS, Cohen O, Wagle N, Winer EP, Zhao J, Brown M. Allele-specific chromatin recruitment and therapeutic vulnerabilities of ESR1 activating mutations. *Cancer Cell*. 2018; **33**(2):173–186.e5.
109. Couse JF, Lindzey J, Grandien K, Gustafsson JA, Korach KS. Tissue distribution and quantitative analysis of estrogen receptor-α (ERα) and estrogen receptor-β (ERβ) messenger ribonucleic acid in the wild-type and ERα-knockout mouse. *Endocrinology*. 1997;**138**(11):4613–4621.
110. Binder AK, Rodriguez KF, Hamilton KJ, Stockton PS, Reed CE, Korach KS. The absence of ER-β results in altered gene expression in ovarian granulosa cells isolated from in vivo preovulatory follicles. *Endocrinology*. 2013;**154**(6):2174–2187.
111. Yager JD, Chen JQ. Mitochondrial estrogen receptors—new insights into specific functions. *Trends Endocrinol Metab*. 2007; **18**(3):89–91.
112. Levin ER. Extranuclear steroid receptors are essential for steroid hormone actions. *Annu Rev Med*. 2015;**66**(1):271–280.

113. Klinge CM. Estrogens regulate life and death in mitochondria. *J Bioenerg Biomembr*. 2017;**49**(4):307–324.
114. Kim JK, Levin ER. Estrogen signaling in the cardiovascular system. Nucl Recept Signal. 2006;**4**:e013.
115. Madak-Erdogan Z, Kieser KJ, Kim SH, Komm B, Katzenellenbogen JA, Katzenellenbogen BS. Nuclear and extranuclear pathway inputs in the regulation of global gene expression by estrogen receptors. *Mol Endocrinol*. 2008;**22**(9):2116–2127.
116. Levin ER. Hormone action and breast cancer. *Cancer Treat Res*. 2009;**147**:1–10.
117. Levin ER. Extranuclear estrogen receptor's roles in physiology: lessons from mouse models. *Am J Physiol Endocrinol Metab*. 2014;**307**(2):E133–E140.
118. Levin ER. Translating extranuclear steroid receptor signaling to clinical medicine. *Horm Cancer*. 2014;**5**(3):140–145.
119. Levin ER, Hammes SR. Nuclear receptors outside the nucleus: extranuclear signalling by steroid receptors. *Nat Rev Mol Cell Biol*. 2016;**17**(12):783–797.
120. Levin ER. Membrane estrogen receptors signal to determine transcription factor function. *Steroids*. 2018;**132**:1–4.
121. Chambliss KL, Wu Q, Oltmann S, Konaniah ES, Umetani M, Korach KS, Thomas GD, Mineo C, Yuhanna IS, Kim SH, Madak-Erdogan Z, Maggi A, Dineen SP, Roland CL, Hui DY, Brekken RA, Katzenellenbogen JA, Katzenellenbogen BS, Shaul PW. Nonnuclear estrogen receptor alpha signaling promotes cardiovascular protection but not uterine or breast cancer growth in mice. *J Clin Invest*. 2010;**120**(7):2319–2330.
122. Schultz-Norton JR, Ziegler YS, Nardulli AM. ERα-associated protein networks. *Trends Endocrinol Metab*. 2011;**22**(4):124–129.
123. Filardo EJ, Thomas P. Minireview: G protein-coupled estrogen receptor-1, GPER-1: its mechanism of action and role in female reproductive cancer, renal and vascular physiology. *Endocrinology*. 2012;**153**(7):2953–2962.
124. Arnal JF, Fontaine C, Abot A, Valera MC, Laurell H, Gourdy P, Lenfant F. Lessons from the dissection of the activation functions (AF-1 and AF-2) of the estrogen receptor alpha in vivo. *Steroids*. 2013;**78**(6):576–582.
125. Hamilton KJ, Arao Y, Korach KS. Estrogen hormone physiology: reproductive findings from estrogen receptor mutant mice. *Reprod Biol*. 2014;**14**(1):3–8.
126. Magnani L, Lupien M. Chromatin and epigenetic determinants of estrogen receptor alpha (ESR1) signaling. *Mol Cell Endocrinol*. 2014;**382**(1):633–641.
127. Kuiper GG, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA. Cloning of a novel receptor expressed in rat prostate and ovary. *Proc Natl Acad Sci USA*. 1996;**93**(12):5925–5930.
128. Hewitt SC, Li L, Grimm SA, Chen Y, Liu L, Li Y, Bushel PR, Fargo D, Korach KS. Research resource: whole-genome estrogen receptor α binding in mouse uterine tissue revealed by ChIP-seq. *Mol Endocrinol*. 2012;**26**(5):887–898.
129. Carroll JS, Liu XS, Brodsky AS, Li W, Meyer CA, Szary AJ, Eeckhoute J, Shao W, Hestermann EV, Geistlinger TR, Fox EA, Silver PA, Brown M. Chromosome-wide mapping of estrogen receptor binding reveals long-range regulation requiring the forkhead protein FoxA1. *Cell*. 2005;**122**(1):33–43.
130. Walter P, Green S, Greene G, Krust A, Bornert JM, Jeltsch JM, Staub A, Jensen E, Scrace G, Waterfield M. Cloning of the human estrogen receptor cDNA. *Proc Natl Acad Sci USA*. 1985;**82**(23):7889–7893.
131. Fanning SW, Mayne CG, Dharmarajan V, Carlson KE, Martin TA, Novick SJ, Toy W, Green B, Panchamukhi S, Katzenellenbogen BS, Tajkhorshid E, Griffin PR, Shen Y, Chandarlapaty S, Katzenellenbogen JA, Greene GL. Estrogen receptor alpha somatic mutations Y537S and D538G confer breast cancer endocrine resistance by stabilizing the activating function-2 binding conformation. *eLife*. 2016;**5**:e12792.
132. Singhal H, Greene ME, Zarnke AL, Laine M, Al Abosy R, Chang YF, Dembo AG, Schoenfelt K, Vadhi R, Qiu X, Rao P, Santhamma B, Nair HB, Nickisch KJ, Long HW, Becker L, Brown M, Greene GL. Progesterone receptor isoforms, agonists and antagonists differentially reprogram estrogen signaling. *Oncotarget*. 2017;**9**(4):4282–4300.
133. Singhal H, Greene ME, Tarulli G, Zarnke AL, Bourgo RJ, Laine M, Chang YF, Ma S, Dembo AG, Raj GV, Hickey TE, Tilley WD, Greene GL. Genomic agonism and phenotypic antagonism between estrogen and progesterone receptors in breast cancer. *Sci Adv*. 2016;**2**(6):e1501924.
134. Börjesson AE, Farman HH, Movérare-Skrtic S, Engdahl C, Antal MC, Koskela A, Tuukkanen J, Carlsten H, Krust A, Chambon P, Sjögren K, Lagerquist MK, Windahl SH, Ohlsson C. SERMs have substance-specific effects on bone, and these effects are mediated via ERαAF-1 in female mice. *Am J Physiol Endocrinol Metab*. 2016;**310**(11):E912–E918.
135. Murakami G, Hojo Y, Ogiue-Ikeda M, Mukai H, Chambon P, Nakajima K, Ooishi Y, Kimoto T, Kawato S. Estrogen receptor KO mice study on rapid modulation of spines and long-term depression in the hippocampus. *Brain Res*. 2015;**1621**:133–146.
136. Dahlman-Wright K, Cavailles V, Fuqua SA, Jordan VC, Katzenellenbogen JA, Korach KS, Maggi A, Muramatsu M, Parker MG, Gustafsson JA. International Union of Pharmacology. LXIV. Estrogen receptors. *Pharmacol Rev*. 2006;**58**(4):773–781.
137. Nilsson S, Mäkelä S, Treuter E, Tujague M, Thomsen J, Andersson G, Enmark E, Pettersson K, Warner M, Gustafsson JA. Mechanisms of estrogen action. *Physiol Rev*. 2001;**81**(4):1535–1565.
138. Rajapaksa G, Thomas C, Gustafsson JA. Estrogen signaling and unfolded protein response in breast cancer. *J Steroid Biochem Mol Biol*. 2016;**163**:45–50.
139. Thomas C, Gustafsson JA. Estrogen receptor mutations and functional consequences for breast cancer. *Trends Endocrinol Metab*. 2015;**26**(9):467–476.
140. Warner M, Huang B, Gustafsson JA. Estrogen receptor β as a pharmaceutical target. *Trends Pharmacol Sci*. 2017;**38**(1):92–99.
141. Chute JP, Ross JR, McDonnell DP. Minireview: nuclear receptors, hematopoiesis, and stem cells. *Mol Endocrinol*. 2010;**24**(1):1–10.
142. Nelson ER, Wardell SE, McDonnell DP. The molecular mechanisms underlying the pharmacological actions of estrogens, SERMs and oxysterols: implications for the treatment and prevention of osteoporosis. *Bone*. 2013;**53**(1):42–50.
143. Gorski J, Wendell D, Gregg D, Chun TY. Estrogens and the genetic control of tumor growth. *Prog Clin Biol Res*. 1997;**396**:233–243.
144. Gorski J, Hou Q. Embryonic estrogen receptors: do they have a physiological function? *Environ Health Perspect*. 1995;**103**(Suppl 7):69–72.
145. Watters JJ, Chun TY, Kim YN, Bertics PJ, Gorski J. Estrogen modulation of prolactin gene expression requires an intact mitogen-activated protein kinase signal transduction pathway in cultured rat pituitary cells. *Mol Endocrinol*. 2000;**14**(11):1872–1881.
146. Hewitt SC, Winuthayanon W, Korach KS. What's new in estrogen receptor action in the female reproductive tract. *J Mol Endocrinol*. 2016;**56**(2):R55–R71.
147. Smith EP, Boyd J, Frank GR, Takahashi H, Cohen RM, Specker B, Williams TC, Lubahn DB, Korach KS. Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. *N Engl J Med*. 1994;**331**(16):1056–1061.
148. Bulun SE, Fang Z, Imir G, Gurates B, Tamura M, Yilmaz B, Langoi D, Amin S, Yang S, Deb S. Aromatase and endometriosis. *Semin Reprod Med*. 2004;**22**(1):45–50.
149. Bulun SE, Monsivais D, Kakinuma T, Furukawa Y, Bernardi L, Pavone ME, Dyson M. Molecular biology of endometriosis: from aromatase to genomic abnormalities. *Semin Reprod Med*. 2015;**33**(3):220–224.
150. Pavone ME, Bulun SE. Clinical review: the use of aromatase inhibitors for ovulation induction and superovulation. *J Clin Endocrinol Metab*. 2013;**98**(5):1838–1844.

151. Zhao H, Zhou L, Shangguan AJ, Bulun SE. Aromatase expression and regulation in breast and endometrial cancer. *J Mol Endocrinol*. 2016;**57**(1):R19–R33.
152. Frasor J, Barnett DH, Danes JM, Hess R, Parlow AF, Katzenellenbogen BS. Response-specific and ligand dose-dependent modulation of estrogen receptor (ER) α activity by ERβ in the uterus. *Endocrinology*. 2003;**144**(7):3159–3166.
153. Harrington WR, Kim SH, Funk CC, Madak-Erdogan Z, Schiff R, Katzenellenbogen JA, Katzenellenbogen BS. Estrogen dendrimer conjugates that preferentially activate extranuclear, nongenomic versus genomic pathways of estrogen action. *Mol Endocrinol*. 2006;**20**(3):491–502.
154. Montano MM, Bianco NR, Deng H, Wittmann BM, Chaplin LC, Katzenellenbogen BS. Estrogen receptor regulation of quinone reductase in breast cancer: implications for estrogen-induced breast tumor growth and therapeutic uses of tamoxifen. *Front Biosci*. 2005;**10**(1–3):1440–1461.
155. Tryfonidis K, Zardavas D, Katzenellenbogen BS, Piccart M. Endocrine treatment in breast cancer: cure, resistance and beyond. *Cancer Treat Rev*. 2016;**50**:68–81.
156. Dasgupta S, O'Malley BW. Transcriptional coregulators: emerging roles of SRC family of coactivators in disease pathology. *J Mol Endocrinol*. 2014;**53**(2):R47–R59.
157. Foulds CE, Panigrahi AK, Coarfa C, Lanz RB, O'Malley BW. Long noncoding RNAs as targets and regulators of nuclear receptors. *Curr Top Microbiol Immunol*. 2016;**394**:143–176.
158. McKenna NJ, Evans RM, O'Malley BW. Nuclear receptor signaling: a home for nuclear receptor and coregulator signaling research. *Nucl Recept Signal*. 2014;**12**:e006.
159. Power RF, Conneely OM, O'Malley BW. New insights into activation of the steroid hormone receptor superfamily. *Trends Pharmacol Sci*. 1992;**13**(8):318–323.
160. Szwarc MM, Lydon JP, O'Malley BW. Steroid receptor coactivators as therapeutic targets in the female reproductive system. *J Steroid Biochem Mol Biol*. 2015;**154**:32–38.
161. Wang L, Lonard DM, O'Malley BW. The role of steroid receptor coactivators in hormone dependent cancers and their potential as therapeutic targets. *Horm Cancer*. 2016;**7**(4):229–235.
162. Pietras RJ, Szego CM. Specific binding sites for oestrogen at the outer surfaces of isolated endometrial cells. *Nature*. 1977; **265**(5589):69–72.
163. Yang SH, Liu R, Perez EJ, Wen Y, Stevens SM Jr, Valencia T, Brun-Zinkernagel AM, Prokai L, Will Y, Dykens J, Koulen P, Simpkins JW. Mitochondrial localization of estrogen receptor β. *Proc Natl Acad Sci USA*. 2004;**101**(12):4130–4135.
164. Song RX. Membrane-initiated steroid signaling action of estrogen and breast cancer. *Semin Reprod Med*. 2007;**25**(3):187–197.
165. Song RX, Chen Y, Zhang Z, Bao Y, Yue W, Wang JP, Fan P, Santen RJ. Estrogen utilization of IGF-1-R and EGF-R to signal in breast cancer cells. *J Steroid Biochem Mol Biol*. 2010;**118**(4-5): 219–230.
166. Haas E, Bhattacharya I, Brailoiu E, Damjanović M, Brailoiu GC, Gao X, Mueller-Guerre L, Marjon NA, Gut A, Minotti R, Meyer MR, Amann K, Ammann E, Perez-Dominguez A, Genoni M, Clegg DJ, Dun NJ, Resta TC, Prossnitz ER, Barton M. Regulatory role of G protein–coupled estrogen receptor for vascular function and obesity. *Circ Res*. 2009;**104**(3):288–291.
167. Pedram A, Razandi M, Kim JK, O'Mahony F, Lee EY, Luderer U, Levin ER. Developmental phenotype of a membrane only estrogen receptor α (MOER) mouse. *J Biol Chem*. 2009;**284**(6): 3488–3495.
168. Li Y, Wang JP, Santen RJ, Kim TH, Park H, Fan P, Yue W. Estrogen stimulation of cell migration involves multiple signaling pathway interactions. *Endocrinology*. 2010;**151**(11): 5146–5156.
169. Flamini MI, Sanchez AM, Genazzani AR, Simoncini T. Estrogen regulates endometrial cell cytoskeletal remodeling and motility via focal adhesion kinase. *Fertil Steril*. 2011;**95**(2):722–726.
170. Sanchez AM, Flamini MI, Zullino S, Gopal S, Genazzani AR, Simoncini T. Estrogen receptor-α promotes endothelial cell motility through focal adhesion kinase. *Mol Hum Reprod*. 2011; **17**(4):219–226.
171. Wu Q, Chambliss K, Umetani M, Mineo C, Shaul PW. Nonnuclear estrogen receptor signaling in the endothelium. *J Biol Chem*. 2011;**286**(17):14737–14743.
172. Razandi M, Pedram A, Jordan VC, Fuqua S, Levin ER. Tamoxifen regulates cell fate through mitochondrial estrogen receptor beta in breast cancer. *Oncogene*. 2013;**32**(27):3274–3285.
173. Marjon NA, Hu C, Hathaway HJ, Prossnitz ER. G protein–coupled estrogen receptor regulates mammary tumorigenesis and metastasis. *Mol Cancer Res*. 2014;**12**(11):1644–1654.
174. Razandi M, Alton G, Pedram A, Ghonshani S, Webb P, Levin ER. Identification of a structural determinant necessary for the localization and function of estrogen receptor α at the plasma membrane. *Mol Cell Biol*. 2003;**23**(5):1633–1646.
175. Song RX, Barnes CJ, Zhang Z, Bao Y, Kumar R, Santen RJ. The role of Shc and insulin-like growth factor 1 receptor in mediating the translocation of estrogen receptor α to the plasma membrane. *Proc Natl Acad Sci USA*. 2004;**101**(7):2076–2081.
176. Song RX, Zhang Z, Chen Y, Bao Y, Santen RJ. Estrogen signaling via a linear pathway involving insulin-like growth factor I receptor, matrix metalloproteinases, and epidermal growth factor receptor to activate mitogen-activated protein kinase in MCF-7 breast cancer cells. *Endocrinology*. 2007;**148**(8):4091–4101.
177. Madak-Erdogan Z, Lupien M, Stossi F, Brown M, Katzenellenbogen BS. Genomic collaboration of estrogen receptor α and extracellular signal-regulated kinase 2 in regulating gene and proliferation programs. *Mol Cell Biol*. 2011;**31**(1):226–236.
178. Madak-Erdogan Z, Charn TH, Jiang Y, Liu ET, Katzenellenbogen JA, Katzenellenbogen BS. Integrative genomics of gene and metabolic regulation by estrogen receptors α and β, and their coregulators. *Mol Syst Biol*. 2017;**13**(4):929.
179. Zheng FF, Wu RC, Smith CL, O'Malley BW. Rapid estrogen-induced phosphorylation of the SRC-3 coactivator occurs in an extranuclear complex containing estrogen receptor. *Mol Cell Biol*. 2005;**25**(18):8273–8284.
180. Maffei L, Murata Y, Rochira V, Tubert G, Aranda C, Vazquez M, Clyne CD, Davis S, Simpson ER, Carani C. Dysmetabolic syndrome in a man with a novel mutation of the aromatase gene: effects of testosterone, alendronate, and estradiol treatment. *J Clin Endocrinol Metab*. 2004;**89**(1):61–70.
181. Robertson KM, O'Donnell L, Jones ME, Meachem SJ, Boon WC, Fisher CR, Graves KH, McLachlan RI, Simpson ER. Impairment of spermatogenesis in mice lacking a functional aromatase (*cyp 19*) gene. *Proc Natl Acad Sci USA*. 1999;**96**(14): 7986–7991.
182. Jones ME, Boon WC, Proietto J, Simpson ER. Of mice and men: the evolving phenotype of aromatase deficiency. *Trends Endocrinol Metab*. 2006;**17**(2):55–64.
183. Simpson ER. Models of aromatase insufficiency. *Semin Reprod Med*. 2004;**22**(1):25–30.
184. Kirma N, Gill K, Mandava U, Tekmal RR. Overexpression of aromatase leads to hyperplasia and changes in the expression of genes involved in apoptosis, cell cycle, growth, and tumor suppressor functions in the mammary glands of transgenic mice. *Cancer Res*. 2001;**61**(5):1910–1918.
185. Mandava U, Kirma N, Tekmal RR. Aromatase overexpression transgenic mice model: cell type specific expression and use of letrozole to abrogate mammary hyperplasia without affecting normal physiology. *J Steroid Biochem Mol Biol*. 2001;**79**(1–5): 27–34.
186. Li X, Strauss L, Mäkelä S, Streng T, Huhtaniemi I, Santti R, Poutanen M. Multiple structural and functional abnormalities in the p450 aromatase expressing transgenic male mice are ameliorated by a p450 aromatase inhibitor. *Am J Pathol*. 2004; **164**(3):1039–1048.

187. Finkelstein JS, Yu EW, Burnett-Bowie SA. Gonadal steroids and body composition, strength, and sexual function in men. *N Engl J Med*. 2013;**369**(25):2457.
188. Siiteri PK, MacDonald PC. Role of extraglandular estrogen in human endocrinology. In: Greep RO, Astwood E, eds. Handbook of Physiology: Endocrinology. Vol 2, Pt 1. Washington, DC, American Physiological Society; 1973;615–629.
189. Huggins C. Endocrine substances in the treatment of cancers. *J Am Med Assoc*. 1949;**141**(11):750–754.
190. Talalay P, Takano GM, Huggins C. Studies on the Walker tumor. II. Effects of adrenalectomy and hypophysectomy on tumor growth in tube-fed rats. *Cancer Res*. 1952;**12**(11):838–843.
191. Herbst AL, Griffiths CT, Kistner RW. Clomiphene citrate (NSC-35770) in disseminated mammary carcinoma. *Cancer Chemother Rep*. 1964;**43**:39–41.
192. Cole MP, Jones CT, Todd ID. A new anti-oestrogenic agent in late breast cancer. An early clinical appraisal of ICI46474. *Br J Cancer*. 1971;**25**(2):270–275.
193. Ward HW. Anti-oestrogen therapy for breast cancer: a trial of tamoxifen at two dose levels. *BMJ*. 1973;**1**(5844):13–14.
194. Manni A, Santen R, Harvey H, Lipton A, Max D. Treatment of breast cancer with gonadotropin-releasing hormone. *Endocr Rev*. 1986;**7**(1):89–94.
195. Jordan VC, Koerner S. Tamoxifen (ICI 46,474) and the human carcinoma 8S oestrogen receptor. *Eur J Cancer*. 1975;**11**(3): 205–206.
196. Cash R, Brough AJ, Cohen MN, Satoh PS. Aminoglutethimide (Elipten-Ciba) as an inhibitor of adrenal steroidogenesis: mechanism of action and therapeutic trial. *J Clin Endocrinol Metab*. 1967;**27**(9):1239–1248.
197. Horsley JS III, Newsome HH, Brown PW, Neifeld JP, Terz JJ, Lawrence W Jr. Medical adrenalectomy in patients with advanced breast cancer. *Cancer*. 1982;**49**(6):1145–1149.
198. Newsome HH, Brown PW, Terz JJ, Lawrence W Jr. Medical and surgical adrenalectomy in patients with advanced breast carcinoma. *Cancer*. 1977;**39**(2):542–546.
199. Newsome HH Jr, Brown PW, Terz JJ, Lawrence W Jr. Medical adrenalectomy and plasma steroids in advanced breast carcinoma. *Surgery*. 1978;**83**(1):83–89.
200. Santen RJ, Santner S, Davis B, Veldhuis J, Samojlik E, Ruby E. Aminoglutethimide inhibits extraglandular estrogen production in postmenopausal women with breast carcinoma. *J Clin Endocrinol Metab*. 1978;**47**(6):1257–1265.
201. Gale KE. Treatment of advanced breast cancer with aminoglutethimide: a 14-year experience. *Cancer Res*. 1982;**42**(8 Suppl):3389s–3396s.
202. Brodie AM, Schwarzel WC, Shaikh AA, Brodie HJ. The effect of an aromatase inhibitor, 4-hydroxy-4-androstene-3,17-dione, on estrogen-dependent processes in reproduction and breast cancer. *Endocrinology*. 1977;**100**(6):1684–1695.
203. Brodie AM, Coombes RC, Dowsett M. Aromatase inhibitors: basic and clinical studies. *J Steroid Biochem*. 1987;**27**(4-6): 899–903.
204. Powles TJ, Davey JB, McKinna A. A feasibility trial of tamoxifen chemoprevention of breast cancer in Great Britain. *Cancer Invest*. 1988;**6**(5):621–624.
205. Key T, Appleby P, Barnes I, Reeves G; Endogenous Hormones and Breast Cancer Collaborative Group. Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. *J Natl Cancer Inst*. 2002;**94**(8):606–616.
206. Kaaks R, Rinaldi S, Key TJ, Berrino F, Peeters PH, Biessy C, Dossus L, Lukanova A, Bingham S, Khaw KT, Allen NE, Bueno-de-Mesquita HB, van Gils CH, Grobbee D, Boeing H, Lahmann PH, Nagel G, Chang-Claude J, Clavel-Chapelon F, Fournier A, Thiébaut A, González CA, Quirós JR, Tormo MJ, Ardanaz E, Amiano P, Krogh V, Palli D, Panico S, Tumino R, Vineis P, Trichopoulou A, Kalapothaki V, Trichopoulos D, Ferrari P, Norat T, Saracci R, Riboli E. Postmenopausal serum androgens, oestrogens and breast cancer risk: the European prospective investigation into cancer and nutrition. *Endocr Relat Cancer*. 2005; **12**(4):1071–1082.
207. Knight WA, Livingston RB, Gregory EJ, McGuire WL. Estrogen receptor as an independent prognostic factor for early recurrence in breast cancer. *Cancer Res*. 1977;**37**(12):4669–4671.
208. McGuire WL, Horwitz KB, Zava DT, Garola RE, Chamness GC. Hormones in breast cancer: update 1978. *Metabolism*. 1978; **27**(4):487–501.
209. McGuire WL, Horwitz KB, De La Garza M. Selecting endocrine therapy in breast cancer. *Curr Top Mol Endocrinol*. 1976;**4**: 28–35.
210. Fuqua AW, Wiltschke C, Zhang QX, Borg A, Castles CG, Friedrichs WE, Hopp T, Hilsenbeck S, Mohshin S, O'Connell PO, Allred DC. A hypersensitive estrogen receptor-α mutation in premalignant breast lesions. *Cancer Res*. 2000;**60**(15):4026–4029.
211. Fuqua SA. The role of estrogen receptors in breast cancer metastasis. *J Mammary Gland Biol Neoplasia*. 2001;**6**(4):407–417.
212. Fuqua SA, Guowei GU, Rechoum Y. Estrogen receptor (ER) α mutations in breast cancer: hidden in plain sight. *Breast Cancer Res Treat*. 2014;**144**(1):11–19.
213. Patel HK, Bihani T. Selective estrogen receptor modulators (SERMs) and selective estrogen receptor degraders (SERDs) in cancer treatment. *Pharmacol Ther*. 2018;**186**:1–24.
214. Boér K. Fulvestrant in advanced breast cancer: evidence to date and place in therapy [published correction appears in *Ther Adv Med Oncol*. 2017;**9**(11):725]. *Ther Adv Med Oncol*. 2017;**9**(7): 465–479.
215. Coen P, Kulin H, Ballantine T, Zaino R, Frauenhoffer E, Boal D, Inkster S, Brodie A, Santen R. An aromatase-producing sex-cord tumor resulting in prepubertal gynecomastia. *N Engl J Med*. 1991;**324**(5):317–322.
216. Ham S, Meachem SJ, Choong CS, Charles AK, Baynam GS, Jones TW, Samarajeewa NU, Simpson ER, Brown KA. Overexpression of aromatase associated with loss of heterozygosity of the *STK11* gene accounts for prepubertal gynecomastia in boys with Peutz-Jeghers syndrome. *J Clin Endocrinol Metab*. 2013;**98**(12): E1979–E1987.
217. Agarwal VR, Takayama K, Van Wyk JJ, Sasano H, Simpson ER, Bulun SE. Molecular basis of severe gynecomastia associated with aromatase expression in a fibrolamellar hepatocellular carcinoma. *J Clin Endocrinol Metab*. 1998;**83**(5):1797–1800.
218. Demura M, Martin RM, Shozu M, Sebastian S, Takayama K, Hsu WT, Schultz RA, Neely K, Bryant M, Mendonca BB, Hanaki K, Kanzaki S, Rhoads DB, Misra M, Bulun SE. Regional rearrangements in chromosome 15q21 cause formation of cryptic promoters for the *CYP19* (aromatase) gene. *Hum Mol Genet*. 2007;**16**(21):2529–2541.
219. Shozu M, Sebastian S, Takayama K, Hsu WT, Schultz RA, Neely K, Bryant M, Bulun SE. Estrogen excess associated with novel gain-of-function mutations affecting the aromatase gene. *N Engl J Med*. 2003;**348**(19):1855–1865.
220. Nagasaki K, Horikawa R, Fujisawa K, Hata I, Shigematsu Y, Tanaka T. A case of female pseudohermaphroditism caused by aromatase deficiency. *Clin Pediatr Endocrinol*. 2004;**13**(1): 59–64.
221. Lin L, Ercan O, Raza J, Burren CP, Creighton SM, Auchus RJ, Dattani MT, Achermann JC. Variable phenotypes associated with aromatase (*CYP19*) insufficiency in humans. *J Clin Endocrinol Metab*. 2007;**92**(3):982–990.
222. Marino R, Perez Garrido N, Costanzo M, Guercio G, Juanes M, Rocco C, Ramirez P, Warman DM, Ciaccio M, Pena G, Feyling JG, Miras M, Rivarola MA, Belgorosky A, Saraco N. Five new cases of 46,XX aromatase deficiency: clinical follow-up from birth to puberty, a novel mutation, and a founder effect. *J Clin Endocrinol Metab*. 2015;**100**(2):E301–E307.
223. Greenblatt RB. Estrogen therapy for postmenopausal females. *N Engl J Med*. 1965;**272**(6):305–308.

224. Wilson RA. The obsolete menopause. *Conn Med*. 1963;**27**: 735–736.
225. Wilson RA, Wilson TA. The fate of the nontreated postmenopausal woman: a plea for the maintenance of adequate estrogen from puberty to the grave. *J Am Geriatr Soc*. 1963;**11**(4):347–362.
226. Wilson RA, Brevetti RE, Wilson TA. Specific procedures for the elimination of the menopause. *West J Surg, Obstet Gynecol*. 1963;**71**:110–121.
227. Wilson RA. Feminine Forever. New York, NY: Evans; 1968.
228. Rogers J. The menopause. *N Engl J Med*. 1956;**254**(16):750–756.
229. Rogers J. The menopause. *N Engl J Med*. 1956;**254**(15):697–704.
230. Santen RJ, Allred DC, Ardoin SP, Archer DF, Boyd N, Braunstein GD, Burger HG, Colditz GA, Davis SR, Gambacciani M, Gower BA, Henderson VW, Jarjour WN, Karas RH, Kleerekoper M, Lobo RA, Manson JE, Marsden J, Martin KA, Martin L, Pinkerton JV, Rubinow DR, Teede H, Thiboutot DM, Utian WH; Endocrine Society. Postmenopausal hormone therapy: an Endocrine Society scientific statement. *J Clin Endocrinol Metab*. 2010; **95**(7 Suppl 1):s1–s66.
231. Stuenkel CA, Davis SR, Gompel A, Lumsden MA, Murad MH, Pinkerton JV, Santen RJ. Treatment of symptoms of the menopause: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2015;**100**(11):3975–4011.
232. Santen RJ, Stuenkel CA, Davis SR, Pinkerton JV, Gompel A, Lumsden MA. Managing menopausal symptoms and associated clinical issues in breast cancer survivors. *J Clin Endocrinol Metab*. 2017;**102**(10):3647–3661.
233. Manson JE, Chlebowski RT, Stefanick ML, Aragaki AK, Rossouw JE, Prentice RL, Anderson G, Howard BV, Thomson CA, LaCroix AZ, Wactawski-Wende J, Jackson RD, Limacher M, Margolis KL, Wassertheil-Smoller S, Beresford SA, Cauley JA, Eaton CB, Gass M, Hsia J, Johnson KC, Kooperberg C, Kuller LH, Lewis CE, Liu S, Martin LW, Ockene JK, O'Sullivan MJ, Powell LH, Simon MS, Van Horn L, Vitolins MZ, Wallace RB. Menopausal hormone therapy and health outcomes during the intervention and extended poststopping phases of the Women's Health Initiative randomized trials. *JAMA*. 2013;**310**(13): 1353–1368.
234. Anderson GL, Chlebowski RT, Aragaki AK, Kuller LH, Manson JE, Gass M, Bluhm E, Connelly S, Hubbell FA, Lane D, Martin L, Ockene J, Rohan T, Schenken R, Wactawski-Wende J. Conjugated equine oestrogen and breast cancer incidence and mortality in postmenopausal women with hysterectomy: extended follow-up of the Women's Health Initiative randomised placebo-controlled trial. *Lancet Oncol*. 2012;**13**(5):476–486.
235. Santen RJ, Song Y, Yue W, Wang JP, Heitjan DF. Effects of menopausal hormonal therapy on occult breast tumors. *J Steroid Biochem Mol Biol*. 2013;**137**:150–156.
236. Santen RJ, Yue W, Heitjan DF. Occult breast tumor reservoir: biological properties and clinical significance. *Horm Cancer*. 2013;**4**(4):195–207.
237. Santen RJ, Yue W, Heitjan DF. Modeling of the growth kinetics of occult breast tumors: role in interpretation of studies of prevention and menopausal hormone therapy. *Cancer Epidemiol Biomarkers Prev*. 2012;**21**(7):1038–1048.
238. Song RX, Mor G, Naftolin F, McPherson RA, Song J, Zhang Z, Yue W, Wang J, Santen RJ. Effect of long-term estrogen deprivation on apoptotic responses of breast cancer cells to 17β-estradiol. *J Natl Cancer Inst*. 2001;**93**(22):1714–1723.
239. Yue W, Wang J, Atkins KA, Bottalico L, Mesaros C, Blair IA, Santen RJ. Effect of a tissue selective estrogen complex on breast cancer: role of unique properties of conjugated equine estrogen. *Int J Cancer*. 2018;**143**(5):1259–1268.
240. Bender CM, Merriman JD, Gentry AL, Ahrendt GM, Berga SL, Brufsky AM, Casillo FE, Dailey MM, Erickson KI, Kratofil FM, McAuliffe PF, Rosenzweig MQ, Ryan CM, Sereika SM. Patterns of change in cognitive function with anastrozole therapy. *Cancer*. 2015;**121**(15):2627–2636.
241. Mendelsohn ME, Karas RH. HRT and the young at heart. *N Engl J Med*. 2007;**356**(25):2639–2641.
242. Shozu M, Simpson ER. Aromatase expression of human osteoblast-like cells. *Mol Cell Endocrinol*. 1998;**139**(1–2):117–129.
243. Lønning PE, Haynes BP, Straume AH, Dunbier A, Helle H, Knappskog S, Dowsett M. Exploring breast cancer estrogen disposition: the basis for endocrine manipulation. *Clin Cancer Res*. 2011;**17**(15):4948–4958.
244. Simpson ER, Brown KA. Minireview: obesity and breast cancer: a tale of inflammation and dysregulated metabolism. *Mol Endocrinol*. 2013;**27**(5):715–725.
245. Morselli E, Santos RS, Criollo A, Nelson MD, Palmer BF, Clegg DJ. The effects of oestrogens and their receptors on cardiometabolic health. *Nat Rev Endocrinol*. 2017;**13**(6):352–364.
246. Qiu J, Bosch MA, Meza C, Navarro UV, Nestor CC, Wagner EJ, Rønnekleiv OK, Kelly MJ. Estradiol protects proopiomelanocortin neurons against insulin resistance. *Endocrinology*. 2018; **159**(2):647–664.
247. Wang HH, Zhou CL, Lv M, Yang Q, Li JX, Hou M, Lin J, Liu XM, Wu YT, Sheng JZ, Huang HF. Prenatal high estradiol exposure induces sex-specific and dietarily reversible insulin resistance through decreased hypothalamic INSR. *Endocrinology*. 2018;**159**(1):465–476.
248. Bedenbaugh MN, D'Oliveira M, Cardoso RC, Hileman SM, Williams GL, Amstalden M. Pubertal escape from estradiol negative feedback in ewe lambs is not accounted for by decreased *ESR1* mRNA or protein in kisspeptin neurons. *Endocrinology*. 2018;**159**(1):426–438.
249. Phumsatitpong C, Moenter SM. Estradiol-dependent stimulation and suppression of gonadotropin-releasing hormone neuron firing activity by corticotropin-releasing hormone in female mice. *Endocrinology*. 2018;**159**(1):414–425.
250. Weems P, Smith J, Clarke IJ, Coolen LM, Goodman RL, Lehman MN. Effects of season and estradiol on KNDy neuron peptides, colocalization with D2 dopamine receptors, and dopaminergic inputs in the ewe. *Endocrinology*. 2017;**158**(4):831–841.
251. Royston SE, Yasui N, Kondilis AG, Lord SV, Katzenellenbogen JA, Mahoney MM. ESR1 and ESR2 differentially regulate daily and circadian activity rhythms in female mice. *Endocrinology*. 2014;**155**(7):2613–2623.
252. Furuta M, Numakawa T, Chiba S, Ninomiya M, Kajiyama Y, Adachi N, Akema T, Kunugi H. Estrogen, predominantly via estrogen receptor α, attenuates postpartum-induced anxiety- and depression-like behaviors in female rats. *Endocrinology*. 2013; **154**(10):3807–3816.
253. Miller NR, Jover T, Cohen HW, Zukin RS, Etgen AM. Estrogen can act via estrogen receptor α and β to protect hippocampal neurons against global ischemia-induced cell death. *Endocrinology*. 2005;**146**(7):3070–3079.
254. Lund TD, Rovis T, Chung WC, Handa RJ. Novel actions of estrogen receptor-β on anxiety-related behaviors. *Endocrinology*. 2005;**146**(2):797–807.
255. Vanacker C, Moya MR, DeFazio RA, Johnson ML, Moenter SM. Long-term recordings of arcuate nucleus kisspeptin neurons reveal patterned activity that is modulated by gonadal steroids in male mice. *Endocrinology*. 2017;**158**(10):3553–3564.
256. Minabe S, Ieda N, Watanabe Y, Inoue N, Uenoyama Y, Maeda KI, Tsukamura H. Long-term neonatal estrogen exposure causes irreversible inhibition of LH pulses by suppressing arcuate kisspeptin expression via estrogen receptors α and β in female rodents. *Endocrinology*. 2017;**158**(9):2918–2929.
257. Hu P, Liu J, Yasrebi A, Gotthardt JD, Bello NT, Pang ZP, Roepke TA. Gq Protein-coupled membrane-initiated estrogen signaling rapidly excites corticotropin-releasing hormone neurons in the hypothalamic paraventricular nucleus in female mice. *Endocrinology*. 2016;**157**(9):3604–3620.
258. Farman HH, Windahl SH, Westberg L, Isaksson H, Egecioglu E, Schele E, Ryberg H, Jansson JO, Tuukkanen J, Koskela A, Xie SK,

Hahner L, Zehr J, Clegg DJ, Lagerquist MK, Ohlsson C. Female mice lacking estrogen receptor-α in hypothalamic proopiomelanocortin (POMC) neurons display enhanced estrogenic response on cortical bone mass. *Endocrinology*. 2016;**157**(8):3242–3252.
259. Grissom EM, Daniel JM. Evidence for ligand-independent activation of hippocampal estrogen receptor-α by IGF-1 in hippocampus of ovariectomized rats. *Endocrinology*. 2016;**157**(8):3149–3156.
260. Ferasyi TR, Barrett PH, Blache D, Martin GB. Modeling the male reproductive endocrine axis: potential role for a delay mechanism in the inhibitory action of gonadal steroids on GnRH pulse frequency. *Endocrinology*. 2016;**157**(5):2080–2092.
261. Stephens SB, Chahal N, Munaganuru N, Parra RA, Kauffman AS. Estrogen stimulation of *Kiss1* expression in the medial amygdala involves estrogen receptor-α but not estrogen receptor-β. *Endocrinology*. 2016;**157**(10):4021–4031.
262. Dubois SL, Wolfe A, Radovick S, Boehm U, Levine JE. Estradiol restrains prepubertal gonadotropin secretion in female mice via activation of ERα in kisspeptin neurons. *Endocrinology*. 2016;**157**(4):1546–1554.
263. Ruka KA, Burger LL, Moenter SM. both estrogen and androgen modify the response to activation of neurokinin-3 and κ-opioid receptors in arcuate kisspeptin neurons from male mice. *Endocrinology*. 2016;**157**(2):752–763.
264. Shults CL, Pinceti E, Rao YS, Pak TR. Aging and loss of circulating 17β-estradiol alters the alternative splicing of ERβ in the female rat brain. *Endocrinology*. 2015;**156**(11):4187–4199.
265. Sárvári M, Kalló I, Hrabovszky E, Solymosi N, Rodolosse A, Vastagh C, Auer H, Liposits Z. Hippocampal gene expression is highly responsive to estradiol replacement in middle-aged female rats. *Endocrinology*. 2015;**156**(7):2632–2645.
266. Kenealy BP, Keen KL, Garcia JP, Richter DJ, Terasawa E. Prolonged infusion of estradiol benzoate into the stalk median eminence stimulates release of GnRH and kisspeptin in ovariectomized female rhesus macaques. *Endocrinology*. 2015;**156**(5):1804–1814.
267. Mittelman-Smith MA, Wong AM, Kathiresan AS, Micevych PE. Classical and membrane-initiated estrogen signaling in an in vitro model of anterior hypothalamic kisspeptin neurons. *Endocrinology*. 2015;**156**(6):2162–2173.
268. Dubois SL, Acosta-Martínez M, DeJoseph MR, Wolfe A, Radovick S, Boehm U, Urban JH, Levine JE. Positive, but not negative feedback actions of estradiol in adult female mice require estrogen receptor α in kisspeptin neurons. *Endocrinology*. 2015;**156**(3):1111–1120.
269. Barron AM, Brown MA, Morgan TE, Pike CJ. Impact of continuous versus discontinuous progesterone on estradiol regulation of neuron viability and sprouting after entorhinal cortex lesion in female rats. *Endocrinology*. 2015;**156**(3):1091–1099.
270. Milne MR, Haug CA, Ábrahám IM, Kwakowsky A. Estradiol modulation of neurotrophin receptor expression in female mouse basal forebrain cholinergic neurons in vivo. *Endocrinology*. 2015;**156**(2):613–626.
271. Almey A, Cannell E, Bertram K, Filardo E, Milner TA, Brake WG. Medial prefrontal cortical estradiol rapidly alters memory system bias in female rats: ultrastructural analysis reveals membrane-associated estrogen receptors as potential mediators. *Endocrinology*. 2014;**155**(11):4422–4432.
272. Yeo SH, Herbison AE. Estrogen-negative feedback and estrous cyclicity are critically dependent upon estrogen receptor-α expression in the arcuate nucleus of adult female mice. *Endocrinology*. 2014;**155**(8):2986–2995.
273. Chen C, Kuo J, Wong A, Micevych P. Estradiol modulates translocator protein (TSPO) and steroid acute regulatory protein (StAR) via protein kinase A (PKA) signaling in hypothalamic astrocytes. *Endocrinology*. 2014;**155**(8):2976–2985.
274. Arimoto JM, Wong A, Rozovsky I, Lin SW, Morgan TE, Finch CE. Age increase of estrogen receptor-α (ERα) in cortical astrocytes impairs neurotrophic support in male and female rats. *Endocrinology*. 2013;**154**(6):2101–2113.
275. MacLusky NJ. Understanding the direct synaptic effects of estradiol. *Endocrinology*. 2013;**154**(2):581–583.
276. Witty CF, Gardella LP, Perez MC, Daniel JM. Short-term estradiol administration in aging ovariectomized rats provides lasting benefits for memory and the hippocampus: a role for insulin-like growth factor-I. *Endocrinology*. 2013;**154**(2):842–852.
277. Clarke SD, Clarke IJ, Rao A, Evans RG, Henry BA. Differential effects of acute and chronic estrogen treatment on thermogenic and metabolic pathways in ovariectomized sheep. *Endocrinology*. 2013;**154**(1):184–192.
278. Santollo J, Marshall A, Daniels D. Activation of membrane-associated estrogen receptors decreases food and water intake in ovariectomized rats. *Endocrinology*. 2013;**154**(1):320–329.
279. Spampinato SF, Merlo S, Molinaro G, Battaglia G, Bruno V, Nicoletti F, Sortino MA. Dual effect of 17β-estradiol on NMDA-induced neuronal death: involvement of metabotropic glutamate receptor 1. *Endocrinology*. 2012;**153**(12):5940–5948.
280. Yang JA, Stires H, Belden WJ, Roepke TA. The arcuate estrogen-regulated transcriptome: estrogen response element-dependent and -independent signaling of ERα in female mice. *Endocrinology*. 2017;**158**(3):612–626.
281. Vinel A, Hay E, Valera MC, Buscato M, Adlanmerini M, Guillaume M, Cohen-Solal M, Ohlsson C, Lenfant F, Arnal JF, Fontaine C. Role of ERα in the effect of estradiol on cancellous and cortical femoral bone in growing female mice. *Endocrinology*. 2016;**157**(6):2533–2544.
282. Zhang Z, Liu J, Veldhuis-Vlug AG, Su Y, Foppen E, van der Eerden BC, Koedam M, Bravenboer N, Kalsbeek A, Boelen A, Fliers E, Bisschop PH. Effects of chronic estrogen administration in the ventromedial nucleus of the hypothalamus (VMH) on fat and bone metabolism in ovariectomized rats. *Endocrinology*. 2016;**157**(12):4930–4942.
283. Connelly KJ, Larson EA, Marks DL, Klein RF. Neonatal estrogen exposure results in biphasic age-dependent effects on the skeletal development of male mice. *Endocrinology*. 2015;**156**(1):193–202.
284. Pepe GJ, Maniu A, Aberdeen G, Lynch TJ, Albrecht ED. Estrogen regulation of fetal adrenal cortical zone-specific development in the nonhuman primate impacts adrenal production of androgen and cortisol and response to ACTH in females in adulthood. *Endocrinology*. 2016;**157**(5):1905–1913.
285. Babischkin JS, Aberdeen GW, Pepe GJ, Albrecht ED. Estrogen suppresses interaction of melanocortin 2 receptor and its accessory protein in the primate fetal adrenal cortex. *Endocrinology*. 2016;**157**(12):4588–4601.
286. Dumitrescu A, Aberdeen GW, Pepe GJ, Albrecht ED. Placental estrogen suppresses cyclin D1 expression in the nonhuman primate fetal adrenal cortex. *Endocrinology*. 2014;**155**(12):4774–4784.
287. Caroccia B, Seccia TM, Campos AG, Gioco F, Kuppusamy M, Ceolotto G, Guerzoni E, Simonato F, Mareso S, Lenzini L, Fassina A, Rossi GP. GPER-1 and estrogen receptor-β ligands modulate aldosterone synthesis. *Endocrinology*. 2014;**155**(11):4296–4304.
288. Qi X, Salem M, Zhou W, Sato-Shimizu M, Ye G, Smitz J, Peng C. Neurokinin B exerts direct effects on the ovary to stimulate estradiol production. *Endocrinology*. 2016;**157**(9):3355–3365.
289. Toda K, Hayashi Y, Ono M, Saibara T. Characterization of ovarian responses to equine chorionic gonadotropin of aromatase-deficient mice with or without 17β-estradiol supplementation. *Endocrinology*. 2016;**157**(5):2093–2103.
290. Van Kempen TA, Gorecka J, Gonzalez AD, Soeda F, Milner TA, Waters EM. Characterization of neural estrogen signaling and neurotrophic changes in the accelerated ovarian failure mouse model of menopause. *Endocrinology*. 2014;**155**(9):3610–3623.
291. Moore AM, Prescott M, Campbell RE. Estradiol negative and positive feedback in a prenatal androgen-induced mouse model of

polycystic ovarian syndrome. *Endocrinology*. 2013;**154**(2): 796–806.
292. Payrits M, Sághy É, Cseko K, Pohóczky K, Bölcskei K, Ernszt D, Barabás K, Szolcsányi J, Ábrahám IM, Helyes Z, Szoke É. Estradiol sensitizes the transient receptor potential vanilloid 1 receptor in pain responses. *Endocrinology*. 2017;**158**(10):3249–3258.
293. Yamagata K, Sugimura M, Yoshida M, Sekine S, Kawano A, Oyamaguchi A, Maegawa H, Niwa H. Estrogens exacerbate nociceptive pain via up-regulation of TRPV1 and ANO1 in trigeminal primary neurons of female rats. *Endocrinology*. 2016; **157**(11):4309–4317.
294. Ghorbanpoor S, Garcia-Segura LM, Haeri-Rohani A, Khodagholi F, Jorjani M. Aromatase inhibition exacerbates pain and reactive gliosis in the dorsal horn of the spinal cord of female rats caused by spinothalamic tract injury. *Endocrinology*. 2014; **155**(11):4341–4355.
295. Greaves E, Collins F, Esnal-Zufiaurre A, Giakoumelou S, Horne AW, Saunders PT. Estrogen receptor (ER) agonists differentially regulate neuroangiogenesis in peritoneal endometriosis via the repellent factor SLIT3. *Endocrinology*. 2014;**155**(10):4015–4026.
296. Zhao Y, Park S, Bagchi MK, Taylor RN, Katzenellenbogen BS. The coregulator, repressor of estrogen receptor activity (REA), is a crucial regulator of the timing and magnitude of uterine decidualization. *Endocrinology*. 2013;**154**(3):1349–1360.
297. Whirledge S, Cidlowski JA. Estradiol antagonism of glucocorticoid-induced GILZ expression in human uterine epithelial cells and murine uterus. *Endocrinology*. 2013;**154**(1): 499–510.
298. Inada A, Fujii NL, Inada O, Higaki Y, Furuichi Y, Nabeshima YI. Effects of 17β-Estradiol and androgen on glucose metabolism in skeletal muscle. *Endocrinology*. 2016;**157**(12):4691–4705.
299. Litwak SA, Wilson JL, Chen W, Garcia-Rudaz C, Khaksari M, Cowley MA, Enriori PJ. Estradiol prevents fat accumulation and overcomes leptin resistance in female high-fat diet mice. *Endocrinology*. 2014;**155**(11):4447–4460.
300. Garrido P, Morán J, Alonso A, González S, González C. 17β-Estradiol activates glucose uptake via GLUT4 translocation and PI3K/Akt signaling pathway in MCF-7 cells. *Endocrinology*. 2013;**154**(6):1979–1989.
301. Jelenik T, Roden M. How estrogens prevent from lipid-induced insulin resistance. *Endocrinology*. 2013;**154**(3):989–992.
302. Camporez JP, Jornayvaz FR, Lee HY, Kanda S, Guigni BA, Kahn M, Samuel VT, Carvalho CR, Petersen KF, Jurczak MJ, Shulman GI. Cellular mechanism by which estradiol protects female ovariectomized mice from high-fat diet-induced hepatic and muscle insulin resistance. *Endocrinology*. 2013;**154**(3):1021–1028.
303. Nanjappa MK, Hess RA, Medrano TI, Locker SH, Levin ER, Cooke PS. Membrane-localized estrogen receptor 1 is required for normal male reproductive development and function in mice. *Endocrinology*. 2016;**157**(7):2909–2919.