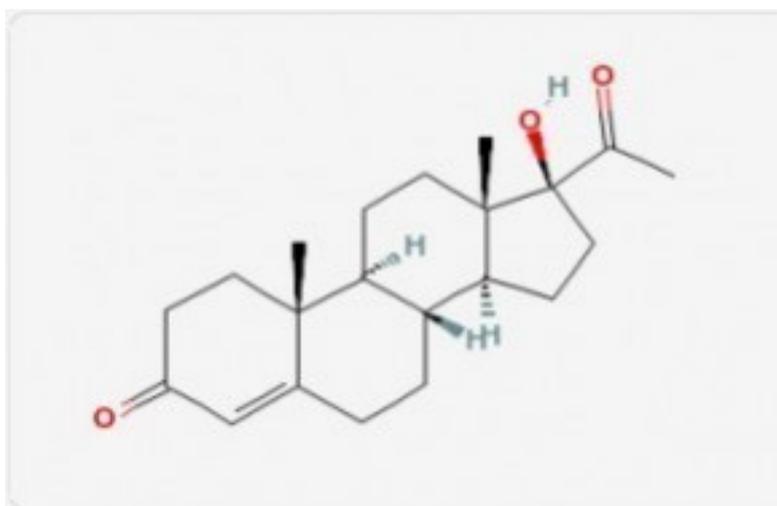
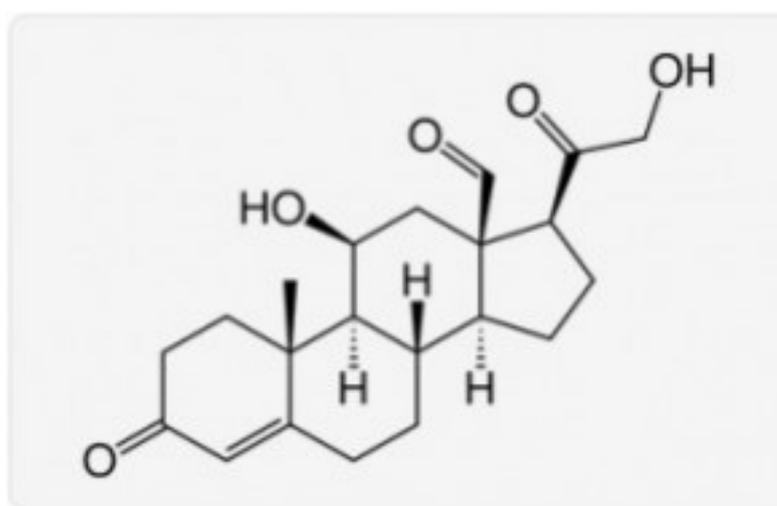


## Salivary 17 $\alpha$ -Hydroxyprogesterone



17 $\alpha$ -hydroxyprogesterone (17-OH progesterone or 17OHP) is a steroid hormone produced in the adrenal gland and gonads. (1,2,3) It is synthesized from progesterone, and it serves primarily as a precursor compound that is converted into cortisol in the adrenal gland, or into androgenic and estrogenic steroid hormones in the gonads. (3,4) 17-OHP is routinely used for the diagnostic assessment of 21-hydroxylase deficiency, which is linked to congenital adrenal hyperplasia, impaired aldosterone synthesis, and fatal salt-wasting. (5,6,7,8,9) 17-OHP exhibits a diurnal rhythm with higher values in the morning and decreasing over the day to a nadir in the evening. (10,11) 17-OHP enters saliva from blood via intracellular mechanisms, and there is excellent correlation between saliva and serum values. (12)

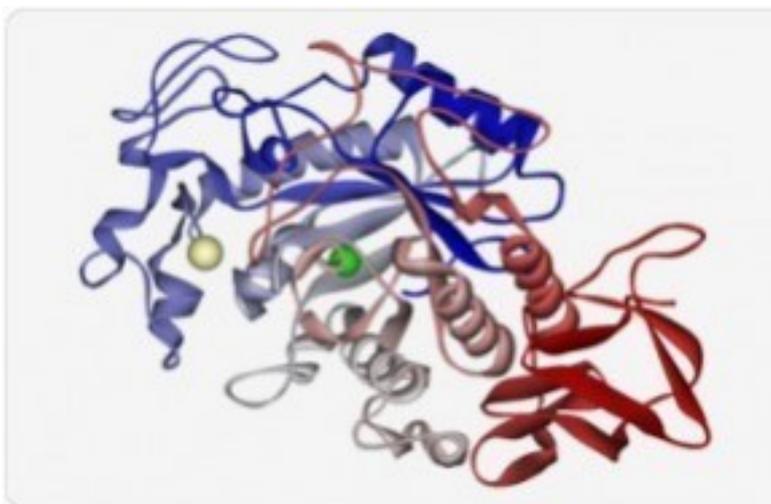
## Salivary Aldosterone



Recent studies have shown that primary aldosteronism (PA) is the most common cause of secondary hypertension with a prevalence of approximately 5-10% among all hypertensive patients and an even higher prevalence among selected patients with advanced stages of hypertension and resistant hypertension. (1) Screening for PA among hypertensive patients is important due to its association

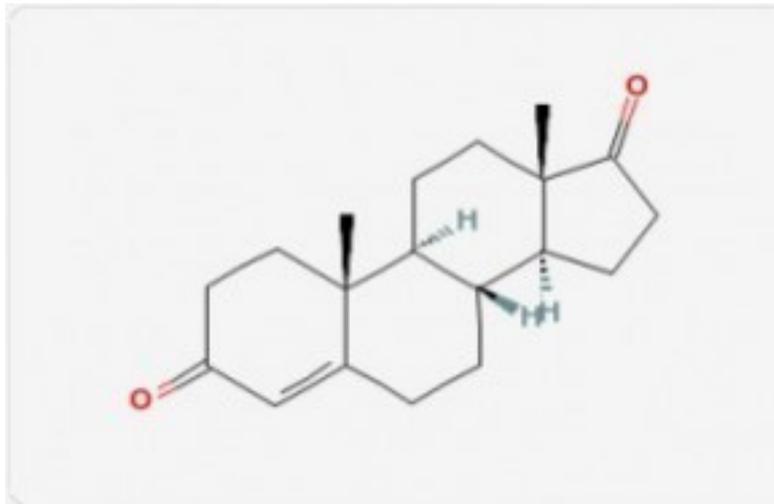
with risk for cardiovascular disease and renal damage, (1) and non-invasive salivary aldosterone measurements have recently been explored as a means to facilitate this screening. Morning salivary aldosterone measurements alone were found to have some ability to discriminate between patients with PA and essential hypertension, and the presence or absence of a diurnal decline showed promise for distinguishing between the two forms of the disease (adenomas vs. bilateral hyperplasia). (2) Dysregulation of circulating aldosterone levels has also been associated with psychiatric disorders, and a recent study has similarly reported a significant negative association between morning salivary aldosterone levels and trait anxiety scores. Those with the high anxiety trait may be associated with an inability to respond with adequate cortisol levels during stress. (3) Produced largely in the adrenal glands, aldosterone is classified as a mineralocorticoid steroid since its classical effect is to regulate the transport of sodium and water across cells of the kidney in exchange for potassium and hydrogen ions, thereby regulating blood volume and pressure. (4,5) Additionally, rapid non-genomic actions and local production of aldosterone have been identified in other tissues, including the heart, vascular system, adrenal gland, and kidney. These non-genomic mechanisms are being studied in connection with a range of diseases including cardiovascular disease, cirrhosis, kidney disease, insulin resistance, and diabetes. (6,7,8) No specific binding protein for aldosterone has been identified in blood. (4) Circulating aldosterone not bound to serum proteins enters saliva by passive diffusion. (9) Salivary aldosterone levels correspond approximately to 30% of those found in plasma, with good correlation found between plasma and non-extracted salivary aldosterone. (10) Salivary aldosterone levels are unaffected by salivary flow rate or hormone-binding proteins. (11) Both salivary and plasma aldosterone increase significantly while standing, compared to being seated; this effect is significantly higher in females than in males. A diurnal rhythm for salivary aldosterone exists for healthy individuals, with highest levels in the morning. (10)

### Salivary Alpha-Amylase



Alpha-Amylase (or  $\alpha$ -Amylase) is a digestive enzyme that hydrolyses alpha-1,4 bonds of large polysaccharides such as starch and glycogen, yielding the smaller by-products of glucose and maltose. (1) Alpha amylase is synthesized in the acinar cells of the saliva glands and stored in secretory granules inside the cells. (2) Its release from the salivary cells is greatly increased in response to taste or chewing motions of the jaw. (3,4,5) Salivary  $\alpha$ -amylase levels are not related to  $\alpha$ -amylase levels in blood, which are derived from pancreatic secretion. (1) In addition to its digestive function, alpha-amylase also plays an important anti-bacterial role in the oral cavity; for this reason it is present in lower levels in non-stimulated saliva between meals. (6,7) Salivary  $\alpha$ -amylase exhibits a diurnal rhythm, with a pronounced decrease within 60 minutes after awakening and a steady increase of activity during the course of the day. (8) Alpha-amylase production in the saliva glands increases in response to psychological and physical stress through interactions with the autonomic nervous system, and it has been found to be a useful as a marker of activity in the autonomic nervous system. (9,10,11) Salivary alpha-amylase levels have been used as a biomarker of ANS activity in various fields of biobehavioral research. (12,13,14,15)

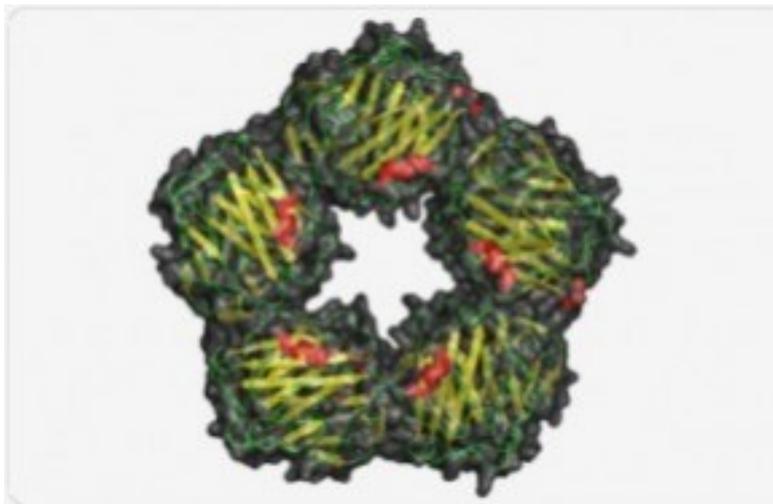
### Salivary Androstenedione



Androstenedione (4-androstenedione; 4-dione; 4-androstene-3,17-dione) is a steroid hormone produced in the adrenal glands and the gonads. (1,2,3) It is synthesized from DHEA or 17OH-progesterone, and it serves principally as the immediate precursor compound that is converted into testosterone or estrone, both of which may then be further converted into estradiol. (4) The same sequence of conversion of DHEA and androstenedione into other steroids also takes place in many peripheral tissues throughout the body, allowing androgens and estrogens to be delivered to the appropriate tissues without leakage of significant amounts into the circulation. (5,6) Levels of androstenedione begin to increase in children at about age 6-8, and it serves as the main source of androgens prior to gonadarche. (7) High levels of androstenedione may confer androgenic risk, especially in females, and estrogenic risks, especially in males. Children and adolescents are particularly vulnerable to the effects of androstenedione conversion to active sex steroids. These effects may disrupt

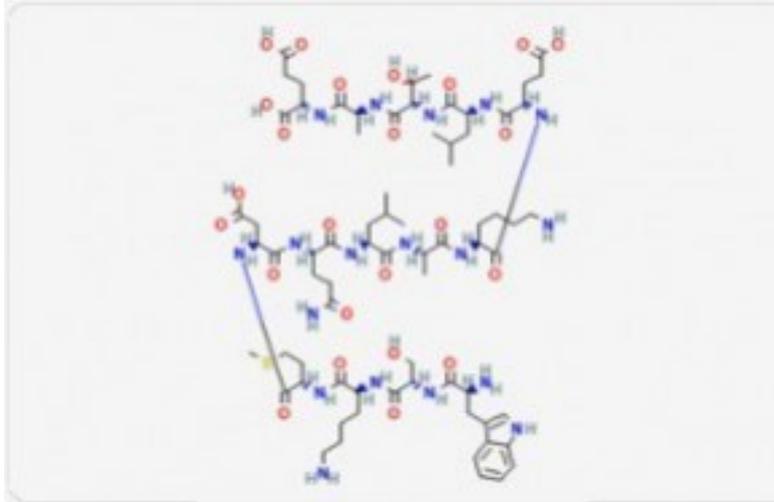
normal sexual development, specifically virilization in girls associated with severe acne, excessive body hair, disruption of the menstrual cycle, and infertility. The conversion of androstenedione to estrogens can cause feminization of boys. (8,9) Elevated levels have been associated with disruptive behavior disorders in children. (7) Measurement of serum androstenedione is used as a marker of androgen biosynthesis. High circulating androstenedione levels are indicated in virilizing congenital adrenal hyperplasia, polycystic ovarian syndrome, and other causes of hirsutism in women. Elevated androstenedione levels may also occur as a result of adrenal or ovarian tumors. (10,11) Androstenedione exhibits a diurnal rhythm similar to that of cortisol, with highest levels in the morning and a nadir in the late evening. (12,13) In blood, androstenedione is not strongly bound to sex hormone binding globulin (SHBG) or albumin. Approximately 95% of circulating androstenedione is available to tissues. (14) Unbound androstenedione enters saliva from blood via intracellular mechanisms, and the correlation between serum and saliva values is highly significant. (15)

### Salivary C-Reactive Protein



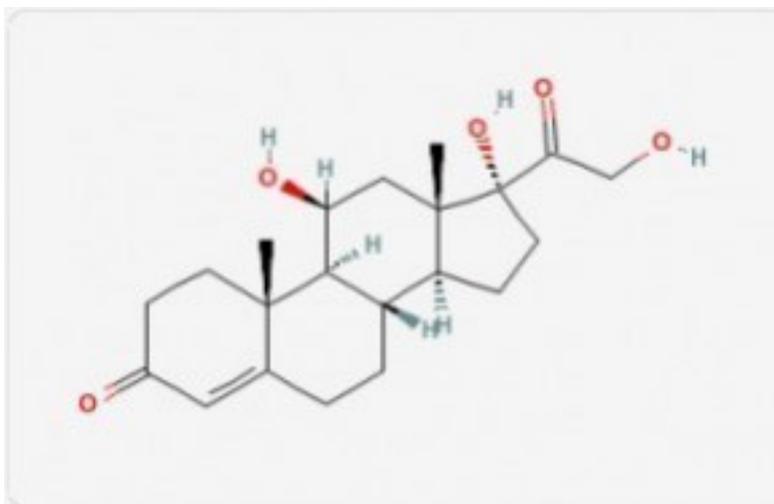
C-Reactive Protein (CRP) is an acute phase protein produced in the liver. Serum CRP measurements are widely used as a bio-marker of inflammation in the body. Elevated levels of serum CRP have been tied to increased risk for heart disease, hypertension, stroke, and other conditions related to inflammation, such as diabetes and autoimmune disorders. (1,2,3,4,5,6,7,8,9) The relationship between serum and salivary levels of CRP is not well understood. Salivary CRP levels have been found to be higher in children with allergic asthma, and in pigs with respiratory viral infections. (10,11) In an unpublished study, sick people admitted to a hospital had average salivary CRP levels 25 times higher than healthy people. (12) Salivary CRP may largely reflect local inflammation in the mouth, but some serum CRP can enter saliva through gingival tissues, especially if periodontal disease is present. (13,14) Ongoing research is investigating the possibility that salivary CRP can be used to monitor inflammation in other parts of the body. (15,16,17,18,19,20)

## Salivary Chromogranin A



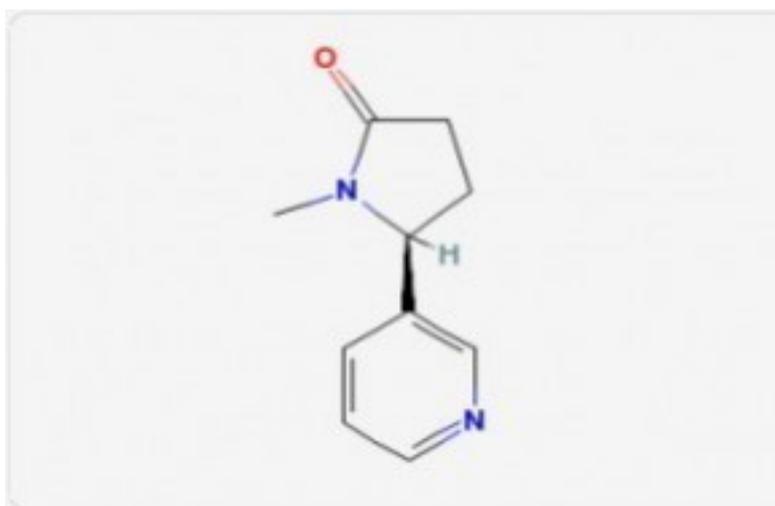
Chromogranin A (CgA, also known as parathyroid secretory protein 1) is an acidic secretory protein belonging to the granin family, found in many types of neuroendocrine tissues. It is stored in secretory vesicles with other types of neurotransmitters and peptide hormones. Granins play an important role in the sorting and aggregation of secretory products in the trans-golgi network, and in the subsequent formation of secretory granules. (1,2,3,4,5) CgA is co-secreted from sympathetic nerves along with the catecholamines, and it has been shown to be useful as a biomarker of sympathetic nervous activity. CgA production has been found in human submandibular saliva glands, and CgA can be measured in saliva. The correlation between saliva and serum CgA is not well understood. (5,6,7) A circadian rhythm for CgA in normal subjects has been reported, with peak values during the night around 11:00 and a nadir in the morning around 8:00. (8)

## Salivary Cortisol



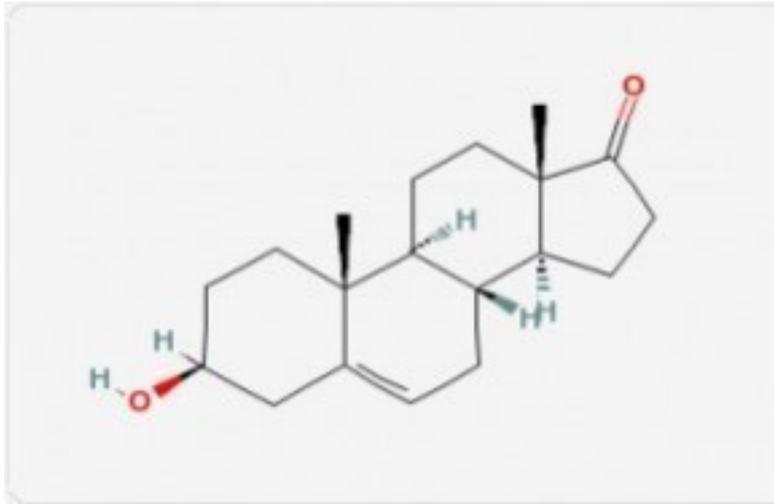
Cortisol (hydrocortisone, Compound F) is the major glucocorticoid hormone produced in the adrenal cortex. Cortisol is actively involved in the regulation of calcium absorption, blood pressure maintenance, anti-inflammatory function, gluconeogenesis, gastric acid and pepsin secretion, and immune function. (1,2,3) Cortisol production has a circadian rhythm. (4) Levels peak in the early morning and drop to the lowest concentration at night. (5) Levels rise independently of circadian rhythm in response to stress. (6) Increased cortisol production is associated with Cushing's syndrome and adrenal tumors, while decreased cortisol production is associated with adrenal insufficiency (e.g., Addison's disease) and adrenocorticotropic hormone (ACTH) deficiency. (7) In the blood only 1 to 15% of cortisol is in its unbound or biologically active form. The remaining cortisol is bound to serum proteins. (8) Unbound serum cortisol enters the saliva via intracellular mechanisms, and in saliva the majority of cortisol remains unbound to protein. (9) Salivary cortisol levels are unaffected by salivary flow rate or salivary enzymes. (10) Studies consistently report high correlations between serum and saliva cortisol, indicating that salivary cortisol levels reliably estimate serum cortisol levels. (11,12,13)

### Salivary Cotinine



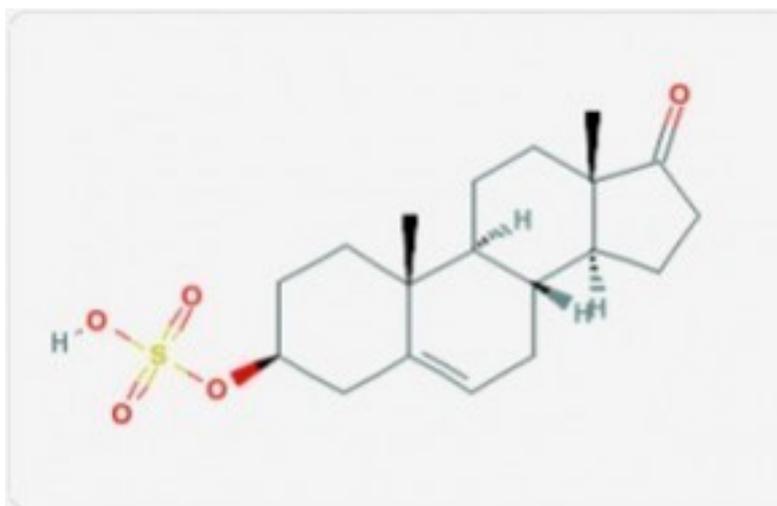
When nicotine from tobacco smoke is taken into the lungs and enters the bloodstream, the principle metabolite produced in the liver is cotinine. Cotinine diffuses easily from blood into saliva, and salivary and blood levels are highly correlated. (1) Cotinine in saliva has a longer half-life than nicotine (more than 10 hours), and the literature has documented it to be a specific and sensitive marker for determining exposure to tobacco and nicotine. (2,3,4)

### Salivary DHEA



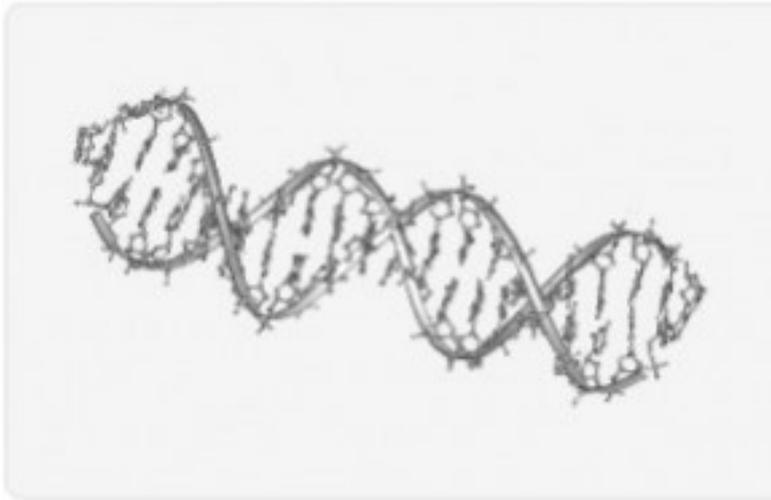
Dehydroepiandrosterone (DHEA; androstenedione; 3 $\beta$ -hydroxy-5-androsten-17-one) is a steroid hormone produced principally in the adrenal cortex. In men, it is estimated that 10-25% of the circulating DHEA is secreted by the testes. (1) DHEA and its sulfated analog DHEA-S serve primarily as precursors that circulate to peripheral tissues, where they are converted to androgens and estrogens. (1,2) This allows androgens and estrogens to be delivered to the appropriate tissues without leakage of significant amounts into the circulation. (3,4,5) In addition to serving as a precursor for other steroid hormones, DHEA is also believed to have some physiological properties of its own. It is known to have anti-glucocorticoid, anti-oxidant, anti-inflammatory, and immunomodulatory effects. (1,5) The mechanisms of these effects are not well understood and currently under investigation. (5,6,7) Circulating levels of DHEA peak around the age of 20 to 30, then decline to only 20-30% of peak level by the age of 70 to 80; it has been explored as a marker of aging and for possible anti-aging therapeutic uses. Low levels have been associated with a range of diseases. (1,5) DHEA is also produced in the brain, where it serves as a protective neurosteroid. (8,9) Like cortisol, DHEA synthesis in the adrenal gland is affected by HPA axis activity and the release of ACTH, and DHEA levels increase in response to stress. (10) Differences in the secretion of the two hormones can exist, however, and changes in the ratio of cortisol to DHEA have been observed in connection with various disorders, including depression, psychiatric conditions, and HIV infection. (11,12,13,14) DHEA exhibits a diurnal rhythm synchronized with cortisol, with highest values in the morning and a nadir in the late evening. (15) In blood DHEA is only weakly bound to albumin or sex hormone binding globulin (SHBG). (16,17) Unbound DHEA enters saliva from blood via intracellular mechanisms, and the serum-saliva correlation is high. (18,19)

Salivary DHEA-S



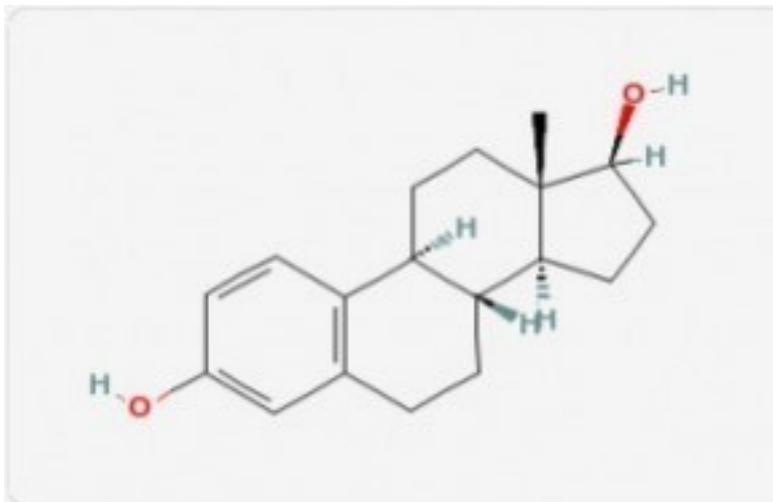
DHEA-S (Dehydroepiandrosterone sulfate or DHEA sulfate) is a steroid hormone produced primarily in the adrenal cortex. It is the sulfated version of the human steroid DHEA, and, like DHEA, it is secreted in response to ACTH. DHEA-S been reported to have a diurnal rhythm, but the findings have varied, and some studies found no variation. (1) DHEA-S in the blood stream has a longer half-life, slower clearance, and is more strongly bound to albumin than DHEA, which may affect synchronicity with the DHEA rhythm. (2,3,4) DHEA-S appears to serve largely as a precursor molecule that is circulated to various target tissues in the body. In those locations, the sulfate is removed to yield DHEA, and the DHEA is then further metabolized into various estrogenic and androgenic compounds. This process allows androgens and estrogens to be delivered to the appropriate tissues without leakage of significant amounts into the circulation. (5,6) DHEA-S is also synthesized directly in the central nervous system, where it is thought to help protect nervous tissues against harmful agents. (7,8) DHEA-S has been investigated for relationships to mental and physical stress and psychological and behavioral disorders. (9,10,11,12,13) DHEA-S is a charged molecule, and it cannot diffuse through the neutral lipid membranes of the salivary cells like the other neutral steroids. The exact mode of entry into saliva is not known. Formerly, it was thought that DHEA-S enters saliva only by squeezing through the tight junctions between cells, and since it is too large to do this readily only small amounts would be present in saliva. (14) More recent work has identified a large family of organic anion transport polypeptides (OATP) that actively transport molecules such as DHEA-S across membranes. It is therefore seems likely that such a mode of entry occurs for DHEA-S into the saliva glands. (15,16) Salivary levels of DHEA-S are quite low—less than 0.1 % of plasma levels in parotid saliva. (14) However, because levels of DHEA-S in blood are 250 and 500 times higher than DHEA in women and men, respectively, (2) the levels found in saliva are high enough to be measurable. Due to the restrictive mode of entry for DHEA-S into saliva, its levels in saliva decrease as salivary flow rates increase. (14) DHEA-S measurements in saliva must therefore be corrected for flow rate. Because of the much higher levels of DHEA-S in blood, it is important to minimize the risk of blood contamination in the saliva samples. Salivary and plasma levels of DHEA-S show a significant positive correlation. (17)

## Salivary DNA Analysis



Salimetrics specializes in saliva research and has expanded to offer genetic testing services, customer support and technical advice. Our DNA specialists use DNA isolation protocols specifically designed for your saliva sample type. Applied Biosystems (AB) TaqMan® technology allows us to target specific species DNA sequences. We offer: Contact us with custom assay project needs. DNA Extraction and Normalization Single Nucleotide Polymorphism (SNP) Genotyping Variable Number Tandem Repeat (VNTR) Analysis Short Tandem Repeat (STR) Analysis

### Salivary Estradiol

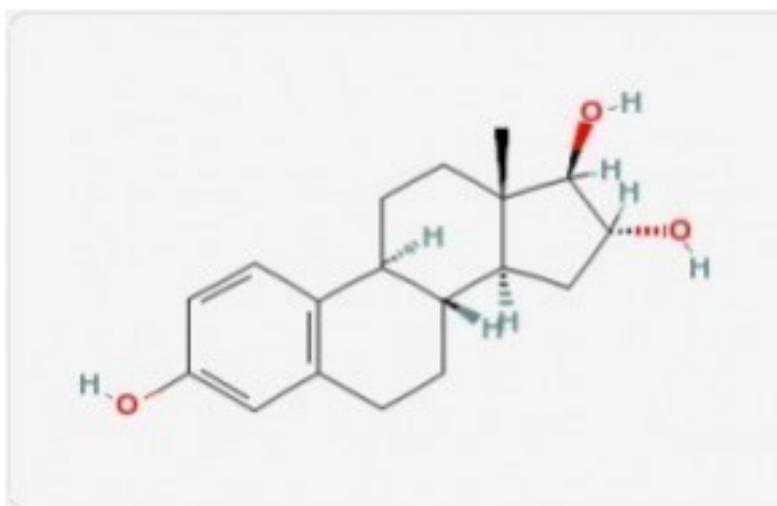


Estradiol (17 $\beta$ -estradiol; E2; 1,3,5(10)-estratriene-3,17 $\beta$ -diol) is one of the three main estrogenic steroid hormones present in humans. It is the most active naturally secreted estrogen. (1) In menstruating women, estradiol is produced primarily by the ovarian follicles from testosterone, with additional amounts produced by extraglandular conversion of testosterone in peripheral tissues.

(1,2,3,4) Concentrations peak mid-cycle, marking ovulation, followed by a rapid decline with a smaller secondary increase during the luteal phase. (5,6) In women of reproductive age, estradiol exhibits a diurnal rhythm where the peaks tend to occur in the early morning; the timing of the peaks is shifted later during the menstrual phase.

There are also ultradian harmonics superimposed upon the basic diurnal rhythm. (7) In post-menstrual women, small amounts of estradiol continue to be made from estrone and testosterone in the peripheral tissues, but estrone, also produced peripherally, replaces it as the predominant form of estrogen. (8,9) In women, estradiol is responsible for the development of secondary sexual characteristics, enhancing breast development and affecting body shape, bones, joints, and fat deposition. (10) In men and pre-pubertal children, estradiol originates principally from extraglandular conversion of androgens; men also have small amounts produced in the testes. (11,12) In men there is no diurnal rhythm to estradiol production. (13) In addition to its role in sexual and reproductive functioning, estradiol also affects other parts of the body, including the cardio-vascular system, the brain, and the immune system. (14,15,16,17) Estradiol also has also been studied for strong relationships with cancers of the breast, ovary, and uterine lining. (18,19,20) In the blood only 1 to 15% of estradiol is in its unbound or biologically active form. The remaining estradiol is bound to serum proteins. Unbound serum estradiol enters saliva via intracellular mechanisms, and in saliva the majority of estradiol remains unbound to protein. (21) The correlation between serum and saliva samples is high. (22,23,24)

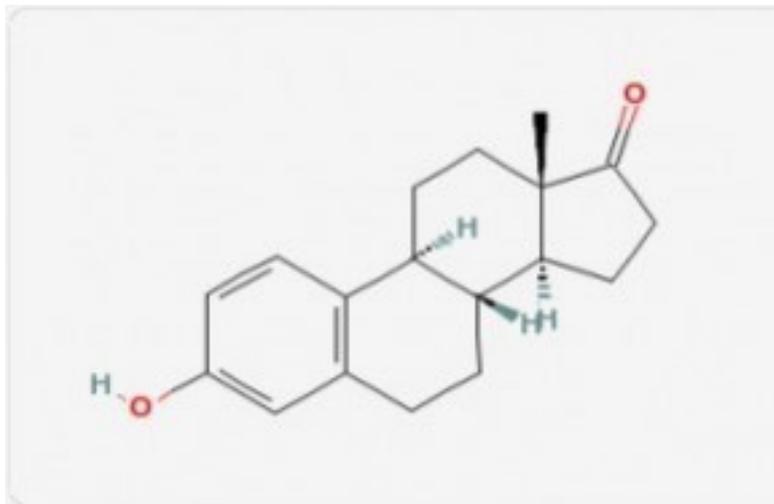
### Salivary Estriol



Estriol (1,3,5(10)-estratriene-3,16 $\alpha$ ,17 $\beta$ -triol; E3) is a female sex steroid hormone largely associated with pregnancy and fetal development. Fetal adrenal DHEA-S is metabolized in the fetal liver to 16 $\alpha$ -hydroxy-DHEA-S, which is then converted to estriol in the placenta. (1,2) By the second trimester, about 90% of the estriol produced is derived from this fetal adrenal DHEA-S. (2) Maternal circulating estriol levels rise progressively during pregnancy, reaching a peak in the third trimester. Production of estriol depends on an intact maternal-placental-fetal unit, and maternal salivary estriol levels have been used to monitor fetal status during pregnancy. (2,3,4,5) Estriol is also used as part of the Tri- or Quad-Screen Test for detection of fetal genetic defects. (6,7) The physiological roles of estriol in non-pregnant women are not well understood and are under investigation. With respect to estrogenic activity, estriol is generally thought to be less potent than estradiol or estrone. However, it has been pointed out that, with regard to nongenomic signaling pathways and functional responses in the pituitary, estriol is a strong estrogen. (8)

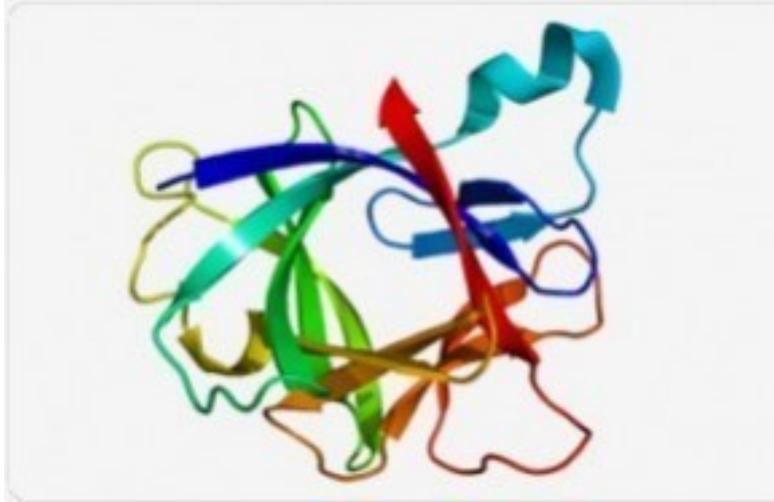
Changes in levels of estriol and the other estrogens that occur due to menopause, pregnancy, and hormone replacement therapy have also been studied extensively for relationships to cancer susceptibility. (9,10,11,12,13,14) Estriol has also been investigated for its relation to autoimmune diseases such as multiple sclerosis and systemic lupus erythematosus, since pregnant women show significant reductions in symptoms during the last trimester of pregnancy. (15,16,17,18,19) Estriol has also been examined for its role in bone and lipid metabolism, and its function as a protective neurosteroid. (20,21) In blood the majority of estriol is bound by serum proteins, with about 14-16 % remaining unbound. (4,22) Unbound estriol enters saliva from blood via intracellular mechanisms, and salivary concentrations closely approximate unbound plasma concentrations. (3,23) There is virtually no protein-bound estriol in saliva. (4) Correlation between serum and saliva samples is highly significant. (24)

### Salivary Estrone



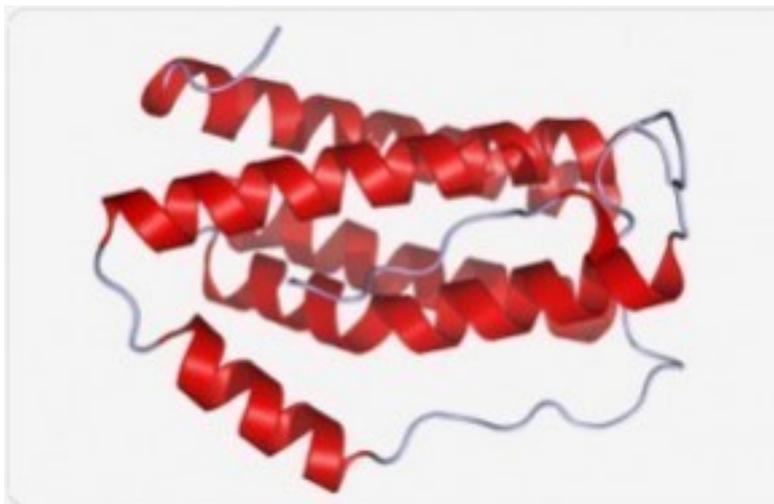
Estrone (3-hydroxy-1,3,5(10)-estratrien-17-one; E1) is one of the three main estrogenic steroid hormones produced in humans. Circulating estrone levels are relatively high at birth in both males and females, decrease postnatally, and increase during puberty. (1) Of the three major estrogens, estrone is predominant after menopause in women. (1) In premenopausal women estrone is primarily produced by conversion of androstenedione in the ovaries, with concentrations peaking in the preovulatory phase and a smaller secondary increase during the luteal phase. (1,2) In post-menopausal women, children, and men, estrone is largely produced by conversion of androstenedione in peripheral tissues. (1,2,3) Estrone is subsequently reduced to estradiol in various peripheral tissues. (3) In non-pregnant women, only about 3% of estrone in the bloodstream is not bound to proteins. (4) Unbound estrone enters saliva from blood via intracellular mechanisms, and in saliva the majority of the estrone remains unbound to protein. The saliva and plasma values are highly correlated. (5)

### Salivary Interleukin-1 Beta



Interleukin-1 $\beta$  (IL-1 $\beta$ ) is one of a family of biologically active small protein molecules known as cytokines. Cytokines are produced by a number of different cell types, including macrophages, monocytes, fibroblasts, and dendritic cells. (1,2,3) IL-1 $\beta$  is an example of a pro-inflammatory cytokine, since it is involved in the body's inflammatory response to acute or chronic infections, or to conditions that cause a persistent low-grade inflammatory state, such as obesity. (4,5) IL-1 $\beta$  is therefore frequently used as a bio-marker of inflammation. (6,7) A study with normal mouse parotid acinar cells has shown that they synthesize IL-1 $\beta$  and store it in secretory granules. The IL-1 $\beta$  is released from the granules following  $\alpha$ - and  $\beta$ -adrenergic stimulation. (8) Relationships between IL-1 $\beta$  levels in blood and saliva are not fully understood.

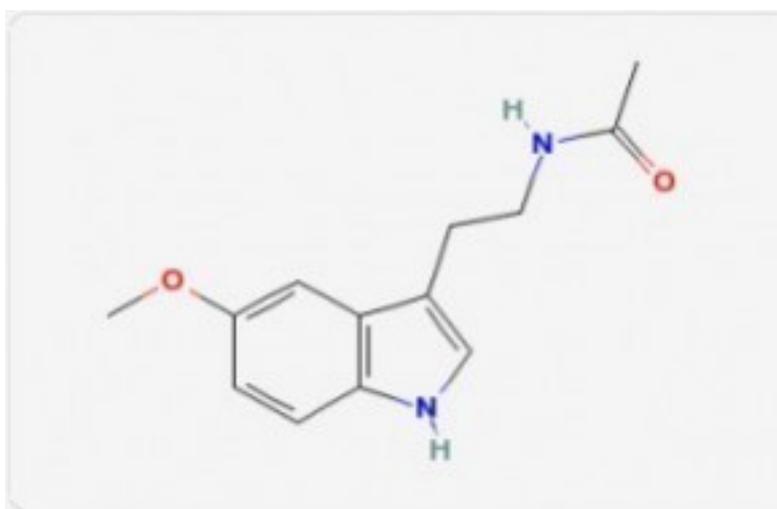
### Salivary Interleukin-6



Interleukin-6 (IL-6) is one of a family of biologically active small protein molecules known as cytokines. It is released by a variety of tissues, including activated leukocytes, adipocytes, and endothelial cells, and it is involved in many processes in the body. IL-6 plays an important role in stimulating the immune response to infection or trauma by inducing the production of acute-phase proteins such as CRP

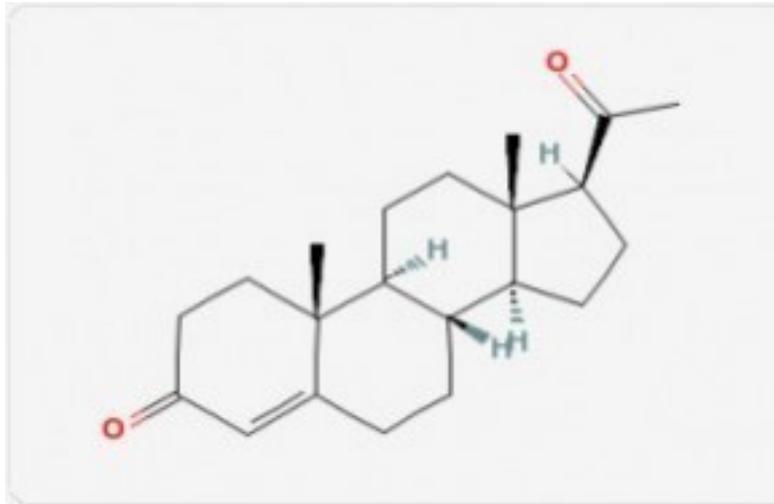
and by fever induction. (1,2,3) In addition to its pro-inflammatory role, IL-6 also has anti-inflammatory properties. (4) Messenger RNA for IL-6 has been found in tissues of healthy human labial salivary glands, and the expression levels of the mRNAs were either up- or down-regulated by adjacent focal infiltrating lymphoid cells. The epithelial cells in the salivary glands are active participants in the autoimmune-mediated process of Sjögren's syndrome, and salivary levels of IL-6 are increased in that disease. (5,6) Salivary IL-6 levels are also increased in periodontal disease. (7) Researchers have found relationships between salivary IL-6 and sleep dysfunction, psychosocial factors, and stress. (8,9,10) A study with mice has shown that normal parotid acinar cells synthesize IL-6 and store it in secretory granules. The IL-6 is released from the granules following  $\alpha$ - and  $\beta$ -adrenergic stimulation. (11)

### Salivary Melatonin



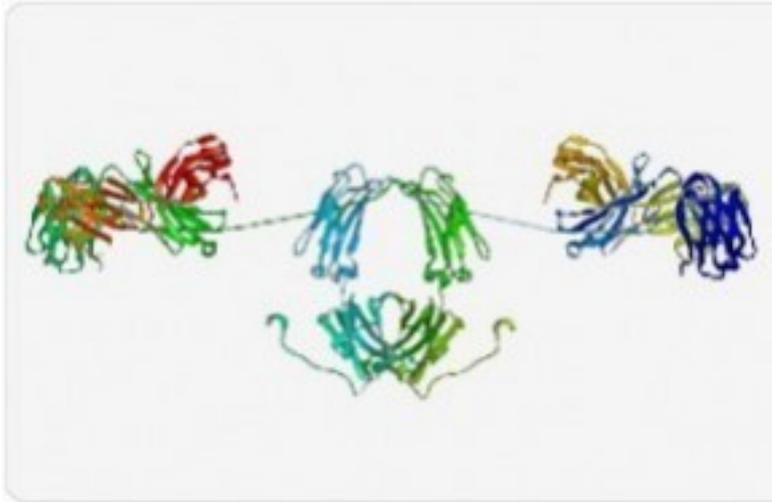
Melatonin (N-acetyl-5-methoxytryptamine) is a naturally occurring hormone found in animals, plants, and microbes. In humans, melatonin is produced primarily by the pineal gland, located in the center of the brain. Melatonin forms part of the system that regulates the sleep-wake cycle by chemically causing drowsiness and lowering the body temperature. Production of melatonin by the pineal gland is inhibited by light and permitted by darkness. Secretion of melatonin, as well as its level in the blood, peaks in the middle of the night and gradually falls during the second half of the night, with normal variations in timing according to an individual's chronotype. (1,2) Melatonin is also important for its ability to scavenge free radicals and to regulate the activity and expression of antioxidant and pro-oxidant enzymes. (3,4,5) In human plasma 61-85% of melatonin is weakly bound to proteins, and there is a close relationship between circulating free and salivary melatonin levels. (6) Melatonin enters saliva from blood either by passive diffusion or active transport. (7) Melatonin is measurable in saliva, and the acrophases of saliva and plasma melatonin rhythms are significantly correlated. (8,9) Plasma and salivary melatonin concentrations increase when moving from a supine to a standing position, and decrease when these positions are reversed, due to changes in plasma volume. (10) Inflammatory processes such as periodontitis trigger an increase in plasma melatonin, which then increases melatonin levels in the oral cavity, where it may increase antioxidant protection. (11)

## Salivary Progesterone



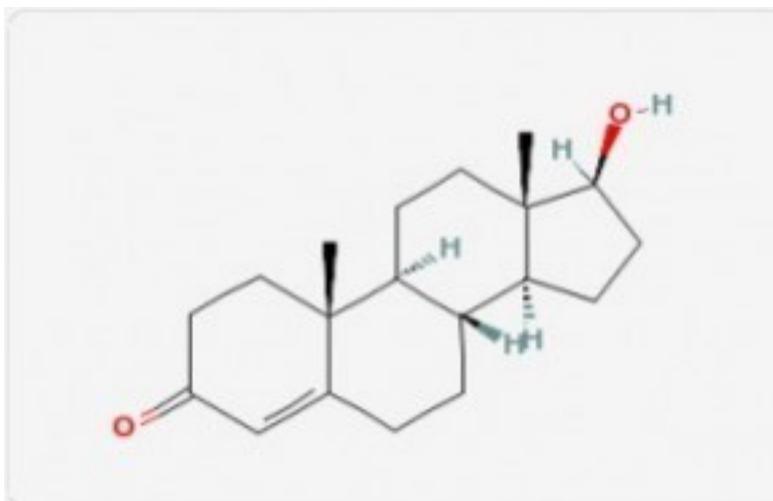
Progesterone (4-pregnen-3,20-dione) is a steroid hormone of primary importance in ovulation, fertility, pregnancy, and menopause. Synthesis of progesterone takes place in the placenta, adrenal glands, and gonads. (1,2,3,4) In normal, non-pregnant women during the mid-luteal phase of the menstrual cycle, progesterone exhibits a prominent circadian rhythm with additional ultradian components. Peak production occurs in the evening around 6 PM. (5) In pregnant women, progesterone also exhibits a similar rhythm during the second and third trimester, with a nadir in the morning and a peak in the late evening. (6) In males, progesterone is thought to play some role in testicular physiology. (7) In addition to its role as a sex hormone, progesterone also serves as a precursor compound for many of the other steroid hormones. Progesterone is also synthesized in the brain and nervous system, where it functions as a neurosteroid that can influence survival and growth of cells, (8,9) and it is involved in brain development and behavior. (8,9,10,11) In blood only 1 to 15% of progesterone is in its unbound or biologically active form. The remaining progesterone is bound to serum proteins. Unbound progesterone enters the saliva via intracellular mechanisms, and the majority of progesterone in saliva is not protein-bound. (12) Correlations obtained between plasma and salivary levels measured in the same subjects have generally been quite high. (13)

## Salivary Secretory Immunoglobulin A



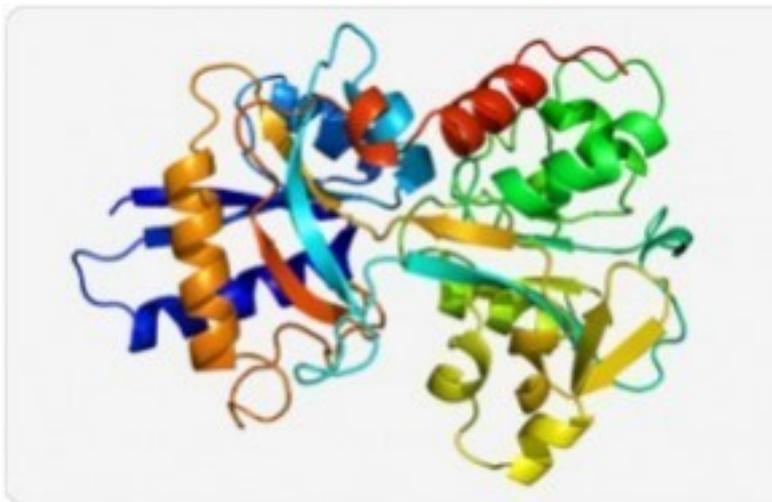
Secretory Immunoglobulin A (SIgA) is a subclass of Immunoglobulin A (IgA), an antibody that plays a critical role in mucosal immunity. SIgA is the main immunoglobulin found in mucous secretions from the tear glands, salivary glands, mammary glands, the respiratory system, the genito-urinary tract, and the gastrointestinal tract. (1) SIgA is not synthesized by mucosal epithelial cells in these structures or derived from blood. Instead, it is produced by B-lymphocytes adjacent to the mucosal cells, then transported through the cell interiors, and released into the secretions from the cells. (2) SIgA plays a key role in protecting vulnerable areas such as the oral cavity, lungs, and gut from invading pathogens. (1) Differences in SIgA levels in the saliva from different glands have been observed in humans, with the highest levels found in the minor saliva glands. (2,3) SIgA exhibits a diurnal rhythm, decreasing from the highest levels in the morning to the lowest in the evening. (4) Levels of SIgA in saliva vary in response to physical and psychological stress through interactions with the autonomic nervous system. (5,6) SIgA levels in saliva are affected by flow rates, with concentrations normally decreasing as flow rates increase. Measurement of flow rates is advisable in order to express SIgA secretion as a function of time. (6)

### Salivary Testosterone



Testosterone is an anabolic steroid hormone synthesized from androstenedione in the Leydig cells of the testes of males and, in smaller quantities, in the ovaries of females. (1) Small amounts are also secreted by the adrenal glands in both sexes. In both men and women a large portion of total testosterone production occurs in peripheral tissues by conversion of circulating DHEA-S, DHEA, and androstenedione. In post-menopausal women, the ovaries and peripheral tissues continue to produce testosterone and other androgens, which then serve as precursors for the synthesis of estradiol in the peripheral tissues. The conversion of precursors into testosterone and the estrogens in peripheral tissues allows these steroids to be delivered to the appropriate tissues without leakage of significant amounts into the circulation, avoiding undesirable effects of high circulating levels. (2,3,4) Testosterone exhibits a diurnal rhythm, with highest levels in the morning and a nadir around midnight. (5,6) In men, testosterone plays an important role in the development of male reproductive tissues including the testis and prostate, as well as promoting secondary sexual characteristics such as increased muscle, bone mass, and hair growth. (7,8) Additionally, testosterone is essential for health and well-being, stamina, sexual function, cardiovascular health, and immune protection. (9,10,11,12) Testosterone measurements are typically used for clinical evaluation of hypogonadism in males and hyperandrogenic states in females. (13,14,15) In blood, only 1 to 15% of testosterone is in its unbound or biologically active form. The remaining testosterone is bound to serum proteins. Unbound testosterone enters the saliva via intracellular mechanisms, and in saliva the majority of testosterone is not protein-bound. (16) The serum-saliva correlation for testosterone is very high for males, but only modest for females, possibly because women's values often fall near the bottom of the measurable range for both serum and saliva immunoassay kits. (17,18)

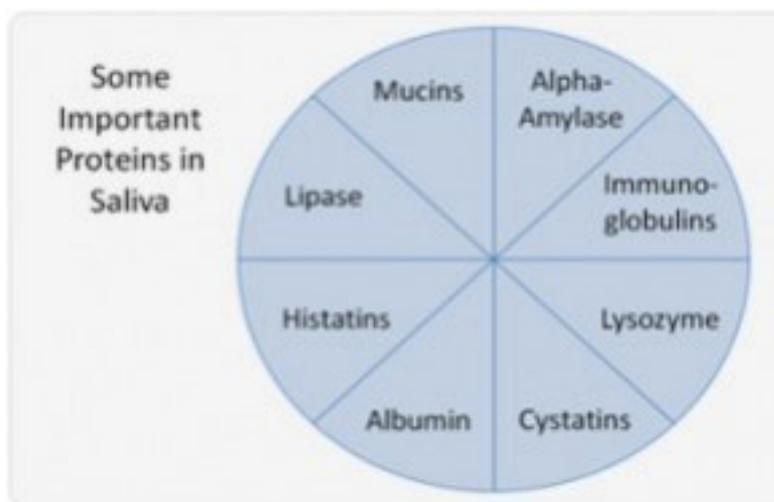
### Salivary Transferrin & Blood Contamination



In the bloodstream the majority of steroid hormones are bound either to non-specific proteins such as albumin or to specific proteins such as corticosteroid binding globulin (CBG) or sex hormone binding globulin (SHBG). These protein molecules are generally too large to diffuse from the blood stream into the saliva glands. Consequently, only the 1-15% of circulating hormone molecules that are

found in an unbound state are free to diffuse from blood into saliva. Steroid hormone concentrations in saliva are therefore much lower than those in blood. If the barrier between the bloodstream and the oral mucosa is compromised by inflammation or microinjury such that there is leakage of blood or plasma into saliva, then there is a possibility that the higher levels of hormones found in the blood stream will contaminate saliva samples, causing falsely high readings. (1) Visual inspection of saliva samples is not adequate to detect blood contamination, since microinjuries to blood vessel membranes may allow hormone molecules and proteins to pass through while still retaining the much larger red blood cells. Dipstick tests for blood, which look for the presence of hemoglobin, are also not a reliable means of screening saliva samples, due to the presence of peroxidases in saliva, which can generate false positive results. (2,3) The Salimetrics Salivary Blood Contamination Enzyme Immunoassay quantitatively measures transferrin, a large protein (mol wt 76,000) present in abundance in blood that is normally present in only trace amounts in saliva. Higher levels of transferrin measured in saliva by this assay indicate the presence of blood contamination and serve as a warning to investigators that samples should be excluded from subsequent quantitative assays for salivary analytes and statistical analyses. (2,3,4) Saliva samples collected from populations that have little or no dental care, or known oral health problems, are especially appropriate for screening with the Blood Contamination Assay Kit.

### Salivary Total Protein



Total protein is a non-specific measure of the total amount of all proteins present in a solution. It is used to examine changes in overall protein secretion in blood or saliva that are associated with disease states, or to look for differences in the ratio of specific proteins (or other analytes) to total protein that exist in different oral fluids or that occur in response to physiological changes or disease states. (1,2,3,4,5,6) Total protein is also sometimes used to normalize concentrations of various salivary proteins such as SIgA in different samples, since concentrations can vary significantly in response to stimulation or alterations of saliva flow. (5) It has been suggested, however, that this practice may be misleading because of differences that exist in the control of secretion of individual salivary proteins among the different salivary glands. (7,8,9) Changes in total protein in saliva and gingival crevicular fluid have

been examined in relation to the presence of periodontal disease, and it may have some use as a marker of certain disease conditions. (10,11) Our laboratory uses a Pierce bicinchoninic acid assay (BCA assay), which is compatible with various chemicals in buffers that can cause interference in other protein determination methods, such as the older Lowry method.