



Rocky Mountain Analytical

Salivary Hormone Analysis

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What is saliva and how do hormones enter saliva?

Saliva is a dilute aqueous fluid (osmolality less than or equal to that of plasma) originating from the salivary glands located under the tongue and along the sides of the mouth. It is a complex mixture of mucins, enzymes, antibodies, electrolytes, and hormones. It serves various functions including digestion, lubrication and protection of the oral mucosa (Vining).

The formation of saliva in a salivary duct begins with electrolytes (particularly sodium) being pumped into the duct by active transport. Water then diffuses into the duct to establish a physiological osmolality. Blood components then enter the duct fluid by one of three processes: active transport, ultrafiltration or passive diffusion (i.e. transfer of molecules down a concentration gradient with no expenditure of energy). Antibodies such as IgA and IgG are pumped into saliva by an energy dependent process. Small molecules like glucose can enter saliva by ultrafiltration through the tight junctions of the cells which line the duct endplates, their rate of entry being inversely related to molecular size. It has been shown that less 1 percent of molecules the size of steroids (MW \approx 300) can pass into saliva via ultrafiltration from interstitial fluid. Unconjugated steroids and other small lipophilic molecules not bound by blood proteins can instead freely negotiate through the membranes of the salivary gland into saliva by passive diffusion. The point here is that in order to see hormones in saliva, they must first have been delivered to tissue, i.e. cell membranes of the salivary apparatus.

Passage of neutral steroids from blood into the salivary ducts is about 10 times faster than the flow rate of saliva (Vining). Because of the rapid passive diffusion of steroids into the saliva ducts, saliva hormones levels are not altered significantly when the flow of saliva is increased with stimulants such as chewing gum. The exception is polar steroids such as DHEA-sulphate (DHEAS). DHEAS is several orders of magnitude more abundant than the unconjugated steroids, and appreciable amounts can still appear in saliva via ultrafiltration. The DHEAS concentration is therefore sensitive to saliva flowrate. Use of saliva stimulants should be avoided if a specimen is being collected for DHEAS.

Bioavailability of Hormones:

In blood 95-99% of the steroids are bound up by binding proteins such as sex hormone binding globulin (SHBG), cortisol binding globulin (CBG), and albumin. The remaining unbound fraction (1-5%) is referred to as “free” hormone, and is generally regarded as bioavailable, since steroid hormone molecules must interact with sites on DNA unencumbered by binding proteins. Numerous studies have shown that endogenous saliva hormone concentrations approximate free hormone concentrations in serum/plasma. When free hormone levels in plasma are measured by means such as equilibrium dialysis, the ratio: saliva hormone level/free plasma hormone level is ≈ 1 (range: 0.4 to 1.5). Free hormone in serum can be estimated as a percentage (usually 1-5%) of total hormone and this same rule of thumb applies for saliva; saliva hormone levels are (1-5%) of serum total hormone levels (See Table 1). The RMA website: www.rmalab.com lists references and abstracts pertaining to the correlation between total and free serum hormone levels and saliva levels. The bottom line is that saliva hormone levels are more or less equivalent to the widely-accepted “bioavailable” hormone levels in blood.

Table 1

Hormone	Typical Total Serum Level	Expected Saliva Level (assumes saliva 2% of total serum)	RMA Measured Saliva Level
Estradiol-female	100 pg/ml	2 pg/ml	0.5-5 pg/ml
Progesterone-female	10 ng/ml	0.2 ng/ml = 200 pg/ml	25-500 pg/ml
Testosterone - male	500 ng/dl = 5 ng/ml	0.1 ng/ml = 100 pg/ml	50-200 pg/ml

Little thought is given to how hormone molecules, floating free in the watery matrix of blood, or bound to proteins, actually reach the nuclei of target cells. The probability that a “free” hormone molecule or a hormone molecule bound to a carrier protein will randomly bump into the cell membrane of a capillary endothelial cell and be “captured” is actually exceedingly small. In reality, we don’t really understand the ways in which steroids penetrate a cell and make their way to the nucleus. For example, there is evidence to suggest that red blood cell-bound steroids are also bioavailable.

It would make sense that hormone carried on red blood cells might be bioavailable, since red cells must wedge their way through capillaries in single file, with portions of their cell membranes in intimate contact with the capillary cell walls for times on the order of 1 second. This sets up the possibility of a very efficient hormone transfer under some circumstances. For example, when hormone creams are applied to the skin, the red cells which pass through the application site likely receive a far larger share of hormone than might otherwise be the case. Since the heart typically pumps the entire blood volume (4-6 litres) every minute or so, these heavily hormone laden red cells will be back to the heart and out to other capillary networks all over the body within the same time window of a minute or so. Hence, with hormone cream use, there is the opportunity for a “hidden” flux of hormone to tissue, a flux which is not measured with conventional blood tests. Saliva hormone levels are inherently more reflective of bioavailability no matter what hormone delivery mode is used, because the saliva hormones represent hormone **which has been delivered to tissue**. Blood levels may be proportional to tissue levels, but they are still indirect measures of hormone delivery to tissue.

ADVANTAGES AND DISADVANTAGES OF SALIVA TESTING

There are numerous advantages to using saliva to test for steroid hormones compared to blood serum or plasma, as well as some disadvantages. These are discussed below:

Advantages of saliva hormone testing:

- ✓ Stress-free
- ✓ Non-invasive (no needles)
- ✓ Less expensive/more convenient for health care provider and patient
- ✓ Flexible collection: time of day/time of month/ location (home vs drawing station)

- ✓ No special processing prior to shipment (e.g. centrifugation, ice-packs)
- ✓ Hormones stable in saliva for prolonged period of time
- ✓ Convenient specimen shipment by Canada Post XPress Post
- ✓ More representative of bioavailable hormone compared to total serum hormone.
- ✓ Wider range of tests available compared to serum

Disadvantages of saliva hormone testing:

- ✗ Technically challenging: pg/ml detection limits in some cases
- ✗ Interfering substances – food, beverages
- ✗ Spurious results with periodontal disease and after toothbrushing
- ✗ Saliva easily contaminated with topical hormones (via lips or hands)
- ✗ Sublingual use of hormones often leads to inaccurate (falsely elevated) results
- ✗ No external testing proficiency program (e.g. CAP)

More technically challenging than serum testing of steroids. Technically, saliva testing is more challenging than blood testing, limiting the number of laboratories that are capable of performing the tests. (Read) Blood levels of steroid hormones are, on average, about 10 to 100 times higher than saliva levels and commercial serum test kits and ranges are designed for these higher levels. For example, salivary estradiol levels range from about 0.5 to 5 pg/ml, close to the sensitivity limit of most commercial kits. Therefore, laboratories performing saliva testing must have the technical expertise to either create their own tests or modify commercial test kits.

Interferences Foods and beverages such as coffee, and drugs taken just before collecting saliva can interfere with test results or cause a transient shift in hormone levels (Lipson). Mucins in saliva can interfere with test results, causing spuriously high levels. Some chewing gums and cottons used for saliva collection contain substances that interfere with some saliva tests (Dabbs), resulting in erroneously high hormone levels. Sugar in regular chewing gum can interfere with some saliva tests. Chewing gum or other physical agents (e.g. parafilm) used to increase the flow of saliva can also cause bleeding of the gingiva, especially if the individual has significant periodontal disease. Small amounts of blood or plasma leaking into saliva from the gingiva can introduce errors in the hormone levels.

Contamination of the saliva collection tube with topical hormones. The saliva can be contaminated inadvertently during collection with hormones present on the hands or lips (from use of topical hormones). This can result in falsely elevated hormone levels. Care must be taken to avoid the use of topical hormones on the face and neck the day before collection. If topical hormones are used, hands must be washed thoroughly to avoid contamination of the tube.

Elevation of results with use of sublingual hormones. Use of sublingual hormone troches can cause spuriously high hormone test results due to direct contamination of the saliva with particles. Individuals who use hormones sublingually at night and collect saliva the following morning will almost certainly get falsely-elevated saliva test results. We recommend consulting the lab before testing, if this delivery mode is used.

No external “Round- Robin” proficiency program. Currently there is no external proficiency program to enable comparison of saliva testing laboratories. This is a significant issue for laboratories which assay saliva directly. This puts the onus on each laboratory to ensure that their methodology delivers the highest possible reproducibility and accuracy. Rocky Mountain Analytical uses a proprietary extraction step before analysis which eliminates many potential sources of interference.

THE IDEAL SALIVA HORMONE TEST REPORT

Ideally, a hormone test report will contain information that serves as a guide to help an individual and his or her health care provider better understand how hormonal imbalances could be affecting health and well being. The hormone test report should also help health care providers and their patients come to a decision about the most effective treatment strategy.

Gender, age, body habitus, menopausal status, menstrual cycle information, and/or surgical status (hysterectomy +/- oophorectomy) all affect hormone levels and should be included on a test requisition and report. The use of hormones (types, doses, delivery systems; oral vs topical) and time of last use all impact test results/expected ranges and should be included in the test report.

The ideal test report includes:

- ✓ Gender/age
- ✓ Height and weight
- ✓ Use of hormones:
 - Types
 - Delivery system (oral vs topical)
 - Dose
 - Time since last use (hours, days)
- ✓ Symptom profile
- ✓ Other medications/supplements

Women:

- ✓ Menopausal status: pre, peri, postmenopausal
- ✓ Menstrual cycle – luteal vs follicular phase
- ✓ Surgical status (hysterectomy/oophorectomy)

The importance of matching symptoms with hormone profiles

The majority of individuals who test their hormones in saliva or blood do so for a reason; they are suffering from symptoms that impact the quality of their life. They recognize that these symptoms are often based on hormonal imbalances. For example, a woman suffering from PMS and fibrocystic breast symptoms, which are getting progressively worse as she approaches menopause, understands that this is due to hormonal changes. She often wants to verify this hormonal imbalance by hormone testing to justify some form of intervention. A man approaching his 50's experiencing low energy, muscle loss, apathy, sagging sex drive and loss of 'get up and go' may recognize that something is wrong and seek hormone testing to determine if his problems are related to dwindling testosterone levels, or an imbalance between testosterone and estradiol.

There is little question that an individual's symptoms are the primary factor motivating them to self educate, seek professional help, and spend money on hormone testing. Documenting these symptoms and understanding their relationship to hormone levels has numerous advantages over just simply knowing the hormone levels. For example, if a woman has normal levels of estradiol, but low progesterone during the luteal phase of her menstrual cycle and she suffers with symptoms of estrogen dominance (fibrocystic breasts, water retention, irritability, PMS) these symptoms help to confirm the hormonal imbalance and point to treatment strategies that can be used to correct the imbalance.

Another reason why it is useful to document symptoms and clinical details is because the health care provider ordering the hormone test often does not fully understand how to interpret the findings. This frequently results in a phone call to the testing laboratory. For a productive and meaningful discussion about the test results, the health care provider and the laboratory professional must both know the patient's age, menopausal status, what hormones they are taking, and their symptoms. If these parameters are not documented on the test report, a time-consuming conversation often ensues in order to reach a mutual understanding of the hormone test results in relation to the patient's primary complaints. Issues are sometimes overlooked in a conversation where both parties lack access to this information.

SUMMARY

In summary, saliva hormone testing is more convenient, less stressful, more cost effective, and more representative of the bioavailable fraction of hormones in blood than serum hormone testing. When hormones are delivered topically, serum hormone testing grossly underestimates both the bioavailable fraction of hormones in blood and the tissue hormone uptake. (This often leads to continued escalation of dosing despite tissue saturation and symptoms of hormone excess. Saliva testing avoids this.) The ideal saliva hormone test report should contain pertinent information not only about an individual's hormone level, but how this relates to symptoms associated with hormone imbalance. Such information helps both health care providers and their patients come to a more educated decision about the most effective treatment strategy (hormonal, nutrition, exercise, stress reduction etc).

APPENDIX:

SALIVARY HORMONE LEVELS WITH TOPICAL/TRANSDERMAL DELIVERY: WHAT DO THE NUMBERS MEAN?

When a hormone is administered topically via skin cream or gel, the saliva level may increase 10-50 times over the normal saliva level for endogenous production of hormone, if saliva is sampled 8-16 hours after application. For example, 30 mg topical progesterone supplementation results in an average rise in salivary levels from about 50 pg/ml (0.05 ng/ml) to between 500 and 3000 pg/ml (0.5 to 3 ng/ml) depending on when the sample is acquired relative to cream application. This increase is dose-dependent and reproducible, but unique to each individual, i.e. the extent of increase may vary from individual to individual. It is seen for all steroid hormones. Furthermore, concurrent measurement of the serum hormone level will reveal no increase or a slight increase. Concern has thus arisen over the validity of saliva hormone testing when hormones are delivered topically, due to a lack of scientific literature on the topic. Out of frustration, many health care providers conclude that saliva testing is subject to some unexplained artifact unique to topical hormone

delivery. In fact, when steroids are delivered topically, serum testing grossly underestimates bioavailable hormone levels and is not reflective of tissue uptake or clinical response. Saliva hormone levels, on the other hand, closely reflect tissue uptake of the hormone, and clinical response, as will be discussed below.

When we apply hormone to an area of skin via cream or gel, we are exposing the red blood cells (RBCs) passing through the capillaries in that skin to very high levels of hormone. Early studies with red blood cells demonstrated that when progesterone was added directly to whole blood about 80% associated with erythrocytes and was removed from serum by sedimentation (Devenuto). Red blood cells can carry hormone both dissolved in their lipid membrane, and in an albumin coating which sheaths each cell (Hiramatsu 1987, Hiramatsu 1991, Koyama, Waddell). If the skin site of hormone application does not drain to the liver, these hormone-laden RBCs will be delivered to the capillary beds of other tissues in approximately 1 minute (a normal heart can recirculate the entire blood volume of 4-6 L in one minute).

The dimensions of the capillary and the RBC, as well as the glycocalyx lining of the capillary set up a situation which is ideally suited to allow a given RBC to offload hormone directly on to the cell membranes of the capillary endothelial cells in the time it takes for that RBC to transit the capillary (≈ 1 second) (Secomb, Koefoed). Hence, following application of hormone cream to the skin, hormone can be delivered directly to tissue via red cells, without having a chance to equilibrate with the surrounding serum. Relative to the diameter of a hormone molecule or a hormone-laden carrier protein, a capillary is enormous, and the chance that a free hormone molecule or carrier protein will “bump into” the capillary wall is much lower. Red cell transport of hormones is **an almost completely overlooked** aspect of hormone transport and bioavailability. This transport mechanism is only now being probed by salivary hormone testing.

Studies show topical delivery of hormones results in increased tissue hormone levels without large increases in serum levels.

Several studies provide evidence that topical hormone supplementation (in this case progesterone) significantly increases tissue levels of hormone without a

parallel increase in serum levels. Three of these studies are discussed briefly below. These studies are important because they emphasize that serum hormone testing does not reflect tissue response when hormones are delivered topically.

In a study by Chang, the effect of topical progesterone and estradiol on breast tissue uptake and cell proliferation was examined in women scheduled for biopsies or reduction mammoplasties (Chang). Women were treated for 10-13 days with a placebo gel or gels containing 1 mg estradiol, 25 mg progesterone, or a combination of estradiol and progesterone. Biopsies were taken and half were analyzed for estradiol and progesterone content; the other half were analyzed for breast cell proliferation by a pathologist. Estradiol and progesterone increased 200 and 100-fold respectively with topical delivery demonstrating tissue uptake. Estradiol increased breast cell proliferation 2-fold, and progesterone decreased breast cell proliferation from baseline, but also suppressed proliferation activated by estradiol. Serum estradiol or progesterone did not increase significantly in any of the treatments despite a remarkable tissue uptake and biological response. These results support the notion that tissue uptake and response can occur with topical hormone delivery without a noticeable effect on serum levels.

A second study by Rachel Miles, MD and associates demonstrated that serum testing of progesterone is not reflective of tissue uptake when progesterone is used as a vaginal suppository (Miles). In this study, progesterone levels in serum and tissue were compared after intramuscular and vaginal delivery of progesterone. After treatment with either intramuscular injection of progesterone or vaginal progesterone suppositories, serum and uterine biopsies were taken to measure blood and tissue uptake of progesterone. Serum levels of progesterone (measured by RIA) were three times higher with intramuscular delivery of progesterone than with vaginal delivery. In striking contrast, tissue uptake in the uterine biopsies was 10 times higher with vaginal delivery of progesterone than with intramuscular injections. For lack of a better understanding, these authors attributed the tissue differences to a 'first vaginal pass effect', a term used to describe local diffusion of progesterone from the vagina to the uterus without significant systemic delivery to other tissues (Bulletti). However, these authors could not prove or disprove this because they

did not biopsy other tissues.

Vaginal delivery of progesterone into the body is, in essence, through the epithelial layer of skin and does not differ in this regard from other forms of topical progesterone delivery. Therefore an additional explanation for the discrepant serum/tissue results is that when progesterone is delivered vaginally it is rapidly delivered to all tissues throughout the body. The manufacturers of Crinone™, a vaginal suppository progesterone gel, make the same claims of a ‘first vaginal pass effect’, yet also claim that women have significant improvement in well being, indicating that progesterone is finding its way to the brain as well as to the uterus. (Fanchin)

A third study performed by a group of Italian investigators showed that progesterone delivered to the nasal cavity caused remarkable changes in uterine morphology but only marginally affected serum progesterone levels (Cicinelli). Following a period of estrogen priming, progesterone was administered as a nasal spray at about 8mg/spray four times per day (total dose of about 30mg). After women used progesterone nasally for a week, uterine biopsies were obtained and analyzed for changes in morphological features. Nasal progesterone induced secretory changes in uterine endometrium, indicating that progesterone found its way from the nose to the uterus. These clinicians would be hard pressed to explain their results by a ‘first pass effect’.

These three studies clearly reveal that when progesterone is delivered topically through the skin, serum testing of steroid hormones does not reflect tissue uptake. Although saliva was, unfortunately, not measured in any of these studies, unpublished results of thousands of saliva tests in our sister laboratory (ZRT Laboratory, Portland, Oregon) reveal that this same concentration (about 15-30mg) of progesterone (or any other steroid hormone) results in a dramatic increase in salivary progesterone. Therefore, when steroid hormones are delivered topically, saliva provides a more realistic and accurate reflection of tissue hormone uptake and biological response.

Slow release estradiol patches are in widespread use, but no one stops to question how only 25 to 50 micrograms of estradiol delivered through the skin

can be effective. It is therefore hard to understand why some physicians refuse to allow their patients to use topical progesterone based on the failure of serum to demonstrate significant increases in hormone levels. Hundreds of thousands of women have benefited enormously from the use of 15 to 30mg topical progesterone/day. Transdermal delivery of hormones is extremely efficient, and this efficiency is simply not reflected by blood testing. Perhaps it is time to question the assumptions we have made about bioavailability and serum testing of steroid hormones, and realize that with saliva testing, we have a new window on delivery of hormones to tissue.

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