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

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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 12 healthy women of reproductive age in the follicular phase of their cycle (days 1-7) after menstruation. The average age was 33.6 ± 2.96 years and average BMI 25.08 ± 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 5 hours of sleep, regular eating according to a recommended menu, and restraint from consuming alcohol. All participants were given explanations about the study and signed informed consent. The study was approved by the ethical committee of the Institute of Endocrinology.

Each volunteer passed two blood-sampling sessions during two consecutive menstrual cycles:

1. Stimulation test by stress and food

Each participant woke up at 6:00 in the morning. They had 700 standard breakfast (two slices of bread, 55 g of breast-meat, chicken slices, 1 slice of fresh cheese; total calories of the breakfast = 515 kcal, total protein content: 20.56 g, total carbohydrate: 47.75 g, total fat: 24.9 g). The participants were then placed in an armchair for 15 minutes, with a light on. Then the participants were asked to undertake a mental arithmetic test (10 minutes) and voice elicitation test (10 minutes). After the blood sampling at 12:00, the participants of the first part of the study received lunch (beef broth soup, furuly, potato-salad, and sauerkraut; total content of the lunch = 479 kcal, total protein content: 45.66 g, total carbohydrate: 102.6 g, total fat: 11.5 g). The lunch was followed by two blood-samplings at 12:30, 13:30 and 13:50.

2. Blank test

Each participant woke up at 6:00 in the morning. They had 700 standard breakfast (two slices of bread, 55 g of breast-meat, chicken slices, 1 slice of fresh cheese; total calories of the breakfast = 515 kcal, total protein content: 20.56 g, total carbohydrate: 47.75 g, total fat: 24.9 g). The participants were then placed in an armchair for 15 minutes, with a light on. Then the participants were asked to undertake a mental arithmetic test (10 minutes) and voice elicitation test (10 minutes). 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 sampling similarly as in the group in the previous part of the study. The schedule of the blood drawings was: 12:00, 13:00 and 13:30.

Blood was taken into a heparinized tube (Vacutainer tubes, 3 ml, Greiner Bio-one) with a sodium acetate and separation gel. Heparin was obtained by centrifugation for 9 minutes at 2500 g at 4 °C, and stored at −20°C.

Cortisol, corticosterone, cortisone and adrenosterone were measured by LC-MS/MS (Sosvorova et al., 2019) and cortisol was additionally measured using an RIA-kit from Immunotech (Czech Republic).

Paired analysis: 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, 10:30, 10:45 and 11:00 for experiment 1; 10:00, 10:30, 10:45 and 11:00 for experiment 2); The RIA method chosen for the measurement of cortisol; The RIA method chosen for the measurement of adrenosterone; and the correlation coefficient. Data were evaluated using IBM SPSS Statistics 25 software.

Results

Stress:

Table 1 shows the profile of cortisol levels when cannulation was being performed as well as when cannulation had been performed 150 minutes after 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 cortisol levels could not be determined accurately and that cortisol levels were not the same as in the blood drawn just after the first sampling.

Corticosterone was also increased already at the time of sampling, while its metabolite adrenosterone was lower (Figure 2). As opposed to the other corticosteroids, 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 corticosterone had reached a plateau in its decline (Figure 3). Similarly, there was an evident decline in adrenosterone but a plateau in its 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., 2019). 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 = 0.605x + 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.

Acknowledgment:

The study was supported by the project MZCR for conceptual development of research organization 00023761 Institute of Endocrinology mduskova@endo.cz.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5