

Cortisol

Physiology

Cortisol is produced in the cortex of the adrenal glands. The lipophilic steroid hormone is released into the circulation and bound to proteins: 90 % to corticoid binding globulin (CBG) and 8 % to albumin. Only 1 –2 % of the total cortisol in the blood is free. Only this part of the cortisol in blood is active on the target cells. The mentioned facts have to be taken into account by regarding correlation studies of cortisol in blood and in saliva. At 145 - 180 ng/ml (400 – 500 nmol/l) of total cortisol plasma level the CBG is saturated. Above this concentration the percentage of free cortisol increases. Therefore the plasma level of total cortisol depends on the CBG concentration. The increased CBG level leads to an elevated cortisol plasma level, but to a normal free cortisol concentration in plasma and saliva. The CBG concentration is affected by various conditions like pregnancy, liver diseases, inflammation, polycystic ovary syndrome and application of different drugs (e.g. contraceptives).

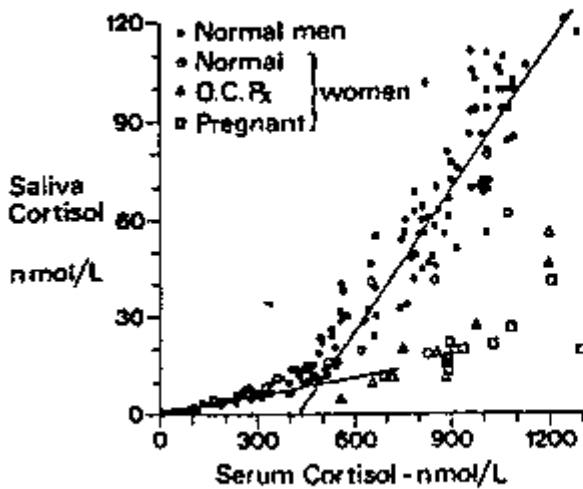


Fig. 1: Relationship between salivary and total serum cortisol in time-matched samples (Vining et al., 1983)

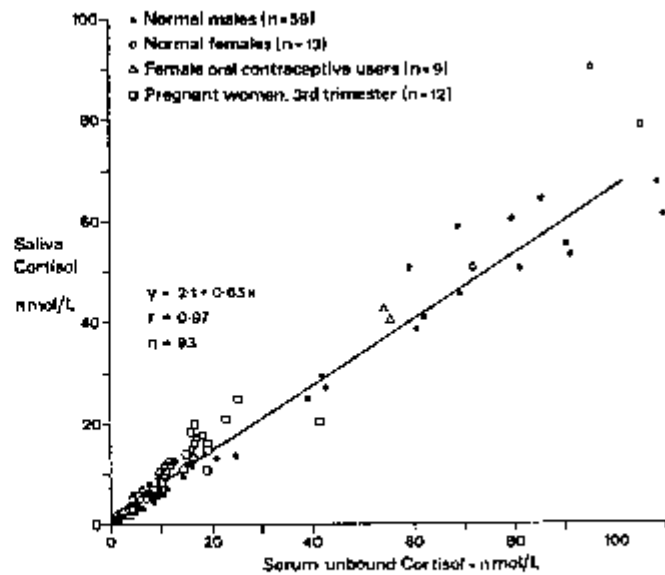


Fig. 2: Relationship between salivary and unbound serum cortisol in time-matched samples (Vining et al., 1983)

Statistics 3

Fig. 22: Correlation of salivary cortisol to cortisol in serum (Vining et al., 1983)

On the left: Relationship between salivary and total serum cortisol in time-matched samples.

On the right: Relationship between salivary and unbound serum cortisol in time-matched samples.

The release of cortisol is regulated by the corticotropin releasing hormone (CRH) from the hypothalamus and the adrenocorticotrope hormone (ACTH) of the pituitary gland in a negative feedback mechanism. This release is influenced by other situations like stress and physical exercise mainly because of the positive feedback of higher adrenaline levels on the ACTH release.

The normal cortisol concentration in saliva of humans during the day is fluctuating with an morning peak 60 to 90 minutes after wake up. The diurnal cycle of salivary cortisol in healthy adult people is shown in the following graph.

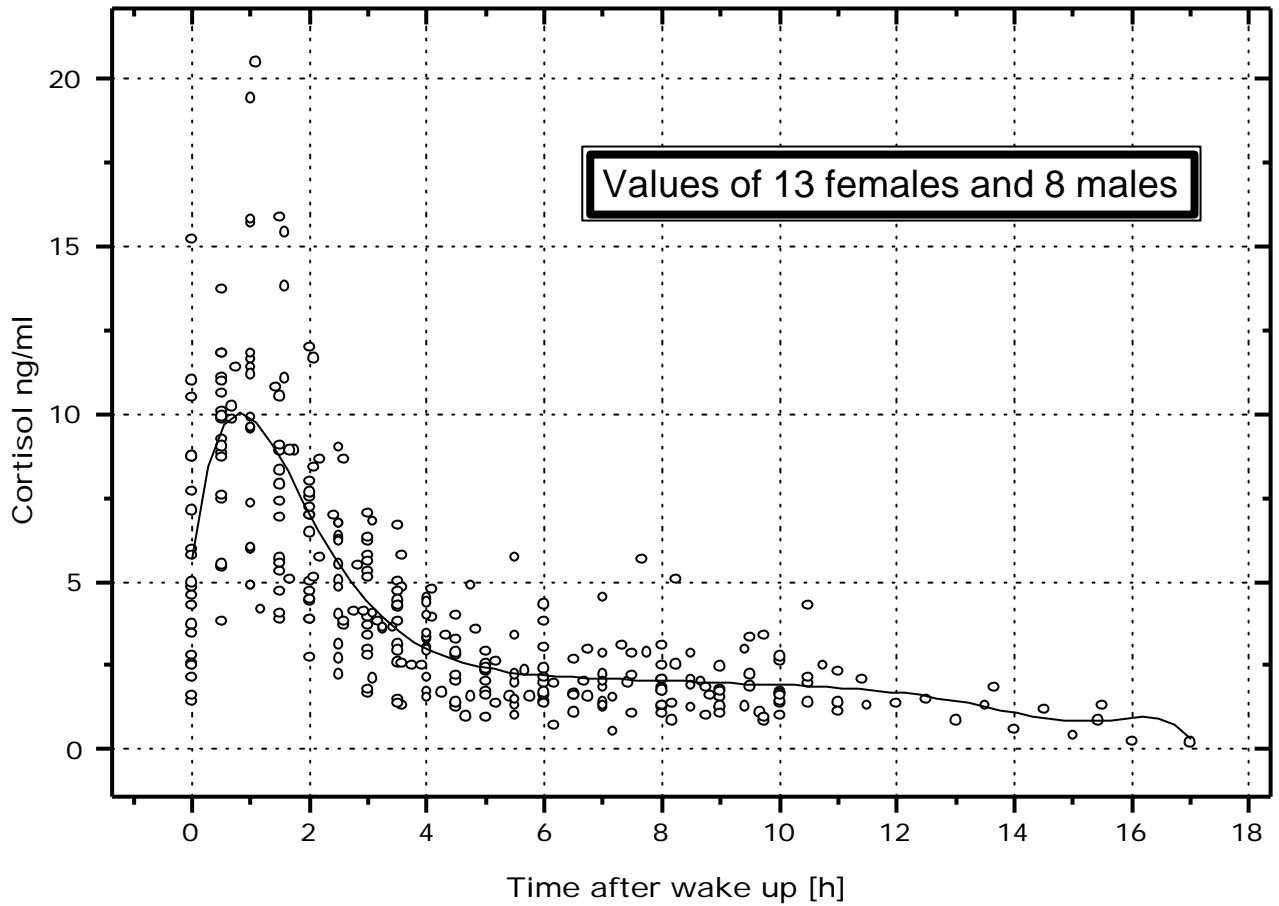


Fig. 23: Diurnal cycle of the salivary cortisol level in relation to the wake-up time.

In the following table some physiological effects of cortisol are listed.

Effects of Cortisol

- Enhancement of the catabolic part of the lipid and protein metabolism and the gluconeogenesis
- antagonism to insulin
- inhibition of the inflammatory and allergic processes
- enhancement of the contractibility of the heart muscle and improvement of the peripheric microcirculation
- enhancement of water excretion by the kidneys
- enhancement of the production of gastric juice
- enhancement of the osteoporotic effect within the bones
- enhancement of the excitability in brain regions
- suppression of the hormone secretion of the anterior part of the pituitary gland (effect on the thyroid gland and the gonads)

Table 8: Effects of Cortisol in mammals.

Indications

In the following table some causes of altered cortisol level are listed.

<p style="text-align: center;">Causes of Altered Cortisol Level</p> <p>Physiological</p> <ul style="list-style-type: none">- elevated during stress situations- elevated during physical exercises- elevated by external application of ACTH- suppressed by application of external corticoids <p>Pathological</p> <p>Hypercortisolism (Cushing Syndrome)</p> <ul style="list-style-type: none">- autonomous tumour of the adrenal cortex- tumour of the pituitary gland (Morbus Cushing, Cushing Disease)- ectopic ACTH-Syndrome <p>Hypocortisolism</p> <ul style="list-style-type: none">- primary insufficiency of the adrenal cortex (Morbus Addison)- secondary (pituitary based) insufficiency of the adrenal cortex- tertiary (hypothalamic based) insufficiency of the adrenal cortex

Table 9: Causes of altered cortisol level in mammals.

Regarding this list it may be concluded that the assessment of cortisol in saliva is recommended in many questions of various scientific fields like endocrinology, psychology, sports medicine, pediatrics, anti-aging medicine, veterinary medicine and others.

Some interesting clinical aspects of the salivary cortisol level are shown in the following paragraphs:

Stress research:

In many studies the endocrinological response of people to different kinds of stress is tested. In order to differentiate peoples reaction there has to be set up a standardized test which will be applied to all participants. The Trier Social Stress Test (TSST) is accepted as such a standardized method. If students perform the TSST every day at the same time for a week they can be divided into two groups regarding their cortisol level during the assay. The "high responder" do have an strong increase of the salivary cortisol level repeated times on following days whereas the "low responder" don't have this increase. But in their study Kirschbaum et al. (1995) didn't find any correlation of the cortisol concentration to psychological scores.

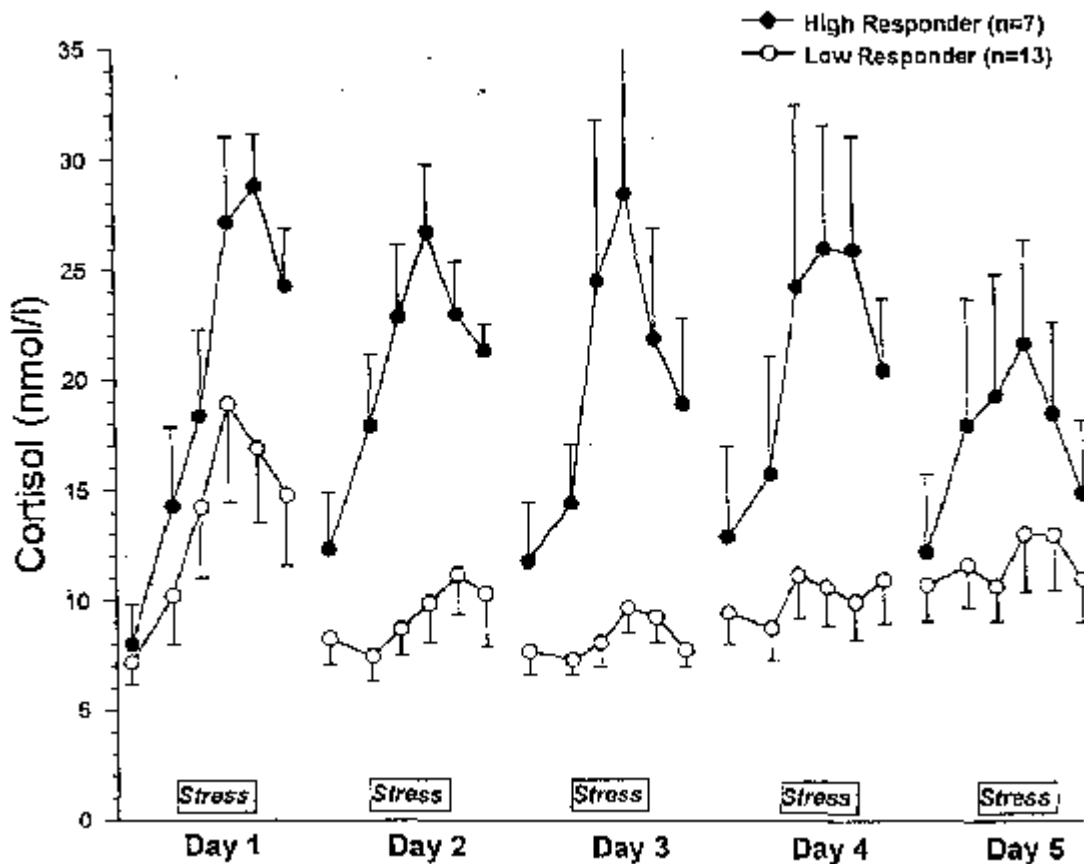


Fig. 24: Cortisol response on standardized stress situations. Students were exposed to the Trier Social Stress Test (TSST) between 4 p.m. and 7 p.m. on 5 days (C. Kirschbaum et al., 1995)

There are hints of some aspects influencing the cortisol reaction on stress in humans: Gender and in women the menstrual cycle seem to have impact on this hormone profile and the application of drugs like oral contraceptives seem to decrease the response of cortisol on stress according to Kirschbaum et al. (1999).

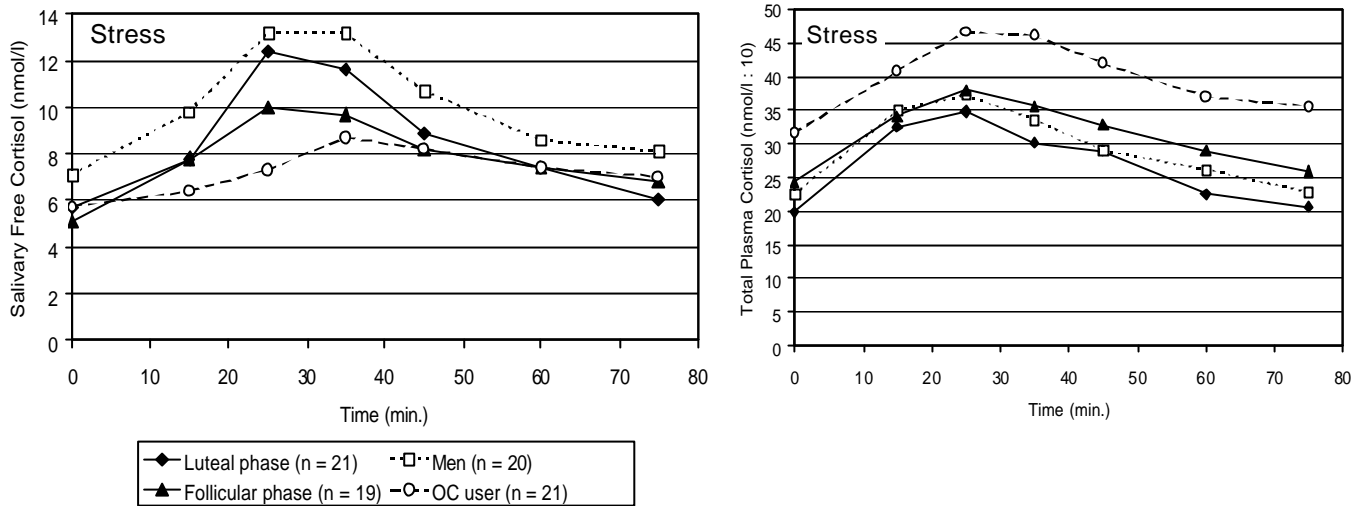


Fig. 25: Impact of gender, menstrual cycle and oral contraceptives during stress on salivary free cortisol and total plasma cortisol. Healthy men and women run through the Trier Social Stress Test (TSST). Basal cortisol level are taken the day before (C. Kirschbaum et al., 1999).

Occupational Medicine

Some common issue in community is the adaptation of people to shift work. There are some people who could bear the change of the working time without any problems, and others do have psychological difficulties during the adaptation time.

In some study (Hennig et al. 1998) it is discussed to differentiate these to groupss of people by the change in the diurnal salivary cortisol profile.

In this study 24 nurses were involved. They collected saliva samples two days at the early working shift (begin at 6:00 am; end at 9:00 pm) and in the following 7 nights of the night shift (begin at 9:00 pm; end at 6:00 am). Salivary cortisol was asesed in each saliva sample.

In figure 26 it is shown that the cortisol morning peak decreased during the night shift, whereas there developed a "night cortisol peak" at 9:00 pm. At the fifth night the cortisol of the night peak exceeded that one of the morning peak. But by looking at the individual cortisol profiles there can be differentiated two kinds of nurses: By 6 of the 24 cortisol profiles even in the 7th night the night cortisol peak didn't exceed the morning peak. These people are called "non-adapter" in figure 27. These 6 nurses did also report that they have psychological difficulties during the adaptation period of the night shift.

It is interesting to know if these non-adapter can be converted to adapter when they are taking melatonin drugs?

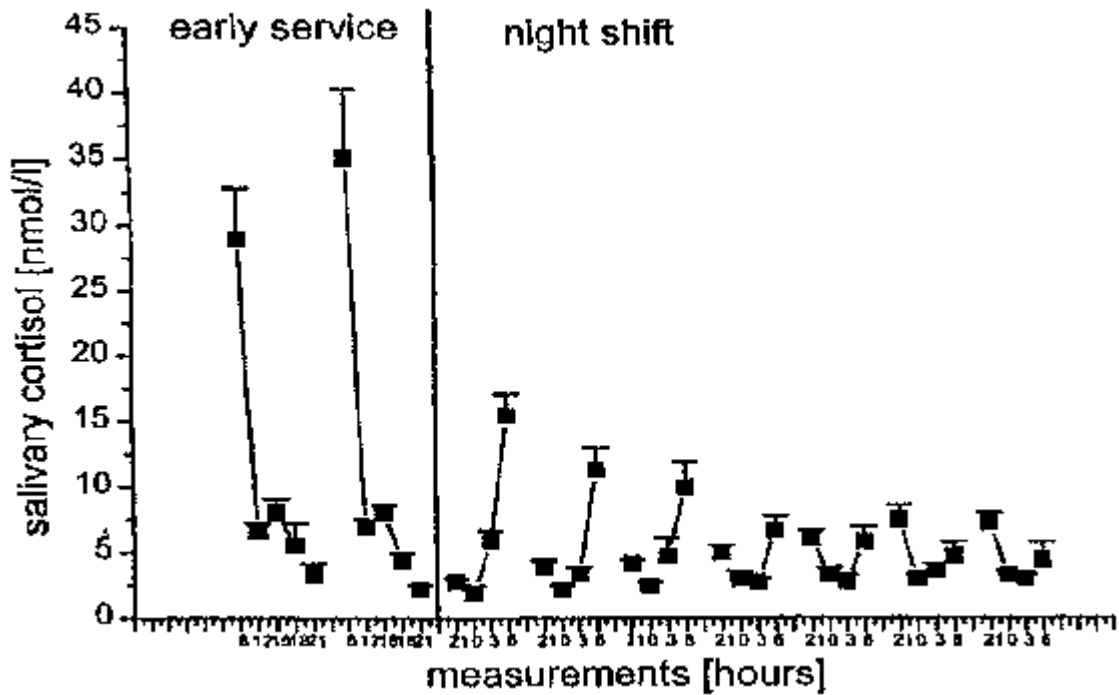


Fig. 26: Diurnal salivary cortisol profile of 24 nurses during two days of the early shift (begin at 6:00 am; end at 9:00 pm) and 7 following days of the night shift (begin at 9:00 pm; end at 6:00 am; J. Hennig et al., 1998)

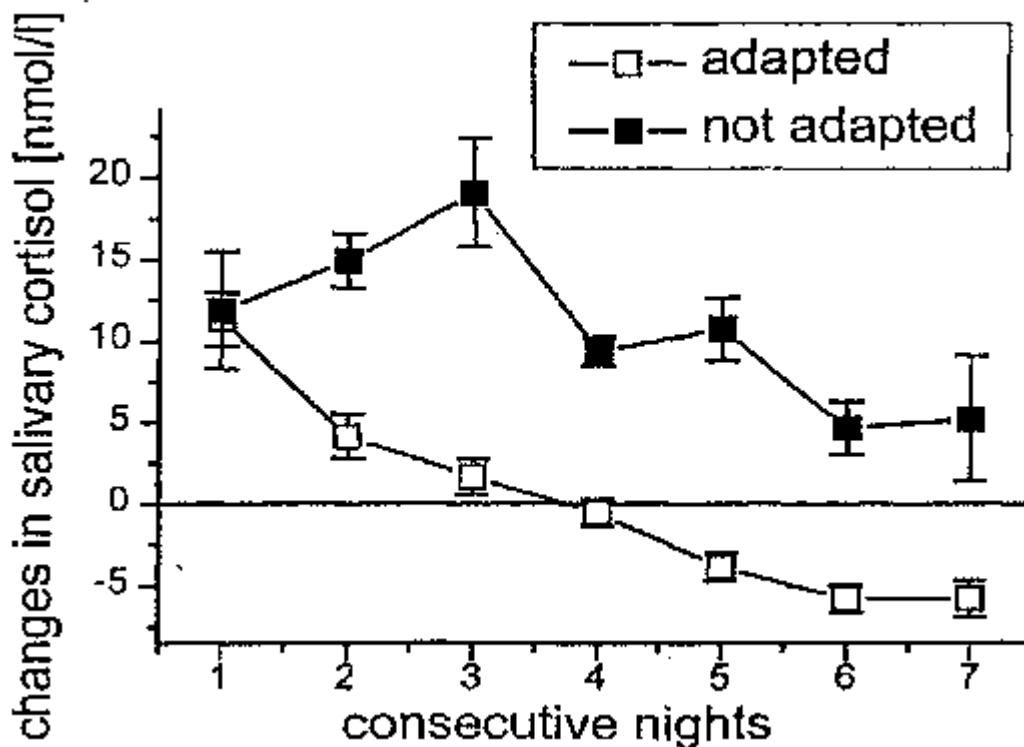


Fig. 27: Difference of the salivary cortisol concentration of the morning peak and the night peak of 24 nurses during 7 days of the night shift (begin at 9:00 pm; end at 6:00 am). In 18 nurses (called "adaptee") after the fifth night the night cortisol peak exceeded the morning peak, whereas in 6 nurses (called "non-adaptee") this didn't happen even in the seventh night (J. Hennig et al., 1998).

Sports medicine:

During physical exercise like a run it is known that the free salivary cortisol concentration is increasing followed by an increase of the free testosterone in man. But there seem to be a difference in the cortisol peak height between well trained people and so called "non-runners".

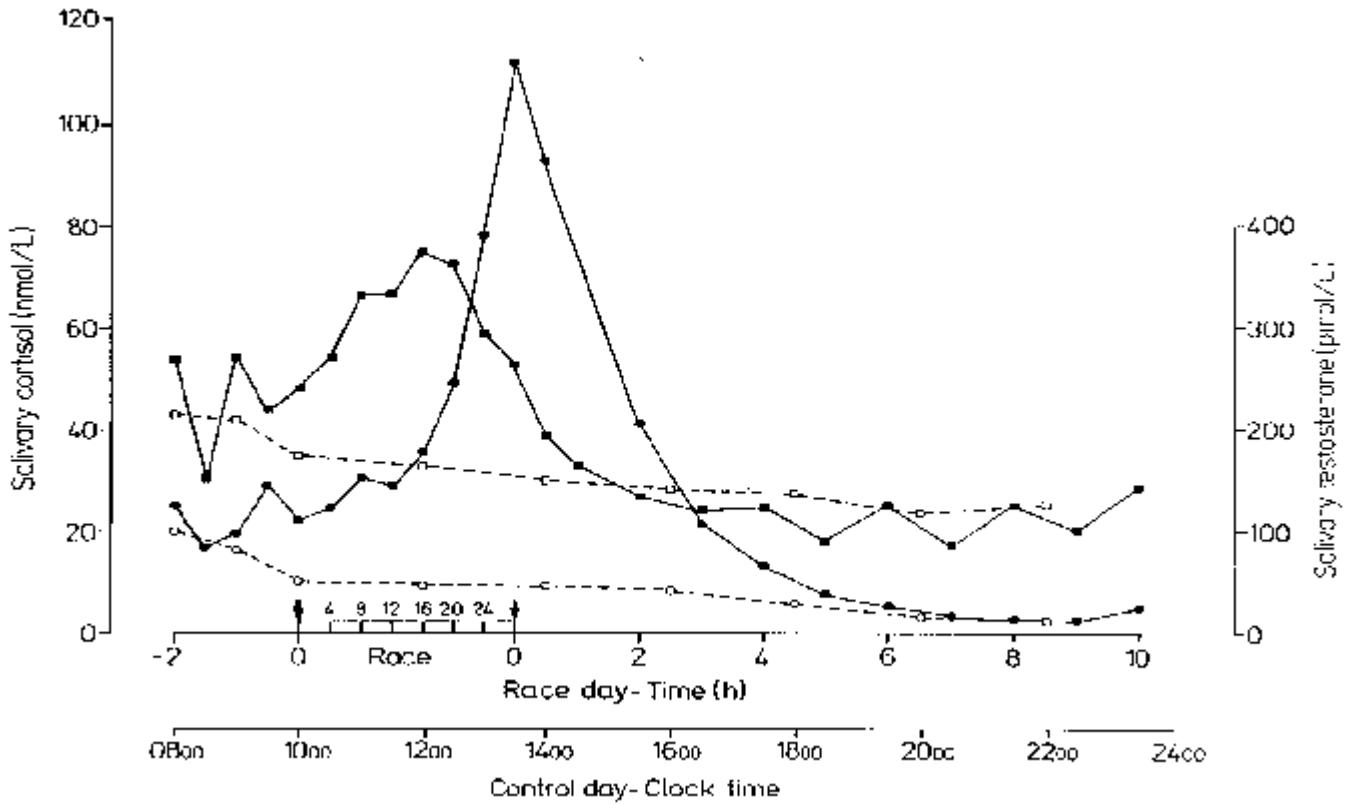


Fig. 28: Salivary cortisol and testosterone level during a marathon run (N. J. Cook et al., 1992)

During Marathon run: • cortisol level (nmol/l) ? testosterone level (pmol/l)
 Control Day: ? cortisol level (nmol/l) ? testosterone level (pmol/l)

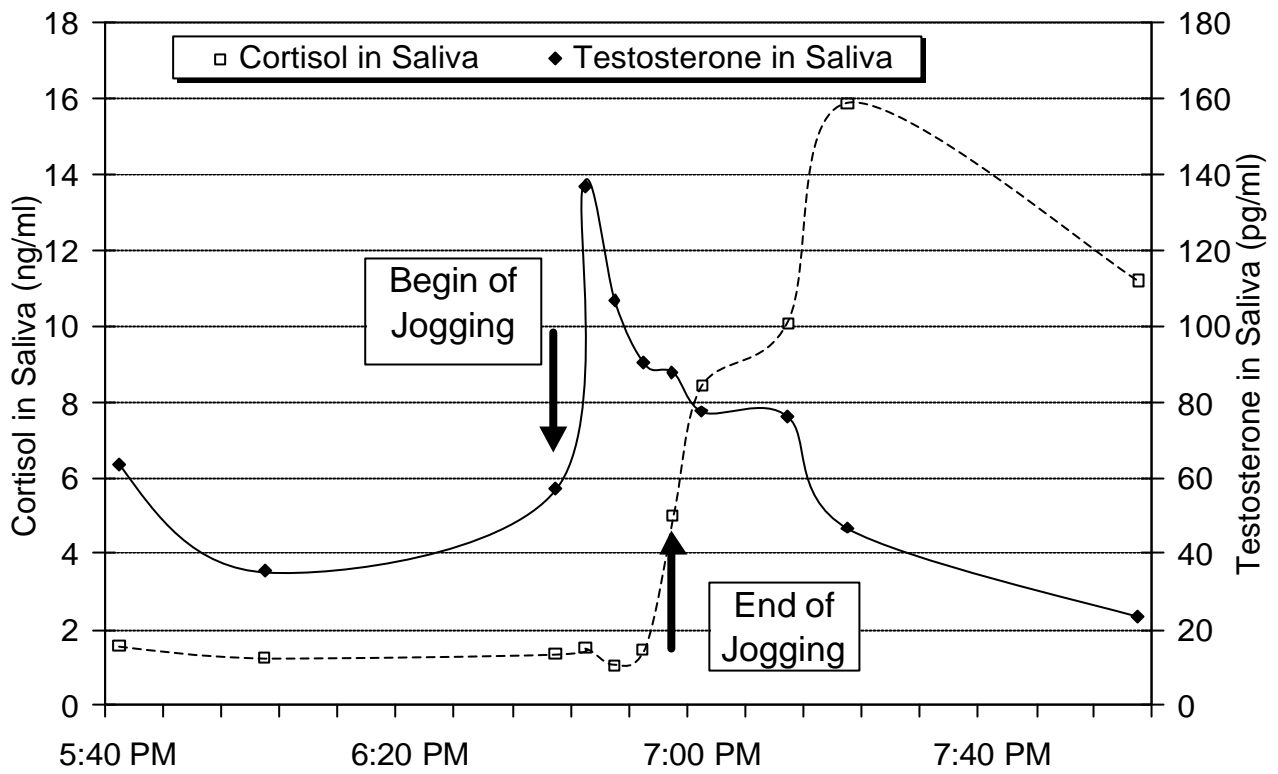


Fig. 29: Salivary cortisol and testosterone level during a 5 km run measured with the LIA of IBL-Hamburg.

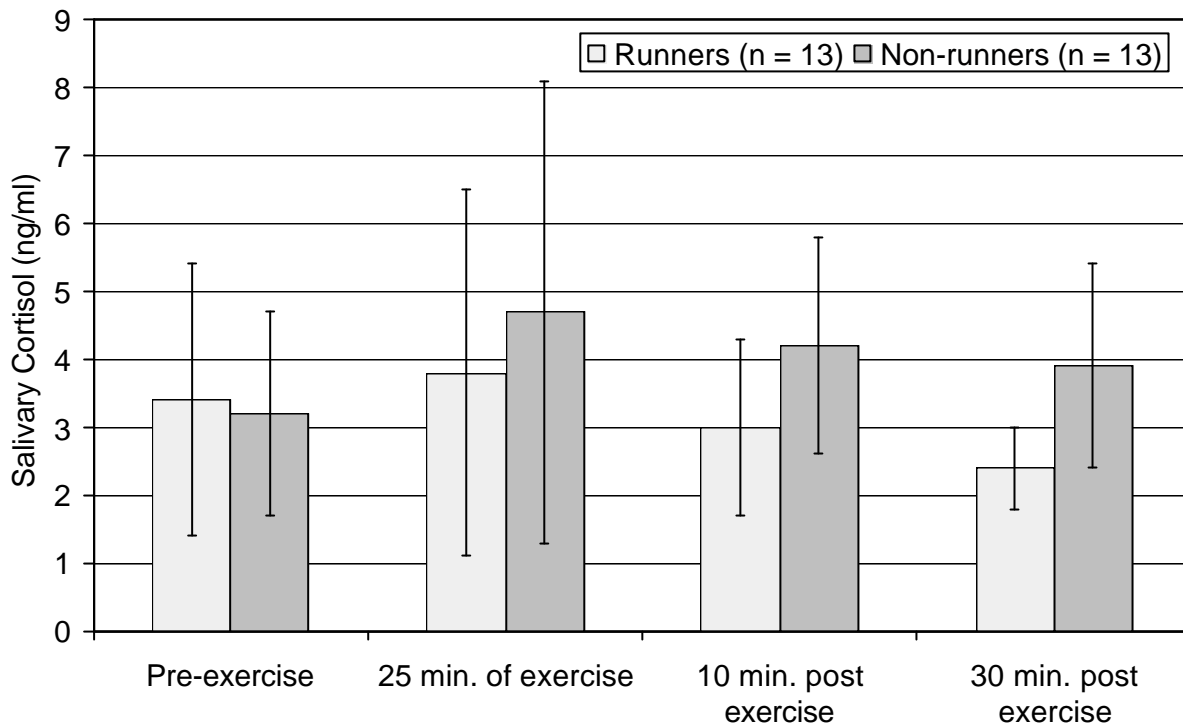


Fig. 30: Salivary cortisol response during physical exercise of trained and non-trained people. (D. L. Rudolph and E. McAuley, 1998). Runners = cross-country runners (ca. 100 km per week for 2 years); non-runners = students; exercise = 30 min. treadmill run (60 % of max. heart rate and max. oxygen consumption)

Endocrinology:

Up to now it is sometimes a problem to discriminate the patients with an early stage of Cushing Disease with healthy persons above all obese humans. Usually for the endocrine diagnosis an assessment of a diurnal cortisol profile or a dexamethason suppression function test (1mg Dexamethason applied at midnight and taking the sample between 8.00 and 9.00 am.) are performed. There are some hints that the discrimination between healthy adults and Cushing syndrome patients is better by assessing the free salivary cortisol levels than the total plasma cortisol.

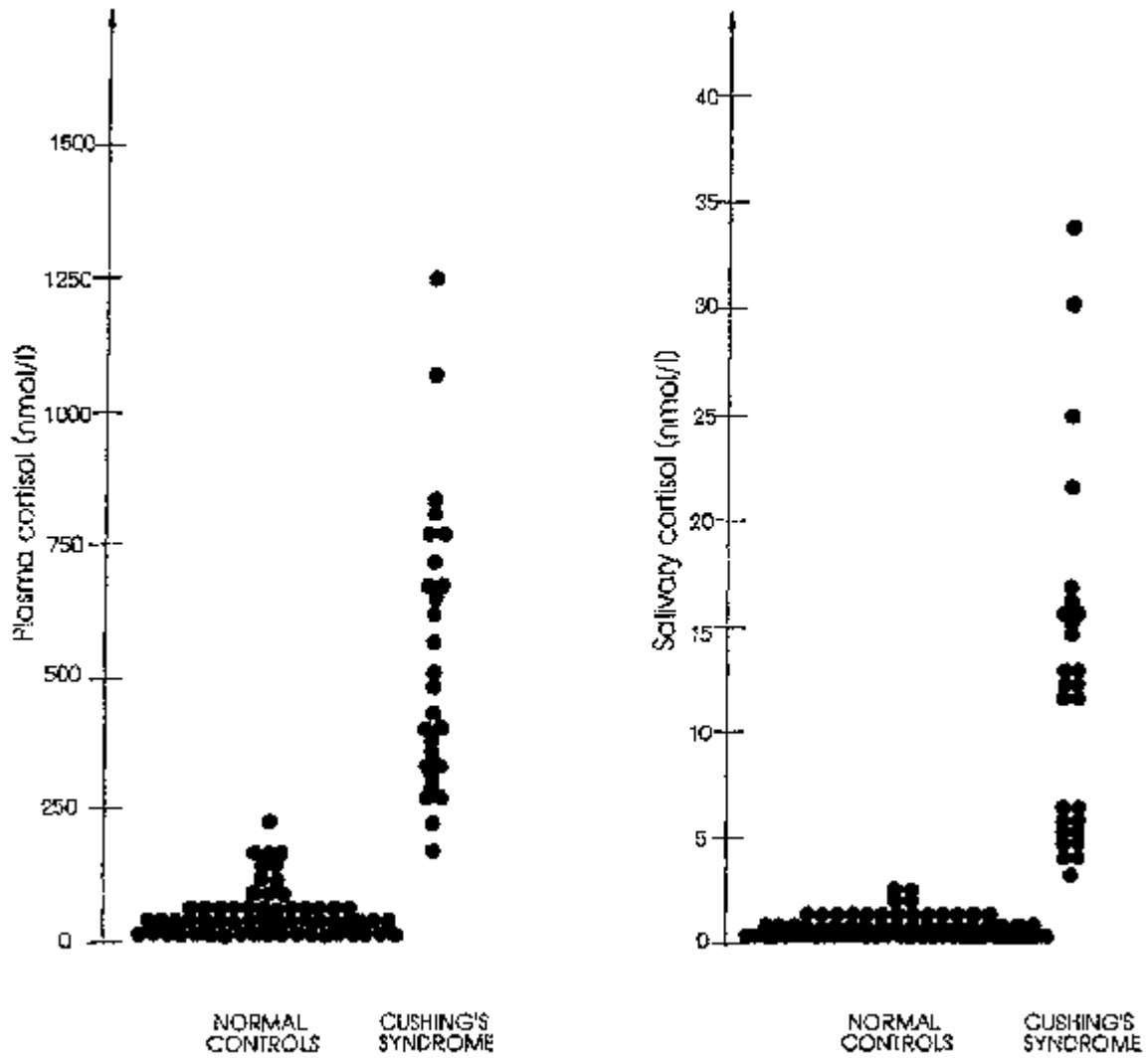
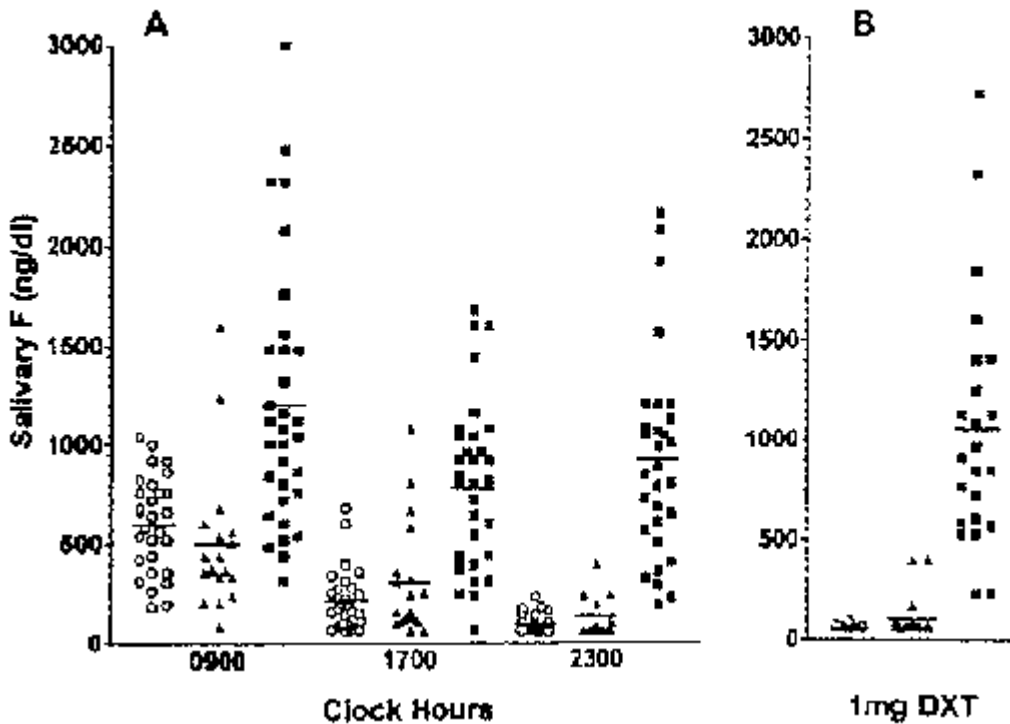


Fig. 31: Saliva and plasma cortisol levels after the Dexamethason-Suppression-Test (Z. Barrou et al., 1996). Application of 1 mg dexamethasone at midnight and taking the plasma and saliva samples between 8 and 9 am; Saliva collection with Salivettes.

It is known that the most important sample by assessing the diurnal cortisol profile is that one taken at midnight. But the best discrimination will be received by performing the dexamethason suppression test. These aspects are also found by using saliva instead of blood.



○ = non obese persons (n = 30) ■ = Cushing patients (n = 33) ▲ = persons with obesity (18)

A. Salivary cortisol level at 9 a.m., 5 p.m. and 11 p.m.

B. Salivary cortisol level between 8 and 9 a.m. after application of 1 mg Dexamethason at midnight

Fig. 32: Saliva cortisol of non-obese and obese persons and of patients with Cushing Disease (M. Castro et al., 1999)

When traveling from the United States to Europe by plane people will have during their first week in Europe two cortisol peaks. One at the past morning time in the United States and the other at the morning in Europe. During the first week stay in Europe the USA morning peak vanishes and that one of Europe increases to the normal level. Maybe the time of this endocrine regulation is related to the jet lag phenomenon in addition to the melatonin hormone profile.

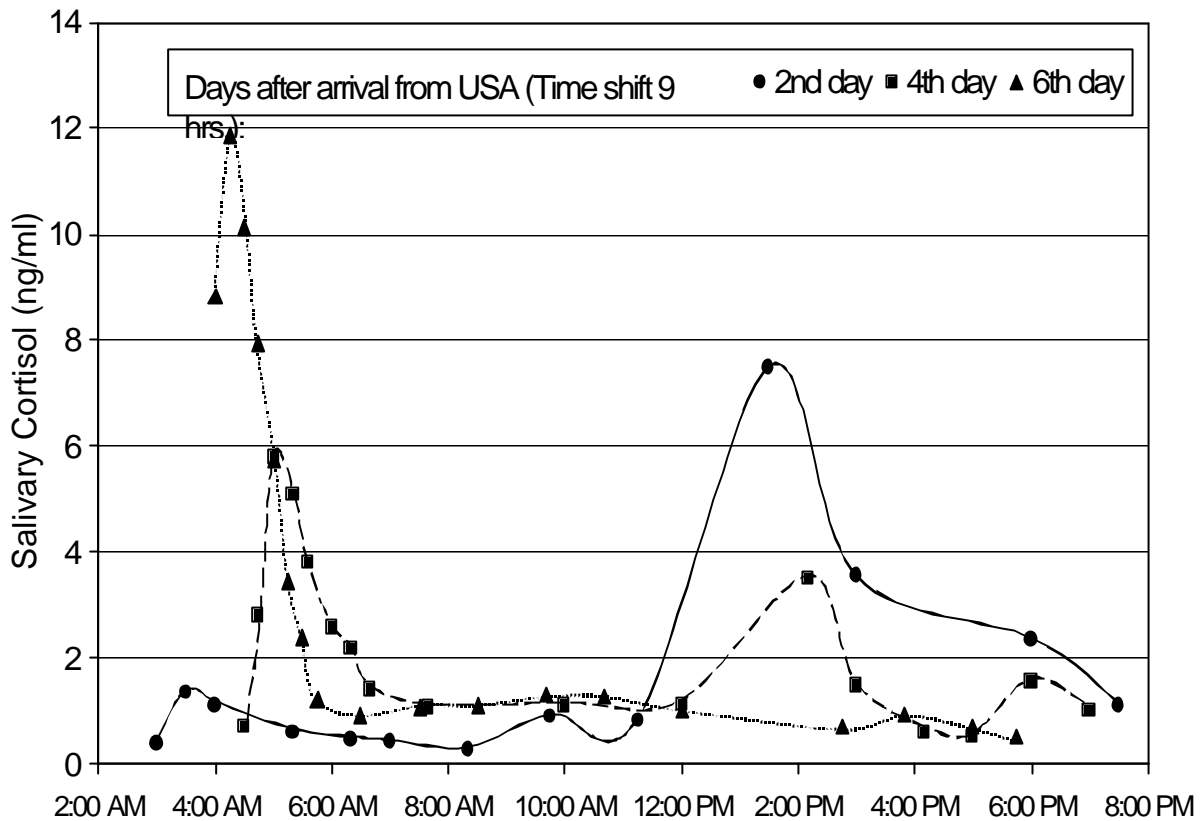


Fig. 33: Salivary cortisol levels after return to Germany from a Trip from the USA measured with the Cortisol LIA from IBL-Hamburg.

Veterinary Medicine

In co-operation with the Clinic for Cattle of the Veterinary School of Hannover, Germany, we assessed the diurnal cortisol profiles of cows. For this study saliva and blood samples of 5 healthy cows which stayed in the center for research studies with an artificial rumen fistula were taken. The saliva samples were collected with Salivettes[®], Sarstedt. The sampling intervals were 30 minutes, beginning at 7:00 am and ending at 4:00 pm.

The cortisol profiles in serum and saliva are shown in figure 34. There is a good correlation between the serum and saliva cortisol values, but all values do have a lower level as in humans. It is striking that the cows didn't have increased cortisol values in the morning but at noon. Maybe, we didn't find a morning peak, because this one might appear earlier in the morning and the cortisol level at 7:00 am is returned to the baseline value?

Up to now, we don't have any explanation for the cortisol peak at noon which appears with the saliva and the serum cortisol, obviously with a great standard deviation. There was not any stressful event at that time for the cows, like feeding or milking time or any manipulation by students.

More investigations on this point are necessary.

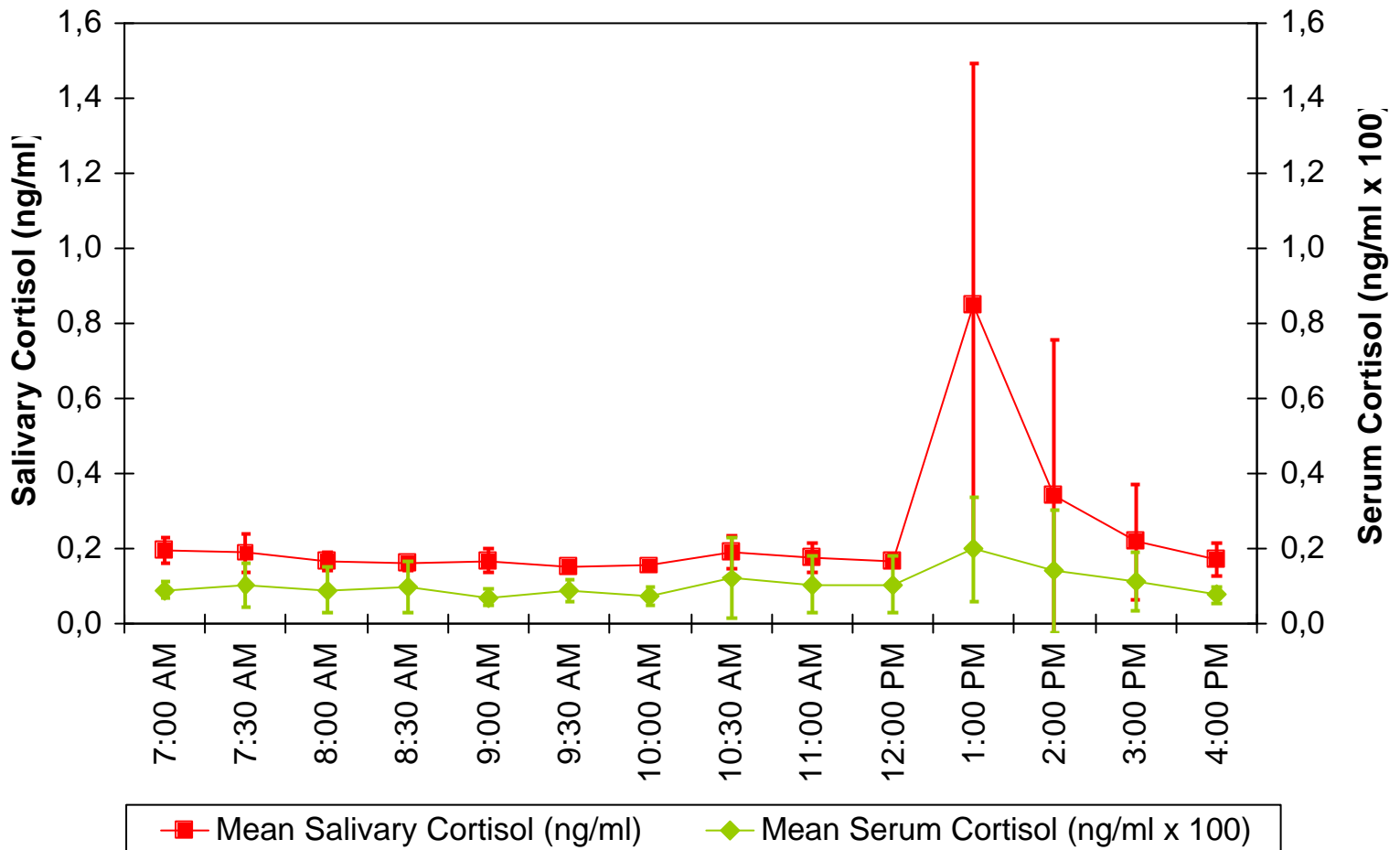


Fig. 34: Diurnal Profile of cortisol in saliva and serum of 5 healthy cows.

In a second study we collected saliva samples of five bovine patients, which underwent a surgical operation because of a dislocation of the abomasum to the left sight at about the same daytime in the late morning.

The saliva samples were collected in Salivettes[®], Sarstedt. The day prior to the operation the samples were collected at the same daytime in 30 minutes intervals for assessing a baseline cortisol level. Of course, we couldn't avoid stress because of the transport and the new surroundings of the cows. At the day of operation we tried to collect the saliva samples prior to, during and after the surgical operation.

Some example of a typical cortisol profiles are shown in figure 35.

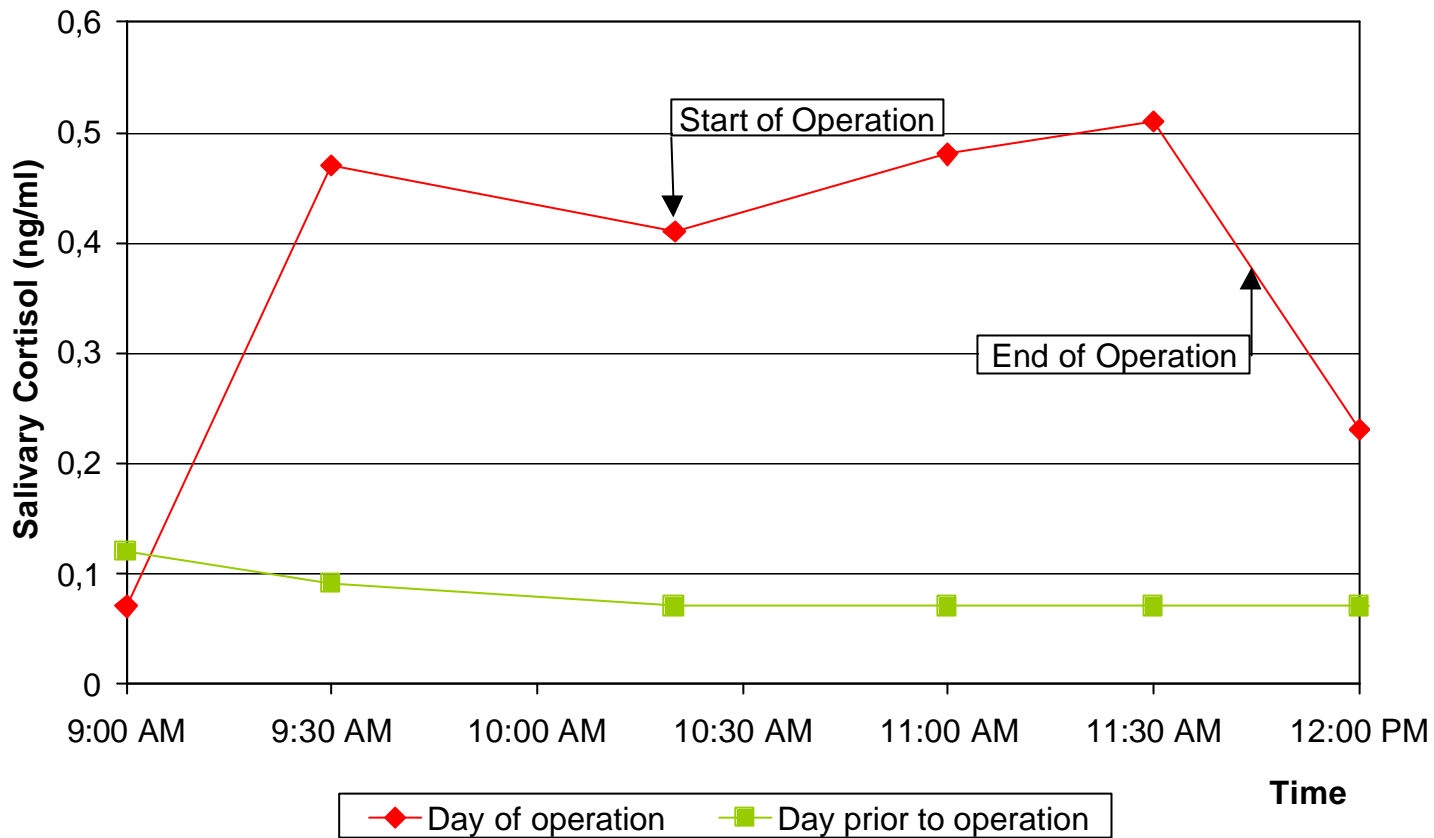


Fig. 35: Salivary cortisol concentration of a cow which underwent a surgical operation because of a dislocation of the abomasum to the left compared to the salivary cortisol level of a reference day.

In figure 36 we calculated the difference between the salivary cortisol concentration of the day of the operation and the day before this event for three cows. It is obvious that the patients react differently on the stress of the surgical operation independent of the time. There was nearly no difference of the cortisol level of cow #2 before and during the operation. The cortisol concentration of cow #5 was increasing long before the start of the operation, maybe because of some stressful manipulation for the preparation of the operation. The cortisol level of cow #4 seems to be mainly affected by the stress of the operation.

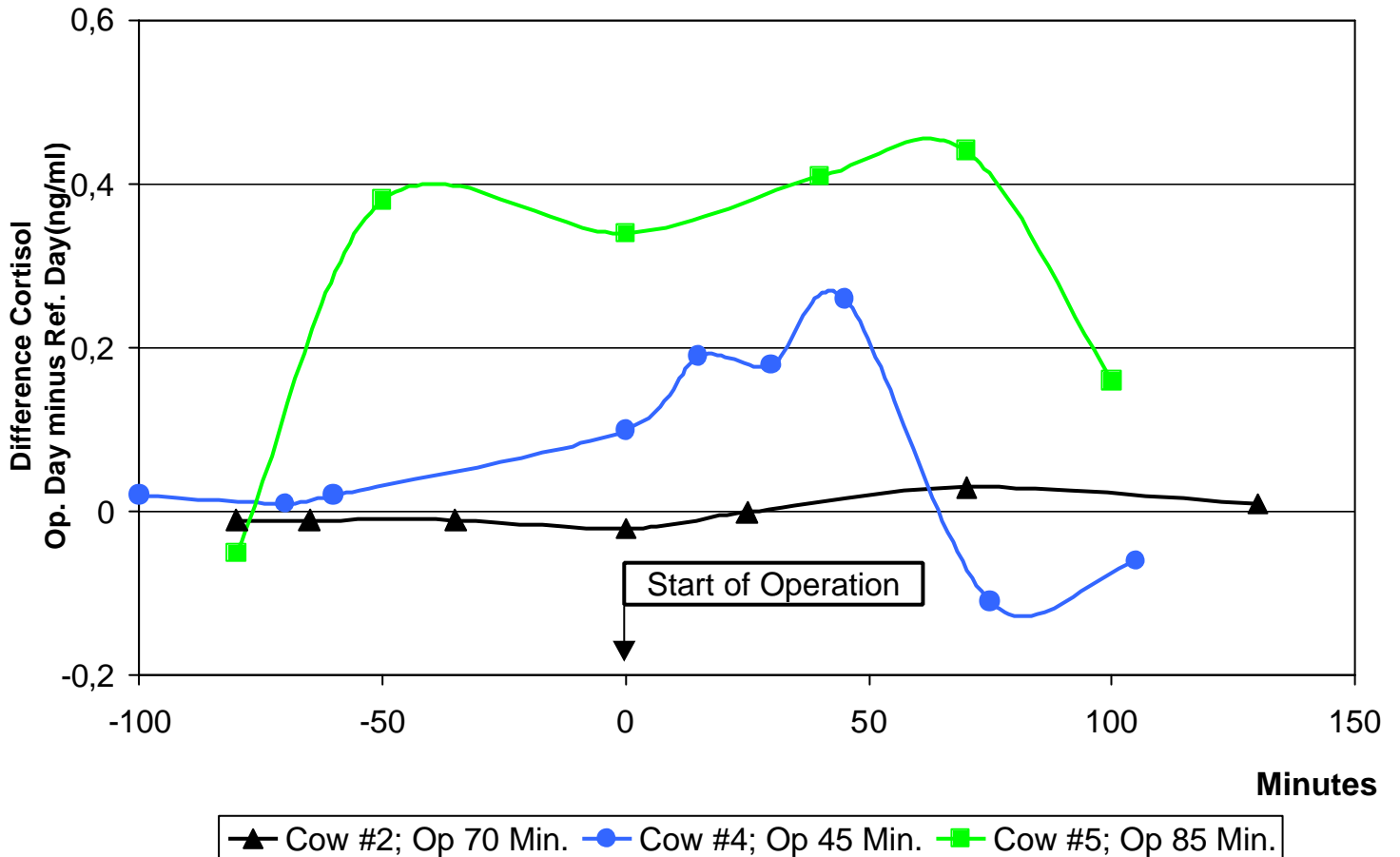


Fig. 36: Difference of the salivary cortisol concentration of three cows, which underwent a surgical operation because of a dislocation of the abomasum to the left, during the time of operation and a reference day.

Normal ranges

In the following the recommended saliva collection panel of some routine laboratories is given. Of course there are a lot of variations in the collection schedule for special studies.

Saliva Collection for Cortisol assessment:

- Circadian panel:
- 4 – 5 samples: immediately after awaking, noon, 4 pm, 10 pm (alternatively: 8 pm and midnight)
 - to evaluate the morning peak: 4 – 6 samples: immediately after awaking and every half hour

The following “values” may only be regarded as a guideline. Of course they depend on the used kit and special population aspects.

	Values (ng/ml)
Wake up time	1.4 – 8.9
Peak (within 90 min. after awake)	5.0 – 17.7
3.5 – 4.5 hrs. after cortisol peak	1.0 – 2.8
7.5 – 8.5 hrs. after cortisol peak	0.5 – 3.1
Dexamethason suppression test*	
cut off (normal/Cushing Disease)	1.0
borderline	0.7 – 1.6
Addisonian	< 0.4

* Application of 1 mg Dexamethason orally at 11 pm; saliva collection next morning immediately after awaking.

Test Characteristics

The following table summarizes the test characteristics of the Free Cortisol in Saliva LIA of IBL-Hamburg (cat.no. RE 620 11)

FREE CORTISOL IN SALIVA

- LIA -

Principle	Competitive Chemiluminescence Immunoassay (LIA)	
Format	12 microtiter strips with 8 wells each	
Sample	20 µl Saliva	
Standards	7 standards, ready for use 0/0.3/0.6/2.0/6.0/15.0/40.0 ng/ml	
Incubation	3 hrs. (18 – 24 °C); 10 min. (18 – 24 °C)	
Substrate	Luminol	
Expected values	Wake up time	1.4 – 8.9 ng/ml
	Cortisol peak (within 90 min. after wake up)	5.0 – 17.7 ng/ml
	3.5 – 4.5 hrs. after cortisol peak	1.0 – 2.8 ng/ml
	7.5 – 8.5 hrs. after cortisol peak	0.5 – 3.1 ng/ml
	Lit. (RIA):	
	Addisonian; Dexam. suppr. Test	< 0.36 ng/ml
	Cushingoid	> 7.20 ng/ml
Sensitivity	0.16 ng/ml	
Precision	Intra assay: 2.9 – 7.7 %	at 0.96 – 8.70 ng/ml
	Inter assay: 5.7 – 11.7 %	at 0.80 – 7.02 ng/ml
Specificity	% cross reactivity (Abraham method)	
	Corticosterone	2.5
	Cortisone	2.0
	Prednisone	1.0
Controls	2 Saliva kit controls	
Automation	Assay is tested on different microtiter plate instruments	
Cat.-No.	RE 620 11	

Comparing this LIA with other immunoassays provided for the assessment of cortisol in saliva some advantages have to be stressed:

The IBL-Hamburg Cortisol in Saliva LIA has :

- the only **FDA clearance** as immunoassay for the assessment of **saliva and plasma** cortisol concentrations
- a high **analytical sensitivity** (0.16 ng/ml)
- a good **specificity** (above all regarding cortisone)
- a good **precision** (functional sensitivity, see fig. below)
- a good **linearity** in the clinical relevant concentration range
- two levels of **saliva controls** included in the kit
- standards, controls and conjugate **ready for use**
- been applied on **automatized instruments**

Sensitivity:

In some Cortisol Saliva Assay Characteristics a very good analytical sensitivity is mentioned (< 0.1 ng/ml). It is important to know that the analytical sensitivity is evaluated by replicate measurements of the zero standard, which consists of a buffer solution and not of saliva components!

More interesting is the reproducibility of the cortisol values of saliva samples (functional sensitivity). Coefficients of variation of replicate measurements below 10 % in the normal cortisol concentration range (i.e. 0.5 – 17 ng/ml) are acceptable. In figure 37 you can see that most salivary cortisol immunoassays don't fulfil this condition in the low concentration range.

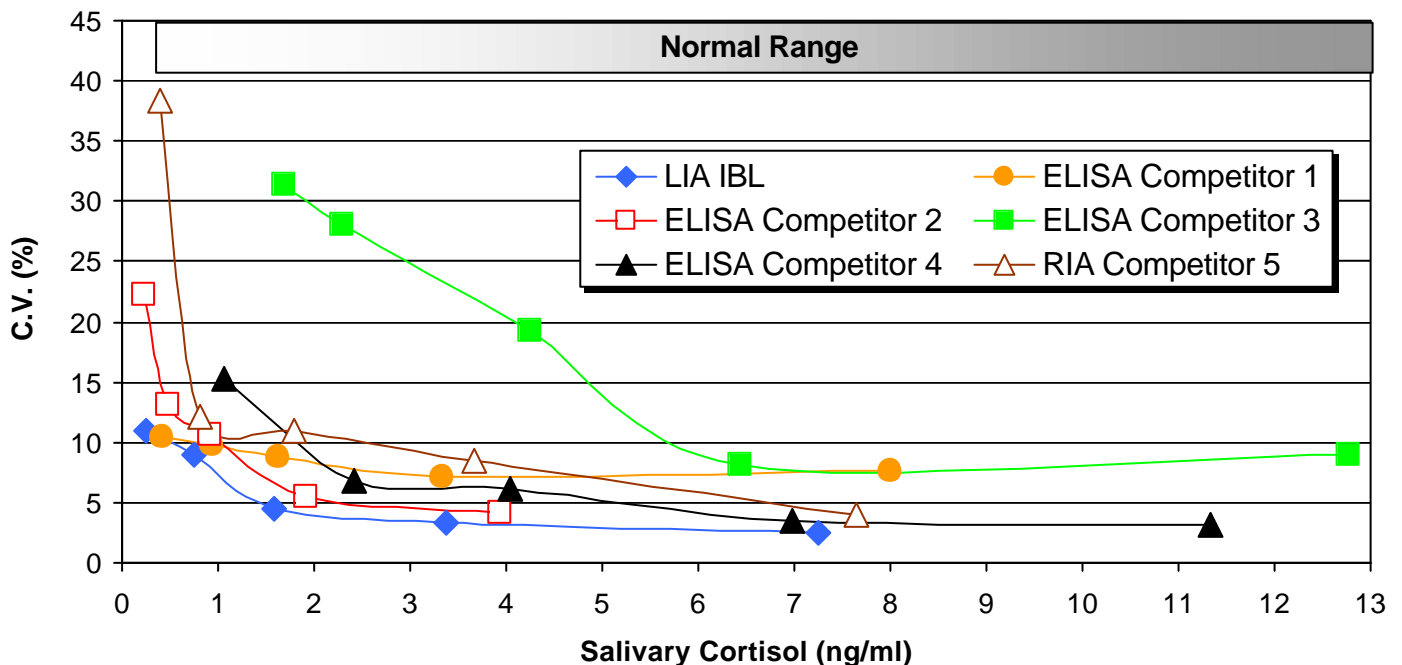


Fig. 37: Functional Sensitivity of various Cortisol in Saliva Immunoassays
The cortisol level in eight replicates of several saliva samples was measured. In the figure the coefficients of variation are plotted against the cortisol concentration.

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