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THE EFFECT OF FASTING ON THE DIURNAL RHYTHM OF RAT ACTH AND CORTICOSTERONE SECRETION

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Abstract — A tight association of feeding, metabolism, and function of the hypothalamo-pituitary-adrenocortical (HPA) axis is well known. Studies of HPA axis responsivity to various diets and food restriction show that not only fasting but also the cyclic circadian input importantly determine these responses. We therefore studied the response of the rat HPA system to different fasting periods (12, 18, 24, 36, and 48 h), all imposed at 6 PM, by measuring blood ACTH and corticosterone concentration. The corresponding metabolic parameters, e.g., blood glucose and free fatty acid (FFA) levels, were also assessed. The obtained results show that fasting altered the normal diurnal rhythm in levels of blood ACTH and FFA, but not corticosterone and glucose. All applied fasting periods increased HPA system activity in comparison with *ad libitum* fed rats. However, the most pronounced elevation in the blood ACTH concentration was observed after 18 h and 36 h of fasting, while the CORT increment was the highest 48 h after food restriction began. As expected, the blood glucose concentration decreased under the influence of all fasting periods, being the lowest after 12 h of overnight fasting. In contrast to that, fasted rats exhibited a normal level of serum FFA early in the morning, an elevated level thereafter, and the peak level 48 h after the onset of starvation.

Key words: ACTH, corticosterone, fasting, glucose, FFA, hypothalamo-pituitary-adrenal system

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INTRODUCTION

Food intake, metabolism, and function of the hypothalamo-pituitary-adrenal (HPA) system are tightly associated in all mammalian species (Dallman et al., 1995). We have previously showed that 48-h fasting causes increase in the blood ACTH and corticosterone concentration (Cvijic et al., 2000; Djordjevic et al., 2003). However, Hanson et al. (1994) demonstrated that not only fasting but also the cyclic circadian input importantly determine the HPA responses. Normally, basal plasma ACTH and corticosterone levels are minimal at lights on in *ad libitum*-fed rats. By contrast, as rats begin to eat towards the end of the light period, plasma ACTH and corticosterone levels rise and peak at lights out, when the major daily bouts of food intake begin (Dallman, 1984). In regularly fed rats, there are also diurnal rhythms in amplitude of the stress response and the sensitivity of ACTH to corticosterone feedback. The HPA response and its sensitivity are of high amplitude at the beginning of the light period and of low amplitude at the onset of dark, after they have voluntarily fasted during the light period (Bradbury et al., 1991). Moreover, if rats are on a restricted feeding regimes, the normal diurnal rhythm in plasma ACTH and corticosterone is altered, as is the magnitude of ACTH responses to stress and corticosteroid feedback efficacy (Akana et al., 1992). The removal of food for 14 h during the light period does not alter responses in the HPA axis, while a brief period of starvation beginning near the onset of darkness markedly alters the response of the HPA axis (Hanson, 1994). Bearing all this in mind, we studied the response of the rat HPA system to different fasting periods (12, 18, 24, 36, and 48 h), all imposed at 6 PM, by measuring blood ACTH and corticosterone concentrations. The corresponding

metabolic parameters, e.g., blood glucose and free fatty acid (FFA) levels, were also assessed.

MATERIALS AND METHODS

Male rats of the Wistar strain (*Rattus norvegicus*), 60-90 days old, weighing 180-220 g were used for the experiments. The animals were acclimated to 22±1°C, maintained under conditions of a 12:12-h light-dark cycle, and given commercial rat food (Subotica, Serbia) and tap water *ad libitum*. They were housed two per cage for 15 days before the start of the experiment.

Thirty animals were completely deprived of food at 6 PM at the beginning of the dark period. Six-animal-groups were one by one successively sacrified at 12, 18, 24, 36, and 48 h after the onset of food deprivation. Regularly fed animals were sacrificed together with each of the experimental groups. The experiments were performed according to the rules for animal care proposed by the Serbian Laboratory Animal Science Association (SLASA).

The rats were decapitated without anesthesia with a guillotine (Harvard-Apparatus, Holliston, MA). Blood was collected from the trunk and divided into two sets of tubes. For obtainment of plasma, EDTA was added in one of the sets. Serum and plasma were frozen for corticosterone and ACTH determination. Plasma ACTH was determined by the chemiluminescent method using an IMMULITE automatic analyzer (DPC, Los Angeles, CA). The values are expressed as pg ACTH/ml plasma. Serum corticosterone was determined with an RIA kit (ICN Biochemicals, Costa Mesa, CA) and the values expressed as μg CORT/ 100 ml serum. Blood glucose concentration was measured with an Exactech glucose analyzer (Medisense Inc., Cambridge, MA) using Dextrostix reagent strips. Serum FFA concentration was determined by the colorimetric method of Ducombe (Ducombe, 1964). The values are expressed as mmol/l serum.

The ANOVA one-way and Tukey's posterior multiple comparison tests were employed for comparison of the experimental groups. The values are expressed as means ± SE of six animals, the level of significance being set at $p<0.05$.

RESULTS

Expectedly, the obtained results show that *ad libitum*-fed control rats have regular HPA activity, such activity being of low amplitude at the beginning of the light period after feeding in the dark and of high amplitude at the onset of darkness. Fasting altered the normal diurnal rhythm in levels of blood ACTH and FFA, but not corticosterone and glucose. Significant differences in plasma ACTH concentration between fed and starved rats occurred at noon and early in the morning (at 12, 18, and 36 h after the onset of starvation; $^{+++}p<0,001$), while they disappeared at the start of the dark period (Fig. 1). Even though the pattern of corticosterone secretion remained unchanged, all applied fasting periods induced a significant increase in blood corticosterone concentration in comparison with the regularly fed rats, with the highest increment 48 h after the food restriction was imposed (Fig. 2, $^{+++}p<0,001$,$^{***}p<0,001$). As expected, the blood glucose concentration decreased under the influence of all fasting periods, being the lowest after 12 h of overnight fasting (Fig. 3, $^{++}p<0,01$). In contrast to that, fasted rats exhibited a normal level of serum FFA early in the morning, an elevated level thereafter, and the peak level 48 h after the onset of starvation (Fig. 4, $^{+++}p<0,001$, $^{*}p<0,05$).

DISCUSSION

As reported previously, the HPA system rapidly responds to fasting being markedly affected by the circadian cycle (Hanson, 1994). Food deprivation during the dark period of the circadian rhythm, the period of maximal HPA activity and food intake in rodents, activates the HPA system. Removal of food prior to the onset of darkness results in a rapid three-fold increase in nocturnal corticosterone secretion above that of *ad lib*-fed controls (Akana et al., 1994). Our present results show that fasting altered the normal diurnal rhythm in ACTH, but not corticosterone secretion. Significant differences in plasma ACTH between fed and starved rats occurred at noon and early in the morning, but they vanished

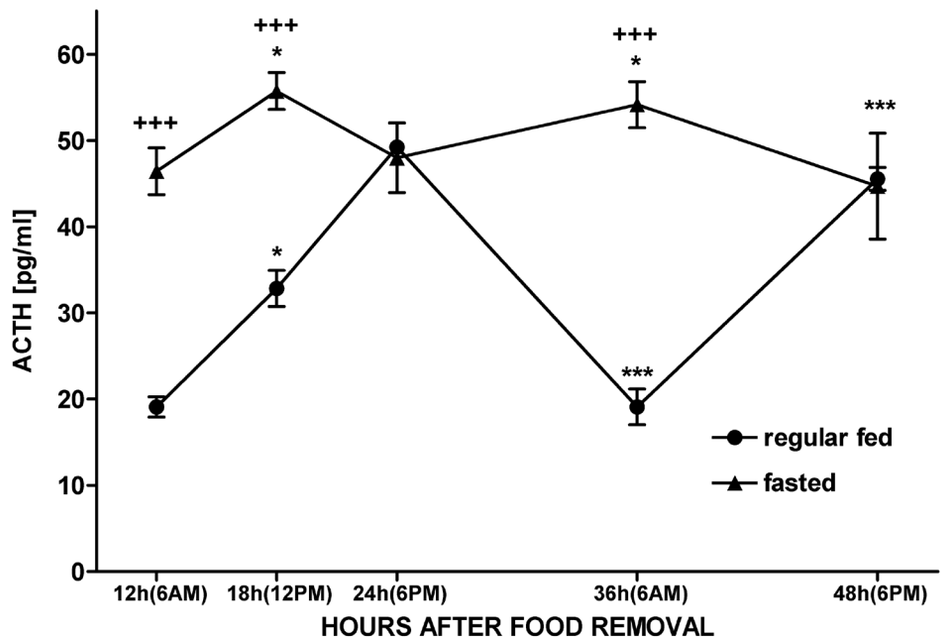

Fig. 1. Plasma ACTH diurnal rhythm in regularly fed (-●-) rats or ones exposed to 12, 18, 24, 36, and 48 h of fasting (-▲-) imposed at 6 PM. Values represent means ± SE; n = 6. *p < 0.05 significant difference between adjacent values; ┼p < 0.05 compared to corresponding control.

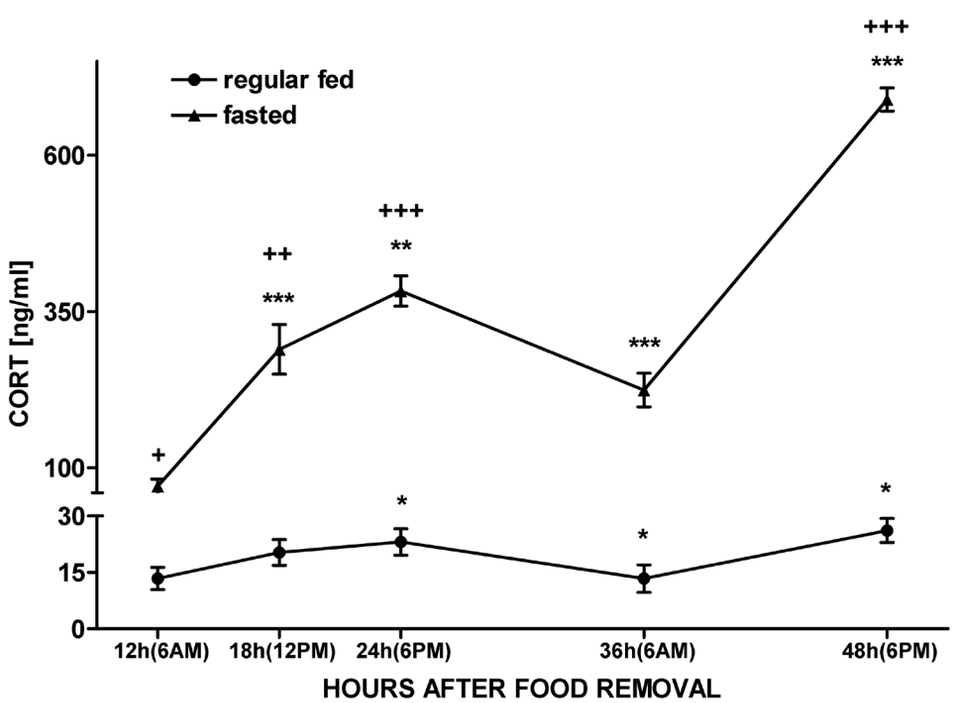

Fig. 2. Serum corticosterone diurnal rhythm in regularly fed (-●-) rats or ones exposed to 12, 18, 24, 36, and 48 h of fasting (-▲-) imposed at 6 PM. *p < 0.05 significant difference between adjacent values; ┼p < 0.05 compared to corresponding control.

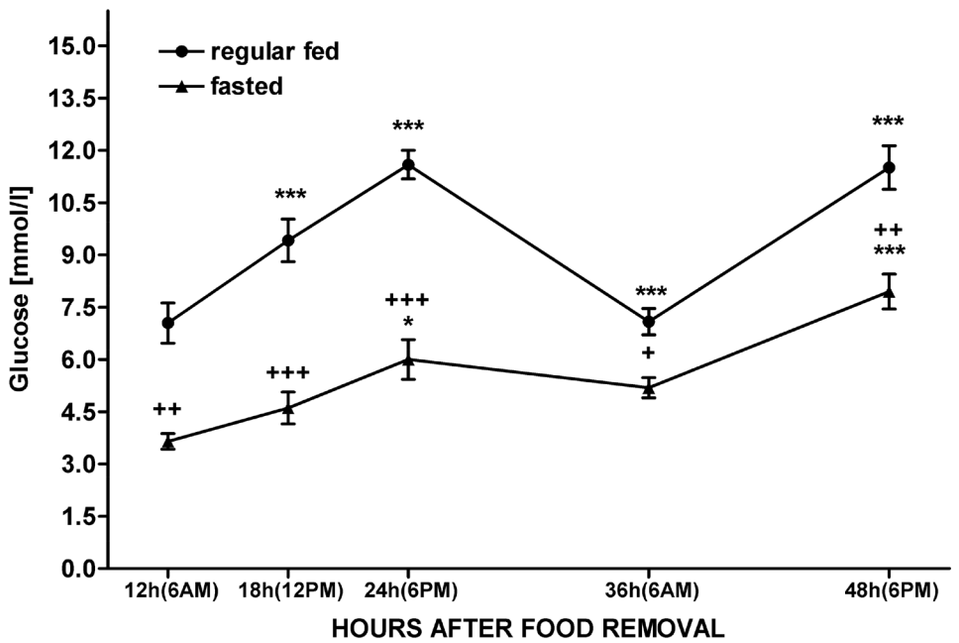

Fig. 3. Blood glucose diurnal rhythm in regularly fed (-●-) rats or ones exposed to 12, 18, 24, 36, and 48 h of fasting (-▲-) imposed at 6 PM. *$p < 0.05$ significant difference between adjacent values; $^{+}p < 0.05$ compared to corresponding control.

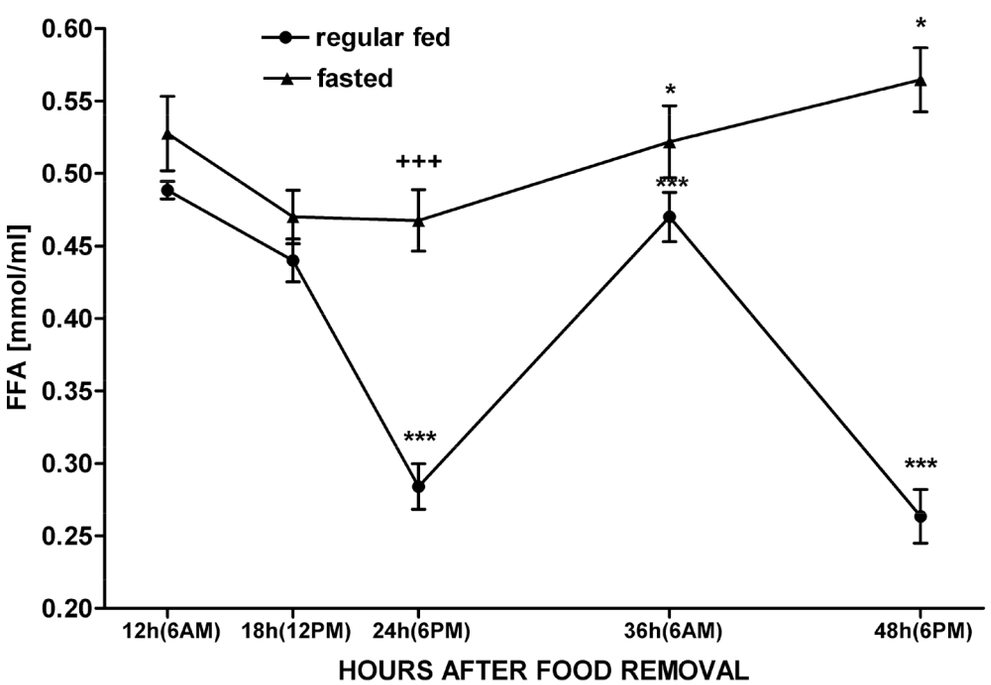

Fig. 4. Serum FFA diurnal rhythm in regularly fed (-●-) rats or ones exposed to 12, 18, 24, 36, and 48 h of fasting (-▲-) imposed at 6 PM. *$p < 0.05$ significant difference between adjacent values; $^{+}p < 0.05$ compared to corresponding control.

at the beginning of the dark period. Corticosterone secretion did not track ACTH well, since it was constantly increased over the 48 h of starvation with two peaks around 6 PM at the onset of the dark phase. It would appear that adrenal corticosterone secretion is not solely regulated by ACTH. According to Dallman et al. (1999), control of adrenal and plasma corticosterone by ACTH is greatly reduced during the second half of the dark period and thereafter. Their results strongly suggest that starvation shifts adrenal regulation of corticosterone synthesis and secretion away from hypothalamic control of ACTH secretion through CRH and AVP. Changes in expression of the four hypothalamic neuropeptides show that starvation-induced changes in CRH and NPY, but not AVP or POMC, occur within the first 6–24 h, suggesting that neurons containing these neuropeptides are early sensors that mediate responses to starvation. Changes of leptin concentrations in starved rats may be a part of the decoupling between adrenal corticosterone and ACTH (Akana, 1994). Leptin receptors are found in the adrenals as well as the hypothalamus (Cao et al., 1997). Moreover, the existence of a positive-feedback loop between FFA and the HPA axis has been postulated. In fact, *in vitro* studies showed a direct stimulatory effect of FFA, especially long-chain unsaturated ones, on the adrenal gland (Widmaier et al., 1995). On the other hand, other authors reported a dual, dose-dependent effect of FFA on cortisol release from adrenal cells; in fact, oleic acid and linoleic acid were able to stimulate glucocorticoid production in the absence of adrenocorticotropic hormone despite high FFA concentrations, which inhibit ACTH action (Matthys and Widmaier, 1998). We detected a peak in the FFA level after 48 h of food deprivation, which perfectly correlates with the large corticosterone peak. Since food was removed toward the end of the light period, it is unlikely that there were large glycogen reserves available for immediate energy. The increase in FFA suggests that energy requirements were met by fat mobilization, fostered by the immediate and marked reduction in insulin. Dallman et al. (1999) reported a decline of the glucose level before 10 PM by approximately 30%, the concentration of glucose being maintained at low but constant levels thereafter, suggesting that by this time hepatic gluconeogenesis was sufficient to maintain the output of this substrate. Hypoglycemia is also a well-known stimulus of HPA function (Fernandez-Real et al., 1997). Changes either in FFAs or in insulin, leptin, or glucose serve as precise afferent signals to the hypothalamus indicating decreasing energy stores. The medial and lateral hypothalamus contains neurons that are directly sensitive to insulin, glucose, and FFA; thus, changes in these would be directly perceived (Levin and Routh, 1996). In conclusion, our present results show that fasting dysregulated normal HPA activity and provoked increase of both ACTH and corticosterone blood levels, but also led to dissociation between ACTH and corticosterone secretion. This suggests that in food-deprived rats, corticosterone secretion is not regulated by ACTH alone, but probably by other metabolic and endocrine factors as well.

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REFERENCES

Akana, S. F., Dallman, M. F., Bradbury, M. J., Scribner, K. A., Strack, A. M. and *C. D. Walker* (1992). Feedback and facilitation in the adrenocortical system: unmasking facilitation by partial inhibition of the glucocorticoid response to prior stress. *Endocrinology* **131**,57-68.

Akana, S. F., Strack, A. M., Hanson, E. S. and *M. F. Dallman* (1994). Regulation of activity in the hypothalamo-pituitary-adrenal axis is integral to a larger hypothalamic system that determines caloric flow. *Endocrinology* **135**,1125-1134.

Bradbury, M. J., Cascio, C. S., Scribner, K. A., and *M. F. Dallman* (1991). Stress-induced adrenocorticotropin secretion: diurnal responses and decreases during stress in the evening are not dependent on corticosterone. *Endocrinology* **128**, 680-688.

Cao, G. Y., Considine, R. V. and *R. B. Lynn* (1997). Leptin receptors in the adrenal medulla of the rat. *Am. J. Physiol.* **273**, E448-452.

Cvijic, G., Djordjevic, J., and *V. Davidovic* (2000). Effect of fasting and refeeding on the activities of monoamine oxidase and antioxidant enzymes in rat hypothalamus and brown adipose tissue. *Gen. Physiol. Biophys.* **19**, 305-316.

Dallman, M. F. (1984). Viewing the ventromedial hypothalamus from the adrenal gland. *Am. J. Physiol.* **246**, R1-12.

Dallman, M. F., Akana, S. F., Bhatnagar, S., Bell, M. E., Choi, S., Chu, A., Horsley, C., Levin, N., Meijer, O., Soriano, L. R., Strack, A.M., and *V. Viau* (1999). Starvation: early signals, sensors, and sequelae. *Endocrinology* **140**, 4015-4023.

Dallman, M. F., Akana, S. F., Strack, A. M., Hanson, E. S. and *R. J. Sebastian* (1995). The neural network that regulates energy balance is responsive to glucocorticoids and insulin and also regulates HPA axis responsivity at a site proximal to CRF neurons. *Ann. N. Y. Acad. Sci*. **771**, 730-742.

Djordjevic, J., Cvijic, G., and *V. Davidovic* (2003). Different activation of ACTH and corticosterone release in response to various stressors in rats. *Physiol. Res. / Acad. Sci. Bohemoslov*. **52**, 67-72.

Ducombe, W. G. (1964). The calorimetric micro-determination of nonesterified fatty acids in plasma. *Clin. Chim. Acta* **9**, 122-125.

Fernandez-Real, J. M., Ricart, W., and *R. Casamitjana* (1997). Lower cortisol levels after oral glucose in subjects with insulin resistance and abdominal obesity. *Clin. Endocrinol*. **47**, 583-588.

Hanson, E. S., Bradbury, M. J., Akana, S. F., Scribner, K. S., Strack, A. M., and *M. F. Dallman* (1994). The diurnal rhythm in adrenocorticotropin responses to restraint in adrenalectomized rats is determined by caloric intake. *Endocrinology* **134**, 2214-2220.

Levin, B. E., and *V. H. Routh* (1996). Role of the brain in energy balance and obesity. *Am. J. Physiol.* **271**, R491-500.

Matthys, L. A., and *E. P. Widmaier* (1998). Fatty acids inhibit adrenocorticotropin-induced adrenal steroidogenesis. *Horm. Metab. Res. Horm. Stoffwechselforsch*. **30**, 80-83.

Widmaier, E. P., Margenthaler, J., and *I. Sarel* (1995). Regulation of pituitary-adrenocortical activity by free fatty acids *in vivo* and *in vitro*. *Prostagland. Leukotr. Essent. Fatty Acids* **52**, 179-183.

УТИЦАЈ ГЛАДОВАЊА НА ДНЕВНО – НОЋНИ РИТАМ ОСЛОБАЂАЊА АСТН И КОРТИКОСТЕРОНА КОД ПАЦОВА

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У овом раду испитиван је одговор система хипоталамус – хипофиза - надбубрежне жлезде (ХХА) на различите периоде гладовања (12, 18, 24, 36 и 48 часова) мерењем концентрације кортикотропина (АСТН) и кортикостерона у крви пацова. Такође је мерена концентрација слободних масних киселина и глукозе у крви, као одговарајућих метаболичких параметара. Резултати показују да гладовање повећава активност ХНА система у поређењу са нормално храњеним пацовима и мења дневно-ноћни ритам ослобађања АСТН, али не и кортикостерона. Највеће повећање концентрације АСТН детектовано је након 18 и 36 часова гладовања, а концентрације кортикостерона и слободних масних киселина 48 часова након почетка гладовања. Као што је и било очекивано, гликемија је била смањена током свих периода гладовања, са најнижим вредностима 12 часова од почетка гладовања.