Reprint from

RECENT ADVANCES IN DOPING ANALYSIS (7)

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Sport und Buch Strauß, Köln, 1999

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In: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck-Engelke (eds.) Recent advances in doping analysis (7). Sport und Buch Strauß, Köln, (1999) 85-94

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Corticosteroids and Exercise-Stress Response

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Introduction

Exercise-stress of varying intensity and duration tends to stimulate variable degrees of responses by the pituitary-adrenocortical and testicular system. Previous investigations to determine these changes based only on absolute concentrations of cortisol in plasma or saliva[1,2,3] have produced conflicting and inconsistent results. Our attempts to profile exercise-stress responses in human stress studies have similarly confirmed that stress indices based solely on absolute concentrations of cortisol (F) and cortisone (E) in urine are unreliable (unpublished data). Therefore, to obtain a consistent and reliable assessment of exercise-stress response we developed a stress-response model based on the concentration ratios of unbound urinary F, E and their primary metabolites of tetrahydrocortisol (THF) and tetrahydrocortisone (THE), using gas chromatography-mass spectrometry. The ratio model was subsequently used to study the effects of exercise in warm and humid conditions and swim-stress on the stress-response profiles of small groups of healthy, drug-free, trained male subjects.

Analytical Methodology

The samples were prepared according to the procedure by Yap et.al [3]. Briefly, the urine was eluted through a plug of XAD-2 resin, washed with demineralised water and the analyte eluted with methanol. After drying under vacuum the resultant dry residue was buffered with aqueous phosphate buffer at pH 7. The analytes were subsequently extracted into diethyl ether, evaporated to dryness under vacuum and derivatized to the methyloxime-trimethylsilyl (MO-TMS) ethers. After a separate clean-up step using Sephadex LH-20 resin swollen in hexane-chloroform, the analytes were evaprated to dryness under vacuum. The dry analytes were reconstituted in iso-octane and analysed by gas chromatography-mass spectrometry.

Human Stress Studies

Exercise-stress tests at VO_2max and submaximal stress levels.

Small groups of healthy, drug-free male athletes and sedentary subjects participated in the study. They were exercise-stressed on a computerised treadmill at VO_2max, 68-75%VO_2 and 51-55%VO_2 in an exercise-laboratory with an ambient temperature of 21^0C and 55% humidity. The control group did not exercise but sat quietly in a separate room during the duration of the stress test. All the subjects were in the 18-22 year old age group. All the experiments were conducted at approximately the same time of each day, for both control and experimental groups, to minimise the circadian rhythmn effects.

Exercise-stress in environmental chamber at 27.6^0C and 64.7% humidity

A group of 8 male footballers were exercise-stressed on a computerised treadmill at 50%VO_2 for 50 min. The subjects wore rectal probes, short pants and thick woolen football jerseys. Both core body and environmental chamber temperatures were electronically monitored every 5 min. The subjects were in the 19 to 25 year old age group.

Swim-stress in 25-metre heated swimming pool (Field Study)

A group of trained teenage male swimmers were swim-stressed at variable swim rates for 1.5 hr. Both the air and water temperatures ranged from 26^0C to 28^0C. The subjects were in the 13-15 year old age group.

In all stress studies urine samples were collected before and after the stress tests. The urines were checked for signs of renal dysfunction using the Diagnostic Multistix (8 SG) reagent dipsticks throughout the study. The subjects drank about 500 ml of filtered bottled water to maintain their hydration status.

Results and discussion

Figures 1-2 show the ion-chromatograms of the mo-tms derivatives of cortisol, cortisone and their metabolites tetrahydrocortisol and tetrahydrocortisone, while Figures 3-5 depict the corresponding EI mass spectra. Figure 5 shows a photograph of an athlete stress-tested on a computerisd treadmill in the exercise laboratory in one of the exercise-stress trials.

Evaluation of the endogenous corticosteroid stress-response profiles of trained subjects depicted in Figures 6-9, revealed that exercise-stress at VO_2max elicited significant changes in the E/F, THE/F, THE/E, and THF/F ratios relative to basal states. Marked changes were also observed in the E/F, THE/THF, THE/F and THE/E ratios in the stress response profiles at VO_2max in the sedentary subjects (Figures 10-13). Application of the ratio stress-response model to other studies showed that exercise in warm and humid conditions also produced significant changes in the E/F, THE/THF and THE/F ratios (Figure 14). An assessment of swim-stress response profiles in a group of trained teenage swimmers derived from field studies demonstrated a trend of increasing positive change approaching statistical significance in the E/F, THE/THF and THE/F ratio profiles (Figure 15).

The overall picture presented by the stress profiles suggests that there may be a causal relationship between training status and physiological stress response. The trend in the stress profiles also suggests that moderate humidity and temperature effects may evoke additional stress-response from the adrenocortical hormones but may not significantly alter the equilibrium state of the testicular hormones. Assessment of the swim-stress profiles indicates that there may be a similar trend in the adrenalcortico response when compared to the exercise-stress profiles derived from acute stress on the treadmill.

The pattern of the responses profiled above suggests that exertion during the swimming activity may be quite vigorous and stressful. However, due to the instantaneous dissipation of built-up heat from the body in the water, the final stress generated may be much less than an equivalent magnitude of exertion out of the water. It is also possible that vigorous activity performed over a longer term in a horizontal position, such as in swimming, combined with heat dissipation in a liquid medium may stimulate the adrenalcortical hormones to respond less acutely compared to the response evoked during a tradmill exercise bout at maximal oxygen uptake.

General Conclusions

1. Exercise-stress can affect the response from the hypothalamic-pituitary axis by altering the enzymatic conversions between F,E and metabolites THF and THE
2. Changes in the stress-response profiles can be affected by the intensity and duration of exercise-stress, environmental conditions and training status or circadian rhythmn.

Recommendations

1. The ratio model may be applied in future studies to facilitate the quantitation of stress responses associated with other adrenalcortical, testicular and luteinizing hormones
2. The model may also be used in future studies for profiling changes in endogenous corticosteroids due to ACTH doping

References

1. O'Connor, P.J., Corrigan, D.L., Medicine and Science in Sports and Exercise, Influence of short-term cycling on salivary cortisol levels,19,224-228, 1987
2. Martin, B.J., Bender, P.R., Chen, H.I., Stress hormonal response to exercise after sleep loss, European Journal of Applied Physiology, 55,210-214,1986
3. Viru, A., Oks,M., Effect of physical exertion on the blood level of bound and free corticoids, Endocrinol.Exp., 6,227-230,1972
4. Yap, B.K.,Johnston, G.A.R.,Kazlauskas, R., Routine screening and quantitation of urinary corticosteroids using bench-top gas chromatography mass-selective detection, Journal of Chromatography-Biomedical Applications, 573, 183-190, 1992

Acknowledgements

Grateful acknowledgement is due to Professor G.A.R.Johnston (Sydney University), Dr.R.P.Weatherby (Southern Cross University), athletes, sedentary subjects, swimming coaches and their trainees who participated in the trials. The authors are also grateful to the Australian Sports Drug Agency (ASDA) for their support and to the staff at the Australian Sports Drug Testing Laboratory (ASDTL) for their expert assistance.

Figure 1: Electron-impact ion chromatograms of cortisol and cortisone mo-tms derivatives

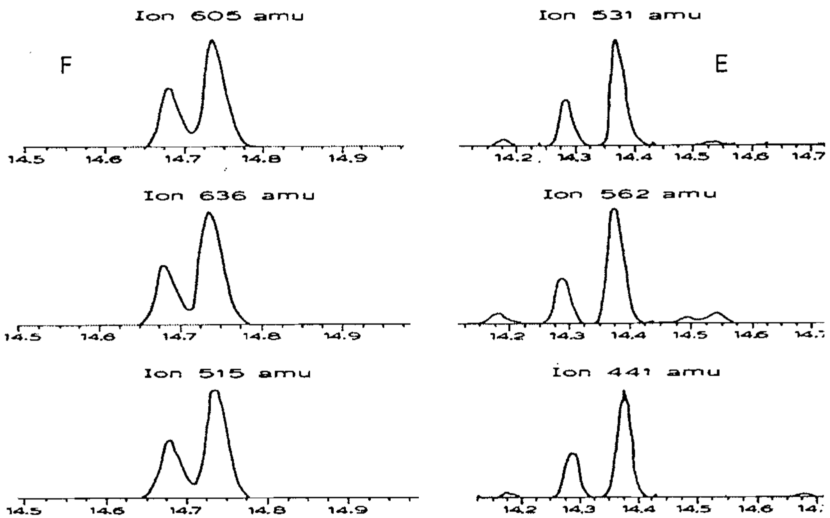


Figure 2: Electron-impact ion chromatograms of tetrahydrocortisol and tetrahydrocortisone mo-tms derivatives

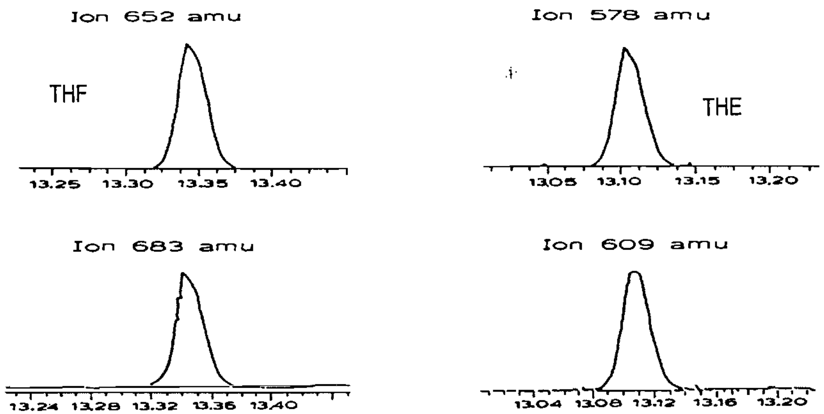


Figure 3: Electron-impact mass spectra of cortisol and cortisone mo-tms derivatives

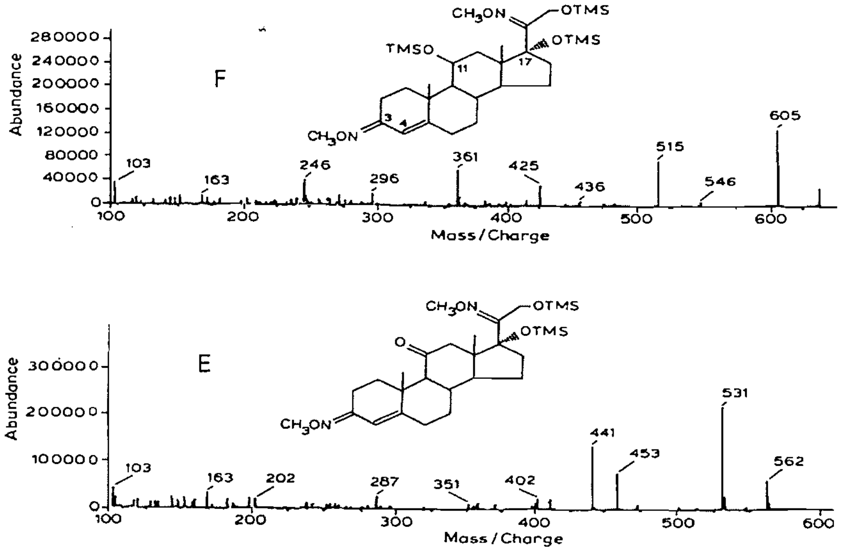


Figure 4: Electron-impact mass spectra of tetrahydrocortisol and tetrahydrocortisone mo-tms derivatives

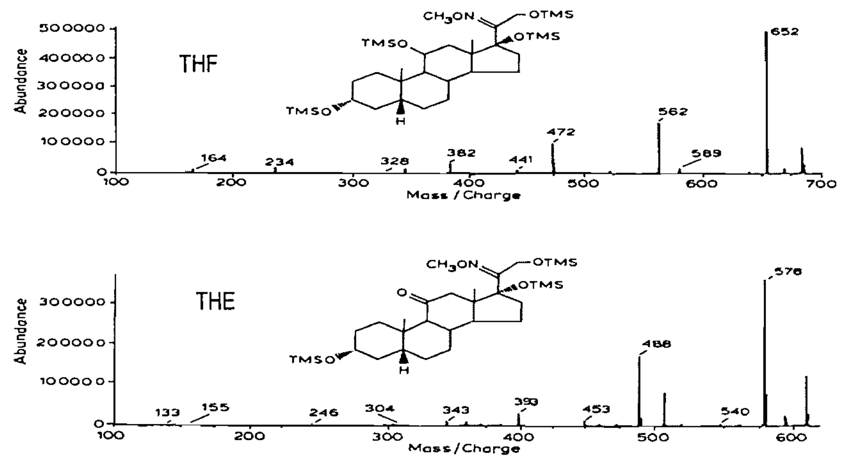
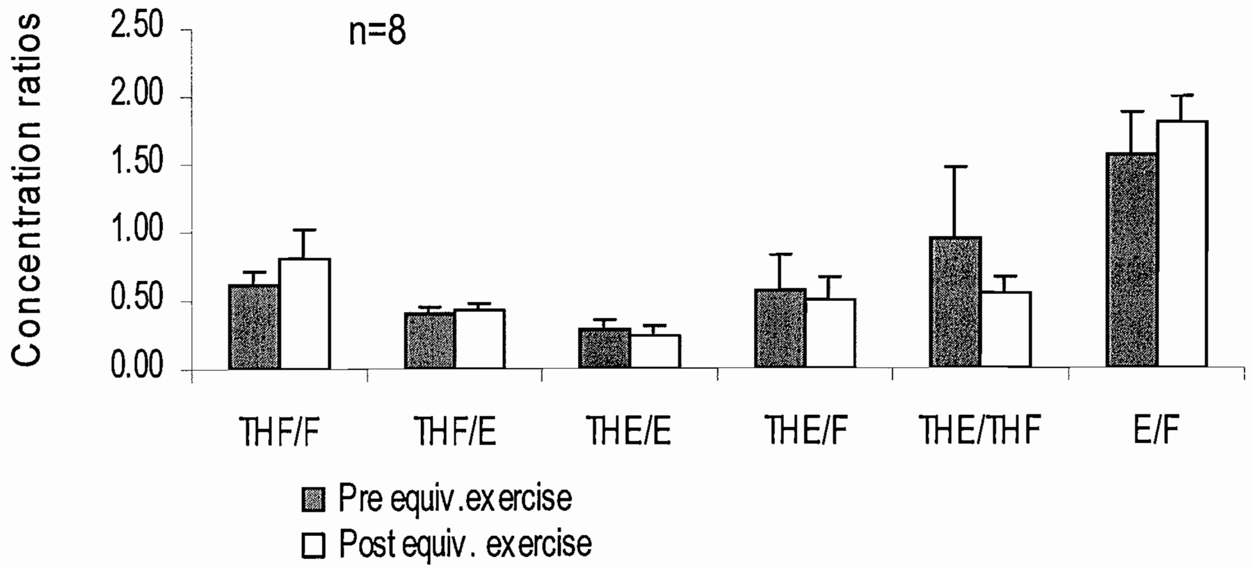


Figure 6 : Mean corticosteroid concentration ratio resting profiles of male athletes



E:Cortisone
F:Cortisol

THE:Tetrahydrocortisone
THF:Tetrahydrocortisol

Figure 5: Human stress studies Exercise-stress trials

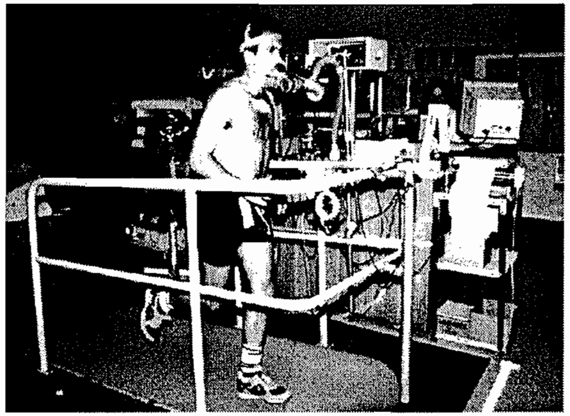


Figure 7: Mean corticosteroid concentration ratio profiles of male athletes exercise-stressed at VO_2 max

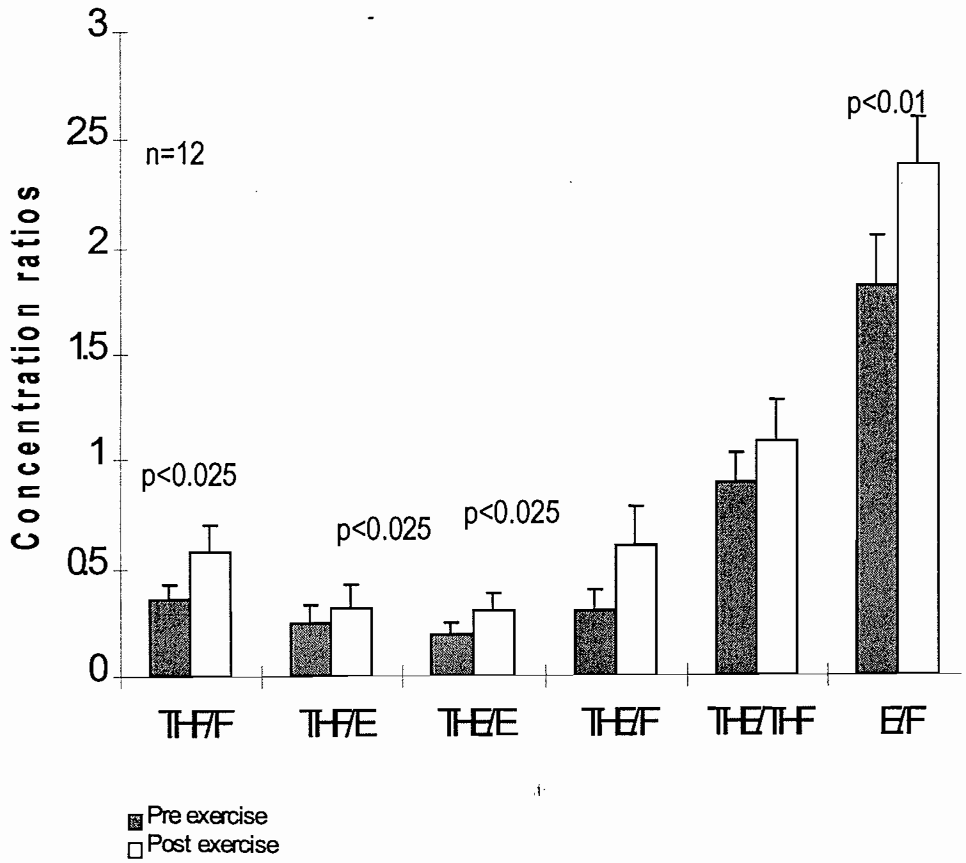


Figure 8: Mean corticosteroid concentration ratio profiles of male athletes exercise-stressed at 68.1+8.1% VO_2

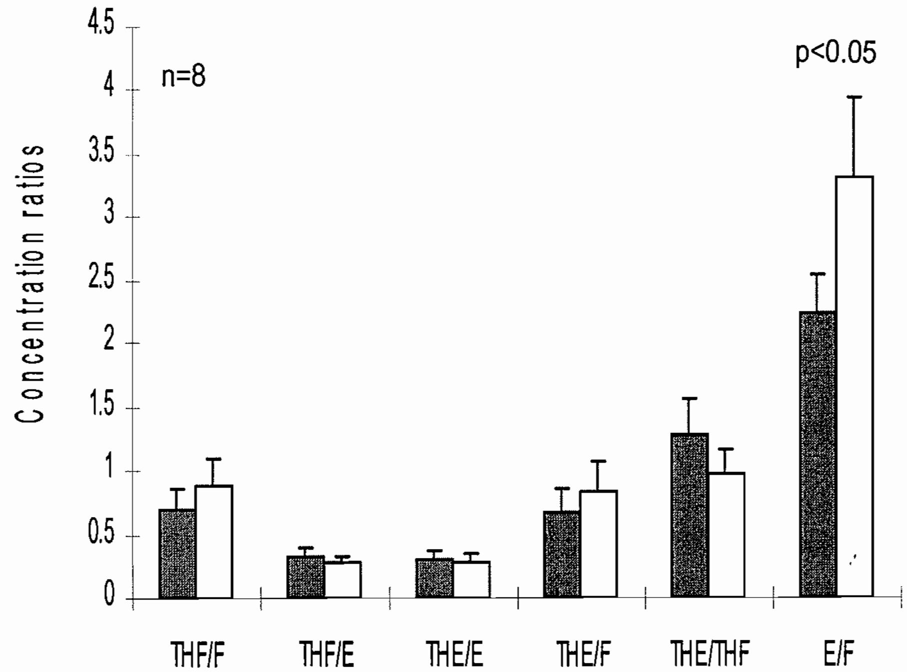


Figure 9: Mean corticosteroid concentration ratio profile of male athletes exercise-stressed at 51.6± 3.0% VO_2

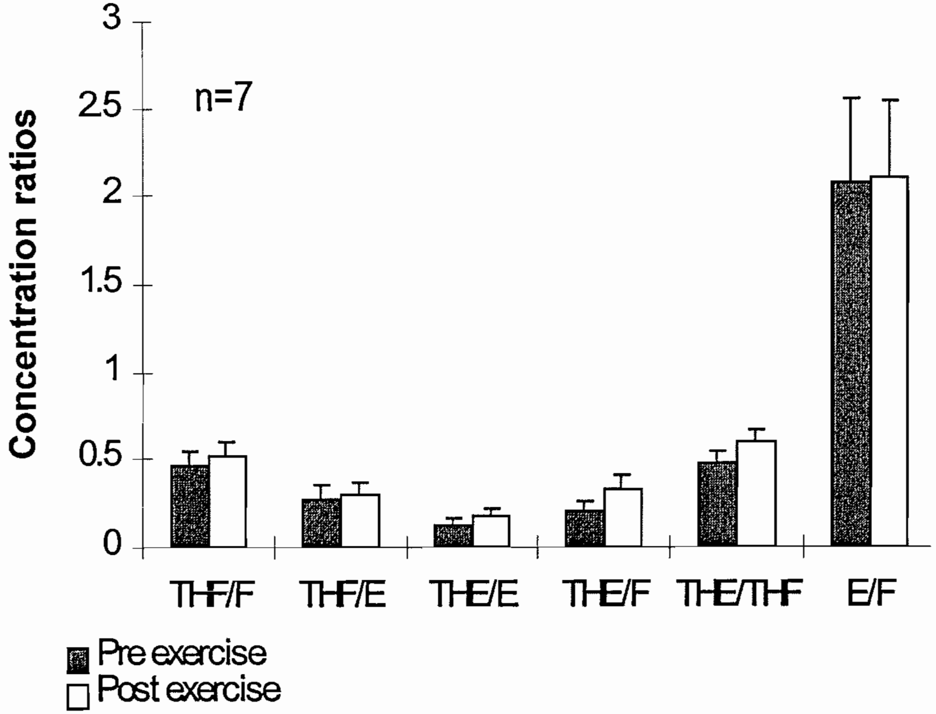


Figure 10: Mean corticosteroid concentration ratio resting profiles of sedentary male subjects

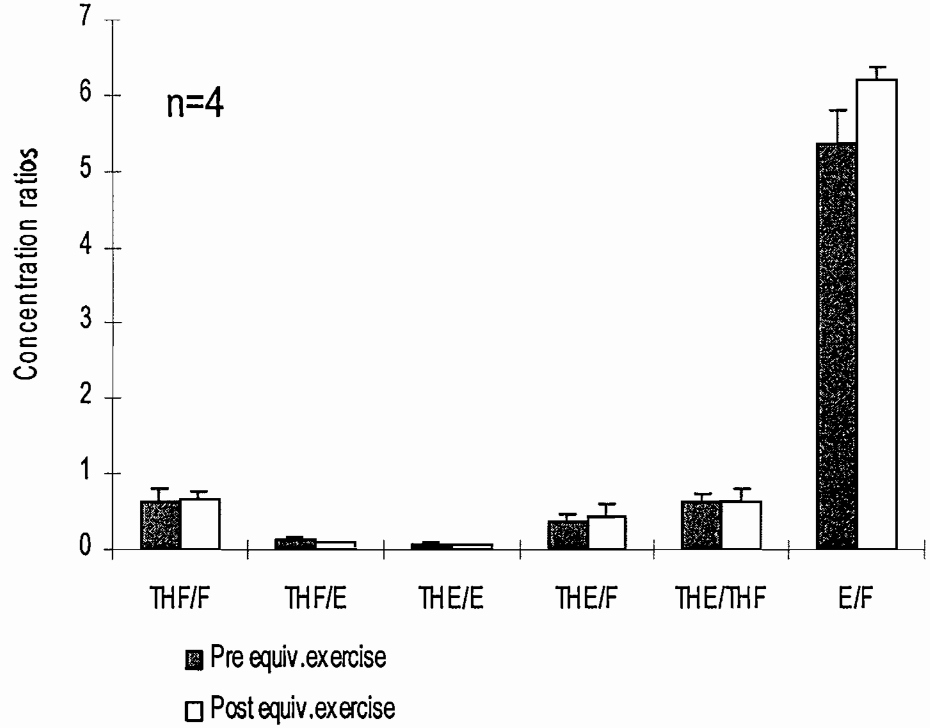


Figure 11: Mean concentration ratio profiles of male sedentary subjects exercise-stressed at VO_2max

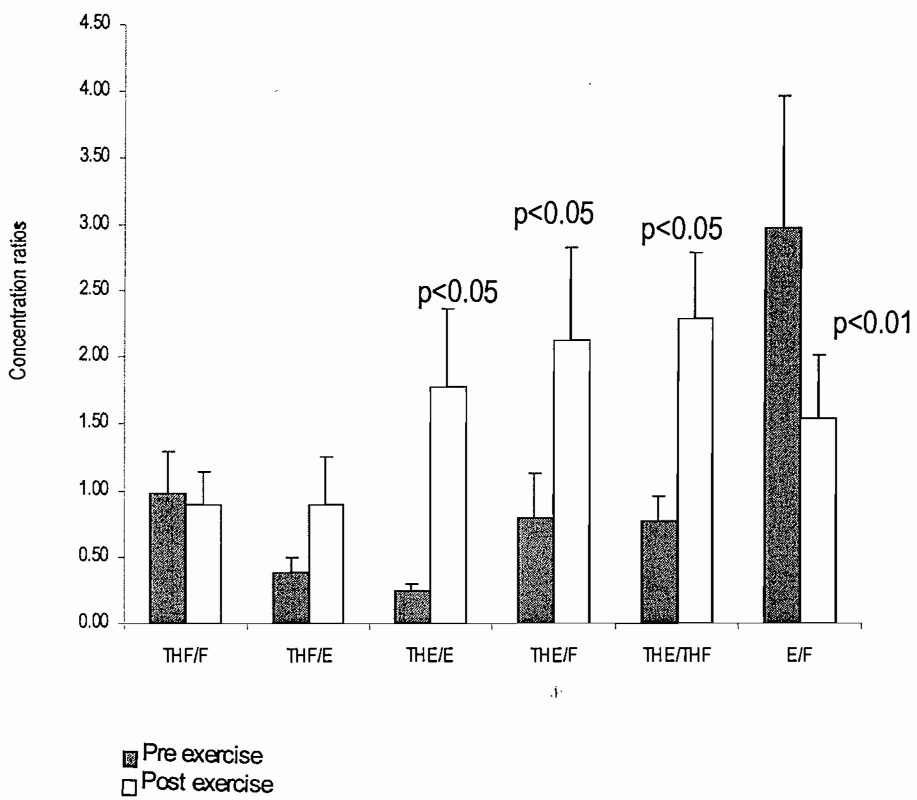


Figure 12: Mean corticosteroid concentration ratio profiles of sedentary male subjects exercise-stressed at 76.5+3.4% VO_2

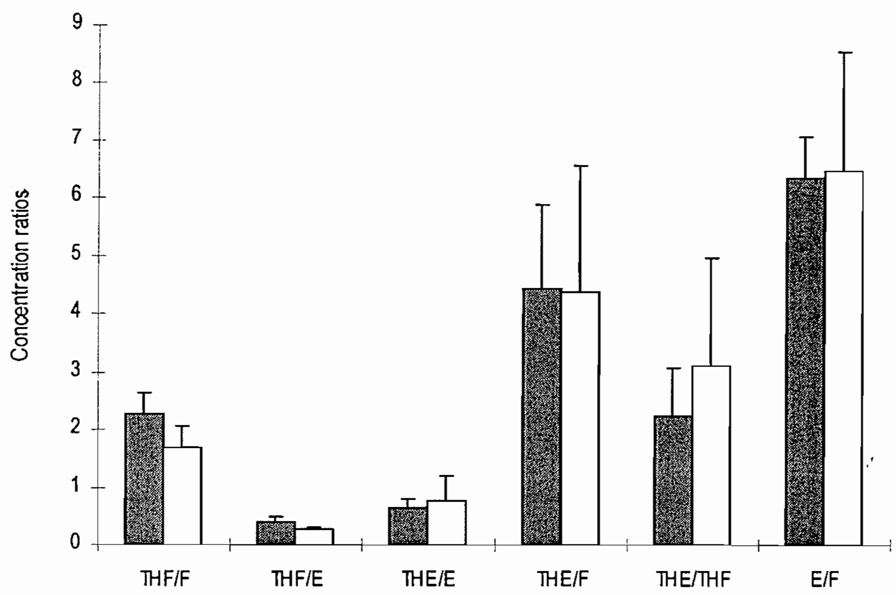


Figure 15: Effect of 1.5 hr training on mean concentration ratio profiles of male swimmers in competition season

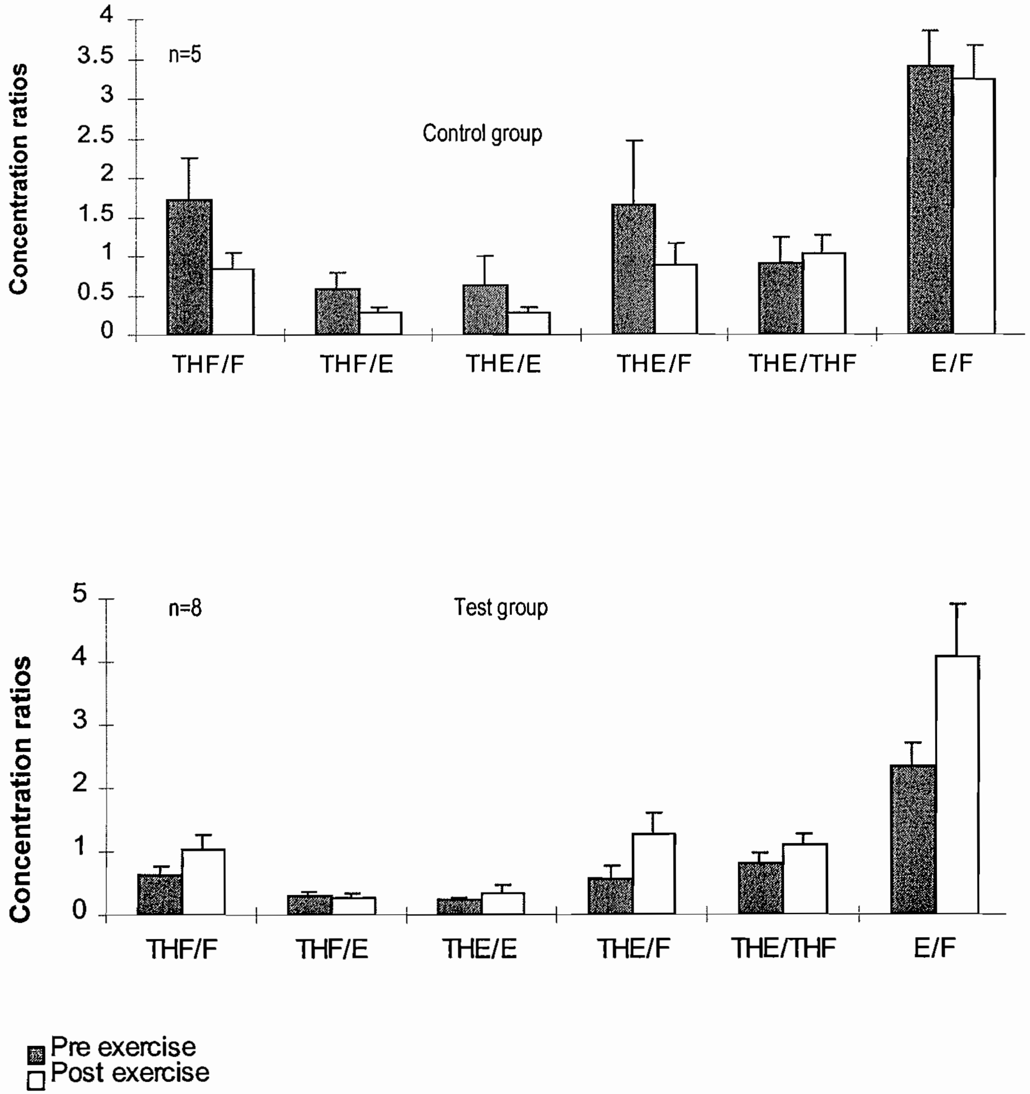


Figure 13: Mean corticosteroid concentration ratio profiles of sedentary male subjects exercise-stressed at 54.9±4.6% VO_2

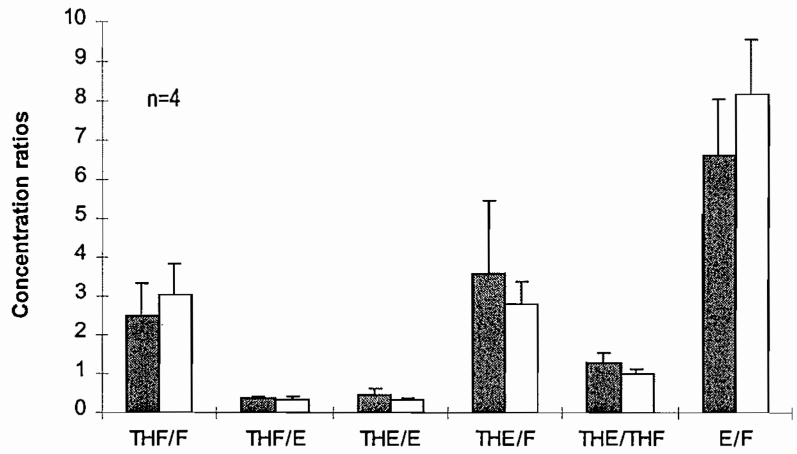


Figure 14: Effect of exercise in warm and moderate humidity on mean concentration ratio profiles of male footballers (core temp:38.2°C)