Saliva Hormones

1. Taking Samples

The taking of saliva samples is non-invasive and possible at any time or space, which is important for the covering of circadian rhythms e.g. of cortisol. Sample shipment is easy due to the considerable stability of parameters (one week at room temperature). Bloodletting stress may alter results, which applies especially for children. Please use only salivettes made of ultrapure polypropylene. The conveyance of the saliva is accomplished with a short straw. Salivettes made of polyethylene or salivettes with cotton or plastic reels should not be used as this will lead to significant impairments in most cases.

2. Interference Factors

Food containing hormones (e.g. milk, dairy products, eggs, meat) as well as drugs containing hormones feign high values. Froth building has to be avoided; should the occasion arise, suck the froth off with the help of the straw. Blood admixture leads to elevated results as blood hormone concentrations are higher (take care that the saliva sample is colorless). Do not use chewing-gum. Any pressure on the teeth has to be avoided (sulcus fluid leads to increased values).

3. Correct Timing

Cortisol and DHEA: 8 a.m., 12 noon, 4 p.m. and 8 p.m. (one sample each)
Sexual hormones: about two hours prior to breakfast, five samples within two hours at intervals of 30 minutes each

To avoid accidental results – some hormones display short-term deviations of concentration – we recommend to collect five samples within two hours, allowing intervals of 30 minutes between two sample drawings, respectively. This procedure should be carried through preferably in the morning, about two hours prior to breakfast. Food may contain substantial amounts of steroid hormones; hence especially milk, dairy products, eggs and meat are to be shunned 12 hours before. Even water should not be drunk during the last five minutes prior to saliva sample drawing.
For the determination of sexual hormones in women with an existing cycle (27/28/29 days), the taking of saliva samples should be accomplished during the second half of the cycle, by preference on the 22nd (+/- 1) cycle day. In case of shorter or longer cycles 5 to 6 days before the start of bleeding. In case of totally irregular cycles: try to test during the second half of the cycle. For control testing avoid application of hormones 24h before collecting the samples. In the laboratory a sample mix containing aliquots of all five samples is produced and used for the test. Drawing only a single sample is strongly warned against (exception: cortisol).

**Melatonin:** several saliva samples at fall-asleep time (DLMO: dim light melatonin onset)

### 4. Measuring Biologically Active Free Hormones

If measuring hormones in the saliva only their biologically active, free part is determined. 95 – 99 % of the blood hormones are bound to proteins such as CBG (corticoid binding globulin), SHBG (sexual hormone binding globulin) or albumin. If bound to proteins hormones are biologically inactive. Hormones in the saliva however exist exclusively in their free form.

#### Cortisol

Checking the circadian rhythm, the capacity of the HHA axis = hypothalamic-pituitary-adrenal axis and the ASI = adrenal stress index (1 x DHEA, 4 x cortisol 8 a.m., 12 noon, 4 p.m. and 8 p.m.)

Cortisol is the most important stress hormone, which is secreted in response to psychological or physical stress. In adjustment to any kind of burden (stress reaction), it shows an enormously wide sphere of action. It influences metabolic activity, enhances blood sugar supply, modulates the psychic responsiveness and is engaged in the immune defense. Basically, it acts anti-inflammatory; however it hampers cellular immune activity. NK cell activity is impeded as well, which causes an increased infection risk. Cortisol is mainly produced during the second half of the night and thus is available for stress management between 7 and 8 o’clock in the morning. In the course of the day, cortisol levels drop enormously, the principal decrease taking place in the hours prior to noon. In the evening only 10 % of the morning level are left. Cortisol levels are not subjected to age specific alterations.

#### DHEA (Dehydroepiandrosterone)

Determination within the scope of adrenal stress index (ASI) complemented by the analysis of the cortisol circadian rhythm

DHEA is chiefly produced in the adrenal cortex and, like cortisol, is regulated by ACTH. In contrast to cortisol levels, DHEA concentrations are strongly age dependent. The highest concentration is reached in young adults; at an old age only 10 % of the initial amount is present. Enduring stress leads to increasingly lower cortisol levels, whereas DHEA levels rise (but may drop later on).
DHEA acts slightly anabolic, encourages the muscular build-up, lowers LDL cholesterol levels, raises HDL cholesterol, improves the overall immune situation and stimulates the cellular immune defense. The DHEA / cortisol ratio should be between 3 and 100.

**Estradiol (E2)**
Clarification of a suspected estrogen dominance along with the determination of progesterone

Estradiol is the main representative of estrogens. Estradiol concentrations in women capable of bearing follow a characteristic monthly profile as well, showing an obvious peak in the middle of the cycle shortly before ovulation. The basic concentration in the female cycle is about the same order of magnitude as the concentration found in men or children.

**Estriol (E3)**
Estriol is also designated as mucous membrane estrogen; it builds up mucous membranes and renders them smooth. Estriol makes up between 60 and 80 percent of all estrogens.

**Progesterone**
Clarification of a suspected estrogen dominance accompanied by the determination of estradiol

Hormone concentrations in women at their reproductive age follow a pronounced monthly rhythm. During the second half of the monthly cycle progesterone concentrations rise significantly to swoop down towards the end of the cycle, thus provoking menstruation. Also during pregnancy a continuous increase in the concentration of this hormone is seen. Progesterone concentrations in men are more or less on the same level as the concentrations observed during the first half of the cycle or those found in post-climacteric women. Short-time deviations are particularly pronounced in women during the second half of the cycle. Interestingly, these short-time deviations frequently happen in parallel to testosterone and also to estradiol deviations, which points to similar regulation mechanisms.

**Testosterone**
Male testosterone concentrations are age dependent. Maximum concentrations are seen in young men aged between 10 and 30 years. Looking at the course of a day, average testosterone concentrations in the evening are about half of the average level seen in the morning. In women neither a marked daily rhythm nor any age dependence are found. However, in women as well, a pronounced short-time rhythm becomes apparent. In both sexes mean saliva concentrations of testosterone are higher than those of estradiol. Intensive physical exercise leads to a zoom of the testosterone level, also in dependence on the athlete’s training condition. After the termination of sportive activities hormone values drop back to a normal level. A lack of physical exercise as well as an immense exertion may cause saliva testosterone concentrations to drop to extremely low values.
Melatonin

Psychically caused sleep disorders, somnipathies due to shift-work, jetlag, depression.

The biosynthesis of the neurohormone underlies a circadian rhythm with high values during the night and low values during the day. DLMO (dim light melatonin onset) as an increase in evening melatonin concentration: 3 (2-8) ng/l about two or three hours prior to going to sleep. It is recommended to take three to five saliva samples every 60 minutes at dim light (< 30 lux, reading-lamp or T.V. set allowed) one hour prior to the expected fall-asleep time.

“Night owl syndrome”: Sleep disorders – the lack of wakening morning light leads to a DLMO delay of about 30 minutes. To be waked up by daylight may help prevent difficulties to fall asleep.

Normal ranges: see report

Material: cortisol, DHEA, melatonin: one salivette
other hormones: five salivettes
(please notice instructions)

Test frequency: weekly

Costs:

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<th>Parameter</th>
<th>price (GOÄ I.I5)</th>
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<tr>
<td>Cortisol</td>
<td>16.76 €*</td>
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<tr>
<td>DHEA</td>
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<td>Estradiol</td>
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<td>Melatonin</td>
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* plus singular expenses (5.55 €) according to § 10 GOÄ (German medical scale of charges )

Specialist contact: Dr. med. Hannelore Raith Phone.: 089 54308-0