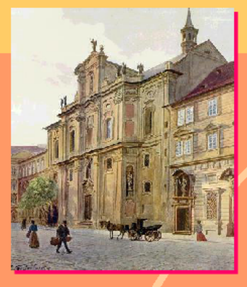
How Does Energy Intake Influence the Levels of Certain Steroids?



Hana Jandíková , Beáta Rácz, Michaela Dušková , Martin Hill , Karel Vondra, Luboslav Stárka

Institute of Endocrinology, Prague

Introduction

Even though the daily profiles of the main sex hormones are well known, small oscillations in their levels that may be connected to food intake have yet to be systematically studied. Glucocorticoids have many various functions in organisms, and they influence the maintenance of the homeostasis. With rhythmic changes of the hormone levels, the hypothalamic-pituitary-adrenal (HPA) axis ensures tissue and organ-specific reactions of the organism to endogenous and exogenous stimuli (Lightman et al., 2008).
According to a study by Stárka et al. (2015), levels of DHEA and of its conjugated form androstenediol and 7α-hydroxy – DHEA decline one to two hours after lunch and dinner. Another recent study has described significant changes of estradiol and SHBG after food intake (Rácz et al., 2015).
The influence of steroid hormones on the food intake is well known. In contrast, however, there are only a few published reports on how the food intake influences levels of steroid hormones.

Objectives

The aim of this study: Analyse the influence of food intake on steroid hormone and melatonin levels.
As stimuli we selected a standard breakfast, oral and intravenous glucose, and psyllium (as a model of mechanical effects of the food on the gastrointestinal tract).

Methods

The study participated eight women with average age of 29.48 ± 2.99 years and BMI of 21.3 ± 1.3 kg/m2. All of the women were pre-menopausal, they were non-smokers, healthy, and they were not using any medication or hormonal contraceptive. Blood samples were collected during the follicular phase (days 1–7 of the menstrual cycle). Five days before they had undergoing the tests, all of the women followed a standard protocol that did not vary much significantly from their normal daily routine (8 hours of sleep, food intake according to a standardized menu). Before the tests they were informed about the study protocol and they signed an informed consent form. The study was approved by the ethical commission of the Institute of Endocrinology in Prague.
Each woman passed the four different tests during four consecutive menstrual cycles:
1) OGTT – an oral glucose tolerance test – 75 g of glucose (Glukopur brand) in 250 ml of unsweetened tee perorally.
2) IVGTT – an intravenous glucose tolerance test – a bolus of 0.33 g of glucose per kg of weight in 20% intravenous solution, administrated to a peripheral vein.
3) a 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).
4) psyllium – a non-caloric fibre, which was meant to simulate mechanical stimulation of the gastrointestinal tract through distention. The women drank 4 g of psyllium in 250 ml water.
An intravenous cannula was inserted into the cubital vein ten minutes before the first blood sampling. Sampling was performed for 120 minutes, with the following schedule:
- the first sampling was performed at 7:30 a.m. after overnight fasting
- subsequent samplings were performed at 20, 40, 60, 90, and 120 minutes

Analytical methods:
Each sample was collected into a plastic tube containing 100 µl of 5% EDTA. Plasma was obtained after centrifugation for 5 min at 2000 rpm at 4 °C, then separated and frozen within half an hour of being drawn from the subject, and stored at –20 °C until analysed.
C-peptide was measured in serum using ECLIA (electrochemiluminiscence immunoassay, Modular E 170 analyser, Roche). The measuring range of the kit (defined by the lower detection limit and the maximum of the master curve) was 0.003–13.3 nmol/l or 0.01–40.0 ng/ml for plasma. Intra- and inter-assay coefficients of variation were 1.5% and 2.3%, respectively. Blood glucose was measured using the enzymatic reference method with hexokinase (Cobas Integra 400 plus analyser, Roche). The measuring range of the kit was 0.12–40 mmol/l (2.16–720 mg/dl). Intra-set and inter-set reproducibility were 1.7% and 2.6%, respectively. Cortisol was measured using an RIA kit (Immunotech, France).
Steroid hormones measured by a GC/MS method
The levels of 37 unconjugated steroids and their polar conjugates were measured using a GC/MS method (Hill et al., 2010). In brief, free steroids were extracted from plasma by diethyl-ether; steroid conjugates were hydrolysed and extracted. The resulting residues were derivatized by methoxyamine hydrochloride and analysed by GC/MS as follows. Steroids were purchased from Steraloids (Newport, RI, USA), Sylon B from Supelco (Bellefonte, PA, USA), methoxylamine hydrochloride from Sigma (St. Louis, MO, USA) and solvents from Merck (Darmstadt, Germany).
Statistical data analysis:
The changes of steroid levels and melatonin were evaluated using a repeated measures ANOVA model consisting of a Subject factor, explaining differences between subjects, and a Stage factor. Due to the non-Gaussian data distribution and non-constant variance, the original data were transformed by a power transformation to attain a symmetric distribution of the data and residuals as well as homoscedasticity (Meloun et al., 2000). The homogeneity of the transformed data was checked by residual analysis as described elsewhere (Meloun et al., 2002, 2004). Then, the significance of the values was evaluated by least significant differences multiple comparisons.

Results

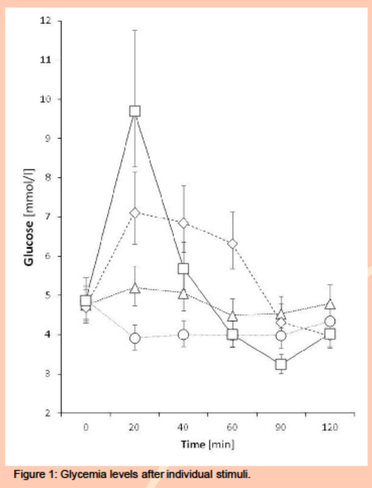


Figure 1: Glycemia levels after individual stimuli.
Δ – standard breakfast
□ – IVGTT
◊ – OGTT
○ – psyllium

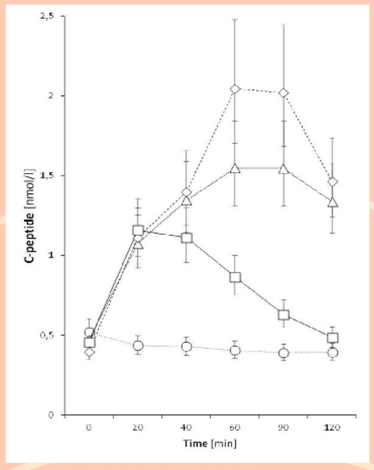


Figure 2: C-peptide levels after individual stimuli.
Δ – standard breakfast
□ – IVGTT
◊ – OGTT
○ – psyllium

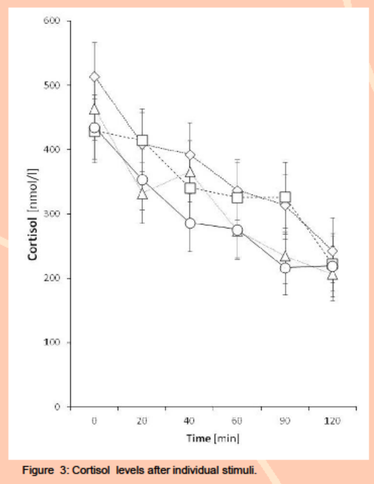


Figure 3: Cortisol levels after individual stimuli.
Δ – standard breakfast
□ – IVGTT
◊ – OGTT
○ – psyllium

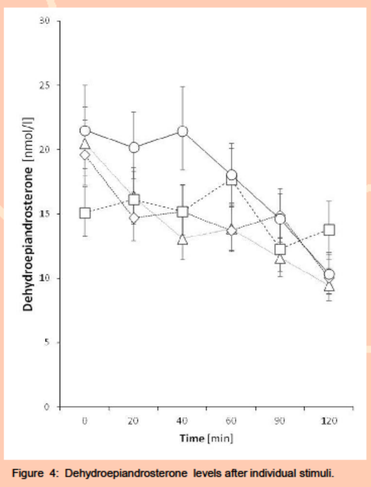


Figure 4: Dehydroepiandrosterone levels after individual stimuli.
Δ – standard breakfast
□ – IVGTT
◊ – OGTT
○ – psyllium

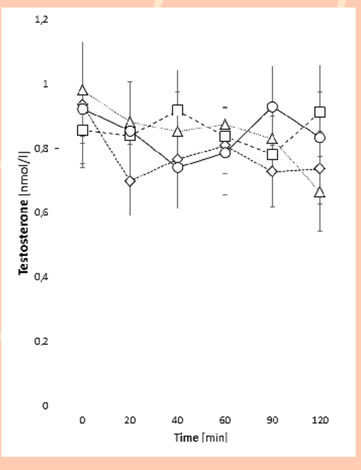


Figure 5: Testosterone levels after individual stimuli.
Δ – standard breakfast
□ – IVGTT
◊ – OGTT
○ – psyllium

In order to better elucidate various findings on the influence of food intake on hormone levels, we studied the influence of several stimuli on the course of hormone levels. As stimuli we chose a standard breakfast, oral glucose, intravenous glucose and psyllium (chosen to follow mechanical effects of food on the gastrointestinal tract). The timeline of samplings was focused on monitoring and analysing of acute and small changes of hormone levels after each stimulus. As we expected, the glycemia and C-peptide levels reflected normal values of a healthy population (Figures 1 and 2).

Cortisol
There was a slowing of the physiological decline in cortisol levels after each of the stimuli, excepting psyllium. This slowing was most pronounced after intravenous glucose, lasting even 60 minutes. After oral glucose and intravenous glucose there was a plateau in cortisol levels, but after breakfast there was an increase in cortisol at 40th minute (Figure 3).

DHEA
After the initial decline there was an increase in DHEA after all stimuli. This increase was most pronounced after intravenous glucose, but this increase was delayed compared to the other stimuli (Figure 4).

Testosterone
The course of testosterone levels did not have any significant relationship to any of the individual stimuli (Figure 5).

Similarly, non-conjugated and conjugated steroids also showed no relationships to individual stimuli, and we were unable to demonstrate a relationship between melatonin and the steroids studied.

Conclusions

Despite the fact that we performed the tests in the morning hours, when steroid hormone levels physiologically start to change due to their diurnal rhythm, we still found that food intake influences some of the hormone levels.

Acknowledgment:

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