Urinary stress variables during exposure to high sustained +Gz

Sqn Ldr Rajat Baijal, Wg Cdr Harish Malik, Dr EM Iyer

Institute of Aerospace Medicine, Indian Air Force, Vimanapura, Bangalore 560 017, India

Fourteen healthy aircrew volunteers were exposed to a simulated aerial combat manœuvre (SACM) with continuous and repetitive peaks of +4 Gz (15 s) and +8 Gz (10 s) in the human centrifuge till volitional fatigue. They performed AGSM while wearing the cutaway anti-G suit when above +4 Gz. Timed and measured urine samples were collected before and after the run using the 'double-void technique'. A significant immediate mean post-run increase of 84.05% in total catecholamines, 121.42% in norepinephrine (NE), 64.28% in vanillyl mandelic acid and 31.55% in 17-oxogenic steroids was noted. The rise in epinephrine (E) excretion rate was not significant. Decrease of excretion rates to near or below pre-run values in the recovery period indicated an acceleration-related stress response. Significant increase of NE/E ratio from 1.87 pre-run to 3.84 post-run showed higher physical work load with absence of predominant psychological stress. SACM tolerance time did not correlate to any of the stress variables. The study shows that high sustained +Gz acceleration elicits a stress response which can be quantified and compared by urinalysis of stress hormones and their metabolites.

Keywords: Acceleration stress, Urinary catecholamines

The variable G environment during SACM involves haemodynamic changes leading to impairment of cerebral blood flow. Intense physical effort (AGSM) increases tolerance to +Gz but requires active effort and leads to exhaustion. The physiologic cost of the combination of these factors occurring at their peak in a controlled situation can be quantified with the urinalysis of the stress hormones.

Material and methods

Subjects. Fourteen healthy male aircrew with mean age 26.65 years (range 24–30 years) were selected for this study. They all had reported for high-G centrifuge training at IAM and were highly motivated.

Controls. The subjects served as their own controls. The pre-run values were used as baseline reference for comparison with the post-run values.

Human centrifuge. The human centrifuge installed at IAM in 1966 is microprocessor-controlled, and has a capability of providing multisegmented G profiles, including SACM. This gives a near-realistic simulation of the in-flight aerial combat environment encountered in a modern high-performance aircraft.

Acceleration stress. The aircrew were subjected to the standard simulated aerial combat manœuvring (SACM) profile employed at IAM for high-G centrifuge training [1].

A warm-up run was given with an onset rate of 1 G/s to a peak of 4 G for 15 s and an offset rate of –0.5 G/s. The SACM included a rapid onset run with an onset rate of 1 G/s up to 4 G for 15 s, then again a build-up at the rate of 1 G/s up to 8 G for 10 s, followed by a deceleration at the rate of –0.5 G/s up to 4 G. This profile continued till the subject felt fatigued and gave a call to terminate the run or there occurred a peripheral light loss (52–56°) or G-LOC when the centrifuge was stopped. The subjects performed AGSM while accelerating from 4 to 8 G and throughout, till they came back to 4 G. The anti-G suit was kept inflated throughout the SACM. SACM tolerance was

taken as the total duration in seconds from the start of the run till its termination.

The subject remained seated in the gondola with its canopy opened, in between the warm-up and SACM run and at the end of the SACM run till his physiological parameters recovered to the preacceleration levels.

Collection of urine sample The double-voiding technique was used for collection of the urine sample.

(i) *Pre-run urine sample* – The subjects emptied their bladder completely about one hour (sample discarded) before the SACM runs. The pre-run sample was collected in a clean urine-collecting jar just before the SACM run.

(ii) *Post-run urine sample* – The immediate post-run urine sample was collected in a clean urine-collecting jar after about 20 min of the cessation of the run. The second post-run urine sample was collected after 120 min of the first post-run urine sample (recovery period). To ensure the availability of an adequate urine volume, the subjects drank 250 ml of water after each void. The exact length of time and the total volume of each sample was noted.

Urinary analysis of hormones and their metabolites. Pre- and post-G-run stored urine samples were analysed for total catecholamine (CA), epinephrine (E), norepinephrine (NE), vanillyl mandelic acid (VMA) and 17-oxogenic steroids (17-OGS).

The total amounts of CA, EP and NE was determined by the fluorometric assay using alumina column isolation technique. Twenty ml sample of urine was used for these estimations.

Urinary assay of VMA and 17-OGS was done spectrophotometrically by the technique recommended by Pisano *et al.* (1962) and Few (1961), respectively. A 5 ml aliquot of each sample was utilized for these estimations.

To ensure that the equipment and reagents being used were of adequate standard, standard curves were established for each procedure at the onset of the study. Random duplicate determinations were done as a check of reliability. As a routine practice, blank and standard solutions were assayed every time a sample was tested.

Statistical analysis The data were analysed using the paired and unpaired Students '*t*-test' and correlation coefficient analysis was used to compare tolerance with the various urinary stress parameters.

Results

The results of the various urinary stress variables pertaining to the 14 aircrew subjects who took part in the HSG study are given below.

Urinary stress variables. For the purpose of statistical analysis and discussion, the values have been reported as excretion rate, viz. µg/min.

Table 1. Catecholamine excretion rates pre-run and immediate post-HSG run

	All subjects (n = 14)
Pre-run	0.069 ± 0.012
Immediate post-run	0.127 ± 0.023
Mean difference	0.058 ± 0.024
Mean percentage change	84.05
t	2.54
P	<0.05

All values expressed as mean ± SE in µg/min and percentage change

Table 2. Epinephrine excretion rates pre-run and immediate post-HSG run

	All subjects (n = 14)
Pre-run	0.026 ± 0.005
Immediate post-run	0.034 ± 0.007
Mean difference	0.008 ± 0.007
Mean percentage change	30.77
t	1.11
P	NS

All values expressed as mean ± SE in µg/min and percentage change
NS Not significant

Table 3. Norepinephrine excretion rates pre-run and immediate post-HSG run

	All subjects ($n = 14$)
Pre-run	0.042 ± 0.008
Immediate post-run	0.093 ± 0.019
Mean difference	0.051 ± 0.018
Mean percentage change	121.42
t	2.795
p	<0.05

All values expressed as mean ± SE in µg/min and percentage change.

Table 4. NE/E ratio pre-run and immediate post-HSG run

	All subjects ($n = 14$)
Pre-run	1.87 ± 0.33
Immediate post-run	3.84 ± 0.86
Mean difference	1.97 ± 0.83
t	2.36
p	<0.05

All values expressed as mean ± SE in µg/min and percentage change.

Table 5. VMA excretion rates pre-run and immediate post-HSG run

	All subjects ($n = 14$)
Pre-run	2.94 ± 0.36
Immediate post-run	4.83 ± 0.93
Mean difference	1.89 ± 0.73
Mean percentage change	64.28
t	2.61
p	<0.05

All values expressed as mean ± SE in µg/min and percentage change.

The changes in the hormonal parameters have been discussed on the basis of 'mean percentage change' and statistical significance has been reported wherever the changes were found to be significant.

(i) *Immediate post-run sample.* Table 1 presents the mean excretion rates of total CA for all the subjects. A significant increase of 84.05% ($p < 0.05$) was found in all the subjects.

Table 6. 17-OGS excretion rates pre-run and immediate post-HSG run

	All subjects ($n = 14$)
Pre-run	10.33 ± 1.36
Immediate post-run	13.59 ± 2.59
Mean difference	3.26 ± 1.25
Mean percentage change	31.55
t	2.61
p	<0.05

All values expressed as mean ± SE in µg/min and percentage change.

Table 7. Recovery period – catecholamine excretion rates pre-run and 2 h post-HSG run

	All subjects ($n = 14$)
Pre-run	0.069 ± 0.012
2 h post-run	0.048 ± 0.008
Mean difference	−0.021 ± 0.008
Mean percentage change	30.43
t	2.54
p	<0.05

All values expressed as mean ± SE in µg/min and percentage change.

Table 8. Recovery period – epinephrine excretion rates pre-run and 2 h post-HSG run

	All subjects ($n = 14$)
Pre-run	0.026 ± 0.005
2 h post-run	0.016 ± 0.003
Mean difference	−0.010 ± 0.005
Mean percentage change	38.46
t	2.29
p	<0.05

All values expressed as mean ± SE in µg/min and percentage change.

Table 2 presents the mean excretion rate of E in the immediate post-run period. The mean percentage increase for all the subjects over the pre-run value was 30.77%, which was not found to be significant. Table 3 presents the mean excretion rate of NE, a significant increase of 121.42% ($p < 0.05$) was found in all the subjects.

Table 4 presents the values of NE/E ratio for all the subjects. There was an overall rise in NE/E ratio, viz. 1.97 ± 0.83 over the pre-run levels in all the subjects. This was statistically significant.

Table 5 presents the mean excretion rate of VMA in the immediate post-run urine sample. An overall increase by 64.28% was seen in all the subjects over the pre-run values. This rise was found to be significant.

Table 6 presents the mean excretion rates of 17-OGS. All the subjects showed a mean percentage increase of 31.55% over the pre-run values. This rise was found to be significant ($p < 0.05$).

(ii) *Recovery period (2 h post-run sample)* Table 7 shows that the excretion rate of CA in the 2 h post-run urine sample returned to below pre-run levels for all the subjects. This decrease of 30.44% was found to be significant ($p < 0.05$).

Table 8 presents the mean E excretion rates in the 2 h post-run sample. A significant decrease of 38.46% ($p < 0.05$) was seen in all the subjects.

Table 9 presents the NE mean excretion rate for all the subjects. They were found to be decreased below the pre-run levels in all the subjects by 23.81%.

The NE/E ratio in the 2 h post-run urine sample is presented in Table 10. The ratio had continued to be greater than the pre-run ratio (NE/E 2 h post-run of 1.34 + 0.92) for all the subjects.

Table 11 presents the VMA excretion rates in the 2 h post-run urine sample. A decline in the excretion rate was seen in all the subjects.

Table 9. Recovery period – norepinephrine excretion rates pre-run and 2 h post-HSG run

	All subjects ($n = 14$)
Pre-run	0.042 ± 0.008
2 h post-run	0.032 ± 0.006
Mean difference	−0.010 ± 0.005
Mean percentage change	23.81
t	1.811
p	NS

All values expressed as mean ± SE in µg/min and percentage change
NS Not significant.

Table 10. Recovery period – NE/E ratio rate is pre-run and 2 h post-HSG run

	All subjects ($n = 14$)
Pre-run	1.87 ± 0.33
2 h post-run	3.21 ± 0.88
Mean difference	1.34 ± 0.92
t	1.46
p	NS

All values expressed as mean ± SE in µg/min and percentage change
NS Not significant.

Table 11. Recovery period VMA excretion rate pre-run and 2 h post-HSG run

	All subjects ($n = 14$)
Pre-run	2.94 ± 0.36
2 h post-run	3.28 ± 0.36
Mean difference	0.34 ± 0.38
Mean percentage change	11.56
t	0.90
p	NS

All values expressed as mean ± SE in µg/min and percentage change.
NS Not significant

Table 12. Recovery period 17-OGS excretion rate pre-run and 2 h post-HSG run

	All subjects ($n = 14$)
Pre-run	10.33 ± 1.36
2 h post-run	9.45 ± 1.44
Mean difference	−0.88 ± 0.95
Mean percentage change	8.52
t	0.92
p	NS

All values expressed as mean ± SE in µg/min and percentage change.
NS Not significant.

Table 12 presents the 17-OGS mean excretion rates for all the subjects. The values had recovered to below the pre-run levels in all the subjects.

Tolerance and urinary stress variables. Tolerance time was correlated to the various urinary stress parameters for all the subjects. No significant correlation was found between tolerance time and any of the parameters assayed in the immediate post-run urine samples.

Discussion

Our study demonstrated a significant rise of 84.05% in total CA excretion levels in the urine sample immediately after the centrifuge run. The values recovered back to below the pre-run levels in the second post-run urine sample. Catecholamine excretion is believed to be a physiological expression of the general stress response. It quantifies the total stress as experienced by the individual. In view of this, all the subjects had exhibited a substantial stress response induced by HSG exposure.

The mean pre-run excretion levels of E and NE were 0.026 ± 0.005 µg/min and 0.042 ± 0.008 µg/min, respectively. These levels are similar to those reported in other studies [2, 3]. In contrast to the expected response and that reported in other studies, the change in E excretion rate showed an insignificant rise of 30.77% in the immediate post-run period. Other studies had also found a significant rise in pre-exposure epinephrine levels and had attributed it to anticipatory stress.

The low mean percentage rise in epinephrine output immediately after the run can be attributed to two factors: anticipatory stress leading to elevated pre-run levels, and a relatively lower level of anxiety during the actual centrifugation. Familiarity with repeated centrifuge runs has been shown to reduce urinary epinephrine excretion [4, 5]. As part of the HSG centrifuge course, our aircrew subjects had been exposed to high sustained acceleration on the centrifuge prior to this study [1]. The presence of psychological stress before the centrifuge run was evident by the significant fall in epinephrine excretion rate below the pre-run values in the second post-run urine sample.

A significant increase of 121.42% in the NE output was noted in the urine sample immediately after the run. This increase in NE excretion rate was higher than that reported in the literature. Goodall [2, 3], in two separate studies had noted 50–100% increase in NE output following centrifugation. The peak acceleration levels used were 12 Gz for 3 min and less than +5 Gz (till subject blackout levels). Sarviharju [6] had found a 34.2% increase in NE excretion levels after 30 min of aerobatics, while Burton *et al.* [7] had observed a 19% increase following inflight aerial combat manœuvres.

Norepinephrine release is known to occur as part of the general stress response. Although NE release is less sensitive to emotional arousal, it has been shown to relate to both physical and mental activity of controlled and appropriate nature. Various forms of physical exercise have been shown to cause enhanced secretion of norepinephrine.

The increase in the immediate post-run output of NE and a subsequent decrease below pre-run levels during the recovery period suggest an acceleration-induced rise in NE excretion rate in this study. The higher mean percentage increase in NE excretion rate noted could be attributed to higher levels of repeated acceleration (+8 Gz) encountered during SACM. In addition, the subjects were required to adopt a maximal sustained straining effort while performing AGSM, in order to maintain vision at these high levels of acceleration. In this respect, the NE output noted during one episode of SACM involving repeated high G levels is comparable to that found by Tarui and Nakumura [8] during seven repetitive flight missions involving aerial combat manœuvring at +6 Gz. They had noted an increase of more than 110% in NE output only after the fourth to sixth flight and had attributed the delayed increase to increasing physical work load.

The NE/E ratio has been used to assess the sympathoadrenal stimulation during inflight studies. It has been reported that a higher value of NE/E ratio suggests higher physical work load. According to Krahenbuhl *et al.* [9, 10], ratios approaching unity indicate the dominance of psychological stress.

In the present study, the immediate post-run NE/E ratio (3.84 ± 0.6) showed a significant increase over the pre-run values (1.87 ± 0.33). The post-run increase, which continued into the recovery period, indicated a higher physical work load in all the subjects during SACM. In other words, it demonstrated that there was an absence of predominant psychological stress during the run. This may be attributed to the familiarity with the centrifuge run acquired over the previous couple of days of the HSG training course [1].

A significant increase of 64.28% was found in the mean excretion levels of VMA which was commensurate with the immediate post-run increase in total catecholamines. These values had recovered to near pre-run levels in the 2 h post-run urine sample.

A significant increase of 31.55% in the mean excretory rate of 17-OGS was noted in the immediate post-run urine sample. The mean pre-run levels of 17-OGS (10.33 ± 1.36 µg/min) were found to be within normal limits of excretion, viz. 5–23 mg per 24 h [11, 12].

The fact that the 17-OGS excretion levels had come to below the pre-run values in the second post-run urine sample indicated that the significant rise was due to the stress response elicited by exposure to high sustained acceleration. Based on the findings of the studies of Burton [7] and others, the stress response may be taken as moderate.

Our observations were in conformity with those of Burton *et al.* [7], who found no correlation between ACM tolerance time and fatigue compared to the sympathetic activity or stress response measured by 17-OHCS excretion. In their study, increases in epinephrine excretion levels were as great after short-duration as after long-duration ACMs.

Vernikos-Danellis *et al.* [13] had also reported the lack of significant correlation between serum cortisol and tolerance among female centrifuge subjects.

Conclusion

In conclusion, the study shows that exposure to a variable high sustained +Gz acceleration profile elicits a moderate stress response in the subjects. This stress response can be quantified by measuring the increase in the output of stress hormones and their metabolites in urine and may be used as an objective indicator of physiologic cost of the HSG exposure. Familiarity with repeated HSG centrifuge runs leads to a reduction in the urinary epinephrine excretion rate. Therefore, it is of importance to define the previous centrifuge experience of the subjects while investigating acceleration stress. The SACM tolerance time is not correlated to the immediate post-run changes, in any, of the urinary stress variables measured.

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