СРПСКИ АРХИВ
ЗА ЦЕЛОКУПНО ЛЕКАРСТВО
SERBIAN ARCHIVES
OF MEDICINE

Address: 1 Kraljice Natalije Street, Belgrade 11000, Serbia
☏ +381 11 4092 776, Fax: +381 11 3348 653
E-mail: office@srpskiarhiv.rs, Web address: www.srpskiarhiv.rs

Paper Accepted[1] **ISSN Online 2406-0895**

Original Article / Оригални рад

Jovana Čukuranović Kokoris[1,♣], Monika Dovenska[2], Biljana Parapid[3], Lazo Pendovski[2], Martin Nikolovski[2], Braca Kundalić[1], Ivana Graovac[1], Rade Čukuranović[1], Verica Milošević[1], Florina Popovska Perčinić[2]

Immunohistomorphometric response of pituitary growth hormone producing cells in rats to prolonged exposure to moderately elevated ambient temperature

Имунохистоморфометријски одговор ћелија које производе хормон раста хипофизе код пацова на продужено излагање умерено повишеној температури околине

[1]University of Niš, Faculty of Medicine, Department of Anatomy, Niš, Serbia;
[2]Ss. Cyril and Methodius University in Skopje, Faculty of Veterinary Medicine, Department of Functional Morphology, Skopje, Republic of North Macedonia;
[3]University of Belgrade, Faculty of Medicine, University Clinical Center of Serbia, Division of Cardiology, Belgrade, Serbia

Received: May 14, 2025
Accepted: May 28, 2025
Online First: May 30, 2025
DOI: https://doi.org/10.2298/SARH250515041C

♣Correspondence to:
Jovana ČUKURANOVIĆ KOKORIS
University of Niš, Faculty of Medicine, 81 Dr Zorana Đinđića Blvd., Niš 18000, Serbia
E-mail: **jovana.cukuranovic.kokoris@medfak.ni.ac.rs**

Immunohistomorphometric response of pituitary growth hormone producing cells in rats to prolonged exposure to moderately elevated ambient temperature

Имунохистоморфометријски одговор ћелија које производе хормон раста хипофизе код пацова на продужено излагање умерено повишеној температури околине

SUMMARY

Introduction/Objective The main objective of this study was to investigate the effect of prolonged exposure (4, 7, 14, 21, 60 days) of rats to moderately high ambient temperature (35 ± 1ºC) on the immunohistomorphometric parameters of pituitary somatropic (GH) cells.

Methods The experiment was conducted on 42 adult Wistar rats, equally divided into six experimental groups (n = 7). Five were continuously exposed to a temperature of 35 ± 1ºC, while the control group was kept at 20 ± 2ºC. GH cells were visualized using the peroxidase-antiperoxidase immunohistochemical method. The morphometric analysis was conducted using the M_{42} multipurpose test system.

Results Rats from all experimental groups had significantly ($p < 0.05$) reduced body mass compared to the control. After 4 and 14 days of exposure to moderate heat, the absolute pituitary weight was significantly ($p < 0.05$) increased by 23.1% and 27.7%, respectively, in comparison with the control. GH cells in all groups were oval and located near capillaries with numerous dark granules. Morphometric analysis of cellular and nuclear volumes of GH cells in the experimental group significantly decreased ($p < 0.05$) compared to the control group.

Conclusion It can be concluded that chronic exposure of adult male rats to moderately high ambient temperatures reduced the immunohistomorphometric parameters of GH cells.

Keywords: moderately high ambient temperature, immunohistomorphometry, somatotrophic cells, rats

САЖЕТАК

Увод/Циљ Основни циљ овог истраживања био је да се испита ефекат продужене изложености (4, 7, 14, 21, 60 дана) пацова умерено високој температури околине (35 ± 1ºC) на имунохистоморфометријске параметре соматропних (ГХ) ћелија хипофизе.

Методе Експеримент је спроведен на 42 одрасла Вистар пацова, подељених у шест експерименталних група ($n = 7$), од којих је пет непрекидно изложено температури од 35 ± 1ºC, док је контролна група држана на 20 ± 2ºC. ГХ ћелије су имунохистохемијски визуелизоване методом пероксидаза-антипероксидаза. Морфометријска анализа је спроведена коришћењем вишенаменског тест система M_{42}.

Резултати Пацови из свих експерименталних група су имали значајно ($p < 0,05$) смањену телесну масу у односу на контролу. После 4 и 14 дана излагања умереној топлоти, апсолутна тежина хипофизе је значајно ($p < 0,05$) повећана за 23,1% односно 27,7%, у поређењу са контролом. ГХ ћелије у свим групама су биле овалног облика и смештене у близини капилара са бројним тамним гранулама. Морфометријска анализа ћелијских и нуклеарних запремина ГХ ћелија у експерименталној групи је значајно смањена ($p < 0,05$) у односу на контролну групу.

Закључак Може се закључити да је хронична изложеност одраслих мужјака пацова умерено високим температурама околине смањила имунохистоморфометријске параметре ГХ ћелија.

Кључне речи: умерено висока температура околине; имунохистоморфометрија; соматотропне ћелије; пацови

INTRODUCTION

High ambient temperatures have become a significant environmental factor in recent decades, directly affecting all biological processes in the body and causing numerous consequences on the functioning of living organisms [1, 2, 3]. Due to climate change and global warming, the impact of high temperatures on growth, food consumption, muscle mass growth [1], bone

mineralization, energy metabolism, and reproduction has become an important aspect of thermophysiology [4].

Exposure to high or low environmental temperatures and continuous exposure to light or darkness are considered environmental stressors to the body [5–8]. In such conditions, a stressogenic reaction occurs, causing the neuroendocrine system response and particularly affecting the hypothalamus-pituitary axis [6, 9, 10]. Earlier works showed that adrenocorticotropic (ACTH) [6, 11], somatotropic (GH) and mammotropic (PRL) [7] cells of the pituitary gland are most sensitive to this kind of stress reaction.

Somatotropic (GH) cells synthesize and secrete growth hormone (GH) into the bloodstream, which is regulated by several circulating hormones and metabolites [12]. The pulsation control of GH secretion and release into the pituitary portal system is regulated by hypothalamic neurons through the release of stimulatory GH-releasing hormone (GHRH) [12], or GH-inhibitory hormone, somatostatin (SRIH) [13]. Growth hormone regulates numerous physiological functions, including protein synthesis, cellular proliferation, body growth [2, 14], neuroendocrine responses, behavior and metabolism through specific populations of neurons [15].

Current climate changes and global warming have also affected the Western Balkans and Southeastern Europe. It is expected that the average annual increase in temperature by 2035 in this region will be by 0.5–1°C, especially during the summer period [11]. Having in mind that during summer, all animals and humans are subjected to longer or shorter periods of warm climate, we hypothesized that the exposure to high ambient temperatures might have a significant impact on the morphofunctional characteristics of GH cells. Therefore, this study aimed to elucidate the potential changes in the immunohistomorphometric characteristics of GH cells after shorter and prolonged exposure of animals to moderately elevated ambient temperature.

METHODS

Experimental animals and experimental design

The experiment was conducted on 2.5-month-old adult Wistar male rats. A detailed description of the experimental protocol can be found in our previous study [11]. Briefly, the experimental animals (n = 7 per group) were continuously exposed (4, 7, 14, 21, 60 days) to moderately high ambient temperature in a special heated chamber with controlled air temperature (35 ± 1^{o}C) and air humidity of 30-40%, while the control group (n = 7) was kept at room temperature (20 ± 2°C). Food and water were given *ad libitum* to all animals throughout the whole experiment.

After the sacrifice, the pituitary gland was removed, measured, and subjected to immunohistochemical staining for GH cell visualization.

Immunohistochemical staining

The rat pituitary glands were fixed in 4% paraformaldehyde, dehydrated in ethanol, cleared in xylol, and embedded in paraffin. The distal part of the glands was cut in a series of seven horizontal 5 μm thick sections through three levels (superior, middle and inferior) [11]. The immunohistochemical localization of pituitary GH-cells was performed by the peroxidase-anti peroxidase (PAP) method. The procedure is described in detail elsewhere [5, 9, 11].

All animal procedures were compliant with EU Directive 2010/63/EU and approved by the Local Animal Care Committee of the Faculty of Veterinary Medicine-Skopje (No. 0201-4506/2 from 7.11.2011).

Stereological measurements

The cellular volume (VcGH; μm^3), nuclear volume (VnGH; μm^3), and volume density of immunopositive GH cells (VvGH; %) were determined on 5 μm thick sections. Measurements were made with the multipurpose test system M_{42} on 50 test fields at x1000 magnification, previously described in detail by Popovska-Perčinić et al. [5]. Digital recordings were made on a DM RB photomicroscope (Leica, Wetzlar, Germany).

Statistical analysis

The morphometric data obtained for each rat were averaged per experimental group, and the standard deviation of the mean (SD) was calculated. One-way analysis of variance (ANOVA) followed by a Tukey test was used to compare differences between the groups. A probability value of 5% or less was considered statistically significant.

RESULTS

Body mass, absolute and relative pituitary weights

The body mass and the absolute and relative pituitary weights are given in Table 1 and Fig. 1. It was found that the body mass in rats exposed to moderate heat for 4, 7, 14, 21 and 60 consecutive days was significantly ($p < 0.05$) reduced by 19.8%, 22.6%, 16.4%, 22.6% and 37.6%,

respectively, compared to the controls. After 4 and 14 days of exposure to moderate heat, the absolute pituitary weight was significantly ($p < 0.05$) increased by 23.1% and 27.7%, respectively, in comparison with the control group. The relative weight of the pituitary gland was significantly ($p < 0.05$) increased by 36.0% only in rats exposed to moderate ambient temperature for 4 days.

Immunohistochemical characteristics of GH cells

The GH cells of the control group were intensively stained, with an oval shape and a prominent spherical nucleus located centrally within the cell cytoplasm (Fig. 2A). The location of GH cells was not significantly changed in the experimental groups. The shape of most cells was oval (Fig. 1B-F). However, there were also some stellate cells with noticeable cytoplasmatic processes (Fig. 1D). Generally, rats exposed for a prolonged time to moderately elevated ambient temperature had smaller GH cells containing darker cytoplasmic areas throughout the entire cytoplasm. They were found mostly arranged in groups (Fig. 1B).

Stereological parameters

In rats exposed to moderate heat for 4, 7, 14, 21 and 60 days, the morphometric analysis of pituitary GH cells showed a significant decrease in their volume ($p < 0.05$) by 18.4%, 25.8%, 14.1%, 24.4% and 19.1%, respectively (Fig. 2A), compared to the control. The nuclear volume of these cells was significantly reduced by 9.5%, 5.3%, 7.4%, 3.2% and 10.6%, respectively, in comparison with the control group (Fig. 2B). The volume density of GH cells was significantly reduced ($p < 0.05$) after 4, 7, and 60 days by 23.7%, 13.2%, and 15.9%, respectively, compared to the control (Fig. 2C).

DISCUSSION

Global warming is characterized by an increase in the average annual temperatures. Western Balkans and Southeastern Europe have experienced prolonged periods of high ambient temperatures during the summer months [11, 16].

In this research, a significant decrease in body weight was found in all groups compared to the control. The largest decrease (-37.6%) was recorded in animals that were exposed to moderately high environmental temperatures for 60 days. Similar results were obtained after exposure of

rats to a temperature of 35 ± 1ºC for 30 days [5], as well as after acute exposure of mice to temperatures between 34°C-38.5°C [17]. Increased water intake and reduced food intake are most probably the cause of body weight reduction, which was observed in rats [18] and broilers [1].

The increased absolute weight of the pituitary gland was observed after the 4th and 14th day of exposure to elevated ambient temperature, whereas increased values of the relative weight were registered only after the 4th day. A similar result was reported in earlier studies after acute [19] and chronic [5] (30 days) exposure of rats to high environmental temperature.

Literature data on the effect of temperature on GH cells are very scarce. Most of them regard acute exposure, such as studies from Vigas et al. [20], which describe the stimulatory effect of high temperature on GH release for several minutes to an hour. The current study aimed to broaden the information on the properties of GH cells after prolonged exposure to moderately high temperatures.

The stereological analysis in this study showed that the cellular and nuclear volumes of GH cells were significantly reduced compared to the control. These results indicate a decreased activity of somatotropic cells during the entire heat exposure period. A decrease in the stereological parameters of GH cells was also observed after 30 days of exposure to moderately high temperatures [5], which is in agreement with our results. The inhibitory effect of GH secretion and reduced blood GH concentration was noticed after acute and chronic stress [21], as well as during short-term acclimation to high temperatures [22]. This can be explained by the increased secretion of somatostatin from the hypothalamus, which serves as a suppressor of GH hormone release [21].

Gusmao et al. [22] and Nestorović et al. [7] came to similar findings after chronic exposure of adult male rats to other types of stressors (immobilization, sound, constant light). The decreased somatotropes' activity might be due to the increased somatostatin blood concentration, known to exhibit an inhibitory effect on the secretion of GH from the pituitary somatotropic cells [23]. Similar results on exposure to a moderately warm ambient environment were also obtained in birds [24]. Studies in rats reported that increased somatostatin synthesis and storage and decreased GHRH mRNA synthesis play a major role in the GH inhibitory effects on glucocorticoids [25]. In addition, it was shown that glucocorticoids are directly involved in the increase of pituitary GHS-R mRNA levels by stimulating GHS-R gene transcription [26]. This might be a possible explanation for the decreased activity of GH cells found in this study since reduced

serum corticosterone concentration was found in our previous research in rats subjected to moderately high temperatures for 7–60 days [11].

CONCLUSION

Prolonged exposure of adult male rats to moderately high ambient temperature has an inhibitory effect on the immunohistochemical and stereological parameters of GH cells.

ACKNOWLEDGEMENTS

The research is supported by the Ministry of Science, Technological Development, and Innovation of Republic of Serbia (Number of grants: 451-03-137/2025-03/200113).

Conflict of interest: None declared.

REFERENCES

1. Ružić Z, Kanački Z, Jokanović M, Vidakovic S, Knežević S, Jović S, et al. The influence of vitamin C and early-age thermal conditioning on the quality of meat and specific production characteristics of broilers during heat stress. Turk J Vet Anim Sci. 2020;44(2):314–22. [DOI: 10.3906/vet-1905-95]
2. Ružić Z, Kanački Z, Stojanović S, Kovačević Z, Knežević S, Todorović S, et al. Rectal temperature and respiration rate as indicators of heat stress in broiler chickens subjected to early-age thermal conditioning and vitamin C supplementation. Turk J Vet Anim Sci. 2023;47(2):160–6. [DOI: 10.55730/1300-0128.4281]
3. Sejian V, Silpa MV, Reshma Nair MR, Devaraj C, Krishnan G, Bagath M, et al. Heat stress and goat welfare: adaptation and production considerations. Animals (Basel). 2021;11(4):1021. [DOI: 10.3390/ani11041021] [PMID: 33916619]
4. Huang Q, Li N, Li Y. Long-term trend of heat waves and potential effects on phytoplankton blooms in Lake Qiandaohu, a key drinking-water reservoir. Environ Sci Pollut Res Int. 2021;28(48):68448–59. [DOI: 10.1007/s11356-021-15414-z] [PMID: 34272668]
5. Popovska-Perčinić F, Ajdžanović V, Pendovski L, et al. Immunohistomorphometric and fluorescent characteristics of rat GH cells after chronic exposure to moderate heat. Mac Vet Rev. 2012;35(2):65–72.
6. Čukuranović-Kokoris J, Ružić Z, Kanački Z, Stojanović S, Paraš S, Milošević V, et al. Effects of vitamin C and early-age thermal conditioning on pituitary ACTH cells in broilers chronically exposed to heat stress: an immuno-histomorphometric and hormonal study. Vet Res Forum. 2024;15(3):125–30. [DOI: 10.30466/vrf.2023.2009320.3981] [PMID: 38770378]
7. Nestorović N, Ristić N, Ajdžanović V, Trifunović S, Milošević V. Morphological and functional changes of pituitary GH and PRL cells following prolonged exposure of female rats to constant light. Exp Appl Biomed Res. 2023;24(3):2019–25. [DOI: 10.2478/sjecr-2019-0063]
8. Jasnić N, Dakić T, Bataveljić D, Vujović P, Lakić I, Jevdjović T, et al. Distinct vasopressin content in the hypothalamic supraoptic and paraventricular nucleus of rats exposed to low and high ambient temperature. J Therm Biol. 2015;52:1–7. [DOI: 10.1016/j.jtherbio.2015.04.004] [PMID: 26267492]
9. Popovska-Perčinić F, Jarić I, Pendovski L, Ristić N, Trifunović S, Milošević V, et al. The effect of moderate heat on rat pituitary ACTH cells: histomorphometric, immunofluorescent and hormonal study. Acta Vet. 2017;67(4):495–507. [DOI: 10.1515/acve-2017-0040]
10. Popovska-Perčinić F, Manojlović-Stojanoski M, Pendovski L, Dinevska-Kjovkarovska S, Miova B, Grubin J, et al. A moderate increase in ambient temperature influences the structure and hormonal secretion of adrenal glands in rats. Cell J. 2021;22(4):415–24. [DOI: 10.22074/cellj.2021.6827] [PMID: 32347034]
11. Čukuranović-Kokoris J, Ajdžanović V, Pendovski L, Ristić N, Milošević V, Dovenska M, et al. The effects of long-term exposure to moderate heat on rat pituitary ACTH cells: histological and hormonal study. Acta Vet. 2022;72(1):1–15. [DOI: 10.2478/acve-2022-0001]
12. Rampersaud A, Connerney J, Waxman DJ. Plasma growth hormone pulses induce male-biased pulsatile chromatin opening and epigenetic regulation in adult mouse liver. Elife. 2023;12:RP91367. [DOI: 10.7554/eLife.91367] [PMID: 38091606]
13. Liguz-Lecznar M, Dobrzanski G, Kossut M. Somatostatin and somatostatin-containing interneurons—from plasticity to pathology. Biomolecules. 2022;12(2):312. [DOI: 10.3390/biom12020312] [PMID: 35204812]
14. Martínez-Moreno CG, Arámburo C. Growth hormone (GH) and synaptogenesis. Vitam Horm. 2020;114:91–123. [DOI: 10.1016/bs.vh.2020.04.001] [PMID: 32723552]
15. Wasinski F, Tavares MR, Gusmao DO, List EO, Kopchick JJ, Alves GA, et al. Central growth hormone action regulates neuroglial and pro-inflammatory markers in the hypothalamus of male mice. Neurosci Lett. 2023;806:137236. [DOI: 10.1016/j.neulet.2023.137236] [PMID: 37030549]
16. Perkins-Kirkpatrick SE, Gibson PB. Changes in regional heatwave characteristics as a function of increasing global temperature. Sci Rep. 2017;7(1):12256. [DOI: 10.1038/s41598-017-12520-2] [PMID: 28947762]
17. Harikai N, Tomogane K, Miyamoto M, Shimada K, Onodera S, Tashiro S. Dynamic responses to acute heat stress between 34 °C and 38.5 °C, and characteristics of heat-stress response in mice. Biol Pharm Bull. 2003;26(5):701–8. [DOI: 10.1248/bpb.26.701] [PMID: 12736516]

18. Kim MG, Oh JS, Kim HK, Leem KH. Effects of exogenous growth hormone administration on dexamethasone-induced growth impairment in adolescent male rats. Exp Ther Med. 2017;14(4):3455–62. [DOI: 10.3892/etm.2017.5017] [PMID: 29042933]

19. Koko V, Djordjevic J, Cvijic G, et al. Effect of acute heat stress on the rat pituitary gland: morphological and stereological study. J Therm Biol. 2006;31(5):394–9. [DOI: 10.1016/j.jtherbio.2006.01.009]

20. Vigas M, Celko J, Koska J. Role of body temperature in exercise-induced growth hormone and prolactin release in untrained and physically fit subjects. Endocr Regul. 2000;34(4):175–80. [PMID: 11137977]

21. Pedroso JAB, Dos Santos LBP, Furigo IC, Spagnol AR, Wasinski F, List EO, et al. Deletion of growth-hormone receptor in hypothalamic neurons affects the adaptation capacity to aerobic exercise. Peptides. 2021;135:170426. [DOI: 10.1016/j.peptides.2020.170426] [PMID: 33069692]

22. Gusmao DO, de Sousa ME, de Sousa LMM, Silva JN, Frazao R, List EO, et al. GH-releasing-hormone neurons regulate the hypothalamic-pituitary-somatotropic axis via short-loop negative feedback. Endocrinology. 2025;166(5):bqaf062. [DOI: 10.1210/endocr/bqaf062] [PMID: 40172534]

23. Čukuranović-Kokoris J, Kundalić B, Pavlović M, Ugrenović S. Pituitary cells in humans during ageing: an immunohistological and morphometric study. J Med Biochem. 2025;44(2):203–10. [DOI: 10.5937/jomb0-54605]

24. Bohler MW, Chowdhury VS, Cline MA, Gilbert ER. Heat-stress responses in birds: a review of the neural components. Biology (Basel). 2021;10(11):1095. [DOI: 10.3390/biology10111095] [PMID: 34827087]

25. Fife SK, Brogan RS, Giustina A, Wehrenberg WB. Immunocytochemical and molecular analysis of the effects of glucocorticoid treatment on the hypothalamic-somatotropic axis in the rat. Neuroendocrinology. 1996;64(2):131–8. [DOI: 10.1159/000127109] [PMID: 8857607]

26. Tamura H, Kamegai J, Sugihara H, Kineman RD, Frohman LA, Wakabayashi I. Glucocorticoids regulate pituitary growth-hormone secretagogue-receptor gene expression. J Neuroendocrinol. 2000;12(6):481–5. [DOI: 10.1046/j.1365-2826.2000.00446.x] [PMID: 10844575]

Table 1. Absolute and relative pituitary weight in animals exposed to moderately high ambient temperature

Group	Absolute pituitary weight (mg)	Relative pituitary weight (mg%)
Control	6.5 ± 0.6	2.5 ± 0.1
4 days	8.0 ± 0.4* (+23.1%)	3.4 ± 0.3* (+36.5)
7 days	6.3 ± 0.5 (-3.1%)	2.4 ± 0.2 (-4.0%)
14 days	8.3 ± 0.6* (+27.7%)	2.9 ± 0.2 (+16%)
21 days	6.4 ± 0.5 (-1.5%)	2.5 ± 0.1 (0.0%)
60 days	5.8 ± 0.5 (-10.1%)	2.6 ± 0.2 (+4%)

The values are expressed as means ± SD;

*$p < 0.05$ *vs.* control

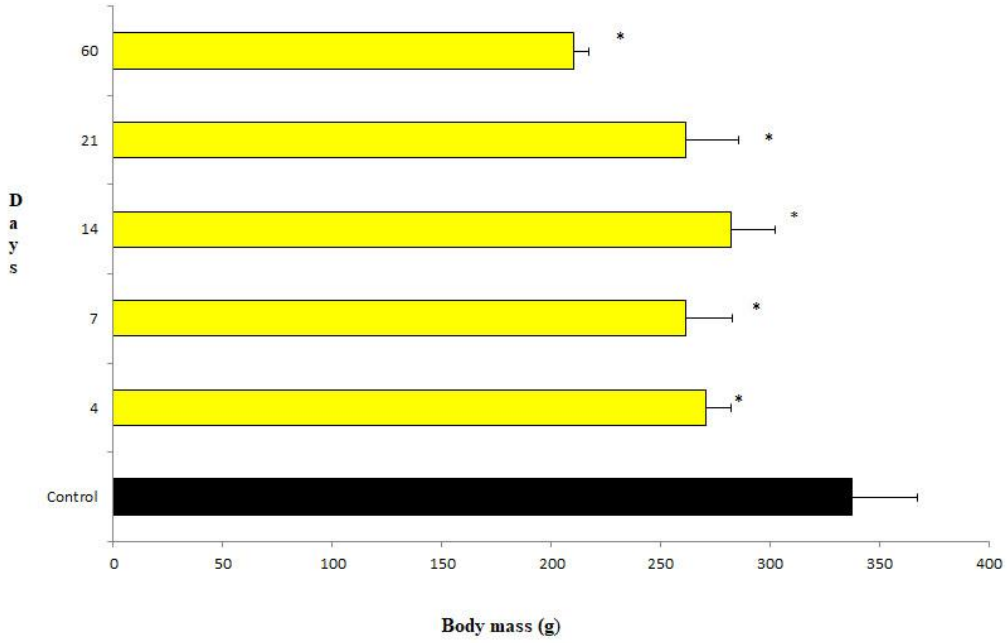

Figure 1. Body mass in animals exposed to moderately high ambient temperature 4, 7, 14, 21, and 60 days; the values are expressed as means ± SD;

*$p < 0.05$ *vs*. control

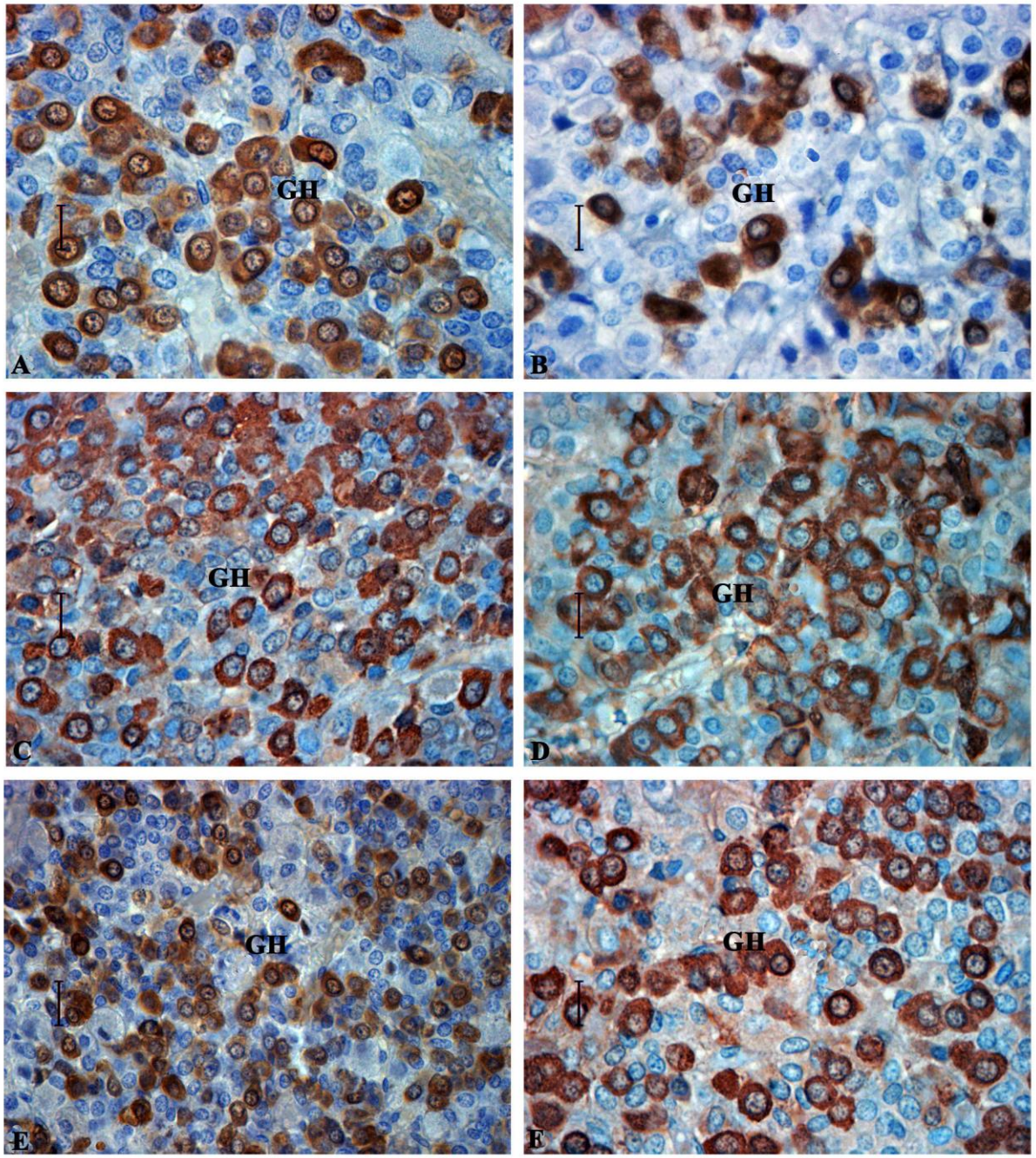

Figure 2. Representative micrographs of immunopositive GH cells in controls (**A**), in animals exposed to moderately high ambient temperature 4 days (**B**); 7 days (**C**); 14 days (**D**); 21 days **(E**); and 60 days **(F**); PAP method, bar 16 μm

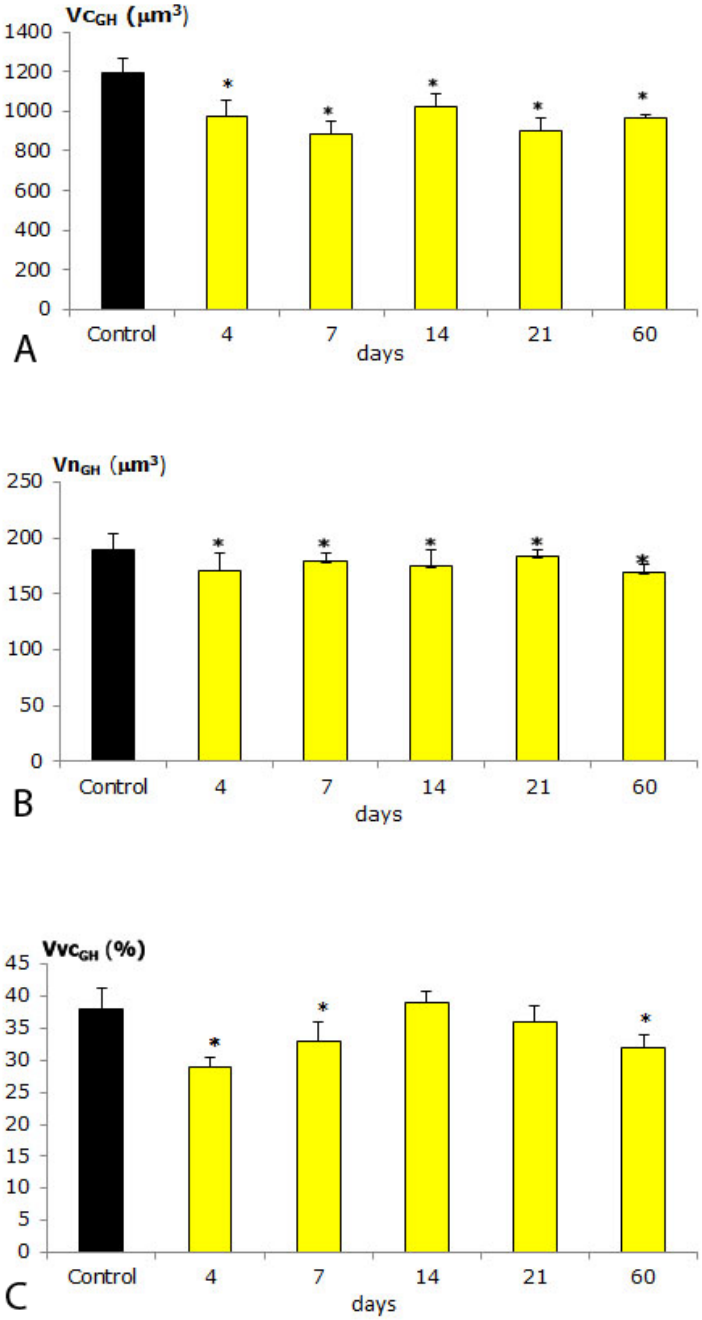

Figure 3. Stereological parameters of immunopositive GH cells in adult male rats after exposure to moderately high ambient temperatures; **A**) Vc-volume of cells (µm^3); **B**) Vn-volume of nuclei (µm^3); and **C**) Vvc-volume of density (%); the values are expressed as means ± SD; *p < 0.05 *vs*. control

