

## REVIEW ARTICLE

## CURRENT CONCEPTS

## The Biology of Human Sex Differences

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EVERYONE IN MEDICINE AND RELATED FIELDS UNDERSTANDS THAT there are marked sex-based differences in the epidemiology, clinical manifestations, course, and therapy of disease.<sup>1</sup> Although very few of these differences are understood in molecular or cellular terms, the explanations must derive from the fundamental biologic differences between the sexes. This article reviews the current understanding of hormonal and genetic differences between the sexes.

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## FERTILITY IN MEN AND WOMEN

Fertility differs considerably between men and women (Table 1). Men are fertile from puberty through at least the 9th decade of life, and some men are fertile into the 10th decade. Although there is some decrease in fecundity, spermatogenesis is active throughout these years. The process preserves germ cells, because the first step in the process of spermatogenesis is actually a mitotic division in which one of the daughter cells is tucked back into the basal epithelium and the other progresses to meiosis and the production of haploid gametes.

Female fertility differs radically from male fertility. Women are fertile only for the 12 hours after the monthly discharge of an egg from the dominant follicle in the ovary. An elaborate endocrinologic sequence underlies this rhythmic fertility.<sup>2</sup> The first source of this cycle is the arcuate nucleus of the hypothalamus; its few cells discharge a decapeptide gonadotropin-releasing hormone in pulses that vary in amplitude and frequency depending on the phase of the menstrual cycle. At the outset of each menstrual cycle, the frequency and amplitude of pulses of follicle-stimulating hormone increase, leading to the ripening of 10 to 12 ovarian follicles. One of these becomes the dominant follicle and the principal source of an extraordinary rise in estrogen during each menstrual cycle. Toward the middle of the menstrual cycle, an acceleration and amplification of pulses of gonadotropin-releasing hormone lead to a surge of luteinizing hormone and follicle-stimulating hormone that triggers ovulation. In the two weeks after ovulation, both estrogen and progesterone are actively secreted by the corpus luteum, the remnant of the dominant follicle after the egg is released. The second source of the rhythm of female fertility is the two-week life cycle of this corpus luteum. If fertilization does not occur, the corpus luteum degenerates, estrogen and progesterone levels fall, and a menstrual period ensues.

The other main difference between male and female fertility is the rapacious apoptosis that occurs in ovarian follicles.<sup>3</sup> Of the 3 million to 4 million follicles present at the time of fetal ovarian differentiation, only a million or so persist at birth; 400,000 to 500,000 at menarche; and none beyond the sixth decade. This process of ovarian attrition, with its accompanying fall in estrogen and progesterone levels, leads to the menopause and its enormous consequences for women's health and risks of disease.

**Table 1. Differences between the Sexes in the Reproductive Strategy.**

Variable	Female	Male
Timing of fertility	12 hr/mo	Constant
Germ cells	Fixed supply plus disappearance by apoptosis	Mitotic replacement
Endocrine pattern	Cyclic	Acylic
Control	Hypothalamus Corpus luteum	Hypothalamus

The mother's role in directly nurturing the fetus during gestation is a special circumstance that may have a very important meaning for one area of impressive disparity between the sexes — autoimmune disorders. Graves' disease, systemic lupus erythematosus, scleroderma, and multiple sclerosis share few clinical features, but these diseases affect women 3 to 10 times as often as men. The time-honored assumption was that gonadal steroids had a major role in this disparity, but the discovery that fetal cells can persist in the mother's circulation for decades after delivery has led to the development of a more compelling hypothesis<sup>4</sup> — the presence of such foreign cells provides antigenic exposure that may be the source of heightened immune reactions in women.

#### HORMONAL DIFFERENCES BETWEEN THE SEXES

The differences between the sexes that underlie and define the differentiation of secondary sex characteristics at puberty are due to the influence of androgens and estrogens. All estrogen is obligatorily synthesized from androgen. In other words, beginning with either acetate or cholesterol, androgens such as androstenedione and testosterone are synthesized in both the ovary and testis and then partially converted to the estrogens estrone and estradiol (Fig. 1). This reaction is irreversible and is catalyzed by aromatase (cytochrome P-450 19), which has strikingly different activities in males and females.

The fact that both sexes make the same steroid hormones means that physiologic differences are necessarily quantitative. To state it another way, the sex-steroid differences between men and women reflect two regulatory decisions: how much androgen is made, and what percentage of that quantity is converted to estrogen. If we focus on

testosterone and estradiol,<sup>6,7</sup> the two most potent representatives of their classes and the principal hormones synthesized in the gonads, two dramatic differences are evident. The testis makes approximately 7000  $\mu\text{g}$  of testosterone per day and converts one quarter of 1 percent to estradiol. At baseline, the ovary makes only 300  $\mu\text{g}$  of testosterone per day, but it converts fully half of it to estradiol.

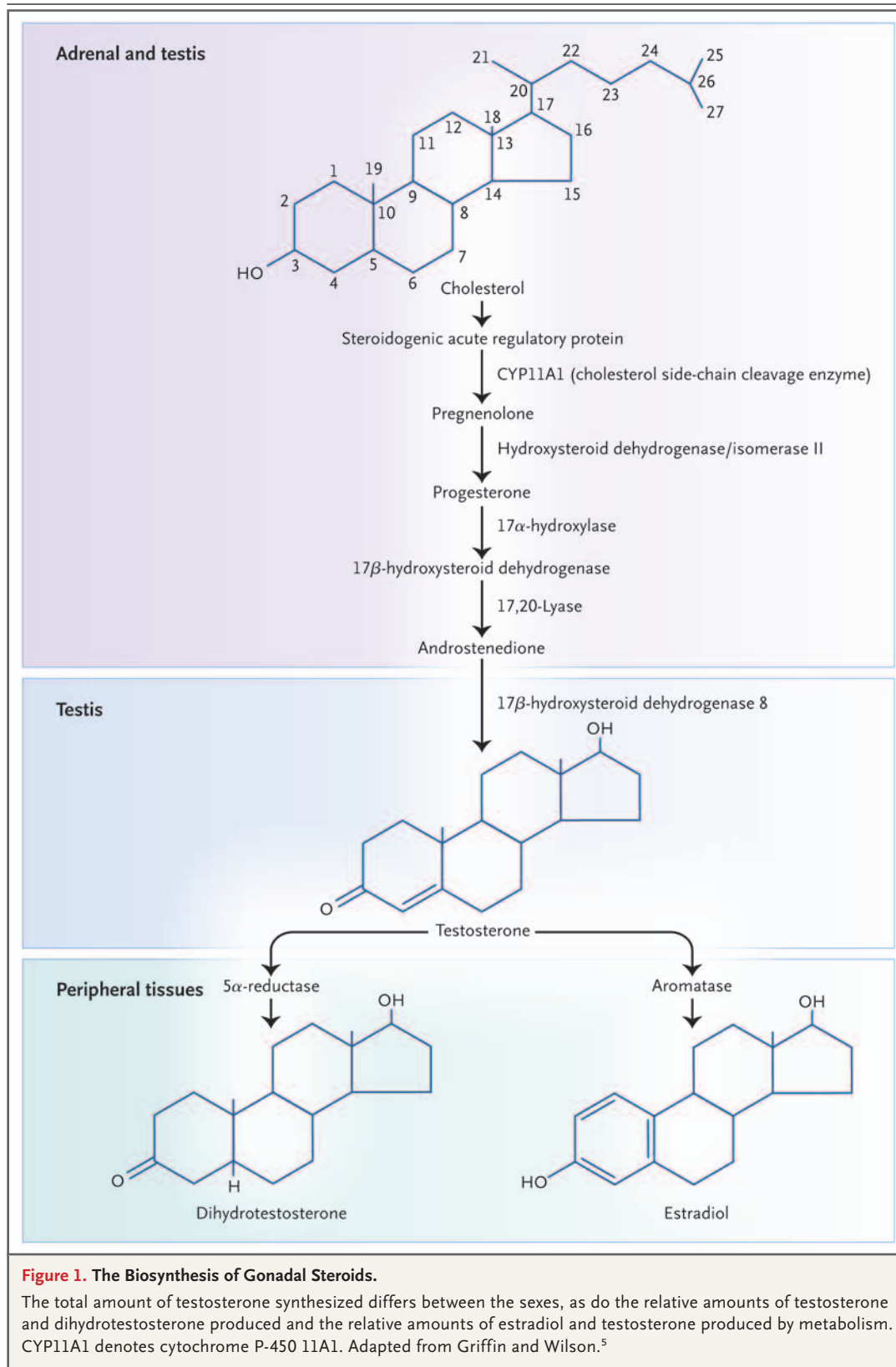
The levels of these two hormones vary throughout the menstrual cycle, with some increase in testosterone production, but as the dominant follicle develops, a larger increase in the percentage of testosterone that is converted to estrogen. Thus, two powerful differences are built into the system. First, men make at least 20 times as much androgen as do women. Second, this contrast is amplified by the fact that the percentage of androgen that is converted to estradiol in a woman is 200 times that in a man. The contrasts are made even starker by a 1000-fold difference in potency; estrogen levels are measured in picograms, but androgen levels are measured in nanograms.

#### TRANSPORT OF SEX HORMONES

The transport of sex steroids in the bloodstream reflects another unique evolutionary arrangement. In the blood, both hormones are bound to a single sex hormone-binding globulin.<sup>8</sup> Androgens lower the level of sex hormone-binding globulin, and estrogens raise it. Furthermore, the hormones are bound with different affinities. Changes in the level of sex steroid-binding globulin have a greater effect on the free androgen level (and thus its biologic expression) than on the corresponding free estrogen level. A given change in the sex hormone-binding globulin level has less of an effect on the availability of estrogen than on that of androgen. Thus, the serum transport of androgens and estrogens contributes to sex-based differences by regulating the relative levels of the free hormones available to other tissues.

#### PERIPHERAL EVENTS

The aromatase gene, *CYP19*, is found in extragonadal tissues, including the brain, prostate gland, breast, bone, liver, and adipose tissue. The genetics of this critical enzyme contrast sharply with those of many other genes. Often, a single gene sequence leads to various protein products as a result of alternative splicing of exons and, thus, varied RNA transcripts. In contrast, the coding



sequence of the *CYP19* gene is one quarter of the gene's length and produces only one protein, the aromatase itself.<sup>9</sup> The greater portion is given to the 11 promoter sites that allow tissue-specific expression. In most tissues, the aromatase has only an intracellular function, converting intracellular androgen to intracellular estrogen. However, both liver and adipocyte aromatase have a major role in determining systemic levels of estrogen. Many conditions are associated with excess aromatase activity (Table 2).

Increased numbers of adipocytes (as in obesity) increase the relative amount of estrogen available, and in certain chronic liver diseases, increased activity of hepatic aromatase can also result in excessive circulating estrogen and gynecomastia. In postmenopausal women, the metabolism of the adrenal hormone dehydroepiandrosterone is a major determinant of the status of sex steroids. Dehydroepiandrosterone is a very weak androgen, but it is a precursor of either more potent androgens or estrogens.<sup>10</sup> In the presence of increased adipocytes, the conversion to estrogen is favored; this step is further enhanced in aging, since aromatase activity increases with advancing age.

The development of secondary sex characteristics at puberty reflects the physiology summarized to this point. Men make predominantly testosterone and convert a fraction of a percent to estrogen. The phenotypic effects of testosterone include male muscularity, deepening of the voice, growth of the beard and prostate gland (mostly caused by dihydrotestosterone), phallic growth, spermatogenesis, sex drive, and erectile function. However, new insights into the importance of the small amount of estrogen in men have been gained from the study of patients who lack either the estrogen receptor or aromatase.<sup>11,12</sup> In both disorders, linear growth is normal during childhood; the early pubertal growth spurt does not occur, but linear growth continues beyond the expected time of conclusion of growth because the epiphyses do not close. Patients with both syndromes have osteopenia. From these observations, we can infer that estrogen in boys is necessary for the pubertal growth spurt, normal bone density, and the completion of growth. We do not know the extent to which these effects require androgen as well. Pubertal girls and women, on the other hand, have dominantly estrogen. This hormone is responsible for breast develop-

**Table 2. Conditions of Excess Aromatase Activity.**

Obesity
Klinefelter's syndrome
Aging
Hyperthyroidism
Liver disease
Endometriosis
Uterine fibroids
Sertoli-cell tumors
Germ-cell tumors
Sex-cord tumors

ment, menstrual flow, the pubertal growth spurt, and the conclusion of growth. However, unlike testosterone in men, estrogen is not responsible for female sex drive, sexual excitement, or sexual satisfaction. The hormonal basis, if any, for these phenomena, is unknown but may be partly due to testosterone.

The intracellular actions of steroid hormones provide an additional source of sex-based differences. The first requirement for a cellular response to a circulating hormone is contact between the ligand and a membrane-bound or intracellular receptor.<sup>13</sup> The human androgen receptor is a member of a superfamily of receptors for steroid hormones, vitamin D, rhodopsin, and other ligands. There are two estrogen receptors in this family, estrogen receptor  $\alpha$  and estrogen receptor  $\beta$ . Studies in mouse models lacking these receptors (knockout mice) have shown that these two receptors have both overlapping and distinct functions.<sup>14</sup>

Intracellular hormone metabolism is another locus of sex differentiation. Many cells contain the aromatase that converts androgen to estrogen. In addition, many tissues have the  $5\alpha$  reductase that converts testosterone to its more potent metabolite dihydrotestosterone.<sup>15</sup> The steroid target tissues also contain proteins known as coactivators and corepressors.<sup>16</sup> These influence the binding of androgens and estrogens to the hormone-binding elements and, hence, to the specific transcription sites in the relevant DNA. Thus, each tissue can construct its own androgenic or estrogenic identity with developmental and potentially behavioral consequences that could not be predicted on the basis of circulating hormone levels.

This may be particularly important in the brain, with respect to sexual behavior, sexual identity, partner choice, and other sex-related events.

#### SEX HORMONES AS SOMATIC HORMONES

A third strategic selection in evolution is the activity of sex hormones in nonreproductive tissues (Table 3). Many tissues of the body are targets for sex hormones, at least as defined by the presence of sex hormone receptors, aromatase and 5 $\alpha$  reductase enzymes, and coactivators and repressors. To a variable degree, these conditions are met in many tissues other than those that have a reproductive role. Estrogen has direct effects on cartilage proliferation, synthesis and calcification of bone matrix, and bone morphogenesis — effects that were formerly attributed to testosterone. Other studies have demonstrated that estrogen has dramatic effects on cardiovascular tissue and function.<sup>17</sup> Some of these effects are typical genomic influences on protein synthesis mediated by transcription induced as a result of the binding of hormones to intracellular receptors and DNA. These actions are generally protective against atherosclerosis and endothelial dysfunction. However, other actions influenced by estrogen, such as vasodilation in response to acetylcholine, occur too rapidly to be explained similarly. They impute the existence of cell-membrane receptors and of nongenomic mechanisms similar to the actions of neurotransmitters. These are additive to the genomic effects in their rapid effects on blood vessels (Fig. 2).

The extrapolation from the presence of androgen and estrogen receptors to the possibility of sex-based differences goes well beyond the cardiovascular system. There are estrogen receptors in bone, specifically in osteoblasts.<sup>18</sup> Under ordinary circumstances, bone formation and bone resorption are coupled and bone density is constant. After menopause, however, bone formation does not keep pace with bone resorption, and there is a continual and progressive loss in bone mineral density — particularly rapid in the first five years after menopause. The resulting osteoporosis, which increases the risk of hip and spine fractures, is a well-known consequence of menopause. Less familiar is the male andropause, a hypothalamically mediated decrease in the production of testosterone by Leydig cells that is less

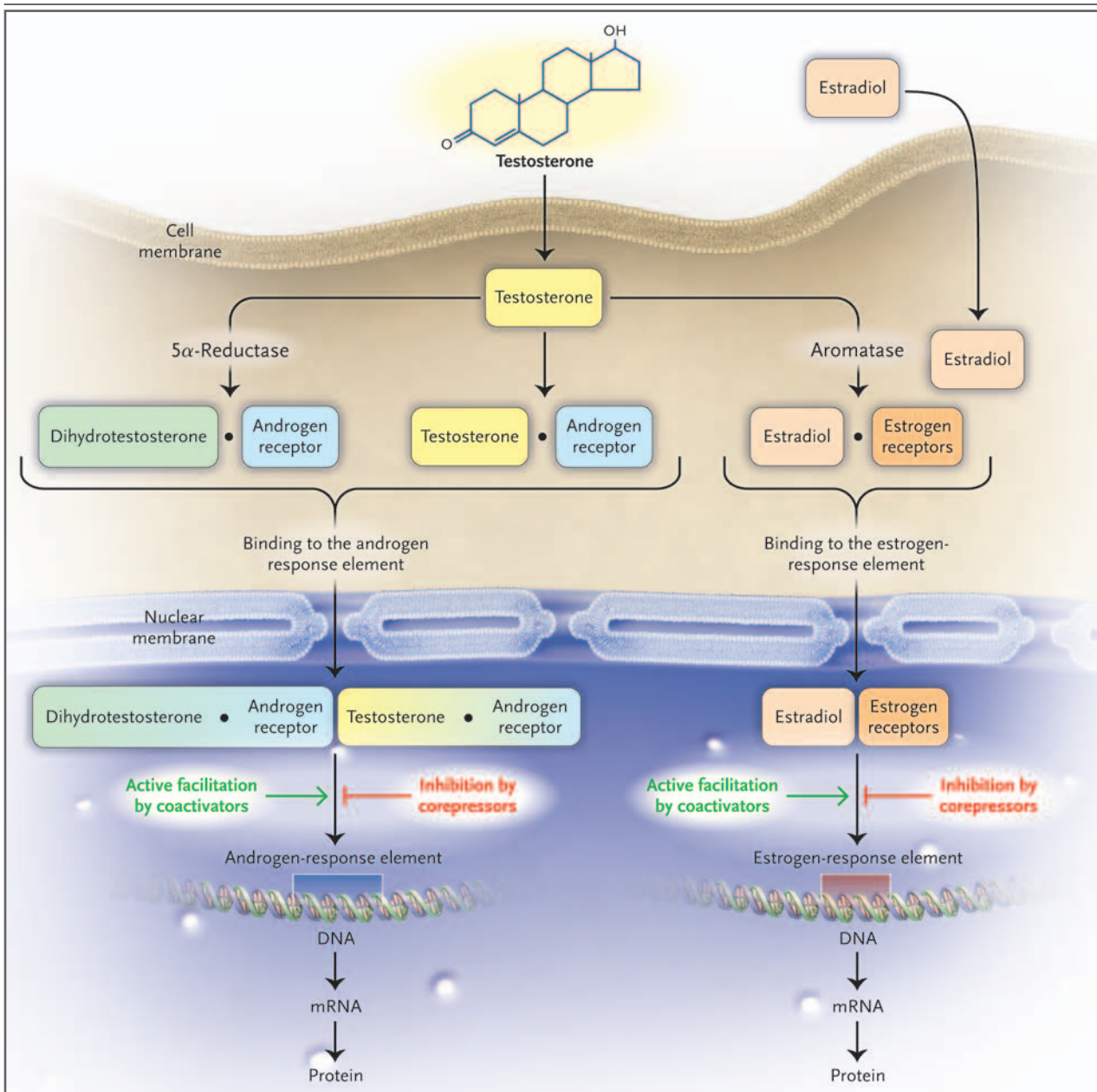
**Table 3. Somatic Tissues Affected by Androgen and Estrogen.**

Androgen	Estrogen
Muscle	Breast
Larynx	Uterus
Beard	Adipose-tissue distribution
Distribution of sexual hair	Heartandvascularendothelium
Bone and cartilage	Bone and cartilage
Immune system	Immune system
Nervous system	Nervous system
Heart	
Red cells	

complete and far more variable than the estrogen deficiency in postmenopausal women.<sup>19</sup> Nevertheless, important consequences include loss of fat-free mass, muscle atrophy and weakness, hypogonadism, and osteoporosis. The age-related increase in the risk of fracture lags about 10 years behind that in women, but the consequences of hip fracture, in particular, are worse in men.

#### GENETIC DIFFERENCES BETWEEN THE SEXES

There are important genetic differences between the 46,XX and 46,XY karyotypes (Table 4). The most obvious is the striking contrast in the size and known patterns of inheritance of the X and Y chromosomes.<sup>20</sup> The X chromosome contains about 5 percent of the DNA in the human genome. The Y chromosome not only is less than half this size but also has a long heterochromatic portion of the long arm that is noncoding. The striking inequality of the two X chromosomes in women as compared with the single X in men is partially reduced by the inactivation of the second X chromosome by random methylation of its genes and conversion of the linear X into a little ball of genetically inert DNA known as the Barr body.<sup>21</sup> This process is thought to occur some time before there are 20 cells in the blastocyst. In such a small cell population, stochastic processes predict that nonrandom inactivation will occur in some persons. In various studies, between 1 and 20 percent of women have nonrandom inactivation of their X chromosome populations. In addition, there is an increase in nonrandomization related to age: skewed inactivation is found in



**Figure 2. Intracellular Actions of Sex Steroids.**

Testosterone enters the cell, some of which is reduced by 5 $\alpha$ -reductase to dihydrotestosterone. Both hormones then bind to the androgen receptor, with dihydrotestosterone having 10 times the binding affinity of testosterone. The resulting ligand–receptor complex then attaches to the androgen-response element — a step facilitated by coactivators and inhibited by corepressors — leading to the transcription of messenger RNA (mRNA), protein synthesis, and androgen effects. Some testosterone entering the cell is aromatized to estradiol through the action of aromatase. Estradiol then binds to its specific receptor. The resulting ligand–receptor complex attaches to the estrogen-response element of chromatin — a step facilitated by coactivators and inhibited by corepressors — leading to mRNA transcription, protein synthesis, and estrogen effects. Each step in the process provides opportunities for the introduction of sex-based differences.

fewer than 7 percent of women under the age of 25 years but in as many as 16 percent after the age of 60 years. Furthermore, not all of the X chromosome is inactivated. The *Xist* gene, a gene that ini-

tiates inactivation on the X chromosome, is transcribed from the inactive X.<sup>22</sup> Approximately 15 percent of genes on the inactive X escape inactivation, and another 10 percent are partially in-

activated, with the percentage inactivated varying from one woman to another.<sup>23</sup> Thus, there is ample scope for double doses of genes carried on the X chromosome to contribute to biologic differences between the sexes. The fetal testis does not actively secrete testosterone until about the 8th to 10th week of gestation. Even after the incomplete inactivation of the second X chromosome, there is ample opportunity for the difference between the XX and XY genotypes to influence early differentiation and later development. Evidence of such an effect has been found in the mouse but not yet in the human.

In contrast to the approximately 1090 genes on the X chromosome, the human Y chromosome contains about 80 genes that can be divided into two broad classes.<sup>24</sup> One class is that of the housekeeping genes, common to the Y and to other chromosomes and primarily involved in the maintenance of cell structure, identity, and physiologic function.<sup>25</sup> The second class consists of genes unique to the Y chromosome and accordingly called the male-specific region. These genes are presumably, and in many places demonstrably, associated with testicular function. The most important of these is the sex-determining region of the Y chromosome (SRY) locus on Yp that directs the differentiation of the fetal gonad into a testis.<sup>26</sup> Bands six through seven on the long arm of the Y chromosome are involved in spermatogenesis, and errors in this area are causes of male-factor infertility. Finally, in contrast to what had long been thought, two regions of the Y chromosome form chiasmata with portions of the short arm of the X chromosome during meiosis. Thus, recombination between the X and the Y chromosomes does occur, if at a low frequency, making a small contribution to sex-based differences. This phenomenon takes on added weight in view of the fact that genes on the Y chromosome have twice the mutation frequency of those on the X chromosome.<sup>27</sup>

#### IMPRINTING

The combination of meiosis in gametogenesis and mitosis in embryogenesis ensures that each autosomal gene locus has both a maternally derived allele and a paternally derived allele that are active. But in the case of at least 50 genes, one of the alleles is turned off by the methylation of cytosine residues, a mechanism superficially similar to X inactivation.<sup>28</sup> In a process that begins during gametogenesis and ends once sex determina-

**Table 4. Contributions of the X and Y Chromosomes According to Sex.**

Variable	Male Sex	Female Sex
Karyotype	46,XY	46,XX
No. of genes	80	1090
Regulation	Some genes active only in testis	Nearly total inactivation of the 2nd X
Sex determination	Testis growth induced by sex-determining region of the Y chromosome	Follicle surrounding oocytes stabilized by the 2nd X

tion is set in the embryo, the uniparental silencing endures throughout the offspring's life. The human genes affected appear primarily to be those influencing growth, with the mother's allele silencing growth-promoting genes and the father's allele suppressing growth-reducing genes. At least one human disorder reflects the power of imprinting.<sup>29,30</sup> When one locus is hemiparental for the father's gene, the result is the Prader-Willi syndrome (characterized by hypotonia, developmental delays, hyperphagia, diminutive hands and feet, incomplete sexual development, and short stature). Conversely, lack of expression of the mother's allele causes Angelman's syndrome (characterized by mental retardation, hypotonia, ataxia, and round-faced).

Mitochondrial inheritance is an additional genetic cause of disparity between the sexes. Mitochondria are present in most cells but derive exclusively from the mother. They reach the zygote through the egg, and the genes they carry are replicated with each cell division but do not undergo recombination with the rest of the genome. Although a single instance of male inheritance of a mitochondrial disease has been reported,<sup>31</sup> its uniqueness underscores the importance of maternal mitochondrial hegemony.

#### SOCIOECONOMIC FACTORS

This article has focused on biologic factors involved in differences between the sexes, but the socio-cultural environment can produce additional differences. For example, the preference for boys in many agrarian societies can lead to preferential nutrition for boys and, at the extreme, abortion of female fetuses and female infanticide. In some societies, girls are subjected to genital mutilation and women may be subjected to horrible physical abuse by husbands and in-laws. In addition, girls and women in some countries and under some

regimes are not allowed to attend school, have secondary citizenship status with no voting rights, and have little control over their own sexuality. These inequalities add to the female burden of illness.

## CONCLUSIONS

Differences between the sexes pervade all clinical experience in medicine. The direct mecha-

nisms of most phenomena involved in sexual dimorphism have not yet been identified. Yet, it is highly likely that these mechanisms will be elucidated through study of systemic or local expressions of the fundamental differences between the sexes in the genetic and endocrine controls described in this article.

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## REFERENCES

1. Wizemann TM, Pardue M, eds. Exploring the biological contributions to human health: does sex matter? Washington, D.C.: National Academies Press, 2001.
2. Bulun SE, Adashi EY. The physiology and pathology of the female reproductive axis. In: Larsen PR, Kronenberg HM, Melmed S, Polonsky KS, eds. Williams textbook of endocrinology. 10th ed. Philadelphia: W.B. Saunders, 2003:587-664.
3. Morita Y, Tilly JL. Oocyte apoptosis: like sand through an hourglass. *Dev Biol* 1999;213:1-17.
4. Bianchi DW. Fetomaternal cell trafficking: a new cause of disease? *Am J Med Genet* 2000;91:22-8.
5. Griffin JE, Wilson JD. Disorders of the testes and the male reproductive tract. In: Larsen PR, Kronenberg HM, Melmed S, Polonsky KS, eds. Williams textbook of endocrinology. 10th ed. Philadelphia: W.B. Saunders, 2003:709-69.
6. Simpson ER, Mahendroo MS, Means GD, et al. Aromatase cytochrome P450, the enzyme responsible for estrogen biosynthesis. *Endocr Rev* 1994;15:342-55.
7. Bulun SE. Aromatase deficiency in women and men: would you have predicted the phenotypes? *J Clin Endocrinol Metab* 1996;81:867-71.
8. Fortunati N. Sex hormone-binding globulin: not only a transport protein. What news is around the corner? *J Endocrinol Invest* 1999;22:223-34.
9. Sebastian S, Bulun SE. A highly complex organization of the regulatory region of the human CYP19 (aromatase) gene revealed by the Human Genome Project. *J Clin Endocrinol Metab* 2001;86: 4600-2.
10. Labrie F, Luu-The V, Lin SX, Simard J, Labrie C. Role of 17 beta-hydroxysteroid dehydrogenases in sex steroid formation in peripheral lutracrine tissues. *Trends Endocrinol Metab* 2000;11:421-7.
11. 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;331:1056-61. [Erratum, *N Engl J Med* 1995;332:131.]
12. Grumbach MM, Auchus RJ. Estrogen: consequences and implications of human mutations in synthesis and action. *J Clin Endocrinol Metab* 1999;84:4677-94.
13. Heinlein CA, Chang C. Androgen receptor (AR) coregulators: an overview. *Endocr Rev* 2002;23:175-200.
14. Couse JE, Hewitt SC, Bunch DO, et al. Postnatal sex reversal of the ovaries in mice lacking estrogen receptors alpha and beta. *Science* 1999;286:2328-31.
15. Wilson JD, Griffin JE, Russell DW. Steroid 5 alpha-reductase 2 deficiency. *Endocr Rev* 1993;14:577-93.
16. Takimoto GS, Graham JD, Jackson TA, et al. Tamoxifen resistant breast cancer: coregulators determine the direction of transcription by antagonist-occupied steroid receptors. *J Steroid Biochem Mol Biol* 1999;69:45-50.
17. Mendelsohn ME, Karas RH. The protective effects of estrogen on the cardiovascular system. *N Engl J Med* 1999;340: 1801-11.
18. Eriksen EF, Colvard DS, Berg NJ, et al. Evidence of estrogen receptors in normal human osteoblast-like cells. *Science* 1988; 241:84-6.
19. Vermeulen A. Andropause. *Maturitas* 2000;34:5-15.
20. Willard HF. The sex chromosomes and X chromosome inactivation. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. The metabolic and molecular bases of inherited disease. 8th ed. New York: McGraw-Hill, 2001:1191-211.
21. Brown CJ. Equality of the sexes: mammalian dosage compensation. *Semin Reprod Med* 2001;19:125-32.
22. Wutz A, Jaenisch R. A shift from reversible to irreversible X inactivation is triggered during ES cell differentiation. *Mol Cell* 2000;5:695-705.
23. Carrel L, Willard HF. X-inactivation profile reveals extensive variability in X-linked gene expression in females. *Nature* 2005;434:400-4.
24. Ross MT, Grafham DV, Coffey AJ, et al. The DNA sequence of the human X chromosome. *Nature* 2005;434:325-37.
25. Page DC. 2003 Curt Stern Award address: on low expectation exceeded; or, the genomic salvation of the Y chromosome. *Am J Hum Genet* 2004;74:399-402.
26. Koopman P. The genetics and biology of vertebrate sex determination. *Cell* 2001; 105:843-7.
27. Skaletsky H, Kuroda-Kawaguchi T, Minx PJ, et al. The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. *Nature* 2003;423: 825-37.
28. Tilghman SM. The sins of the fathers and mothers: genomic imprinting in mammalian development. *Cell* 1999;96: 185-93.
29. Knoll JH, Nicholls RD, Magenis RE, Graham JM Jr, Lalonde M, Latt SA. Angelman and Prader-Willi syndromes share a common chromosome 15 deletion but differ in parental origin of the deletion. *Am J Med Genet* 1989;32:285-90.
30. Kantor B, Makedonski K, Green-Finberg Y, Shemer R, Razin A. Control elements within the PWS/AS imprinting box and their function in the imprinting process. *Hum Mol Genet* 2004;13:751-62.
31. Cann RL, Stoneking M, Wilson AC. Mitochondrial DNA and human evolution. *Nature* 1987;325:31-6.

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