SHORT COMMUNICATION

THE EFFECT OF LONG TERM SWIM TRAINING ON PHYSIOLOGICAL STRESS LEVELS IN THE RAT

I Webster *PhD*, EF Du Toit *PhD*, B Huisamen *PhD*

University of Stellenbosch, South Africa

Corresponding author: Ingrid Webster
email: iwebster@sun.ac.za | tel: +27 21 938 9386 | fax: +27 21 938 9476

ABSTRACT

Swim training is used in rats as an exercise model of cardioprotection, for skeletal muscle studies, for rehabilitation studies in muscle and neural atrophy. However, acute swimming is also used to induce psychological stress in rats in many studies. There is little data on the effect of long term swim training on the stress levels in rats. Male Wistar rats were randomly divided into sedentary (Sed) or exercised (Ex) groups. Ex groups were exercise trained by swimming for a period of 8 weeks, 5 days per week, starting at 5 minutes per day and incrementally increasing to 60 minutes per day. At the end of the 8 weeks the open field test was performed and blood corticosterone levels were measured by RIA to determine whether the swim training protocol had any effects on stress behaviour and hormone levels of the rats. Neither the behavioural studies nor the corticosterone levels showed any increase above control levels in the groups investigated. Corticosterone levels for Sed (133.3 ± 37.1 ng/ml) and Ex (130.4 ± 30.1 ng/ml) groups were similar, and this was also reflected in the behavioural data collected. In our study, long term swim training did not elicit a physiological stress response in the rat model at the end of the 8 weeks training program.

KEYWORDS

Stress, long-term exercise, behavior, coticosterone, rat.

INTRODUCTION

For decades exercise has been described as both a preventative measure and a prophylactic for many diseases and ailments. Swim training (long term chronic swimming) has been used in rats as an exercise model to elicit a number of beneficial physiological and metabolic responses. It is used as it is an easily controllable way of determining duration and intensity of exercise in the rat. These physiological and metabolic benefits include enhancement of immunity[1], improved resistance to myocardial ischaemia and reperfusion damage[2, 3], improved recovery after spinal cord injury[4] and halting of the deleterious effects of aging[5, 6].

However, swimming has also been used to elicit stress responses in rats[7]. This stress is usually elicited by an acute forced swim session of between a few minutes to half an hour[8]. Psychological stress (e.g. foot shock, forced swim test) raises levels of corticosterone in the rat[9], which in turn leads to physiological changes that include increased myocardial infarct size[10], increased memory loss and cognition[11,12] and impaired immune response[13].

Long term exercise training has been shown to decrease corticosterone levels in Long Evans rats during[9] and directly after exercise[16], however there is, to our knowledge, no information in the literature regarding the effect of long term swim training on the psychological or physiological stress response of the rat at rest. Thus we set out to determine whether chronic swim training had an effect on corticosterone levels or elicited a change in behaviour of rats in the open field.

MATERIALS AND METHODS

Animal model

Male Wistar rats weighing between 200-220 g were used in all experiments. The project was approved by the CEAR (Committee of Experimental Animal Research) of the Faculty of Health Sciences, University of Stellenbosch and complied with the guidelines of the South African Medical Research Council for the humane use of laboratory animals. The rats were allowed free access to food (standard rat chow) and water, and maintained in the University of Stellenbosch (US) Central Research Facility at 22°C with a 12 hour day/night cycle. Adequate measures were taken to minimize pain or discomfort.

Swim training program

Each group was randomly divided into exercise trained and sedentary groups. Training comprised swimming at set times of the day, each day. Training duration was started at 5 minutes per day to minimize stress and was increased by 5 minutes increments each day to a maximum of 60 min per day. Rats were exercised for a minimum period of 8 weeks, 5 days per week in order to elicit metabolic changes[5, 14], with a 2 day recovery period in between. Water temperature was kept at 30°C for the duration of the swim session, and the rats were dried after swimming before being reintroduced into their housing cages. Rats were sacrificed 24 hours after the last bout of exercise and the hearts rapidly excised.

Behavioural studies

The behaviour of the rats was documented the day before rats were sacrificed for the blood sample collection. Each rat was placed in the open field for 5 minutes to test for differences in anxious-like behaviour and activity. This was done 24 hours after the last bout of swimming exercise.

The open field test is designed to measure behavioural responses such as locomotor activity, hyperactivity, and exploratory behaviour. The open field is also used as a measure

Table 1: *Table to show parameters measured in the open field test to measure stress levels in behaviour of the rats. Sed (control sedentary rats), Ex (control exercised rats)*

		Frequency of movement (no. of times)		Time Spent (seconds)	
	Distance covered (cm)	Into inner zone	Into outer zone	In inner zone	In outer zone
Sed	1657 ± 144.6	8.7 ± 1.3	9.5 ± 1.3	27.44 ± 3.5	272 ± 3.4
Ex	1810 ± 141.4	12.4 ± 1.9	13.5 ± 2.0	42 ± 9.0	257 ± 9.1

of anxiety. Rats tend to avoid brightly illuminated, novel, open spaces. Open field testing is a once off trial test with little or no impact on the animal's subsequent behaviour. The apparatus for the open field test is a square enclosure (1 m by 1 m) made of black Perspex.

To analyze exploratory and locomotor activities as an indication of stress in the rat, animals were placed in the left rear quadrant of an open field. The number of line crossings and the total distance covered by the rat were measured over 5 minutes. These are classical measures of locomotor and exploratory activities. The more time the rat spends in the inner zone of the open field, and the more exploratory the rat is, the less stressed it is perceived to be.

Each rat was placed individually in a corner of the field and its behaviour recorded for 5 minutes. All activity was recorded using a video camera mounted above the open field and scored later by an advanced motion-recognition software package (Noldus Ethovision version 3.1 software) that detects and analyzes the movements of the rat. The video image of the open field arena was partitioned into 36 equal-size squares; 24 border squares and 12 centre zone squares.

Total distance, average speed, and time spent in various parts of the field (e.g. the border areas vs. the open, middle area) were measured and analyzed. Testing was carried out in a temperature, noise and light controlled room. During the test procedure silence was maintained in the test room. The behavioural tests were performed from 1-1:30 pm daily to ensure that normal daily fluctuations in corticosterone, circadian rhythm and activity did not affect the results[15]. The rats were placed in a cage in the testing room an hour before the test in order for them to acclimatize to the new environment. The open field was cleaned with 70% ethanol after each rat had been tested. Each rat was tested individually and in a separate test room. Throughout the entire testing-session, the sequence of events and procedures was always the same and the test circumstances (handling, room-features, equipment used) were as standardised and controlled as possible. The entire test procedure lasted approx. 20 minutes per animal, and was recorded on videotape to allow analysis at a later time. Rats were sacrificed the following day and blood collected for analysis of corticosterone levels.

Corticosterone levels

Rats were sacrificed the day after the behaviour tests were performed, 24 hours after the last bout of swimming. Blood was collected from the thoracic cavity, placed in BD Vacutainer® serum separation tubes and spun down at 2000 g for 10 minutes at 4ºC for separation of the serum. Serum was stored at -80ºC for later analysis. An ImmuChem Corticosterone 125I RIA kit (MP Biomedicals) was used to determine levels of corticosterone in the serum.

RESULTS

Distance covered in the open field

There were no differences in the average distance covered between the 2 groups of animals. Sedentary rats moved an average of 1657 ± 144.6cm in 5 minutes compared to Exercised (1810 ± 141.4cm) rats. See Table 1.

Frequency of movement between inner and outer zones of the open field

No differences were found in the frequency of movement from the inner zone into the outer zone and vice versa. Values are given as number of crossings from the inner to the outer zone during a 5 minute period or from the outer to the inner zone during a 5 minute period.

Sedentary rats moved from the inner into the outer zone 9.5 ± 1.3 times and Exercised rats crossed 13.5 ± 2 times during the 5 minutes. See Table 1.

Sedentary rats moved from the outer to the inner zone 8.7 ± 1.3 times and Exercised rats crossed 12.4 ± 1.9 times during the 5 minutes. See Table 1.

Time spent in inner and outer zones of the open field

As with frequency of movement and distance traveled, there were no significant differences found in the time spent in the two zones between groups. Sedentary rats spent 27.44 ± 3.5 sec in the inner zone and 272 ± 3.4 sec in the outer zone. Exercised rats spent 42.0 ± 9.0 sec in the inner zone versus 257.8 ± 9.1 sec in the outer zone. See Table 1.

Corticosterone levels

Stress increases the levels of circulating corticosterone in the rat (the equivalent of cortisone in humans). Elevated levels of this hormone are indicative of stress in animals.

The serum corticosterone levels were measured in each rat, to determine whether the swim training had any effects on stress levels in the animals at rest. Sedentary (133.3 ± 37.1 ng/ml) and Exercised (130.4 ± 30.1 ng/ml) rats had similar stress hormone levels in the rested state. See Figure 1.

Behavioural studies and blood corticosterone levels in these animals suggest that the swim training did not elicit a stress response in these rats.

DISCUSSION

Contrary to the effects of acute swim stress on rats[16, 17], the long term chronic swim training elicited no stress response in rats at rest as reflected by either behaviour or corticosterone levels in our rats. The open field test is a measure of the amount of

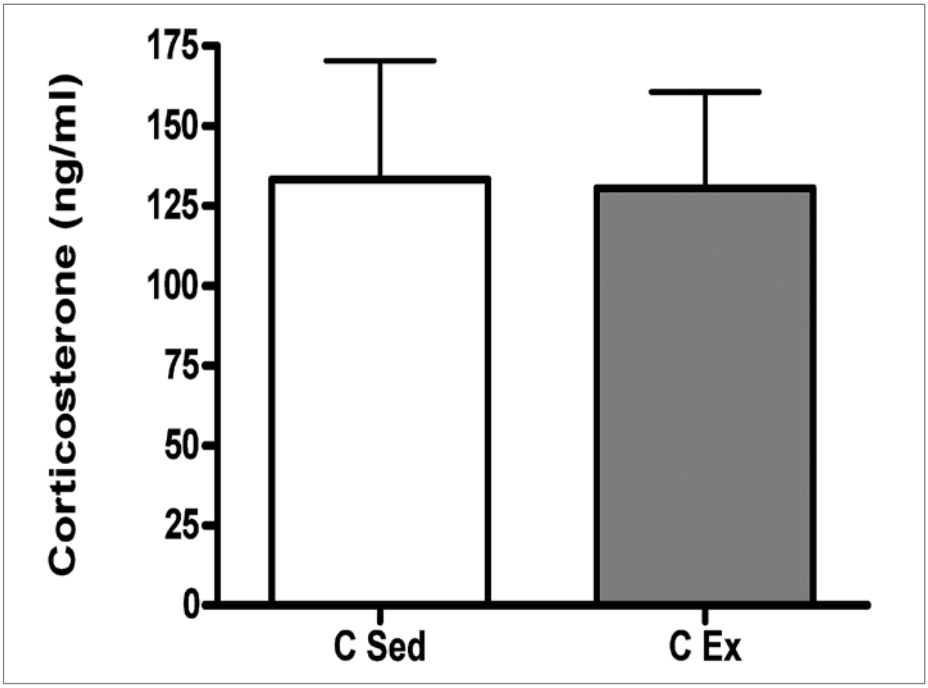

Figure 1: *Serum corticosterone levels of exercised and sedentary groups. n = 10 per group*

stressful behaviour displayed by the rats[18]. Although we found no significant differences in behaviour between the groups, the exercised group tended to move into the inner and outer zone and remain in the inner zone longer than sedentary group. Although these differences were not significant, it suggests that they may have been less stressed than the other groups.

Contarteze *et al.*[16] have shown that only acute swimming elicits elevations in the two stress hormones ACTH and corticosterone in rats post exercise. The forced swim test is used as a model of stress and depression in neurological studies[17] where rats are subjected to a once off swim for 15 minutes. It has been shown that during these tests, Wistar rats display more passive behaviour compared to other rat strains[17]. In our model, rats were acclimatized to the swim training, starting with swim duration of 5 minutes per day and increasing the duration incrementally daily. Acute exposure to swimming rather than trained or chronic swimming has been shown to increase stress hormone levels in rats[19]. Stress was minimized during the initial training period since the aim of this study was to elucidate the effects of long term exercise, and by minimizing or eradicating the effects of compounded acute psychological and physiological stress, only one variable, exercise, could then be studied. Thus in conclusion, long term swim training did not increase corticosterone levels or stress behavior in rats.

ACKNOWLEDGEMENTS

Funding: National Research Foundation, The Harry Crossley Foundation, University of Stellenbosch.

Technical Assistance: Lelanie Marais, Jacky Faure.

REFERENCES

1. Kaufman JC, Harris TJ, Higgins J and Maisel AS. Exercise-induced enhancement of immune function in the rat. Circulation. 1994;90:1: p525-32
2. Margonato V, Milano G, Allibardi S, Merati G, de Jonge R and Samaja M. Swim training improves myocardial resistance to ischemia in rats. Int J Sports Med. 2000;21:3: p163-7
3. Freimann S, Scheinowitz M, Yekutieli D, Feinberg MS, Eldar M and Kessler-Icekson G. Prior exercise training improves the outcome of acute myocardial infarction in the rat. Heart structure, function, and gene expression. J Am Coll Cardiol. 2005;45:6: p931-938
4. Smith RR, Shum-Siu A, Baltzley R et al. Effects of swimming on functional recovery after incomplete spinal cord injury in rats. J Neurotrauma. 2006;23:6: p908-19
5. Iemitsu M, Miyauchi T, Maeda S et al. Aging-induced decrease in the PPAR-alpha level in hearts is improved by exercise training. Am J Physiol Heart Circ Physiol. 2002;283:5: p1750-60
6. Iemitsu M, Maeda S, Jesmin S, Otsuki T and Miyauchi T. Exercise training improves aging-induced downregulation of VEGF angiogenic signaling cascade in hearts. Am J Physiol Heart Circ Physiol. 2006;291:3: p1290-8
7. Salman H, Bergman M, Weizman A et al. Effect of diazepam on the immune response of rats exposed to acute and chronic swim stress. Biomed Pharmacother. 2000;54:6: p311-5
8. Cox RH, Hubbard JW, Lawler JE, Sanders BJ and Mitchell VP. Cardiovascular and sympathoadrenal responses to stress in swim-trained rats. J Appl Physiol. 1985;58:4: p1207-1214
9. Scheuer DA and Mifflin SW. Repeated intermittent stress exacerbates myocardial ischemia-reperfusion injury. Am J Physiol. Feb 1998;274:2 Pt 2: p470-475
10. Scheuer DA and Mifflin SW. Chronic corticosterone treatment increases myocardial infarct size in rats with ischemia-reperfusion injury. Am J Physiol. Jun 1997;272:6 Pt 2: p2017-2024
11. Lupien SJ, Gaudreau S, Tchiteya BM et al. Stress-induced declarative memory impairment in healthy elderly subjects:

relationship to cortisol reactivity. J Clin Endocrinol Metab. 1997;82:7: p2070-5
12. Lupien SJ, Wilkinson CW, Brière S, Ménard C, Ng Ying Kin NM and Nair NP. The modulatory effects of corticosteroids on cognition: studies in young human populations. Psychoneuroendocrinology. 2002;27:3: p401-16
13. Sterzer P, Wiegers GJ and Reul JM. Long-term in vivo administration of glucocorticoid hormones attenuates their capacity to accelerate in vitro proliferation of rat splenic T cells. Endocrinology. 2004;145:8: p3630-8
14. Venditti P, Bari A, Di Stefano L and Di Meo S. Effect of T3 on metabolic response and oxidative stress in skeletal muscle from sedentary and trained rats. Free Radic Biol Med. 2009;46:3: p360-6
15. Richter L. The effect of central administration of corticotrophin-releasing factor on the behaviour and neurochemistry of rats. M.Sc. thesis, University of Stellenbosch, 2004
16. Contarteze RVL, De Barros Manchado F, Gobatto CA and Rostom De Mello MA. Stress biomarkers in rats submitted to swimming and treadmill running exercises. Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology. Nov 2008;151:3: p415-422
17. Armario A, Gavaldà A and Martí J. Comparison of the behavioural and endocrine response to forced swimming stress in five inbred strains of rats. Psychoneuroendocrinology, 1995;20:8: p879-890
18. Walsh RN and Cummins RA. The open-field test: A critical review. Psych Bullet. May 1976;83:3: p482-504
19.