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SOME BIOCHEMICAL INDICES OF MILD PHYSICAL STRESS: A PRELIMINARY STUDY

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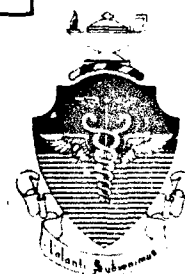
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**USAF SCHOOL OF AEROSPACE MEDICINE
Aerospace Medical Division (AFSC)
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NOTICES

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The voluntary informed consent of the subjects used in this research was obtained in accordance with AFR 169-3.

The Office of Public Affairs has reviewed this report, and it is releasable to the National Technical Information Service, where it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Toward establishing an analytical capability for quantifying the physical demands of job stress, the effectiveness of an experimental protocol was ascertained in a group of healthy men. The protocol featured measurement of epinephrine (E), norepinephrine (NE), and cortisol in blood drawn moments before and after 3 discrete levels of treadmill exercise. An added feature was testing of subjects with differing degrees of physical fitness (as adjudged by exercise habits). Although ways of enhancing the protocol's effectiveness		

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20. ABSTRACT (Continued)

→ were identified, the following definitive findings emerged: (1) plasma NE concentrations were the most sensitive to workload and differentiated fitness subgroups, (2) plasma E levels were slightly less sensitive to workload and did not differentiate as well fitness subgroups, and (3) evidence of adrenocortical stimulation was found only at the highest exercise level, and only for the less fit subjects. Exercise-induced changes in plasma NE correlated well ($r=0.861$) with concomitant changes in blood lactate (reported elsewhere), suggesting the 2 indices might collectively distinguish physical from cognitive or emotional demands in various work environs. ←

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SOME BIOCHEMICAL INDICES OF MILD PHYSICAL STRESS: A PRELIMINARY STUDY

Numerous reports have documented the use of blood constituents for quantifying physiologic response to exercise. A cursory review of the literature suggests major attention has focused on exercise intensities ranging from moderate to severe. Little attention seems to have been given to biochemically quantifying responses to less strenuous exercise. Interest in moderate levels of exercise is reflected by the growing concern over the adverse effects of job stress. Concern is directed not only toward immediate physical, cognitive, and emotional demands, but also toward effects on job performance and long-term health effects. This report addresses the immediate physical aspect of work demands.

The present investigation was undertaken as an exploratory endeavor, with principal objectives to examine the effectiveness of a proposed protocol to quantify the relatively mild levels of exercise as might be experienced by workers in physically demanding occupations, and to recommend improvements to the protocol. The protocol specified the testing of subjects on a treadmill at 3 workload levels and the measurement of 3 hormones in venous blood collected shortly before and immediately after each workload. The following hormones were measured: plasma cortisol and plasma epinephrine (E) as indices of adrenocortical (1) and adrenomedullary (2) secretion, respectively, and plasma norepinephrine (NE) as a mixed indicator of adrenomedullary secretion and of sympathetic nerve stimulation (3).

The choice of experimental subjects in this study permitted the proposed protocol to examine differences in the response of individuals of differing degrees of physical fitness.

METHODS

Nine healthy men (ages 22-46 years) served as experimental subjects. By design, the subjects were assigned to 3 groups, each comprised of 3 individuals and each representing a different degree of physical fitness. The approximate degree of fitness was not ascertained by measurement of aerobic capacity but was presumed from the subjects' regular participation in exercise activities. Group N (nonjogging) was made up of men who did not engage in a regular program of exercise. Group S (semi-jogger) subjects participated in various forms of strenuous exercise, but not on a regular basis. Group J (jogger) was comprised of men who jogged at least 3 miles on alternate workdays. The mean ages of subjects in Groups N, S, and J were 41, 34, and 36 years, respectively.

The test protocol consisted initially of inserting a small cannula (5.08 cm in length) into an antecubital vein. After allowing time for the subjects to recover from the cannulation, a "resting" blood sample was drawn. Then, over the next 90 minutes, each individual was subjected to 3 exercise levels, each of 10-minute duration and each separated from the preceding exercise period by a 10-minute rest period. Blood samples were drawn shortly before and immediately

after each exercise period. Exercise levels were randomized within each subject. A treadmill maintained at 3.6 mph with grades of 0°, 5°, and 10° was used for producing exercise levels denoted herein ex-1, ex-2, and ex-3, respectively. These levels translate to approximate energy expenditures of 47, 83, and 128 cal/kg/min, respectively.

Each blood sample was split at the time of collection, with one aliquot taken for hormone analyses and the other for analyses reported elsewhere (4). Blood for hormone assays was immediately placed in tubes containing reduced glutathione and EGTA (ethylene-glycol-bis-tetraacetic acid), centrifuged at 5°C and 2000 rpm for 10 minutes, and the plasma stored at -80°C until analyzed. All analyses were carried out with commercially available assay kits in general accord with the recommendations of the supplier. Kits marketed by Clinical Assays (Cambridge, Mass.) and Upjohn Diagnostics (Kalamazoo, Mich.) were used for cortisol and catecholamine (NE and E) determinations, respectively.

Statistical testing was accomplished primarily through repeated measurements analyses of variance, followed up with appropriate subtests (paired and unpaired Student's t tests) using pooled variance estimates from the general analysis (5).

RESULTS

One of the J subjects became indisposed during the experiment and was withdrawn from further consideration. The overall response of the other 8 subjects is summarized in Table 1. The relatively mild levels of exercise produced substantial and significant increases in the concentration of the catecholamines, but not cortisol. The exercise-induced increments in the 2 catecholamines were comparable at ex-3, increasing by about 200%. The significant postexercise drop in cortisol is contrary to the typical response to exercise and other stressors (1); moreover, the uniform magnitude of the drop suggests a common influence, such as the circadian drop known to occur during the midmorning hours in which this experiment was conducted.

TABLE 1. PLASMA HORMONE LEVELS (Mean $\pm$ S.D., No.=8) BEFORE AND AFTER EXERCISE

	Exercise level	Pre exercise	Post exercise	Change	
Plasma NE (pg/ml)	ex-1	455 $\pm$ 125	552 $\pm$ 69	+98	} (+130)*
(Resting level 310 $\pm$ 80)	ex-2	461 $\pm$ 143	702 $\pm$ 88	+240 ^a	
	ex-3	439 $\pm$ 104	1386 $\pm$ 361	+947 ^a	
Plasma E (pg/ml)	ex-1	42 $\pm$ 32	83 $\pm$ 64	+41 ^b	} (+41)
(Resting level 44 $\pm$ 32)	ex-2	44 $\pm$ 24	84 $\pm$ 51	+40 ^b	
	ex-3	50 $\pm$ 33	140 $\pm$ 66	+90 ^a	
Plasma cortisol (μ g/dl)	ex-1	11.9 $\pm$ 6.5	10.9 $\pm$ 5.3	-1.0 ^c	} (+0.9)
(Resting level 12.2 $\pm$ 6.8)	ex-2	12.4 $\pm$ 5.3	11.4 $\pm$ 5.1	-1.0 ^b	
	ex-3	10.9 $\pm$ 4.4	9.8 $\pm$ 3.5	-1.1 ^c	

^ap<0.001

^bp<0.05

^cp<0.01

*Pooled S.D. of the difference from the analysis of variance (used in the tests for significant changes).

To ascertain whether the fitness subgroups differed in their response to graded workloads, it was deemed inappropriate to base the assessment on pre-exercise-to-postexercise changes, since an apparent and variable carryover effect was detected in preexercise data. For this reason, statistical appraisal of subgroup responses to exercise was based not on preexercise-to-postexercise changes, but on resting-to-postexercise changes. Accordingly, results of that appraisal are summarized in Figure 1. All 3 fitness groups demonstrated a significant

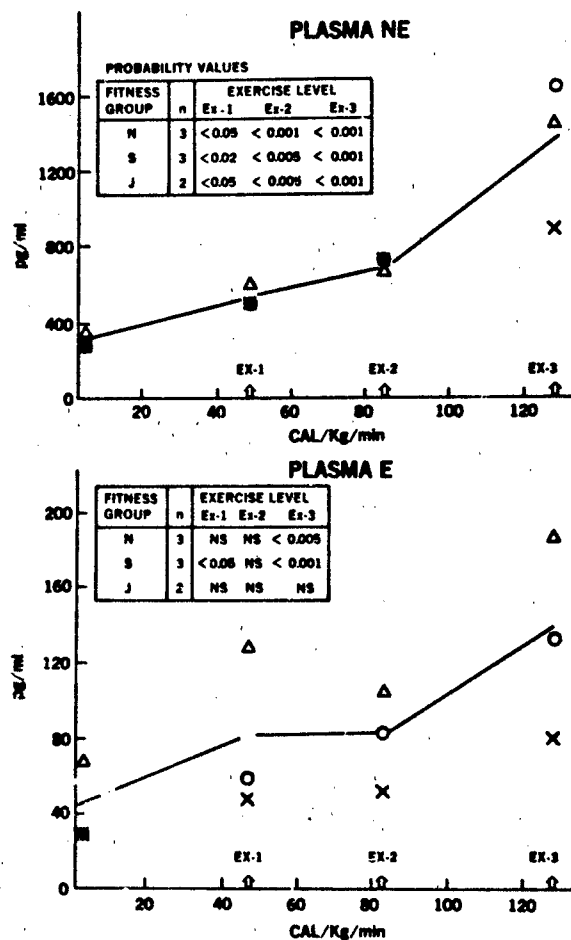


Figure 1. Plasma catecholamine (NE and E) means at rest and at 3 workloads in subjects of varying degrees of physical fitness. Solid lines denote overall response (No.=8). Responses of subjects in fitness groups N (No.=3), S (No.=3), and J (No.=2) are denoted o, Δ , and x, respectively. Probabilities are associated with differences between resting and post-exercise levels.

increase in plasma NE concentration at each of the 3 workloads. Plasma E was significantly elevated in only 3 cases; all of these occurring in the less-fit groups, and primarily at the highest exercise. The lack of a significant change in group J may be due to the extremely small sample.

Further tests were done (not shown in Figure 1) to compare the postexercise concentrations of the 3 groups at each workload, separately. In the case of plasma NE, differences were found only at the highest workload; group N was greater than group S ($P < .05$), and both were greater than group J ($P < .001$). For plasma E, only one difference was found, also at ex-3; group S was greater than group J ($P < .05$). Failure of plasma E to differentiate the fitness groups may be attributed in part to fairly large intersubject variability.

Pertinent information emerges upon examining preexercise data of the exercise test which immediately followed ex-3 (i.e., for cases where either ex-1 or ex-2 followed ex-3); for those data reflect changes in the blood hormone levels during "recovery" from that highest exercise level. As shown by the individual values presented in Table 2, 6 of the 8 subjects exercised at ex-3 prior to either of the other levels. Relative to plasma catecholamine concentrations just prior to ex-3, concentrations 10 minutes after ex-3 suggested that recovery was, on the average, about 71% complete for NE and 62% for E. While those recovery percentages reflected the very short half-life known to exist for those catecholamines in circulating blood (6), they also demonstrated the need to extend the rest periods by a few minutes.

TABLE 2. CHANGES IN PLASMA HORMONE LEVELS 10 MINUTES AFTER EXERCISE AT EX-3

Subject I.D.	Fitness group	Cortisol ($\mu\text{g/dl}$)			NE (pg/ml)			E (pg/ml)		
		Pre ex-3	Post ex-3	Next Pre	Pre ex-3	Post ex-3	Next Pre	Pre ex-3	Post ex-3	Next Pre
D.B.	N	7.0	6.0	11.6	491	1655	623	38	104	50
S.R.		11.9	10.3	9.5	444	1826	616	56	166	84
J.W.		9.5	9.3	10.9	380	1491	517	47	129	69
F.R.	S	7.8	7.5	12.0	334	1177	767	24	86	36
J.M.	J	7.4	7.5	6.7	318	948	380	27	82	27
R.G.		13.3	12.2	9.5	634	831	452	31	79	42

Note: The 2 subjects not shown in this table performed ex-3 last and therefore did not have data 10 min after ex-3.

A different and perhaps more important reason for extending rest periods (as well as for having one after the final test or the treadmill) is suggested by the cortisol data appearing in Table 2. While all 6 subjects manifested the aforementioned drop in plasma cortisol immediately after exercise, the cortisol levels of 3 subjects rose during the subsequent rest period. Moreover, of the 3 subjects who did not show an increase in cortisol, 2 comprised the most physically fit group.

It was of interest to compare the catecholamine data summarized in Table 1 with blood lactate data obtained for the same subjects, but reported elsewhere (4). Based on preexercise-to-postexercise changes, correlations between E and lactate and between E and NE were not especially striking (the coefficients were 0.551 and 0.647, respectively). In contrast, a coefficient of 0.861 was found between changes in lactate and NE (Fig. 2). The coefficients are a combination of within- and between-subjects correlation.

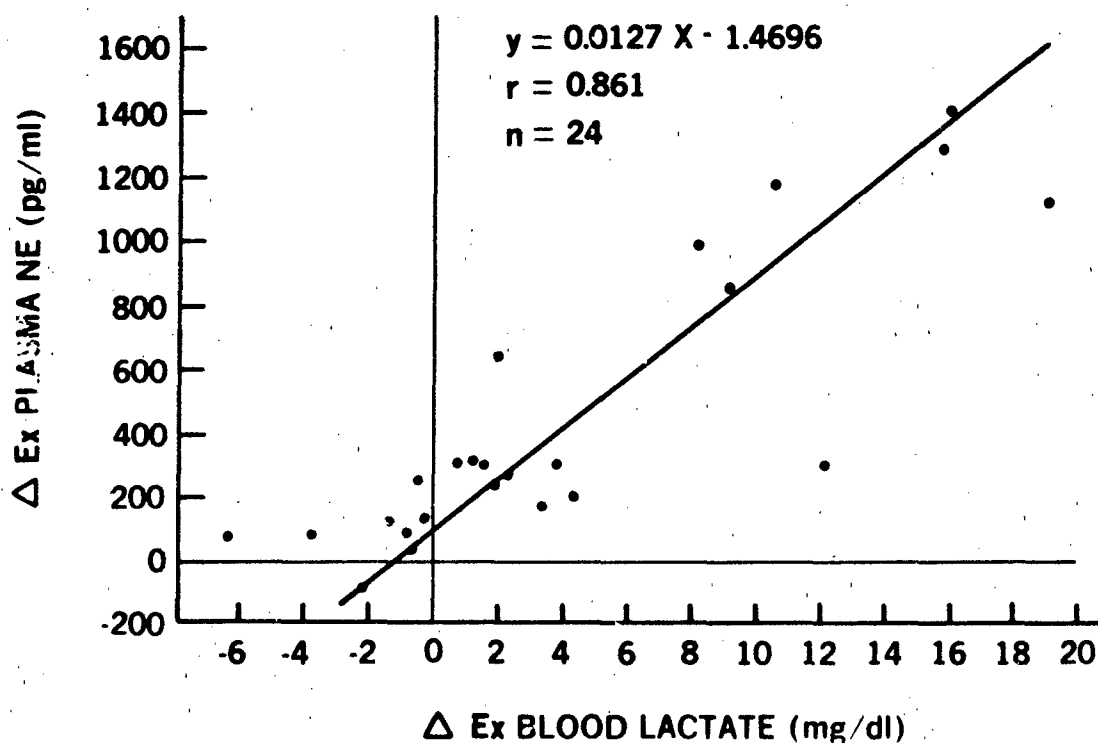


Figure 2. Correlation of preexercise to postexercise changes (ΔEx) in plasma NE and blood lactate.

DISCUSSION

Notwithstanding the exploratory nature of this study, definitive results emerged. The protocol was found reasonably effective in quantifying the response of healthy individuals to mild workloads, and evidence was found suggesting how the protocol's effectiveness could be enhanced by relatively minor changes.

The present protocol was initially formulated because a literature search failed to uncover a suitable reference for the desired application. Most reports specified severe workload scales, and no mention was made of rest periods between workloads to eliminate or minimize carryover (cumulative) effects. Those rest periods are essential when quantifying the biochemical/physiologic response to specific work levels or demands.

Increasing the duration of rest periods by 5 to 10 minutes would accomplish the dual purpose of allowing more time for the elevated catecholamine levels to return to preexercise levels and for adrenocortical stimulation to be reflected by elevated cortisol levels. Admittedly, failure to take the latter factor into consideration is attributed to the seemingly not-so-well publicized lag-time required for stressor stimuli to pass through the hypothalamus-pituitary-adrenal axis (7). On the other hand, prior consideration was given to the relatively short half-life of the catecholamines in circulating blood, but 10 minutes proved inadequate for full recovery. With respect to the lag-time issue, it would be interesting to speculate how this factor might impact on published studies which report no significant change in plasma cortisol levels in subjects (including experimental animals) immediately after exposure to known or suspected stressors.

Another refinement in the protocol that might enhance the stress-sensitivity of plasma cortisol is to conduct experiments in the latter part of the workday, a time when the circadian drop in cortisol levels is less marked. The benefit of afternoon experimentation seems obvious, i.e., less of the adrenocortical response would likely be neutralized by the circadian drop. Although the circadian influence was not unequivocally demonstrated in the present data, its presence is suggested by two facts. First, of the 24 pairs of preexercise/post-exercise blood samples collected in this study, only 2 failed to show a lower cortisol concentration in the postexercise sample. Second, in the 2 subjects who should have been the least affected by exercise, cortisol continued to drop 10 minutes after ex-3 (Table 2).

Randomization of workloads to eliminate order effects is predicated on the premise that carryover effects are virtually absent. Although lengthening of rest periods in future evaluations should make the randomization of workloads inconsequential with respect to the catecholamines, this would not be true for cortisol since its half-life in circulating blood is much longer. For this reason, a further refinement would be to specify progressive (not randomized) increments in workloads.

A requirement of the present protocol we feel should not be compromised is venous cannulation. Use of the cannula improves plasma catecholamine measurements in two crucially important respects. First, the very short half-life of the

catecholamines in circulating blood necessitates the collection of samples in the shortest time possible; with a cannula in place, that time span is 10 seconds or less. Second, after allowing sufficient time for blood catecholamine levels to normalize after insertion of the cannula, a further psychologic effect on those levels from subsequent venous collections should be negligible; this would likely not be true for separate, multiple venipunctures.

A more accurate and thorough assessment of the exercise sensitivity of the 3 blood indices examined here must await an appraisal which incorporates the refinements discussed in this report. From present results, however, there seems little doubt that plasma NE will prove to be the most sensitive indicator of varying workload intensities and of varying degrees of physical fitness. Although the present data suggested plasma E might prove almost as effective as NE in discerning differences in workload, its relatively large intersubject variability makes it appear less likely that it might also discern differences in fitness. On the other hand, evidence was found suggesting plasma cortisol, as a delayed response (Table 2), might prove almost as effective as plasma NE for differentiating degrees of fitness, but no evidence was found to suggest that adrenocortical stimulation--even as a delayed response--occurred at workloads below level 3.

Blood lactate results obtained from present experimentation were reported elsewhere (4) as part of a separate study of the feasibility of using fingertip blood for quantifying physical demands placed on individuals in remote job sites (such as inflight). From the correlation reported here between blood lactate and plasma NE, it seems probable that the two indices will prove useful in future job-stress appraisals which seek to differentiate physical from cognitive and/or emotional demands. For example, since demands of the latter types frequently evoke sympathoadrenomedullary (and even adrenocortical) responses without appreciably affecting muscle activity, then the finding in a given job-stress appraisal of relative high concentrations of NE with essentially unaltered lactate levels would suggest the workload to be largely cognitive or emotional (not physical) in nature.

CONCLUSIONS

An experimental protocol formulated for biochemically quantifying relatively mild physical workloads was found effective in several respects, but specific improvement areas in the protocol were identified which should enhance its usefulness. Of the biochemical parameters examined, plasma NE concentrations were found to be the most sensitive to differences in workload and in physical fitness of subjects. Exercise-induced changes in plasma NE correlated closely with recently reported blood lactate changes in the same subjects, a relationship which suggests a means of differentiating physical from mental and/or psychologic demands in future job-stress appraisals.

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