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Short Communication

The effect of chronic food restriction on immunopositive ACTH cells in peripubertal female rats

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Abstract. In peripubertal female rats, we have previously found that 50% food restriction (FR) increases plasma IL-6, haptoglobin and both alanine transaminase (ALT) and alkaline phosphatase (AST) aminotransferases, indicating the existence of an inflammatory response. To study whether such FR influences the hypothalamic-pituitary-adrenal (HPA) axis, we examined by immunohistochemistry the morphofunctional features of pituitary adrenocorticotropic (ACTH) cells. In FR rats the volume and volume density of ACTH cells as well as plasma ACTH levels were increased by 17.6%, 12.5% and 13.4%, respectively, in comparison with controls ($p < 0.05$). We concluded that chronic FR is a systemic stressor in young females, capable to stimulate the HPA axis, probably as a result of IL-6 action.

Key words: Food restriction — Female rats — ACTH cells — ACTH

Chronic food restriction (FR) represents a continuous physiological stress of particular concern for young mates in the phase of rapid growth, with relatively high metabolic requirements. We previously found increased serum levels of the cytokine interleukin IL-6, acute phase protein haptoglobin (Uskoković et al. 2009) and the aminotransferases, alanine transaminase (ALT) and aspartate transaminase (AST) (Grigorov et al. 2004) in female Wistar rats exposed to 50% FR for 6 weeks in the peripubertal period. This FR led to a significant reduction in body weight to about 50% that of controls (Grigorov et al. 2004). Overall this indicated the existence of inflammatory conditions and adaptive responses during chronic severe FR. The hallmark characteristic of the stress response is multidirectional immuno-neuro-endocrine interaction (Besedovsky and del Rey 1996). Activation of the hypothalamic-pituitary-adrenal (HPA) axis through different pathways, including those of cytokines such as IL-6, has a key role in this process. IL-6 is known to contribute to stimulation of anterior pituitary hormone secretion (Spangelo and MacLeod 1990) and it induces adrenocorticotropic hormone (ACTH) release and corticosterone responses (Lenczowski et al. 1997). Giovambatista et al. (2000) showed that undernourished female rats displayed hypercorticosteronemia and enhanced adrenal glucocorticoid content. However, controversy exists regarding the ACTH responses. While Giovambatista et al. (2000) observed similar basal ACTH concentrations in well-nourished and undernourished rats, several authors found decreased ACTH levels following different types of diet-restriction in rats (Han et al. 1995; Avraham et al. 2002; Chacon et al. 2005). Han et al. (1998) demonstrated that hyperadrenocorticism in diet-restricted rats could occur as the consequence of an enhanced sensitivity of adrenal cells to ACTH. These data and our findings regarding the significant increase of serum IL-6 (Uskoković et al. 2009) prompted us to undertake the present study to assess the morphological characteristics of ACTH cells in the pituitary gland and changes in ACTH plasma levels, after exposure of peripubertal female rats to chronic 50% FR.

Peripubertal female Wistar rats were caged individually in a *vivarium*, with a 12/12 h light/dark regime, at room temperature (20 ± 2°C) and 50% relative humidity. All rats were free of infection. The animals were fed commercial rat food *ad libitum* until 1 month of age, when they were divided into two groups of ten animals each. Control animals continued to receive unlimited food, whereas experimental FR animals were fed daily for 6 weeks with 50% of the

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amount of food consumed by the controls. All animals were sacrificed after 6 weeks, when the body weight for FR rats was 110.5 ± 6.6 g (mean ± standard error of mean, SEM) and for the control group 225.2 ± 19.3 g. The experimental protocols were approved by the Animal Care Committee of the Institute for Biological Research (Belgrade, Serbia) in conformity with the recommendation provided in the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (ETS No. 123, Appendix A).

Pituitary glands were excised, weighed in air, fixed in Bouin's solution and embedded in paraffin. Serial 5 μm thick tissue sections were treated with xylol and serial alcohol dilutions. Pituitary ACTH cells were localized by the peroxidase-antiperoxidase-complex (PAP) method of Sternberger et al. (1970). Measurements were performed on the widest portion of the pituitary gland and immunocytochemically-labeled ACTH cells were analyzed

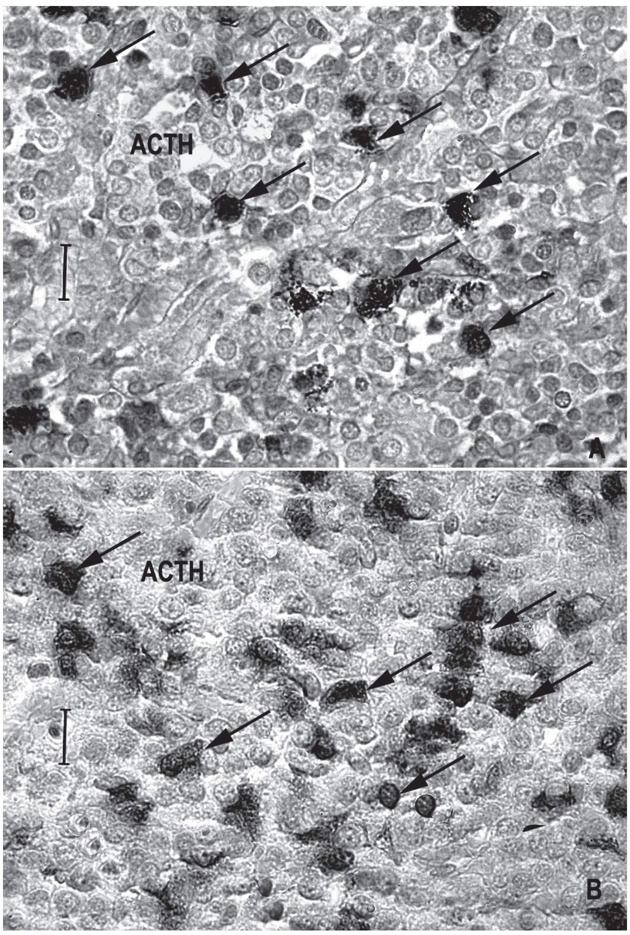

Figure 1. Immunohistochemically labeled ACTH cells in control (**A**) and FR rats (**B**). Peroxidase-antiperoxidase-complex (PAP) method; magnification ×63.

Table 1. The effects of 50% food restriction (FR) for 6 weeks in peripubertal female rats on absolute and relative pituitary weights

Group	Absolute pituitary weight (mg)	Relative pituitary weight (mg/100 g body weight)
Control	5.5 ± 0.1	2.6 ± 0.2
FR	5.0 ± 0.1 (−9.1%)	4.6 ± 0.4* (+76.9%)

All values are means ± SD (n = 10/group), * $p < 0.05$ *vs.* control.

using the M_{42} multipurpose test system (Weibel 1979). According to the obtained data, cell volume (volume of the single cells; V_c; μm^3), volume of the nuclei (V_n; μm^3) and volume density (percentage of immunoreactive cells – related to all other hormone secreting cells in anterior pituitary; V_{VC}; %) were calculated for ACTH-immunoreactive cells. Levels of ACTH were determined in undiluted plasma by the Immulite method (DPC, Los Angeles, USA). Morphometric and hormonal level data obtained for each group were averaged *per* experimental group and the standard deviation of each means (SD) was calculated. Student's t-test was used to determine the significance of differences between mean values (control *vs.* FR). Probability values of 5% or less were considered statistically significant**.**

The absolute and relative weights of pituitary glands from the control and FR peripubertal female rats are given in Table 1. The relative pituitary weight was increased in the FR group by 76.9% ($p < 0.05$), in comparison with the corresponding controls. Immunohistochemically labeled ACTH cells in control rats were angular or stellate in shape, localized between capillaries, with cytoplasmatic processes between neighboring cells. The nucleus followed the shape of the cell body. Small, specific secretory granules were present primarily at the cell periphery. The shape and localization of the ACTH immunopositive cells in FR rats were not significantly different from those in the control group (Fig. 1A, B). However, the morphometric parameters, i.e. the volume of ACTH cells and their volume density in FR rats, were increased ($p < 0.05$) by 17.6% and 12.5% respectively, in comparison with control values (Fig. 2A, B). In accordance, plasma ACTH level was 13.4% higher ($p < 0.05$) in FR rats (Fig. 2C).

These results clearly demonstrate that in peripubertal female rats chronic 50% FR stimulates the stress (HPA) axis response, as evidenced by increased morphofunctional parameters of ACTH cells. The data also suggest that applied FR could be considered as a systemic (immunological) stressor in young females. The changes in the secretory pattern of ACTH reported here and of IL-6 in Uskoković et al. (2009) may be part of neuroendocrine and metabolic mechanisms evolved to maximize survival during prolonged dietary restriction (Chacon et al. 2005).

It is known that concentrations of IL-6 and ACTH are strongly correlated (Lenczowski et al. 1997) and that IL-6 plays a role in the differing adrenal response between the sexes, making a larger contribution to stimulating the HPA axis in female mice (Bethin et al. 2000). This is in accordance with our unpublished results which revealed that, under the same conditions, there was a significantly smaller increase in IL-6 concentration in FR male rats than in females and nonsignificant changes in ACTH level compared with well-nourished mates. Since pituitary corticotropic and adrenocortical cells have IL-6 receptors (Bethin et al. 2000), the implicated gender specific differences suggests the utility of further analysis of IL-6-ACTH associations in determining the female predominance seen in many human inflammatory diseases. We believe that the specificity of their relation depends on sex, age and duration/severity of FR and could be the reason for the modulatory ACTH level observed in different diet-restricted studies. Moreover, Mutschler and Kiefer (2010) reported that stress-related chronic stimulation of the HPA axis could be a pathophysiological mechanism in the development of body weight disorders. Whether the obtained activation of the HPA axis is a benefit or not during FR is a matter for our future studies.

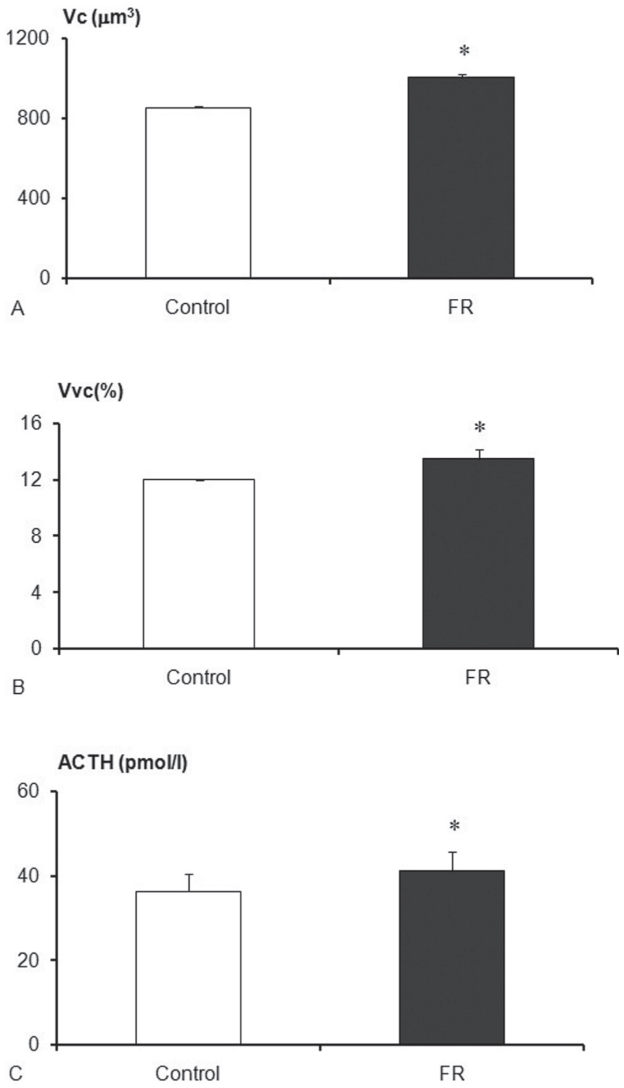

Figure 2. **A**. Cellular volume (V_c) of immunopositive ACTH cells. **B.** Relative volume density (V_{vc}) of ACTH cells expressed as a percentage of total gland tissue. **C**. Plasma concentrations of ACTH (pmol/l) in control and FR peripubertal female rats. All values are means ± SD (n = 10/group), * $p < 0.05$ *vs.* control.

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