**Endocrine,** *vol. 13, no. 1, 123–129, August 2000* 0969–711X/00/13:123–129/$11.75 

# Biological Effects of Single and Repeated Swimming Stress in Male Rats

## *Beneficial Effects of Glucocorticoids*

Yan Hu, Erdal Gursoy, Arturo Cardounel, and Mohammed Kalimi

*Department of Physiology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA*

**We have examined the biological effects of single (45 min at 22°C) and repeated swimming stress (45 min at 22°C for 7 d) using male Sprague-Dawley rats. Repeated swimming for a week resulted in a significant inhibition in total body weight (25%) as compared to control unstressed animals. There was significant increase in adrenal and kidney relative weight and decreases in relative thymus weight in repeated swimming-stressed animals as compared to control animals. Repeated swimming stress resulted in almost threefold increase in plasma corticosterone levels with concomitant dramatic decrease in total glucocoticoid receptor (GR) levels in liver, thymus, and heart as compared to control unstressed animals. Interestingly, single swimming stress resulted in a significant elevation in lipid peroxidation levels in the liver and heart. In contrast, there was no change in the lipid per oxidation levels in the liver and heart between chronic stressed and control unstressed animals. Finally, both single and repeated swimming-stress animals had almost 50% reduction in plasma triglyceride levels as compared to control unstressed animals. It is concluded that elevated plasma corticosterone levels by downregulating GR during repeated swimming stress exerts beneficial effects in rats by retarding the total body weight gain and lowering plasma triglyceride levels without affecting free-radicals-induced oxidative stress.**

**Key Words:** Swimming stress; glucocorticoid receptor; lipid peroxidation; triglycerides.

## Introduction

Our laboratory has an ongoing interest in studying the biological effects of stress *(1,2)*. It is generally believed that the stress responses in human and animals are largely mediated through the HPA axis stimulation and release of glucocorticoids *(3)*. Glucocorticoids are produced in response to many common stressors such as restraint, swimming, ether, and hypoglycemia, and are involved in the feedback inhibition of HPA axis activity during stress *(1–9)*. Glucocorticoids by stimulating gluconeogenesis and inhibiting glucose uptake in peripheral tissues are essential for general adaptive responses to acute and repeated stress.

Although common pathway of HPA axis stimulation is involved in the responses to various kinds of stressors, we postulate that response intensity and thus the biological responses during stress vary according to the type and duration of stress. In the present study, we determine the biological effects of single acute and repeated swimming stress, which involves moderate struggling and immobility in terms of body weight gain, relative tissue weights, plasma corticosterone levels, GR levels, plasma triglycerides, and lipid peroxidation in heart and liver.

## Results

Results presented in Fig. 1 (C, C+C) show that 7 d repeated-swimming-stressed animals gain less (almost 25%) total body weight as compared to controls unstressed animals.

Repeated swimming stress caused a significant decrease in relative thymus weight and increase in adrenal and kidney weights when compared to control or acute stressed (Table 1, C, A, C+C) animals. Repeated-swimming-stress administration had no observable effect on the relative liver, heart, testes, or spleen weight (Table 1, C, and A, C+C) when compared to control or acute stressed animals.

Figure 2 (C, A, C+C) shows that acute or 7 d repeated swimming stress resulted in almost three- to fourfold increase in plasma corticosterone levels as compared to control animals.

Repeated swimming stress seemed to significantly decrease plasma triglyceride levels as compared to control untreated animals Table 2 A, C+C, C shows an almost 50% reduction in plasma triglycerides levels in both acute and repeated-swimming-stress animals as compared to control animals.

Received February 29, 2000; Revised May 10, 2000; Accepted May 30, 2000.

Author to whom all correspondence and reprint requests should be addressed: Dr. M. Kalimi, Box 551, MCV station, Richmond, Virginia 23298-0551, E-mail: Mkalimi @ hsc.vcu.edu

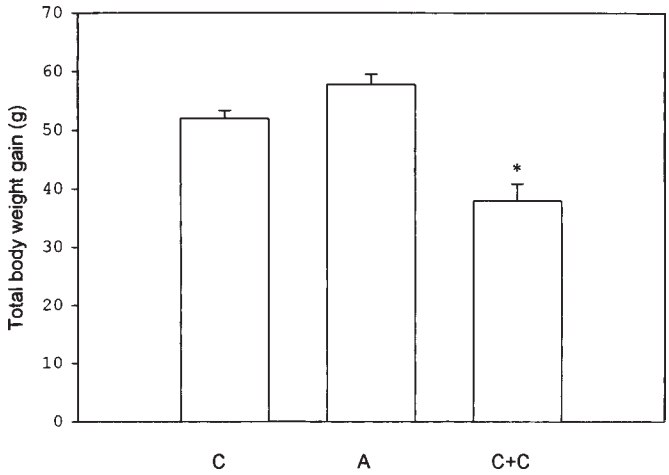


**Fig. 1.** Body weight gain in control (C), acute-swimming-stressed (A), and repeated-swimming-stressed (C+C) rats. Each bar represents mean ± SEM of two experiments (total $n = 12$). *Significantly different from control levels ($p < 0.05$).

Table 1
Effects of Swimming Stress on the Relative Tissue Weight

| | C[a] | A | C + C |
|---|---|---|---|
| Relative adrenal weight mg/100 g bw | 74.4 ± 5.3 | 69.9 ± 5.2 | 92.2 ± 3.1* |
| Relative thymus weight g/100 g bw | 0.21 ± 0.01 | 0.2 ± 0.01 | 0.13 ± 0.01* |
| Relative spleen weight g/100 g bw | 0.26 ± 0.02 | 0.29 ± 0.02 | 0.24 ± 0.05 |
| Relative liver weight g/100 g bw | 4.1 ± 0.1 | 4.3 ± 0.2 | 4.0 ± 0.3 |
| Relative heart weight g/100 g bw | 0.35 ± 0.01 | 0.34 ± 0.01 | 0.38 ± 0.01 |
| Relative kidney weight g/100 g bw | 0.78 ± 0.04 | 0.92 ± 0.07 | 0.89 ± 0.06* |
| Relative testes weight g/100 g bw | 1.34 ± 0.01 | 1.33 ± 0.03 | 1.29 ± 0.01 |

[a]C: Control animals, A: acute swimming stressed animals, C + C: chronic swimming stressed animals. Each point represents the mean ± SEM ($n = 12$).

*Significantly different from control levels ($p < 0.05$).

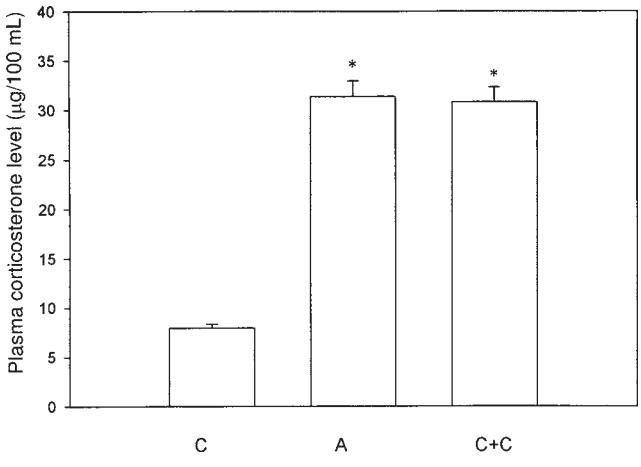


**Fig. 2.** Plasma corticosterone levels in control (C), acute-swimming-stressed (A), and repeated swimming stressed (C+C) rats. The plasma corticosterone levels were measured as described in Methods. Each point represents the mean ± SEM of two experiments (total $n = 12$). *Significantly different from control levels ($p < 0.05$).

Data presented in Figs. 3A and B(C, A) show that acute swimming stress to rats resulted in significant elevation in malonaldehyde levels (that represents lipid peroxidation levels) in liver (Fig. 3A) and heart (Fig. 3B) in comparison to controls. Interestingly, repeated stressed animals had almost similar levels of lipid peroxidation in both liver and heart to that observed for control unstressed animals (Figs. 3A, B, C+C, C).

Figure 4A (C, A, C+C) demonstrates that both acute and repeated swimming-stress animals had almost 25–30% decrease in total GR levels (both cytosolic and nuclear) in liver when compared to the control unstressed animals. Interestingly, heart, and thymus [(Figs. 4B and C (C, A, C+C)] showed more than 60% declined in their total GR levels when compared to the control unstressed animals.

## Discussion

In the present work using male Sprague-Dawley rats, we have examined the biological effects of acute and 7 d

Table 2
Plasma Triglyceride Levels

| | Triglyceride (mg/100 mL) |
|---|---|
| C[a] | 34.8 ± 1.8 |
| A | 17.2 ± 1.4* |
| C + C | 20.4 ± 1.7* |

[a]C: Control animals, A: acute swimming stressed animals, C + C: chronic swimming stressed animals. Each point represents the mean ± SEM ($n$ = 12).

*Significantly different from control levels ($p < 0.05$).

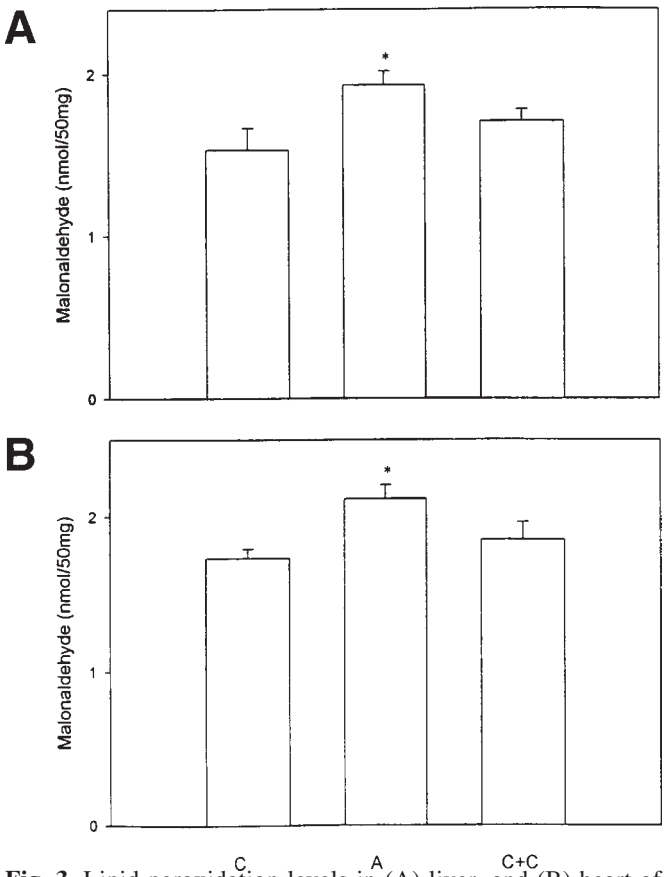


**Fig. 3.** Lipid peroxidation levels in (A) liver, and (B) heart of control (C), acute stressed (A), and repeated-swimming-stressed (C+C) rats. Lipid peroxidation was determined as described in Methods. Each bar represents the mean ± SEM of two experiments (total $n$ = 12). *Significantly different from control levels ($p < 0.05$).

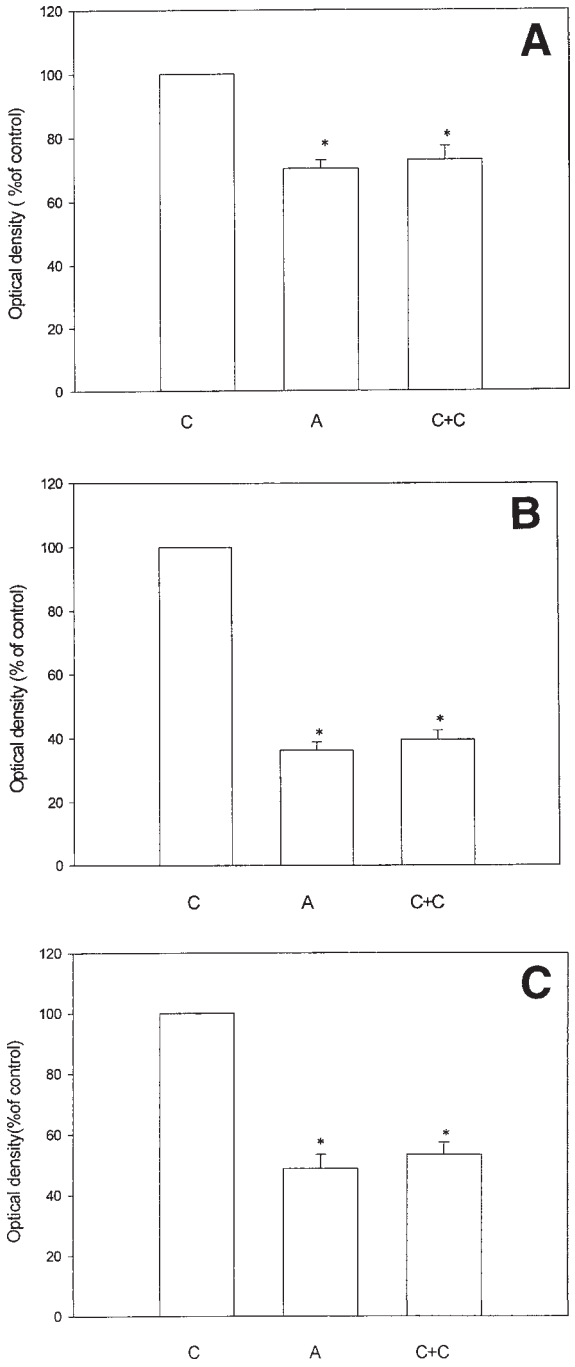


**Fig. 4.** Immunoblots studies of glucocorticoid receptor in (A) liver, (B) thymus, and (C) heart of control (C), acute stressed (A), repeated-swimming-stressed (C+C) rats. The homogenates were analyzed by Western blot as described in Methods. Each bar represents the mean ± SEM of 4–6 samples.

repeated swimming stress on plasma corticosterone levels, total GR, plasma triglycerides, and lipid peroxidation levels in heart and liver. Seven d of repeated stress resulted in significant inhibition in the body weight gain of animals as compared to control untreated animals. Interestingly, tissue weight showed distinct tissue-specific pattern in their relative weights. For example, there was a significant decrease in the relative weight of thymus, while kidney and adrenal showed a significant increase in their relative weights. On the other hand, relative weights of liver, spleen, heart, and testes remained unchanged when compared to control unstressed animals. Hardly any information is available regarding the effects of repeated swimming stress on body weight and tissue weights. Previously, we and other investigators *(2,3,6)* have reported increased adrenal

weights on repeated immobilization stress, while changes in thymus weight on repeated immobilization stress in rats remains inconclusive *(2,4,6)*. Lowly *(14)* and Herman et al. *(3)* reported no change in thymus weight while Alexandrova and Farkas *(2)* found involution of thymus gland in rats exposed for 18 d to various stressors. Thus, results obtained by us suggest that repeated swimming stress inhibits total body weight gain, decreases thymus weight, and increases adrenal weight.

We observed that plasma corticosterone levels increased almost three- to fourfold by both acute and repeated swimming stress. In addition, for the first time we determined total glucocorticoid receptor (cytoplasm and nuclear) during acute and repeated swimming stress using GR antibody. Previously, most GR determinations during various stress regimens were carried out using binding assays, which measured only free cytoplasm GR *(2,14)*. Our results showing decreased total (bound plus free) glucocorticoid receptor levels are in agreement with those reported by measuring only free cytoplamic GR *(2,4)*. Interestingly, we found that there is a downregulation of total GR in many GR tissues such as liver, thymus, and heart with both acute as well as repeated swimming stress. Thus, it seems that chronic swimming stress maintains persistently elevated plasma corticosterone levels in rats after binding to GR and subsequent downregulation of GR exhibits three classical glucocorticoid effects: inhibition in body weight gain, involution of the thymus gland and adrenal hypertrophy. We believe that the downregulation of GR observed by us may be an adaptive and protective response against adverse effects of glucocorticoids during repeated swimming stress.

Inconclusive results showing either changes or no effects have been reported regarding plasma triglyceride levels with chronic stress *(15,16)*. Our report is the first demonstrating that both acute and repeated swimming stress resulted in remarkable decrease in plasma triglycerides levels. The attenuation in plasma triglyceride levels may be likely owing to the degradation of triglycerides to free fatty acids and decreased lipogenesis under chronic stress conditions. The observed decrease in plasma levels of triglyceride observed by us is more likely mediated by elevated plasma corticosterone levels. This postulation is based on the well-known catabolic actions of glucocorticoids on body fat and protein to provide crucial carbohydrate balance during acute and chronic stress.

In the present study, we found that while acute swimming stress increased lipid peroxidation levels, and susceptibility to oxidative stress in both liver and heart, repeated swim stress seemed to adapt animals against free-radical-induced oxidative stress damage and kept lipid peroxidation levels to the control unstressed level. This is particularly surprising because there is growing evidence that stress can lead to elevated production of free radicals with subsequent lipid peroxidation *(17–19)*. We speculate that the unchanged lipid peroxidation levels following stress in our study may represent the beneficial effect of swimming stress.

As universally accepted, the most dramatic and pronounced endocrine reaction to stress in rodents is increased glucocorticoid secretion. The glucocorticoids are known to play crucial role in the organism's adaptation to stress. However, the biological role of glucocorticoid excess is far from clear at the present time. For example, elevation in plasma glucocorticoid levels during acute stress is believed to be vital for survival of animals by potentiating protective cellular homeostasis. On the other hand, prolonged and excess glucocorticoid exposure to animals during various types of chronic stress have shown potentially beneficial effects. This has provided the concept that adaptation to chronic stress could at times be protective. Otherwise it can result in various pathophysiological dysfunctions including obesity, hyperlipidemia, insulin resistance, aging, and cancer *(20,21)*. The mechanisms of protection or injury in chronic stress remain to be elucidated *(20,21)*. In other words, when and how excess glucocorticoid (GCs) secretion in vivo exerts desirable beneficial effects vs GCs potentially detrimental pathophysiological effects remains to be determined.

In this regard, Sapolsky et al. *(22)* have proposed the "glucocorticoid cascade hypothesis of stress and aging," noting that prolonged and excess secretion of glucocorticoids can have deleterious consequences on hippocampus, muscle, bone, and the immune system of animals. We postulate that the biological effects of glucocorticoid excess depends on the nature, duration, and intensity of stressors. This is based on swim the stress model in our present study and two other types of stressful interventions reported by us and others, namely, restraint stress *(1,2)* and caloric deprivation stress *(23–25)*.

Recently, we have shown *(2)* that 2 mo of (2 h daily) restraint stress to male Sprague-Dawley rats resulted in adaptation to repeated stress, within 2 mo, by lowering the plasma corticosterone levels. This was accompanied by stabilization of the significant inhibition of total body weight gain noted during the first 30 d of repeated restraint stress *(1)*. Interestingly, we observed a significant elevation in the total glucocorticoid receptor levels in liver, thymus, and spleen. This was associated with increased lipid peroxidation levels in the liver and heart of repeatedly stressed animals *(2)*. Based on the above, we postulated that a repeated restraint stress regimen by maintaining higher glucocorticoid receptor levels stabilizes inhibition of weight gain. However, the subsequent elevation in lipid peroxidation levels with increasing free radical damage represents an adverse effect of glucocorticoid excess. On the other hand, our results employing moderate swim stress of 7 d duration, showed a contrasting downregulation of glucocorticoid receptors. This was associated with lower body weight gain, and decreased lipid peroxidation levels. Thus,

decrease in reactive oxygen species associated with decreased in lipid peroxidation can represent a beneficial effect of glucocorticoid excess.

The above postulate, as an explanation for the beneficial effects of chronic swim stress is supported by similar results obtained using caloric restriction stress *(23–25)*. Caloric deprivation, as is true for chronic swim stress, increases corticosterone output in caloric-deprived rodents, although caloric restriction has shown increased longevity and retardation of degeneration of various aging tissues in rodents and primates *(23–25)*. In this regard, studies using rodents and primates have observed three important features in caloric restricted animals; (i) elevation in plasma corticosterone levels, (ii) inhibition in total body weight gain, and (iii) decreased lipid peroxidation levels in various tissues *(23–25)*. Thus, the benefits of elevated corticosterone levels in caloric-restricted animals, which lowers body weight gain and maintains lean body mass, may exert this effect by decreasing free-radical-induced tissue damage that can result in prolongation of aging *(23–25)*. Although other hormones and growth factors besides glucocorticoids may be involved in the observed beneficial effects of caloric restriction, the crucial role of glucocorticoids is also emphasized by the observations that (i) adrenalectomy inhibits the beneficial effects of caloric deprivation *(26)* and (ii) cortisol is shown to increase the life span of cultures of human embryonic lung fibroblats (WI-38) cells in vitro *(26)*.

## Materials and Methods

### *Materials*

#### *Chemicals*

[1,2-$^3$H] Corticosterone (52 Ci/mmol) was purchased from Du Pont Company (Wilmington, DE). Corticosterone antibody, unlabeled corticosterone, leupeptin, PMSF, and aprotinin were obtained from Sigma (St. Louis, MO). Glucocorticoid receptor antibody was purchased from Santa Cruz (Santa Cruz, CA). All other chemicals used were of analytical grade.

#### *Animals*

All procedures involving animals were conducted in conformity with the guidelines of Institutional Animal Care and Use Committee of Virginia Commonwealth University and the National Institute of Health (NIH) *Guide for the Care and Use of Laboratory Animals* [DHHS Publication No. (NIH) 80–23, Revised, Office of Science and Health Reports, Bethesda, MD 20205].

Male Sprague-Dawley rats weighing 200–220 g were used. Sprague-Dawley rats showed elevated basal and prolonged stress-induced plasma cortiocosterone levels; therefore, they are good experimental models to study stress-related biological effects. Male animals were used to avoid variable steroids levels observed during the regular estrous cycle of female animals. Rats were kept one per cage in an animal room separated from the laboratory under standard conditions of 12-h light, 12-h dark cycle (lights on at 7 AM) and temperature (22 ± 1°C) for 1 wk before and throughout the experiments. Animals were fed rodent chow and water ad libitum.

### *Methods*

#### *Swimming Stress*

To prevent variation in plasma corticosterone level from day to day, we carried out our swimming-stress experiments daily between 10 AM and 11 AM. The plasma corticosterone level is lowest during the morning in rats. Therefore, we chose the morning hours to perform our experiments in order to get the maximum stress response. The swimming stress was performed daily for 45 min by putting the animal in 10 × 20 in. water tank at the water temperature of 22°C and forcing it to swim. Preliminary experiments showed that the peak level of plasma corticosterone was reached within 45 min of swimming stress. Therefore, we used 45 min stress protocol in our experiments. The experiment lasted for 7 d. All the animals were randomly divided into three groups. Six animals ($n = 6$) were used for each group.

Protocol: Group 1: Unstressed animals (control); Group 2: Acute stressed animals, single 45 min swimming stress; Group 3: Chronic stressed animals. The animals were forced to swim for 45 min daily for 7 d.

Following each stress session, animals were dried by paper towel and were returned to their home cages and were able to access freely food and water for the remainder of the day. The body weight of animals was determined at the beginning of the experiment and prior to decapitation. All animals were decapitated between 10 AM–11 AM in the laboratory. After decapitation, about 3 mL trunk blood was collected into tubes containing 100 μL of 0.3 *M* EDTA and centrifuged at 1500*g* for 20 min at 4°C using the Sorvall RC-3 centrifuge. The supernatant plasma was collected and stored at –20°C until subsequent analysis. The liver, testes, adrenal glands, spleen, heart, and kidney were rapidly removed, cleaned from connective tissues, etc., dried with paper towels, and weighed.

#### *Preparation of Tissue Homogenate*

The liver, testes, heart, and thymus were mixed with 3 mL ice-cold 0.1 *M* phosphate buffer pH 7.5 containing 5% SDS, 1% beta mercaptoethanol, a cocktail of protease inhibitors (0.1 m*M* phenylmethyl-sulfonylfluoride, 1 μ*M* leupeptin, and 1 μ*M* aprotinin), and 10 m*M* sodium molybdate per gram of tissue. The mixtures were then homogenized using a Polytron tissue homogenizer (Brinkmann Instruments, Westbury, NY). The homogenate was centrifuged for 30 min at 14,000 rpm using an Eppendorf centrifuge 5415 C (Brinkmann Instruments, Westbury, NY). Protein concentration was measured using standard

Bio-Rad protein assay based on Bradford dye-binding procedure with bovine serum albumin as standard *(10)*. The supernatant was collected, used as homogenate, and stored at –75°C for future use. At the time of the analysis, samples were thawed, recentrifuged again with an Eppendorf centrifuge at 14,000 rpm for 30 min.

*Western Blotting*

SDS-PAGE was performed by the method of Laemmli *(11)*. Blotting followed a modified protocol by Towbin *(12)*. Samples were mixed with an equal volume of SDS sample buffer (0.5 *M* Tris-HCl, pH 6.8, 10% glycerol, 10% SDS, 50 m*M* DTT, and 0.005% bromophenol blue) and heated in boiling water bath for 4 min; 7.5% separating and 4% stacking SDS-polyacrylamide gels were prepared as described by Laemmli *(11)*. Samples (adjusted to about 100 μg of protein) were loaded onto gel. Electrophoresis was done at 165 V using a Mini-Protean II slab gel apparatus. After electrophoresis, gels were allowed to equilibrate in 20% methanol blotting buffer for 15 min. Immunoblotting was carried out by transferring proteins from slab gels to Immun-Lite membranes (cat. no. 162-0170, Bio-Rad, Hercules, CA) using electrophoretic transfer apparatus (Mini Transblot, BioRad) at 100 V for 2 h in cold room (–20°C). The membranes were blocked overnight in the cold room with 10% nonfat dry milk in Tris-buffered saline (TBS, 20 m*M* Tris, 500 m*M* NaCl, pH 7.5). The membranes were then incubated for 1 h at room temperature with GR polyclonal antibody (this antibody reacts with both occupied and unoccupied glucocorticoid receptor), diluted 1:200 in TTBS (TBS, 0.05% Tween-20). After the membranes were washed with TTBS four times for 15 min, the membranes were incubated with conjugate secondary antibody [goat anti-rabbit IgG (H+L)-alkaline phosphatase conjugate, cat. no. 170-6518, Bio-Rad] diluted 1:3000 in TTBS containing 1-% non-fat dry milk. After four 15 min washes with TTBS, the membranes were detected with chemiluminescent substrate kit (cat. no. 170-6534, Bio-Rad), that is, the membranes were incubated with chemiluminescent substrate for 5 min and exposed to film (BioMax MR film, Eastman Kodak, Rochester, NY) for 25 min. Quantitation of GR was performed by densitometric scanning of autoradiograms exposed within the linear range of the X-ray film using the Pharmacia LKB/Ultrasca XL 1D analysis program. Optical density (OD) reading for the GR band were determined from samples run in at least three different blots.

*Lipid Peroxidation Determination*

The liver and heart (approx 200 mg) were immediately homogenized in ice cold 1.15% KCl using a Polytron homogenizer (Brinkmann Instruments), to make 10% homogenate. The determination of the lipid peroxidation levels in the above tissues was performed by the thiobarbituric acid (TBA) method *(13)*. Three milliliters of 1% phosphoric acid followed by 1 mL of 0.6% 2-TBA were added to a 0.5 mL of 10% homogenate. The mixture was heated in a boiling water-bath for 45 min and then cooled. Four milliliters of *n*-butanol was added to the cooled mixture for extraction. After the extraction, the *n*-butanol layer was separated by centrifugation at 2000*g* for 10 min. The *n*-butanol layer was removed and the optical density of the *n*-butanol layer was measured spectrophotometrically at 535-nm wavelength. The TBA values were expressed as nmol of malonaldehyde per 50 mg wet tissue.

*Plasma Corticosterone Measurements*

Plasma samples were thawed and diluted 50-fold in diluent (0.1% BSA, 0.05 *M* Tris-HCl, 0.1 *M* NaCl and 0.1% $NaN_3$, pH 8.0). The mixture was heated in boiling water for 5 min to dissociate protein-bound corticosterone in plasma, and then centrifuged at 1500*g* for 15 min. The supernatant was further diluted 10-fold in diluent and used for corticosterone determination. Plasma corticosterone was measured by radioimmunoassay using an antiserum developed in rabbit against corticosterone-21-thyroglobulin (Sigma, St. Louis, MO). The working concentration of antiserum was 10-fold diluted. The assay was carried out according to the protocol provided by Sigma. To 0.1 mL plasma sample or standard corticosterone solution containing 10 ng/mL, 5 ng/mL, 2.5 ng/mL, 1.25 ng/mL and 0.625 ng/mL, 0.5 mL working antiserum was added in polypropylene test tubes. The samples were incubated for 30 min at room temperature. Then 0.1 mL saturating concentration of [$^3$H]corticosterone was added to each test tube and incubated for 1 h at 37°C water bath. Following cooling the tubes for 30 min at 4°C, 0.2 mL cold dextran-coated charcoal suspension (0.5% charcoal, 0.25% dextran in 0.05 *M* Tris-HCl buffer, pH 8.0, containing 0.1 *M* sodium chloride and 0.1% sodium azide) were rapidly added and incubated at 0°C in ice-water. Test tubes were centrifuged at 1500*g* for 15 min at 4°C. The supernatant was removed from each tube and put into new vials. The scintillation cocktail (Budget-Solve, Research Products International Corp., Mount Prospect, IL) was added to each vial. Beckman LS 100c counter (Beckman Instruments, Palo Alto, CA) determined the amount of radioactivity present in each vial with 65% efficiency for tritium. The triplet was used for each sample. The plasma concentration of corticosterone was presented as μg/100 mL of plasma.

*Measurement of Plasma Triglycerides*

Triglycerides in whole plasma were determined using the Boehringer Mannheim Diagnostic Laboratory Systems (Indianapolis, IN). Dr. Philip Anderson, Clinical Chemical Section, and Medical College of Virginia Hospitals graciously carried out these assays.

*Statistical Analysis*

Results are presented as the mean ± SEM. Analysis of variance (ANOVA) was used to determine differences among groups using a SigmaStat software package computer. A Student–Newman–Keuls (SNK) test was used to

compare groups. Statistical differences were considered significant if $p$ was less than 0.05.

## References


1. Al-mohaisen, M., Cardounel, A., and Kalimi, M. (2000). *Steroids* **65,** 8–15.
2. Yan, H., Cardounel, A., Gursoy, E., Anderson, P., and Kalimi, M. (2000). *Biochem. Pharmacol.* **59,** 753–762.
3. Herman, J. P. and Cullinan, W. (1997). *Trends Neurosci.* **20,** 78–84.
4. Alexandrova, M. and Farkas, P. (1992). *J. Steroid Biochem. Molec. Biol.* **42,** 493–498.
5. Herman, J. P., Adams, D., and Prewitt, C. (1995). *Neuroendocrinology* **61,** 180–190.
6. Gomez, F., Lahmame, A., de Kloet, E. R., and Armario, A. (1996). *Neuroendocrinology* **63,** 327–337.
7. Kiss, A. and Aguiler, G. (1993). *Brain Res.* **630,** 262–270.
8. Marti, J. and Armario, A. (1993). *Eur. J. Pharm.* **236,** 295–299.
9. Armario, A., Gavalda, A., and Marti, J. (1995). *Psychoneuro endocrinology* **20,** 879–890.
10. Bradford, M. M. (1976). *Anal. Biochem.* **72,** 248–254.
11. Laemmli, U. K. (1970). *Nature* **227,** 680–685.
12. Towbin, H., Staehelin, T., and Gordon, J. (1979). *Proc. Natl. Acad. Sci. USA* **76,** 4350–4354.
13. Ohkawa, H., Ohishi, N., and Yagi, K. (1979). *Anal. Biochem.* **95,** 351–358.
14. Lowy, M. T. (1991). *J. Steroid Biochem. Molec. Biol.* **39,** 147–154.
15. Hershock, D. and Vogel, W. (1989). *Life Sci.* **45,** 157–165.
16. Tsopanakis, C. and Tesserommatis, C. (1991). *Pharmacol. Biochem. Behv.* **38,** 813–816.
17. Ruiz de Gordoa, J. C., Santafe, J., Segarra, J., and Santisteban, A. (1994). *Psychosom. Med.* **56,** 486–492.
18. Zezerov, A. E., Ivanova, S. M., and Ushakov, A. S. (1998). *Kosm. Biol. Aviakosm. Med.* **21,** 39–49.
19. Steinberg, D. (1997). *J. Biol. Chem.* **272,** 20,963–20,969.
20. Munck, A. and Toth, N. (1992). *Mol. Cell Endocrinol.* **90,** C1–C4.
21. McEwen, B. (1992). *Progress in Brain Res.* **93,** 365–383.
22. Sapolsky, R., Krey, L., and McEwen, B. (1993). *Endo. Rev.* **7,** 284–302.
23. Leaky, J., Chen, M., Manjgaladze, M., Turturro, A., Duffy, P., Pipkin, J., and Hart, R . (1994). *Annal. New Acad. Sci.* **719,** 175–194.
24. Sohol, R. and Weindruch, R. (1996). *Science* **273,** 59–66.
25. Masoro, E. (1993). *J. Am. Geriatr. Soc.* **41,** 994–999.
26. Paschkov, L. and Schwartz, A. (1996). *Carcinogenesis* **17,** 209–212.
27. Cristafalo, V. (1970). Aging in cell and tissue culture, New York; Plenum Press, pp. 83–92.