THE EFFECT OF STRESS, DIET AND ANALYTICAL METHODS ON THE LEVELS OF CORTICOID METABOLITES

Michaela Dušková, Lucie Sosvorová, Jana Vítků, Hana Jandíková, Tereza Chlupáčová, Jose De Cordeiro, Luboslav Stárka

Institute of Endocrinology, Prague

Introduction

Measuring plasma cortisol levels is a common task of clinical biochemistry laboratories, and not just when ordered by endocrinologists. However, in the pre-analytical phase there are various influences that can distort the information sought. These influences include stress brought about by the sampling, poorly-timed sampling, not taking the sampling after fasting, and choosing a method that is incompatible with the desired aims. Errors in the pre-analytical phase significantly complicate the interpretation of results, and could lead to an incorrect diagnosis.

Objectives

The aim of this study:
Elucidate optimal conditions for blood sampling as well as the choice of analytical methods for measuring of corticoids.

Methods

The study was performed using 10 healthy women of reproductive age in the follicular phase of their cycle (days 1–7 after menstruation). The average age was 33.6 ± 2.56 years and average BMI 25.06 ± 1.3. The women had no chronic diseases, were non-smokers, and did not use hormonal contraceptives or any other medications. Before starting the study, they were advised to maintain a balanced regimen of 8 hours of sleep, regular eating according to a recommended menu, and restrain from consuming alcohol. All participants were given explanations about the study and signed informed consent. The study was approved by the ethical commission of the Institute of Endocrinology.

Each woman passed two tests during two consecutive menstrual cycles:
1) Stimulation test by stress and food
Each participant woke up at 6:30 in the morning. They had at 7:00 standard breakfast (two slices of bread, 50 g of breast-meat chicken slices, 1 slice of fresh cheese; total caloric content of the breakfast was 515 kcal, total protein content: 20.58 g, total carbohydrates: 47.75 g, total fat: 24.9 g). The test started at 10:00, when cannula was inserted into the forearm or cubital vein. The time schedule – according to which the blood was drawn – was: 10:00, 10:15, 10:30, 10:45 and 11:00.
After the blood sampling at 12 o'clock, the participants of this part of the study received lunch (beef broth soup, turkey, potato dumplings, and sauerkraut; total content of the lunch was 679 kcal, total protein content: 45.55 g, total carbohydrates: 100.4 g, total fat: 11.5 g). The lunch was followed by blood drawings at 12:30, 13:00 and 13:30.

2) Blank test
Each participant woke up at 6:30 in the morning. They had at 7:00 standard breakfast (two slices of bread, 50 g of breast-meat chicken slices, 1 slice of fresh cheese; total caloric content of the breakfast was 515 kcal, total protein content: 20.58 g, total carbohydrates: 47.75 g, total fat: 24.9 g). The peripheral cannula was inserted at 7:30 into the forearm or cubital vein. The blood was drawn according to the same schedule as it was in the first part of the study, it means, that at 10:00 was drawn the first blood sample, which was followed by additional samples at 10:15, 10:30, 10:45 and 11:00. In contrast to the stimulation test, in the blank test participants did not have any lunch at 12 o'clock, but they were submitted to samplings similarly as the group in the previous part of the study. The schedule of the blood drawings was: 12:00, 12:30, 13:00 and 13:30.

Blood was taken into a Vacuette Serum Clot Activator tubes (a plastic tube with a clotting activator and separation gel). Serum was obtained by centrifugation for 5 minutes at 2000 g at 4 °C, and stored at –20 °C.
Cortisol, cortisone, corticosterone and aldosterone were measured by LC-MS/MS (Sosvorová et al., 2015), and cortisol was additionally measured using an RIA kit from Immunotech (Czech Republic).

Statistical analyses: The relationships between dependent variables and the effects of sampling time were evaluated using a repeated measures ANOVA model consisting of the following factors: Time (10:00, 10:15, 10:30, 10:45 and 11:00 for experiment 1); (11:00, 12:00, 12:30, 13:00 and 13:30 for experiment 2) and Subject (explaining inter-individual variability). The ANOVA model was followed by least significant difference (LSD) multiple comparisons. To eliminate skewed data distribution and heteroscedasticity, the original data were transformed by Box-Cox transformation to attain a Gaussian distribution and constant variance before further processing. Simple regression was used to compare the two different analytical methods, with the RIA method chosen as the reference since it is commonly used during routine cortisol measurements. The statistical software Statgraphics Centurion, version XV from Statpoint Inc. (Herndon, Virginia, USA) was used for all statistical analyses.

Results

Figure 1: Influence of the time of blood drawing after cannulisation on cortisol and cortisone levels.
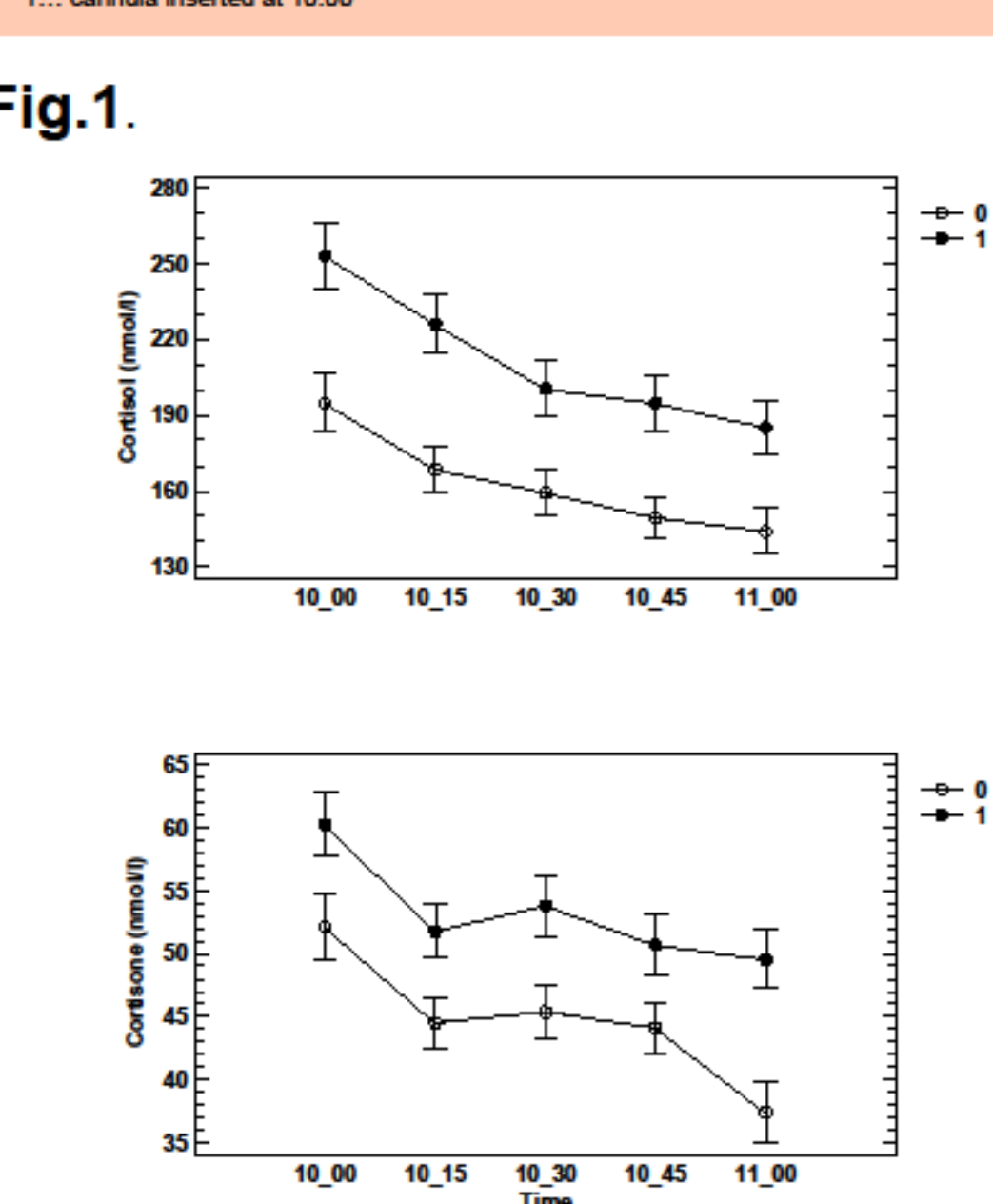0...cannula inserted at 8:00
1... cannula inserted at 10:00

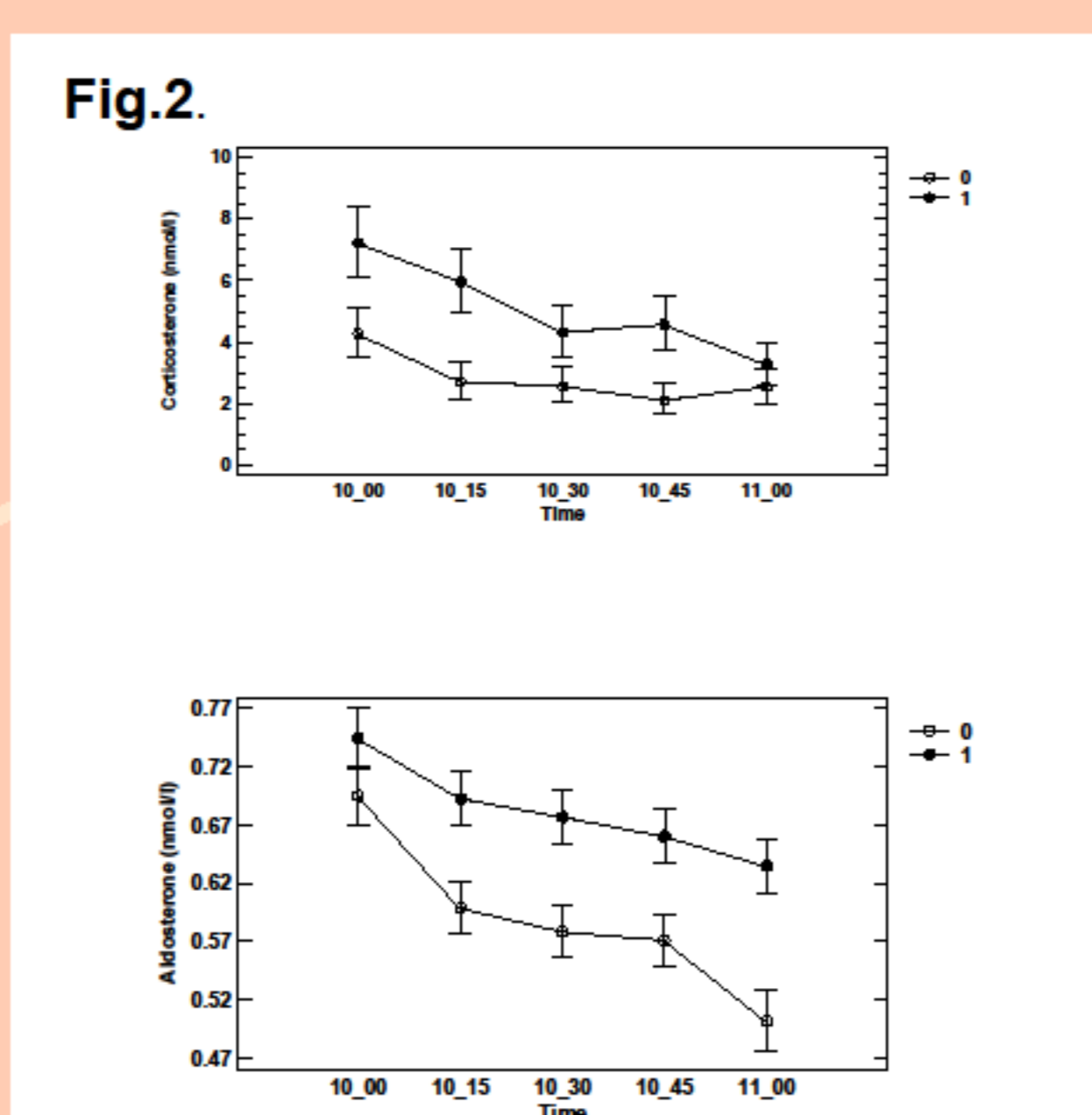Figure 2. Influence of the time of blood drawing after cannulisation on levels of corticosterone and aldosterone.

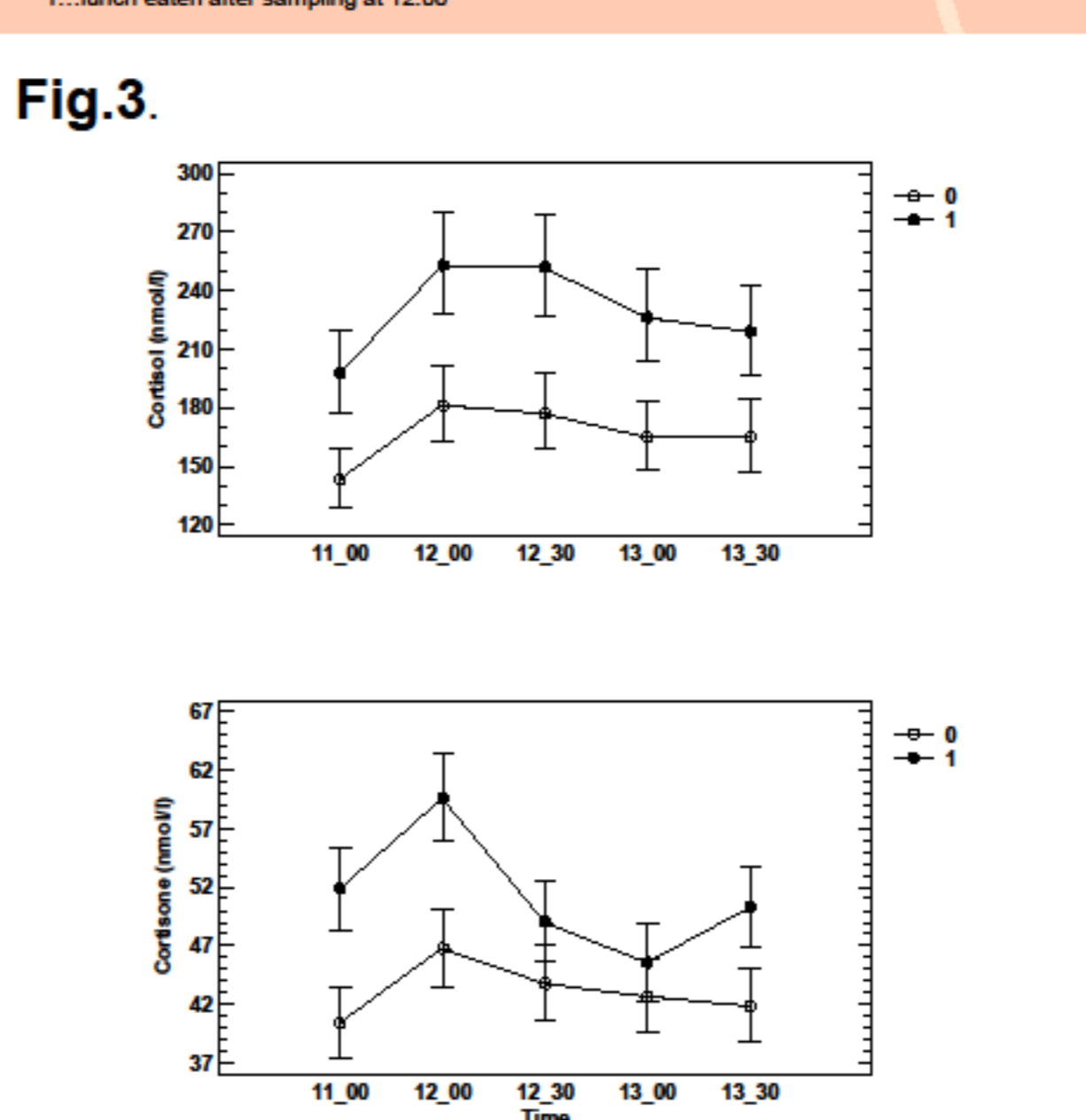Figure 3.: Cortisol and cortisone levels before lunch and then at half-hour intervals.
0...no lunch
1...lunch eaten after sampling at 12:00

Figure 4: Levels of corticosterone and aldosterone before lunch and then at half-hour intervals after lunchtime
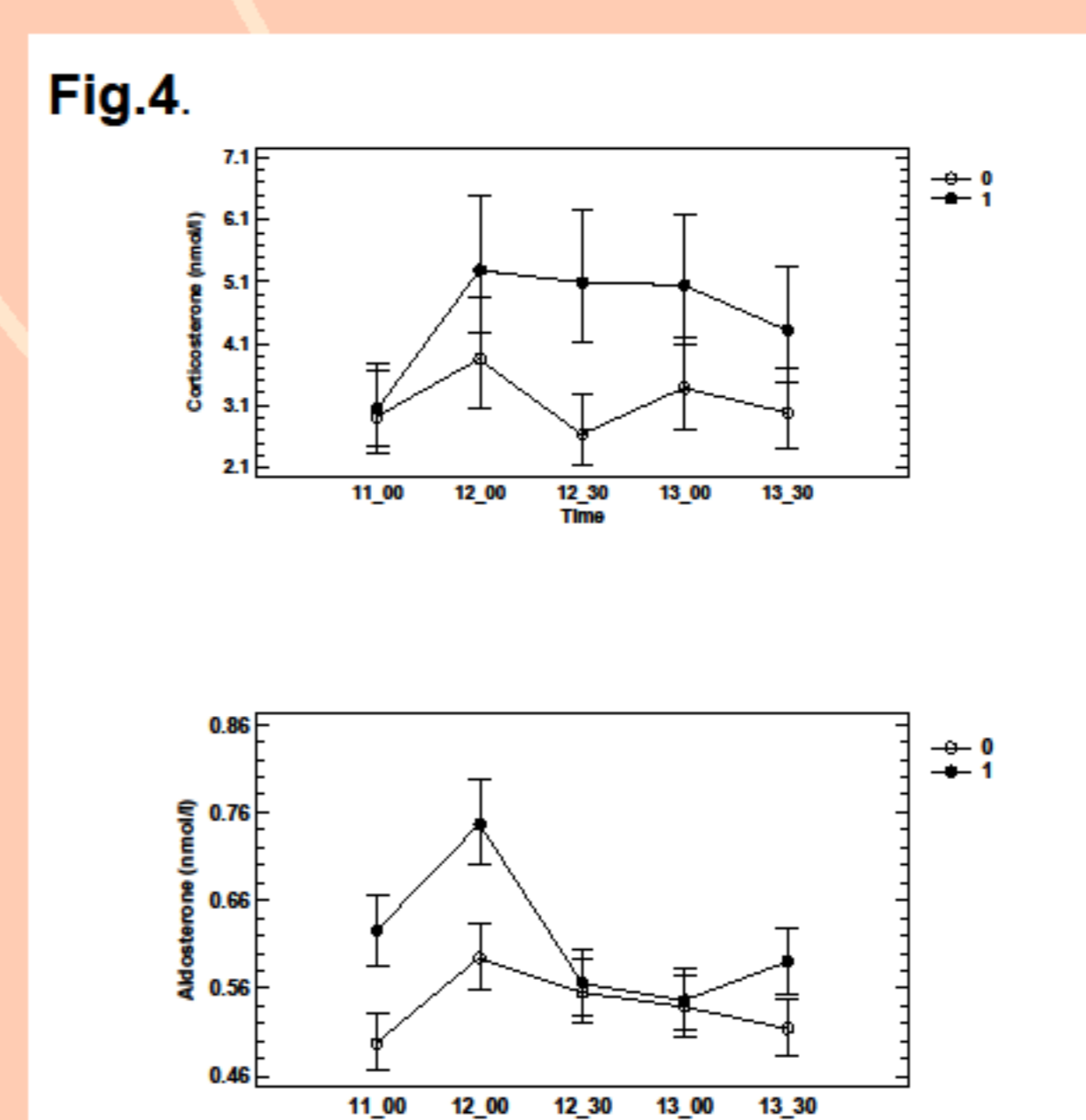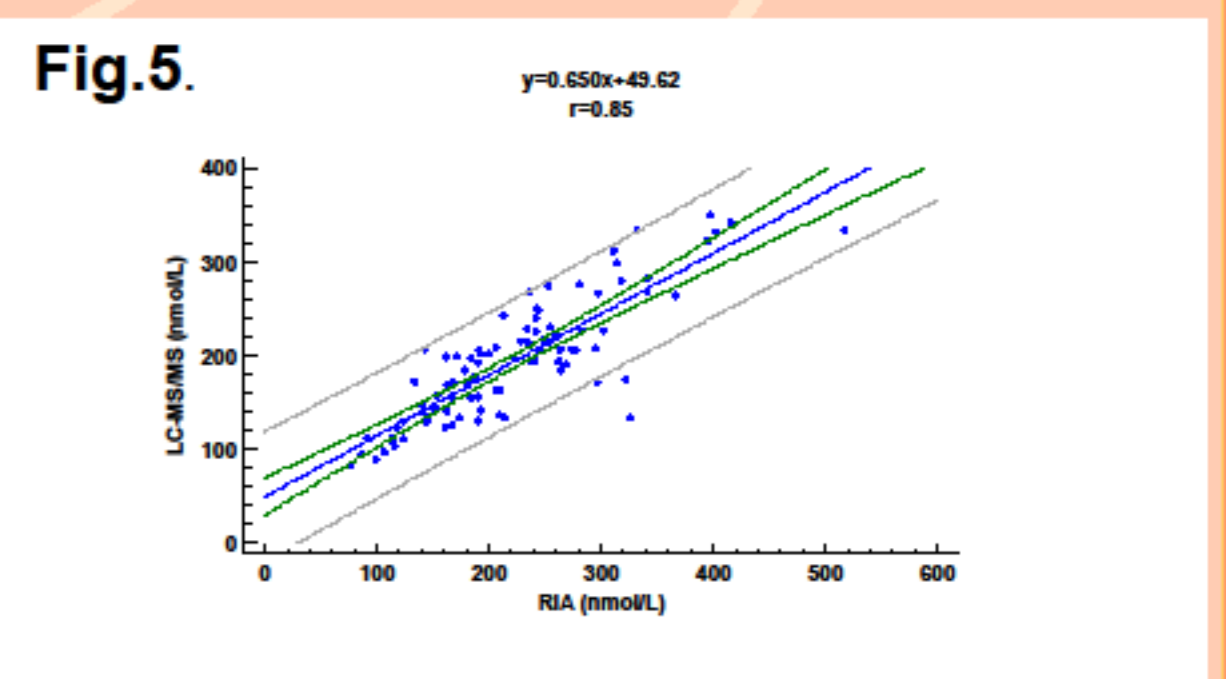
figure 5. Comparison of the LC-MS/MS and RIA methods. Simple regression for cortisol, RIA was selected as the reference method and LC-MS/MS as the test method.

Fig.1.   Fig.2.   Fig.3.   Fig.4.   Fig.5.

Stress :
Figure 1 shows the profile of cortisol levels when cannulation was being performed as well as when cannulation had been performed 150 minutes before the first blood sampling. Values just after inserting the cannula were significantly higher than values when calm. This reflects the fact that blood drawing can invoke minor or even fairly high stress in some patients. Higher levels of plasma cortisol lasted at least 1 hour after the first sampling. These results indicate that merely the knowledge that blood will be drawn can stimulate higher cortisol levels, not just the needle insertion itself. Cortisone levels were also increased, similarly as for cortisol (Figure 1).
Corticosterone was also increased already at the time of sampling, while increase in its metabolite aldosterone was later (Figure 2). As opposed to the other corticoids tested, the effect of stress on corticosterone disappeared after an hour (Figures 1 and 2).

The main lunchtime meal
The effects of the main meal of the day – lunch at noontime – were interesting. All corticoids tested had a marked increase between 11 and 12 o'clock, which could reflect a physiological preparation for eating as part of the circadian rhythm. After eating there was an evident decline in cortisone levels, while its precursor cortisol had rather a plateau in its decline (Figure 3). Similarly, there was an evident decline in aldosterone but a plateau in the decline of its precursor corticosterone (Figure 4).

Choice of analytical method
We compared cortisol in 90 plasma samples measured by the commercial RIA kit from Immunotech and a published LC-MS/MS method (Sosvorova et al., 2015). The RIA method, which was used as the reference method, showed strong correlation with the LC method (r=0.85), with the regression approximated by the equation y = 0.650x + 49.62 (Figure 5). The slope of the regression line indicates some overestimation of cortisol levels when using RIA.

Conclusions

Not just endocrinologists but also other specialists often require cortisol measurements from biochemical laboratories. Determining the correct levels of cortisol, especially in the differential diagnosis of hypocortisolism, hypercortisolism and normal functioning of the hypothalamic-pituitary-adrenal axis, require maintaining the proper conditions even in the pre-analytical phase of sampling. It is necessary to take into account the daily rhythm of cortisol and avoid sampling in the first hours after waking (if not specifically measuring CAR), as well as taking into account food intake and the stress of blood drawing. Finally, the choice of proper analytical method should be made with knowledge of their limitations.