



# LCMS Saliva Steroid & Steroid Synthesis Inhibitor Profile

ZRT Laboratory now offers a comprehensive LC-MS/MS saliva assay that measures the levels of 18 endogenous steroid hormones (see Steroid Hormone Cascade diagram on the next page) including estrogens, progestogens, androgens, glucocorticoids, and mineralocorticoids. In addition to endogenous hormones the new assay quantifies the level of melatonin and the synthetic estrogen ethinyl estradiol, present in most birth control formulations, as well as several synthetic aromatase inhibitors (anastrozole and letrozole) and the 5 $\alpha$ -reductase inhibitor finasteride.

The LC-MS/MS assay expands beyond the 5-steroid panel of parent hormones (estradiol, progesterone, testosterone, DHEAS, and cortisol) currently tested by immunoassay (IA) at ZRT Laboratory. Testing the levels of both upstream precursors and downstream metabolites of these parent active steroids, listed above and shown in the diagram on the next page, will help determine which steroid synthesis enzymes are low, overactive, blocked by natural or pharmaceutical inhibitors, or defective due to metabolic dysfunctions (e.g., Polycystic Ovarian Syndrome (PCOS), Premenstrual Dysphoric Disorder (PMDD), luteal dysfunction, overexpression of aromatase, and estrogen dominance) and inborn errors of metabolism such as Congenital Adrenal Hyperplasia (CAH).

## Hormones and Hormone Synthesis Inhibitors Tested by LC-MS/MS in Saliva

### Estrogens

These include the endogenous estrogens estradiol, estrone, and estriol, plus the synthetic estrogen ethinyl estradiol (EE). Estradiol is the most potent of the endogenous estrogens, being 5 and 10 times more potent than estrone and estriol, respectively, in its activation of cellular estrogen receptors. EE, a synthetic steroid present in most oral steroidal birth control formulations, is a highly potent estrogen mimetic that is about 2x more potent than estradiol in activating cellular estrogen receptors, which is why only very low concentrations are needed for it to elicit profound potentially adverse hyper-estrogenic effects. Additionally, EE induces lower ovarian synthesis and bioavailability of endogenous estradiol,

## Tests Included

### Estrogens

Estradiol (E2), Estriol (E3), Estrone (E1), Ethinyl Estradiol (EE)

### Progestogen Precursors and Metabolites

Pregnenolone Sulfate (PregS), Progesterone (Pg), Allopregnenolone (AlloP), 17-OH Progesterone (17OHPg)

### Androgen Precursors and Metabolites

Androstenedione (Adione), Testosterone (T), Dihydrotestosterone (DHT), DHEA (D), DHEA-S (DS), 7-Keto DHEA (7keto)

### Glucocorticoid Precursors and Metabolites

11-Deoxycortisol (11DC) Cortisol (C), Cortisone (Cn)

### Mineralocorticoid Precursors and Metabolites

Corticosterone (Ccn), Aldosterone (Ald)

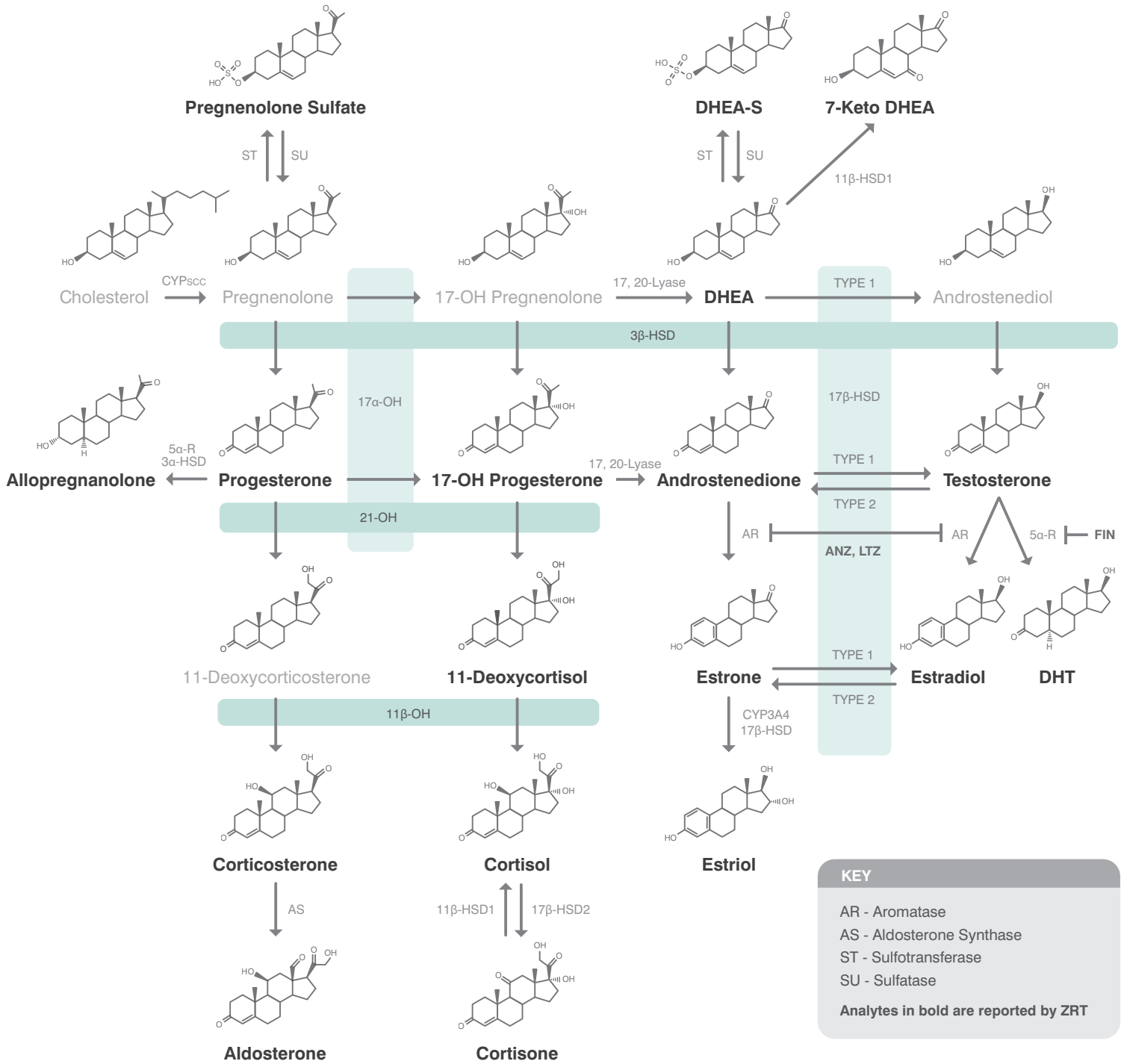
### Other

Melatonin (Mel)

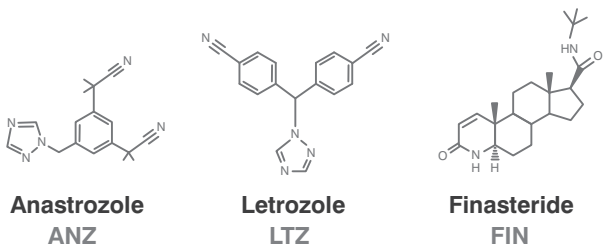
### Steroid Synthesis Inhibitors

Anastrozole (ANZ), Finasteride (FIN), Letrozole (LTZ)

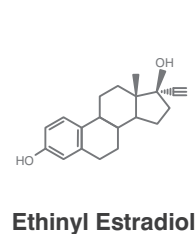
# Saliva Steroid Cascade



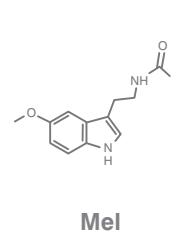
## Steroid Synthesis Inhibitors



## Synthetic Contraceptive Estrogen



## Melatonin





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# Hormone Testing

## Minimally-invasive home test kits

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progesterone and testosterone by suppressing gonadotropin (LH and FSH) synthesis and increasing serum binding proteins (e.g., SHBG, CBG) that decrease the free fraction (salivary level) and potential tissue bioavailability of the parent steroids. No other laboratories currently offer EE testing, which allows for assessment of its estrogenic activity and potential for adverse symptoms experienced by a subset of women who do not tolerate birth control formulations containing EE.

### Progestogens

These include progesterone and its precursor (pregnenolone sulfate) and downstream metabolites of progesterone that are precursors to glucocorticoids (17-hydroxyprogesterone) or belong to a class of neuroactive steroids (allopregnanolone) that bind to and modify the activity of GABAA receptors in the brain to induce a calming (anxiolytic) effect. Pregnenolone sulfate is also a neuroactive steroid but has opposite (stimulating/anxiogenic) actions in the brain. Individual differences in the level of progesterone and its precursor and downstream metabolites, either from endogenous production during the luteal phase or with different types of progesterone or pregnenolone therapies, will help shed light on defective endogenous pathways of hormone synthesis (e.g., low progesterone and allopregnanolone during the mid-luteal phase and high 17-OH progesterone and low cortisol and aldosterone in individuals with CAH) or exogenous supplementation with oral vs. topical progesterone or pregnenolone. Pregnenolone, which sits at the top of the cascade and is considered the mother of all other downstream hormones, can take many different routes of metabolism to downstream parent steroids and their metabolites that depend on individual differences in expression of enzymes of steroid hormone synthesis. The LC-MS/MS salivary assay will shed light on which enzymatic pathways are most active with pregnenolone or other downstream parent steroid precursor therapies.

### Androgens

These include precursors DHEA, DHEA-S, 7-keto DHEA, androstenedione, and the more active androgens, such as testosterone, and its more potent metabolite dihydrotestosterone.

The aromatase inhibitors anastrozole and letrozole, used commonly in breast cancer patients as well as men and women supplementing with testosterone therapy to block excessive conversion to estrogens, will help determine the extent to which these antagonists effectively block testosterone conversion to estradiol. Finasteride is included in the assay to determine its effectiveness in blocking 5 $\alpha$ -reductase conversion of testosterone to the more active dihydrotestosterone and progesterone to allopregnanolone, both of which could have beneficial or adverse effects depending on individual sensitivity to these metabolites or propensity for converting the parent steroid to excessive amounts of metabolites that cause adverse symptoms. 7-keto DHEA, used as a supplement, increases 7-keto DHEA levels in the circulation. Unlike DHEA, 7-keto DHEA is not metabolized to downstream androgens (e.g., androstenedione, testosterone, DHT) or estrogens. The LC-MS/MS test is the first commercial product that measures the level of 7-keto DHEA relative to DHEA. DHEA and 7-keto DHEA are present naturally in saliva in near equal amounts. Supplementation with DHEA significantly raises the DHEA/7-keto DHEA ratio and increases downstream androgen and estrogen metabolites in most individuals, making it possible to identify those using DHEA supplementation vs. those with naturally high DHEA from adrenal hyperplasia. Herbal adrenal adaptogens may also more likely simultaneously increase both DHEA and 7-keto, differentiating this type of supplementation from DHEA itself. Supplementation with 7-keto DHEA increases 7-keto DHEA only and lowers the DHEA/7keto-DHEA ratio, making it possible to identify those taking this alternative form of DHEA and quantitate its bioavailable fraction in saliva relative to dosing.

### Glucocorticoids

These include the cortisol precursors 17-hydroxyprogesterone and 11-deoxycortisol, and its downstream and inert metabolite cortisone. Testing the relative amounts of cortisol and cortisone will elucidate the activities of 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (converts cortisone to cortisol) and type 2 (inactivates cortisol to inert cortisone). Cortisol testing, in concert with cortisone, will also help to evaluate how hormone therapies (e.g., thyroid, estrogens), adrenal adaptogens, or other nutritional

interventions alter the conversion of bioactive cortisol to its inert metabolite cortisone, or vice versa. Testing the levels of these cortisol precursors in concert with its upstream precursors and the androgens will help differentiate high androgen symptoms caused by PCOS from those resulting from CAH.

### **Mineralocorticoids**

These include aldosterone and its precursor corticosterone, a weak glucocorticoid and mineralocorticoid. Corticosterone is converted to aldosterone by aldosterone synthetase in the mitochondria of the Zona glomerulosa, the outermost region of the adrenal cortex. Corticosterone and aldosterone measurements are useful for differentiating CAH, caused by 21-hydroxylase deficiency (results in low aldosterone and cortisol), from that of the more rare form of CAH caused by 17-hydroxylase deficiency (results in normal or high corticosterone and aldosterone but low cortisol and sex steroid hormones). Aldosterone is important for regulating sodium and potassium balance to control water retention or loss, blood pressure and blood volume. Low or high levels of these may help define the causes for hypoaldosteronism, associated with salt wasting, low blood pressure, and adrenal dysfunction potentially linked to CAH. Alternatively, hyperaldosteronism, associated with water retention, salt sensitivity, and high blood pressure may help shed light on other endocrine dysfunctions.

### **LC-MS/MS vs. Immunoassay**

LC-MS/MS assays and immunoassays (IA) for steroids are both accurate over physiological ranges in adult females and males. Ranges for the parent steroids (estradiol, progesterone, testosterone, DHEAS, and cortisol) measured by LC-MS/MS show near quantitative equivalence to IA tests over physiological ranges in adult males, and females with normal menstrual cycles, demonstrating excellent accuracy of both the salivary steroid IA and LC-MS/MS assays performed at ZRT Laboratory. This allows a less expensive, but equally accurate and reliable prescreening of the parent steroids by IA and, if needed, a more comprehensive LC-MS/MS screening for more complex endocrine challenges, where levels of hormones may be outside of the normal adult physiological range due to metabolic dysfunctions and insufficient or excessive hormone therapy.

A preference for the LC-MS/MS assay may include males or females with very low estradiol and testosterone levels due to age (e.g., prepubertal, postmenopausal) or use of a steroid synthesis inhibitor such as an aromatase inhibitor or a contraceptive synthetic steroid like EE that blocks estradiol, progesterone, and testosterone synthesis by inhibiting LH and FSH synthesis. It may also include women or men on steroid hormone therapies that convert the parent steroid to more active metabolites (e.g., progesterone to allopregnanolone and testosterone to dihydrotestosterone) that could elicit adverse side effects if produced in excessive amounts.

Another advantage of the LC-MS/MS assay is that it expands beyond the parent active steroids to provide insight into reasons why the parent steroids might be high or low due to precursor steroids or downstream metabolites controlled by enzymes that create or metabolize the parent steroids (e.g., elevated 17-OH progesterone and low cortisol and aldosterone in individuals with late onset CAH).

ZRT is the only laboratory that offers a commercial test that quantifies the synthetic steroid EE as well as the most common synthetic steroid synthesis inhibitors. While these may have the desired effect to prevent pregnancy and reduce endogenous hormone synthesis, respectively, excessive bioavailable levels of them, as measured in saliva, due to abnormally slow or rapid metabolism and clearance may lead to serious and even life-threatening secondary side effects and adverse symptoms and conditions (e.g., stroke with excessive levels of synthetic estrogens not detected by a conventional IA for estradiol, or depression and suicide risk with very low conversion of parent steroids, testosterone and progesterone, to neuroactive steroids). Identifying hypersensitive individuals who poorly metabolize and clear these synthetics, over-inhibit synthesis of endogenous hormones, and react adversely to them should provide rationale for dose adjustment or changing any hormone therapies, bioidentical or synthetic, or other medications that may be causing problems.

### **How Does the Salivary LC-MS/MS Assay Differ from Conventional Serum Immunoassays?**

Saliva is an ideal body fluid to use for steroid hormone assays because it is noninvasive, easy to collect at any time or place, and the steroid hormones in saliva are representative of the small fraction of hormones circulating in the bloodstream that are bioavailable to tissues. This bioavailable fraction represents about 1-3% of the total hormone circulating in the bloodstream that breaks away from blood binding proteins, enters tissues, binds unique receptors and is responsible for triggering specific responses characteristic of the hormone.

Serum/plasma steroid IAs or even serum GC- or LC-MS/MS steroid assays measure the total level of steroid in the bloodstream, not the small fraction that is released from the steroid-binding proteins in the bloodstream into saliva and tissues, or the bioavailable fraction that is representative of the active level of hormone at the cellular level. The actual bioavailable fraction of the steroid in serum can vary considerably depending on the level of binding protein(s) for a specific steroid, which may depend on the level of other hormones and the individual variability in the liver's capacity to manufacture the hormone binding proteins. These serum binding proteins are present to help stabilize the steroids, which

would otherwise be metabolized and eliminated rapidly. Each of the hormone categories have specific serum binding proteins. Estrogens, particularly estradiol as well as phytoestrogens found in plants and synthetic estrogens, induce liver synthesis of the estrogen and androgen (testosterone and DHT) binding proteins Sex Hormone Binding Globulin (SHBG), Cortisol Binding Globulin (CBG) that binds and sequesters progesterone and cortisol, and Thyroid Binding Globulin (TBG) that binds and protects the thyroid hormones T3 and T4 from degradation that would occur in minutes. These hormone binding proteins affect the bioavailability of estrogens, progesterone, androgens, and the thyroid hormones triiodothyronine (T3) and thyroxine (T4). Total steroid serum assays, IAs or those performed by GC- or LC-MS/MS, do not measure these subtle differences in the bioavailability of the steroid hormones, which requires more sophisticated separation techniques or measurement of the binding protein (e.g., SHBG) to obtain a rough estimation of the free/bioavailable fraction of the total hormone. Methods based on separation of free and protein bound steroids in serum by centrifugation through a membrane are possible, but only commercially available in a handful of laboratories. Both serum methods for detecting the putative bioavailable fraction of steroids are flawed in that they do not account for the higher salivary, capillary blood, and tissue levels of all categories of steroid hormones seen with topical hormone delivery.

Saliva steroid measurements have the advantage that the salivary gland serves as an ultrafiltration to separate bound from free (bioavailable) steroids, much like the methods used to separate serum bound steroids through a membrane. Therefore, LC-MS/MS saliva testing provides a unique opportunity to simultaneously evaluate the full cascade of bioavailable steroids, starting with pregnenolone to their parent steroids and more active metabolites, as well as synthetic steroids and inhibitors of steroid hormone synthesis, in one run. To perform the tests offered by LC-MS/MS would require 23 separate IAs, most of which are not commercially available.

## How Does the Salivary LC-MS/MS Assay Differ from Urine GC- or LC-MS/MS Assays?

What is measured in urine assays, aside from free cortisol and cortisone, are inactive steroid conjugates (mostly sulfate and glucuronide) that were once active parent steroids available to target tissues. These urine steroid conjugates are not bioactive, like the steroids measured in saliva or blood, and will not enter target cells; they are only scheduled for elimination in urine or bile. Very little of the steroid conjugates that form and circulate in the bloodstream before elimination in urine or bile pass through the plasma membranes of the salivary gland or other tissues and enter the tissues/cells. While steroid hormone conjugates tested

in urine provide a good estimate of their total synthesis throughout a time period (usually 24 hours), they are only inert and quickly eliminated into urine as waste-by products by the kidneys. Urine steroid metabolites are not representative of the amount of total unconjugated steroid present in serum/plasma, or the fraction that enters tissues and elicits a response characteristic of the hormone, as seen with salivary hormones. Urine does, however, have the advantage that the inert steroid conjugate waste is somewhat reflective of the total steroid production over the time course of collection; but it also does not measure a large fraction of steroid hormones excreted through bile/feces, which is more likely occurring with topical hormone therapy.

## Tests Included in the Profile

### Estradiol

Estradiol is the predominant, and the most potent, circulating estrogen. Bioavailable estradiol, which represents about 2% of the total protein-bound estradiol in the bloodstream<sup>1</sup>, exits the bloodstream in capillary beds and enters target cells such as the brain, breasts, uterus, bone and heart. There, it binds to the estrogen receptors and activates an estimated 250+ genes that induce the synthesis of enzymes and other functional proteins involved in regulating a multitude of actions, including neurotransmitter synthesis and action in the brain, and activation of cell growth and proliferation and synthesis of progesterone receptors in the estrogen/progesterone target tissues that allows for subsequent counter-response to the growth promoting actions of estrogens. In reproductive age women, an excess of estradiol relative to progesterone, known as “estrogen dominance”, can explain many symptoms including endometrial hyperplasia, pre-menstrual syndrome, fibrocystic breasts, and uterine fibroids<sup>2</sup>. Perimenopausal women can also experience symptoms of estrogen dominance, which include weight gain, fibrocystic and tender breasts, uterine fibroids, irritability, and water retention. With the onset of menopause, low estradiol levels lead to hot flashes, night sweats, vaginal dryness, sleep disturbances, foggy thinking, and bone loss. In men, too much estradiol, relative to testosterone, leads to feminizing effects such as breast enlargement and can result in a functional testosterone deficiency.

### Estriol

Estriol is the weakest of the three major naturally-occurring estrogens in women. It is a product of the metabolism of estrone and estradiol. Because of its weak estrogenic activity, estriol is sometimes preferred for intravaginal use as an alternative to systemic estrogen therapy for the treatment of urogenital atrophy in postmenopausal women<sup>3</sup>. It is also used in anti-aging skin creams as a form of topical estrogen replacement to counteract the effects of age-related estrogen loss on skin. Estriol is the major

estrogen found in the maternal circulation during pregnancy<sup>4</sup>; 90% of this circulating estriol is the product of metabolism of DHEA from the fetal adrenals, and so maternal estriol levels are used as an indicator of fetal health. In non-pregnant women, estriol levels are similar in both pre- and post-menopause and are also similar to levels in men. Salivary estriol has been found to be predictive of increased risk of preterm labor in pregnant women<sup>5</sup>. In non-pregnant women, it is most commonly used for monitoring of levels in women using estriol-containing supplements as part of hormone replacement therapy.

### **Estrone**

Estrone is one of the three main circulating estrogens in humans. Like estradiol, estrone is secreted by the ovaries, but it is also predominantly produced in peripheral tissues by the action of aromatase on its precursor androstenedione<sup>6</sup>. Its estrogenic activity is intermediate to that of estriol, the weakest estrogen, and estradiol, the strongest. Estrone is converted to the more potent estradiol in tissues by the action of 17 $\beta$ -hydroxysteroid dehydrogenase, and through this conversion it represents the main source of circulating estradiol in postmenopausal women and in men. Estrone is the predominant circulating estrogen in postmenopausal women, compared to estradiol which predominates in premenopausal women. This is because ovarian estradiol production declines significantly post-menopause while estrone production from androstenedione changes minimally compared to premenopause. The aromatization of androstenedione to estrone increases with increased body weight, since aromatase is prevalent in fat tissue. This increased availability of estrone contributes to the rise in circulating estradiol with increasing body mass index in obese postmenopausal women<sup>7</sup>.

### **Ethinyl estradiol**

Ethinyl estradiol is an estrogen receptor agonist commonly used in combined oral contraceptives. It is a synthetic derivative of estradiol. In the liver it stimulates the synthesis of SHBG, increasing SHBG levels by 2- to 4-fold in women, which has the effect of binding more circulating testosterone and reducing free testosterone concentrations by 40-80%<sup>8</sup>. The suppression of free testosterone levels may impact female sexual desire<sup>9</sup>.

### **Pregnenolone sulfate**

Pregnenolone sulfate is a neurosteroid that enhances the glutamate N-methyl-D-aspartate (NMDA)-receptor function and inhibits receptors for glycine, GABA, thereby regulating the excitation-inhibition balance in the CNS<sup>10</sup>. It enhances learning and memory and promotes nerve cell survival<sup>11</sup>. Along with other endogenous neurosteroids, it has been implicated in the development of neuropsychiatric disorders such as schizophrenia, depression, and anxiety. Trials of the therapeutic use of pregnenolone, the precursor of pregnenolone sulfate, in

schizophrenia have shown some success<sup>12</sup>. Low plasma levels of pregnenolone sulfate have been observed in people with generalized social phobia<sup>13</sup>.

### **Progesterone**

Progesterone's primary function during the menstrual cycle is to induce a secretory endometrium ready for implantation of a fertilized egg. Levels therefore increase during the luteal phase of the cycle after ovulation<sup>14</sup>. If no implantation occurs, progesterone returns to follicular phase levels. If a pregnancy results, progesterone continues to rise to very high levels and carries out a variety of functions necessary to sustain the pregnancy<sup>15</sup>. In some patients with infertility, ovulation may occur but luteal phase levels of progesterone are inadequate. Luteal phase deficiency is a result of inadequate progesterone production by the corpus luteum. During menopause, ovarian progesterone production dwindles, resulting in postmenopausal levels similar to those seen in men. Progesterone has wide-ranging physiological effects, including neuroprotection, maintenance of skin elasticity, and development of bone tissue. Progesterone also counteracts the proliferative effects of estrogen on the endometrium. When samples are collected after transdermal application of progesterone, saliva progesterone levels are higher than serum, indicating distribution of progesterone to tissues<sup>16</sup>.

### **Allopregnanolone**

Allopregnanolone is a potent positive allosteric modulator of the GABAA receptor<sup>17</sup>. Allopregnanolone has a wide variety of effects, including antidepressant, anxiolytic, stress-reducing, pro-sleep, sedative, neuroprotective, cognitive, and analgesic<sup>18</sup>. Allopregnanolone is the main metabolite of progesterone.

### **17-OH Progesterone**

The hormone 17-hydroxyprogesterone is produced by the adrenal glands. 17-OHPg is converted to cortisol, which is released in varying amounts, but at particularly high levels during times of physical or emotional stress. A cortisol deficiency can occur in certain people which can lead to an increase in 17-OH progesterone in the blood. LCMS saliva testing has allowed accurate determination of 17-OH progesterone along with other androgens, correlating with serum levels and allowing useful steroid profiling in disorders of steroid metabolism<sup>19</sup>. High levels of 17-OHPg can indicate CAH<sup>20</sup>. CAH is a glandular disorder that results in the adrenal glands being unable to create enough cortisol, which may consequently increase the production of DHEA and testosterone.

### **Androstenedione**

Androstenedione is secreted predominantly by the adrenal gland and production is controlled, in part, by adrenocorticotrophic hormone (ACTH). It is also produced in the testes and ovaries from DHEA-S. It is a weak androgen and an intermediate in the

biosynthesis of testosterone and estrone from DHEA. It has been found to have some estrogenic activity. Elevated androstenedione levels can cause symptoms or signs of hyperandrogenism in women<sup>21</sup>. Significant elevations of androstenedione may be indicative of androgen-producing adrenal or gonadal tumors<sup>22</sup>. Androstenedione is converted to estrone by the action of aromatase in fat tissue.

## Testosterone

In men, levels of testosterone begin to decline with age, usually beginning around the mid-40s. The decline in testosterone production by the testes can be more precipitous in some men than others. Excessive weight gain, stress, lack of exercise, and many medications can further reduce testosterone levels, leading to symptoms that include low libido, irritability, depression, loss of muscle mass and strength, weight gain, erectile dysfunction, osteoporosis, and adverse changes in the blood lipid profile. Testosterone levels in saliva are an accepted method for assessment of hypogonadism in men<sup>23</sup>. In women, high testosterone, often caused by ovarian cysts, leads to conditions such as excessive facial and body hair, acne, and oily skin and hair<sup>24</sup>. Low testosterone in postmenopausal women, seen particularly after surgical removal of the ovaries, leads to female symptoms of androgen deficiency including loss of libido, thinning skin, vaginal dryness, and loss of bone and muscle mass<sup>25</sup>.

## Dihydrotestosterone (DHT)

Dihydrotestosterone is an endogenous androgen that is formed from testosterone via 5 $\alpha$ -reductase activity in certain tissues including the prostate gland, seminal vesicles, epididymis, skin, hair follicles, liver, and brain. DHT, relative to testosterone, is more potent as an agonist of the androgen receptor. Inhibition of 5 $\alpha$ -reductase activity to reduce prostatic DHT levels is used to treat benign prostatic hyperplasia (BPH)<sup>26</sup>. DHT has been used clinically as treatment for low testosterone levels in men. DHT is biologically important for sexual differentiation of the male genitalia during embryogenesis. Circulating levels of DHT are low in relation to testosterone. Deficiency in 5 $\alpha$ -reductase results in incompletely virilized males which is clinically supported by an elevated ratio of testosterone to DHT<sup>27</sup>.

## DHEA & DHEA-S

Dehydroepiandrosterone (DHEA), a hormone produced by the adrenal glands, is the precursor for estrogens and testosterone, and is therefore normally present in significantly greater quantities than all the other steroid hormones. It is mostly found in the circulation in the form of its sulfate ester, DHEA sulfate (DHEA-S), levels of which in saliva are higher and more stable than those of DHEA. Its production is highest in the late teens to early 20s and declines gradually with age in both men and women<sup>28</sup>. Levels of DHEA-S reflect adrenal gland function. Low DHEA-S

indicates adrenal insufficiency and/or androgen deficiency and can be associated with reduced libido and general malaise. High DHEA-S levels are seen in hyper-adrenal states such as CAH, and in polycystic ovarian syndrome; high levels in women are associated with masculinizing effects because of its local metabolism to testosterone. DHEA supplementation has been successfully used to treat adrenal insufficiency<sup>29</sup> and can restore normal levels of testosterone in women with androgen deficiency, particularly as a consequence of ovary removal<sup>30</sup>. Because of its conversion to potent estrogens and androgens, levels should be closely monitored during supplementation to avoid excess. DHEA is a supplement commonly used topically and orally. It is marketed as a dietary supplement for weight loss, increased metabolism, and memory enhancement.

## 7-Keto DHEA

7-keto DHEA (also known as 7-oxo DHEA) is a steroid produced by metabolism of DHEA. It is not directly converted to testosterone or estrogen. 7-keto DHEA is rapidly absorbed when given as a supplement and converted to its sulfate derivative. It is commonly used to produce the metabolic effects of DHEA while avoiding metabolism into estrogens or androgens, and clinical research supports its role in benefiting metabolism and weight management. Endogenous 7-keto DHEA may have some anti-cortisol activity through enzyme competition<sup>31</sup> which in the case of hypercortisolism may be beneficial to the adverse effects of cortisol on metabolic syndrome. Most studies on 7-keto DHEA are on improving the metabolic rate where there appears to be improvement in metabolism despite being on a low caloric diet. There is also limited information that 7-keto DHEA may act to increase levels of T3 while patients are on a caloric restricted diet<sup>32</sup>.

## 11-Deoxycortisol

11-deoxycortisol is an adrenal hormone. It is the end product of 17-hydroxyprogesterone (17OHPg) through 21-hydroxylase synthesis and is the immediate precursor of cortisol. Levels of these cortisol precursors and the enzymes that stimulate cortisol synthesis from them are typically increased when ACTH levels are increased<sup>33</sup>, which can occur with Cushing syndrome, adrenal carcinoma, ACTH-producing tumors, or 11 $\beta$ -hydroxylase deficiency, a more rare form of CAH<sup>34</sup> than seen with 21-hydroxylase deficiency, which constitutes > 95% of all CAH cases

## Cortisol

Under the direction of the hypothalamus and pituitary, and controlled by a negative feedback loop, the zona fasciculata of the adrenal cortex is stimulated to produce cortisol in response to circadian peaks and troughs in ACTH synthesis in addition to various types of stressors such as emotional/psychological, physical (injury, exercise), chemical, pathological (viruses,

bacteria, etc.). The feedback loop is commonly referred to as the hypothalamic-pituitary-adrenal (HPA) axis. In a normal non-stressed state cortisol production is at its highest upon waking and declines steadily during the day, reaching its lowest point at bedtime<sup>35</sup>. Cortisol has a wide range of effects on mind and body and interacts with the reproductive, thyroid and immunological systems. As part of the stress response, it prepares the body for “fight or flight” by regulating epinephrine (adrenaline) biosynthesis, and in doing so it can suppress the production of other hormones, including those involved with reproduction, and some immune functions. When cortisol levels remain high as a result of chronic exposure to stressors, this suppression of other systems is maintained for longer than normal and can result in susceptibility to infection, hypothyroidism, bone loss, infertility, and low libido. On the other hand, lower than normal cortisol levels can result from adrenal insufficiency or HPA axis dysfunction, and are associated with decreasing attention span, fatigue, allergies, and blood sugar imbalances<sup>36</sup>. Salivary cortisol measurements are considered the gold standard in testing stress, in screening for Cushing’s disease<sup>37</sup>, and in evaluations of adrenal insufficiency, such as Addison’s disease.

### **Cortisone**

Salivary cortisone<sup>38</sup> is an inert form of cortisol, as is estrone to estradiol. Simultaneous testing for cortisol and cortisone assists in diagnosing acquired or inherited abnormalities of 11 $\beta$ -hydroxy steroid dehydrogenase, affecting the cortisol to cortisone ratio<sup>39</sup>. Deficiency of 11 $\beta$ -HSD results in a state of mineralocorticoid excess because cortisol, not cortisone, acts as a mineralocorticoid receptor agonist.

### **Corticosterone**

Corticosterone is a precursor molecule for aldosterone. It is produced from 11-deoxycorticosterone and is converted to aldosterone in the mineralocorticoid pathway. Corticosterone is the first intermediate in the mineralocorticoid pathway with significant corticoid activity. The utility of measuring this intermediate is for the diagnosis of disorders of steroid synthesis, such as CAH<sup>40</sup>.

### **Aldosterone**

Aldosterone is a hormone produced in the outer section of the adrenal glands. It plays a central role in the regulation of blood pressure, mainly by acting on organs to increase the amount of salt reabsorbed into the bloodstream and to increase the amount of potassium excreted in the urine. Aldosterone also causes water to be reabsorbed along with sodium, which increases blood volume and therefore blood pressure<sup>41</sup>. Measurement is useful for investigating primary and secondary aldosteronism<sup>42</sup>. The most common cause of high levels is excess production, which could be from an adrenal tumor. Low aldosterone levels are found in Addison’s disease, which is characterized by a general loss of adrenal function resulting in low blood pressure, lethargy,

and an increase in potassium levels in the blood<sup>43</sup>.

### **Melatonin**

Melatonin is a hormone that is produced from the pineal gland in a circadian pattern and plays a role in the initiation of sleep. The production and release of this hormone is connected to the time of day, ideally increasing when it is dark and decreasing when it is light. Melatonin and cortisol follow opposite circadian patterns but are not cross-regulated in a negative feedback manner. The production of melatonin decreases with age<sup>44</sup>. Treatment with melatonin may be useful in people with circadian rhythm sleep disorders, such as delayed sleep phase disorder, jet lag, shift worker disorder, and the non-24-hour sleep-wake disorder most commonly found in totally blind individuals<sup>45</sup>.

### **Anastrozole**

Anastrozole is a medication that inhibits the enzyme aromatase to suppress testosterone conversion to estrogens. used in combination with other treatments for suppressing testosterone conversion to estrogens. It can be used in combination with other treatments, typically men using testosterone therapy to prevent conversion to estrogens; and in breast cancer and prostate cancer patients to inhibit endogenous estrogen production that could stimulate estrogen-sensitive tumor growth. It is most often used for hormone-receptive breast cancer<sup>46</sup>. It works by binding to the aromatase enzyme and blocking the conversion of androgens to estrogens in peripheral tissues. Off-label it is commonly used to decrease the production of estrogen in men<sup>47</sup> and is also used as part of a treatment plan for women with endometriosis<sup>48</sup>.

### **Finasteride**

Finasteride is a 5 $\alpha$ -reductase inhibitor used to block the formation of the potent androgen DHT from its precursor, testosterone. It is used to treat benign prostatic hyperplasia as well as male pattern baldness in men and women, and excessive facial or body hair growth in women. Serum testosterone levels increase as a result of the reduced conversion to DHT, but the increase is not usually outside the normal range<sup>49</sup>. Treatment with finasteride has been linked with sexual side effects<sup>50,51</sup>.

### **Letrozole**

Letrozole is an aromatase inhibitor used as an adjuvant treatment for hormone-dependent breast cancer. It inhibits peripheral estrogen production in fat tissues, where it prevents the conversion of testosterone into estradiol. It has similar safety and efficacy to anastrozole<sup>52</sup>.



## References

1. Worthman CM, Stallings JF, Hofman LF. Sensitive salivary estradiol assay for monitoring ovarian function. *Clin Chem* 1990;36:1769-73.
2. Wong YF, Mao K, Panesar NS, et al. Salivary estradiol and progesterone during the normal ovulatory menstrual cycle in Chinese women. *Eur J Obstet Gynecol Reprod Biol* 1990;34:129-35.
3. Capobianco G, Donolo E, Borghero G, et al. Effects of intravaginal estriol and pelvic floor rehabilitation on urogenital aging in postmenopausal women. *Arch Gynecol Obstet*. 2012;285:397-403.
4. Hampson E, Phillips SD, Soares CN, Steiner M. Steroid concentrations in antepartum and postpartum saliva: normative values in women and correlations with serum. *Biol Sex Differ*. 2013;4:7.
5. Soghra K, Zohreh S, Kobra AK, Reza MM. Single measurement of salivary estriol as a predictor of preterm birth. *Pak J Biol Sci*. 2014;17:730-4.
6. Simpson ER. Sources of estrogen and their importance. *J Steroid Biochem Mol Biol*. 2003;86:225-30.
7. Jones ME, Schoemaker M, Rae M, et al. Changes in estradiol and testosterone levels in postmenopausal women after changes in body mass index. *J Clin Endocrinol Metab*. 2013;98:2967-74.
8. Zimmerman Y, Eijkemans MJ, Coelingh Bennink HJ, et al. The effect of combined oral contraception on testosterone levels in healthy women: a systematic review and meta-analysis. *Hum Reprod Update*. 2014;20:76-105.
9. Warnock JK, Clayton A, Croft H, et al. Comparison of androgens in women with hypoactive sexual desire disorder: those on combined oral contraceptives (COCs) vs. those not on COCs. *J Sex Med*. 2006;3:878-882.
10. Wu FS, Gibbs TT, Farb DH. Pregnenolone sulfate: a positive allosteric modulator at the N-methyl-D-aspartate receptor. *Mol Pharmacol*. 1991;40(3):333-6.
11. Weng JH, Chung BC. Nongenomic actions of neurosteroid pregnenolone and its metabolites. *Steroids*. 2016;111:54-59.
12. Ritsner MS, Bawakny H, Kreinin A. Pregnenolone treatment reduces severity of negative symptoms in recent-onset schizophrenia: an 8-week, double-blind, randomized add-on two-center trial. *Psychiatry Clin Neurosci*. 2014;68(6):432-40.
13. Heydari B, Le Mellédo JM. Low pregnenolone sulphate plasma concentrations in patients with generalized social phobia. *Psychol Med*. 2002;32(5):929-33.
14. Ishikawa M, Sengoku K, Tamate K, et al. The clinical usefulness of salivary progesterone measurement for the evaluation of the corpus luteum function. *Gynecol Obstet Invest* 2002;53:32-7.
15. Meulenberg PM, Hofman JA. Salivary progesterone excellently reflects free and total progesterone in plasma during pregnancy. *Clin Chem* 1989;35:168-72.
16. Du JY, Sanchez P, Kim L, et al. Percutaneous progesterone delivery via cream or gel application in postmenopausal women: a randomized cross-over study of progesterone levels in serum, whole blood, saliva, and capillary blood. *Menopause*. 2013;20:1169-75.
17. Bäckström T, Bixo M, Johansson M, et al. Allopregnanolone and mood disorders. *Prog Neurobiol*. 2014;113:88-94.
18. Schüle C, Nothdurfter C, Rupprecht R. The role of allopregnanolone in depression and anxiety. *Prog Neurobiol*. 2014;113:79-87.
19. Mezzullo M, Fazzini A, Gambineri A, et al. Parallel diurnal fluctuation of testosterone, androstenedione, dehydroepiandrosterone and 17OHprogesterone as assessed in serum and saliva: validation of a novel liquid chromatography-tandem mass spectrometry method for salivary steroid profiling. *Clin Chem Lab Med*. 2017;55:1315-1323.
20. de Groot MJ, Pijnenburg-Kleizen KJ, Thomas CM, et al. Salivary morning androstenedione and 17 -OH progesterone levels in childhood and puberty in patients with classic congenital adrenal hyperplasia. *Clin Chem Lab Med*. 2015;53:461-8.
21. Baxendale PM, Jacobs HS, James VH. Plasma and salivary androstenedione and dihydrotestosterone in women with hyperandrogenism. *Clin Endocrinol (Oxf)*. 1983;18:447-57.
22. Young MC, Walker RF, Riad-Fahmy D, Hughes IA. Androstenedione rhythms in saliva in congenital adrenal hyperplasia. *Arch Dis Child*. 1988;63:624-8.
23. Arregger AL, Contreras LN, Tumilasci OR, et al. Salivary testosterone: a reliable approach to the diagnosis of male hypogonadism. *Clin Endocrinol (Oxf)* 2007;67:656-62.
24. Baxendale PM, Jacobs HS, James VH. Salivary testosterone: relationship to unbound plasma testosterone in normal and hyperandrogenic women. *Clin Endocrinol (Oxf)* 1982;16:595-603.
25. Miller KK. Androgen deficiency in women. *J Clin Endocrinol Metab*. 2001;86:2395-401.
26. Bartsch G, Rittmaster RS, Klocker H. Dihydrotestosterone and the concept of 5 alpha-reductase inhibition in human benign prostatic hyperplasia. *World J Urol* 2002;19:413-425.

27. Okeigwe I, Kuohung W. 5-Alpha reductase deficiency: a 40-year retrospective review. *Curr Opin Endocrinol Diabetes Obes.* 2014;21:483-7.
28. Baulieu EE, Thomas G, Legrain S, et al. Dehydroepiandrosterone (DHEA), DHEA sulfate, and aging: contribution of the DHEAge Study to a sociobiomedical issue. *Proc Natl Acad Sci USA.* 2000;97:4279-84.
29. Allolio B, Arlt W, Hahner S. DHEA: why, when, and how much-DHEA replacement in adrenal insufficiency. *Ann Endocrinol (Paris).* 2007;68:268-73.
30. Arlt W. Androgen therapy in women. *Eur J Endocrinol* 2006;154:1-11.
31. Sedláková B, Dušátková L, Zamrazilová H, et al. 7-oxygenated derivatives of dehydroepiandrosterone and obesity. *Prague Med Rep.* 2012;113:147-55.
32. Hampl R, Stárka L, Janský L. Steroids and thermogenesis. *Physiol Res.* 2006;55:123-31.
33. Lindner JM, Suhr AC, Grimm SH, et al. The dynamics of a serum steroid profile after stimulation with intravenous ACTH. *J Pharm Biomed Anal.* 2018;151:159-163.
34. Taylor DR, Ghataore L, Couchman L, et al. A 13-Steroid Serum Panel Based on LC-MS/MS: Use in Detection of Adrenocortical Carcinoma. *Clin Chem.* 2017;63:1836-1846.
35. Gozansky WS, Lynn JS, Laudenslager ML, Kohrt WM. Salivary cortisol determined by enzyme immunoassay is preferable to serum total cortisol for assessment of dynamic hypothalamic-pituitary-adrenal axis activity. *Clin Endocrinol (Oxf)* 2005;63:336-41.
36. Jerjes WK, Cleare AJ, Wessely S, et al. Diurnal patterns of salivary cortisol and cortisone output in chronic fatigue syndrome. *J Affect Disord.* 2005;87:299-304.
37. Cardoso EM, Arregger AL, Tumilasci OR, Contreras LN. Diagnostic value of salivary cortisol in Cushing's syndrome (CS). *Clin Endocrinol (Oxf)* 2009;70:516-21.
38. Taylor RL, Machacek D, Singh RJ. Validation of a high-throughput liquid chromatography-tandem mass spectrometry method for urinary cortisol and cortisone. *Clin Chem* 2002;48:1511-19.
39. Zhang Q, Chen Z, Chen S, et al. Intraindividual stability of cortisol and cortisone and the ratio of cortisol to cortisone in saliva, urine and hair. *Steroids.* 2017;118:61-67.
40. Tonetto-Fernandes V, Lemos-Marini SH, Kuperman H, et al. Serum 21-deoxycortisol, 17-hydroxyprogesterone, and 11-deoxycortisol in classic congenital adrenal hyperplasia: clinical and hormonal correlations and identification of patients with 11 beta-hydroxylase deficiency among a large group with alleged 21-hydroxylase deficiency. *J Clin Endocrinol Metab* 2006;91:2179-84.
41. Atherden SM, Corrie JE, Jones DB, et al. Development and application of a direct radioimmunoassay for aldosterone in saliva. *Steroids.* 1985;46:845-55.
42. Vaidya A, Mulatero P, Baudrand R, Adler GK. The Expanding Spectrum of Primary Aldosteronism: Implications for Diagnosis, Pathogenesis, and Treatment. *Endocr Rev.* 2018;39:1057-1088.
43. Michels A, Michels N. Addison disease: early detection and treatment principles. *Am Fam Physician.* 2014;89:563-8.
44. Graham D, McLachlan A. Declining melatonin levels and older people. How old is old? *Neuro Endocrinol Lett.* 2004;25:415-8.
45. Arendt, J. Melatonin: characteristics, concerns, and prospects. *J Biol Rhythms* 2005;20: 291-303.
46. Margolese RG, Cecchini RS, Julian TB, et al. Anastrozole versus tamoxifen in postmenopausal women with ductal carcinoma in situ undergoing lumpectomy plus radiotherapy (NSABP B-35): a randomised, double-blind, phase 3 clinical trial. *Lancet.* 2016;387:849-56.
47. Glaser RL, York AE. Subcutaneous Testosterone Anastrozole Therapy in Men: Rationale, Dosing, and Levels on Therapy. *Int J Pharm Compd.* 2019;23:325-339.
48. Abu Hashim H. Potential role of aromatase inhibitors in the treatment of endometriosis. *Int J Womens Health.* 2014;6:671-80.
49. Steiner JF. Clinical pharmacokinetics and pharmacodynamics of finasteride. *Clin Pharmacokinet.* 1996;30:16-27.
50. Lee S, Lee YB, Choe SJ, Lee WS. Adverse Sexual Effects of Treatment with Finasteride or Dutasteride for Male Androgenetic Alopecia: A Systematic Review and Meta-analysis. *Acta Derm Venereol.* 2019;99:12-17.
51. Corona G, Tirabassi G, Santi D, et al. Sexual dysfunction in subjects treated with inhibitors of 5-reductase for benign prostatic hyperplasia: a comprehensive review and meta-analysis. *Andrology.* 2017;5:671-678.
52. Smith I, Yardley D, Burris H, et al. Comparative Efficacy and Safety of Adjuvant Letrozole Versus Anastrozole in Postmenopausal Patients With Hormone Receptor-Positive, Node-Positive Early Breast Cancer: Final Results of the Randomized Phase III Femara Versus Anastrozole Clinical Evaluation (FACE) Trial. *J Clin Oncol.* 2017;35:1041-1048.