(1)

The Effects of Graded Exercise on Plasma Proenkephalin Peptide F and Catecholamine Responses at Sea Level

1989

William J. Kraemer, Joeseph E. Dziados, Scott E. Gordon, Andrew C. Fry, and Katy L. Reynolds

Running Head: Sea Level Responses of Catecholamines and Peptide F

M5-90

Exercise Physiology Division, U.S. Army Research Institute of Environmental Medicine, Natick, MA 01760 and Center for Sports Medicine, The Pennsylvania State University, University Park, PA 16802 U. S. A.

Correspondence to: William J. Kraemer, Ph.D.
Center for Sports Medicine
Greenberg Sports Complex
The Pennsylvania State University
University Park, PA 16802 U.S.A.

KRAEMER, W.J., J.E. DZIADOS, S.E. GORDON, A.C. FRY, AND K. REYNOLDS. The effects of graded exercise on plasma proenkephalin Peptide F and catecholamine responses at sea level. PEPTIDES 0(0) 0000-0000, 19__. The purpose of this study was to evaluate the effects of graded treadmill exercise on plasma proenkephalin Peptide F immunoreactivity (ir) and catecholamine responses at sea level [50 m]. Little data exists regarding the sea level responses of plasma Peptide F ir to exercise. Thirty-five healthy male subjects performed a graded exercise test on a motor-driven treadmill at the relative exercise intensities of 25, 50, 75, and 100 % of $\dot{V}O_{2max}$. Significant ($p < 0.05$) increases above rest were observed for plasma Peptide F ir and norepinephrine at 75% and 100% of the $\dot{V}O_{2max}$ and at 5 min into recovery. Significant increases in plasma epinephrine were observed at 75 and 100 % of $\dot{V}O_{2max}$. Whole blood lactate significantly increased above resting values at 50, 75 and 100% of the $\dot{V}O_{2max}$ and at 5 min into recovery. These data demonstrate that exercise stress increases plasma Peptide F ir levels at sea level. While the exercise response patterns of Peptide F ir are similar to catecholamines and blood lactate responses, no bi-variate relationships were observed. These data show that sea level response patterns to graded exercise are similar to those previously observed at moderate altitude [2200 m].

Key Words: endogenous opioid peptides; lactate; epinephrine; norepinephrine; aerobic exercise

The adrenal medulla is a significant source of peripherally synthesized and released proenkephalin fragments and catecholamines, primarily epinephrine (2,13,23,24). While the exercise stress responses of plasma catecholamines have been previously studied (8,9,12,14,15,21,22,25,28), the concomitant responses of proenkephalin peptides to exercise stress are less understood.

Initial studies had demonstrated that exercise stress increased plasma concentrations of proenkephalin Peptide F ir in a similar response pattern to epinephrine and norepinephrine levels in untrained subjects (18). Conversely, trained endurance athletes demonstrated inverse relationships between plasma Peptide F ir and epinephrine concentrations as exercise intenstiy was progressively increased to maximal intensity (18). However, these data were obtained at moderate altitude [2200 m].

Recently, it was demonstrated that plasma Peptide F ir concentrations were not responsive to a single 10 min bout of exercise (80-85% $\dot{V}O_{2max}$) at sea level but did respond to the same exercise intensity at high altitude [4300 m] (20) . These data raise the question whether the previous observations of increased plasma Peptide F ir levels are specifically related to altitude adaptations in the adrenal medulla. Thus, it is unclear how exercise stress influences plasma concentrations of proenkephalin peptides, more specifically Peptide F, at a sea level altitude. The purpose of this investigation was to examine plasma concentrations of Peptide F ir and concomitant catecholamine responses to graded exercise stress in physically active males at sea level.

METHOD

Thirty-five male subjects volunteered for the investigation. The physical characteristics of the subjects were mean ± 1 SD: age 22.97±3.77 (yrs), height 175.92±6.07 (cm), weight 75.42±8.97 (kg), body fat 16.78±6.32 (%), maximal oxygen consumption 54.06±5.10 ($ml \cdot kg \cdot min^{-1}$). Subjects were recreationally active. No competitive athletes were utilized as subjects in this investigation. After informed consent was obtained each subject was familiarized with all of the experimental procedures utilized in this study. Preliminary medical evaluations by a physician revealed that subjects had no history of any endocrine disorder, nor were any of the subjects on medications. Subjects refrained from any strenuous exercise and caffeine for 24 hrs prior to all experimental testing. Subjects were allowed only water 8 hrs prior to testing.

Body composition was evaluated by hydrostatic weighing (7), with residual volume determined using an oxygen dilution method (32). Maximal oxygen consumption ($\dot{V}O_{2max}$) was performed using a continuous, progressive, treadmill exercise test protocol to volitional fatigue (3). An on-line metabolic system and ECG [Lead II configuration] was utilized for cardiorespiratory data aquisition (4). These data were then used to monitor the relative exercise intensities of 25, 50, 75 and 100 % of the $\dot{V}O_{2max}$ used for the test. The exercise test was performed using the same system. Each submaximal stage of the relative exercise test was seven minutes in duration and oxygen consumption was maintained within a ± 0.5% of the target exercise intensity.

Thirty minutes prior to commencing the ~~the relative~~ exercise test an indwelling 20 gauge teflon cannula was placed into a superficial arm vein and kept patent with a continuous flow of isotonic saline (30 $ml \cdot hr^{-1}$). A resting blood sample was obtained prior to exercise and subsequent samples were obtained at the end of each exercise stage and at 5 and 15 minutes into recovery. Blood samples were centrifuged and all plasma samples were stored at -120°C until analyzed.

Hemoglobin was analyzed in triplicate using a cyanmethemoglobin method (*Sigma Chemical* Co., St. Louis, MO), and hematocrit was analyzed in triplicate using a standard microcapillary technique. Changes in plasma volume, pre- to post-exercise were calculated from changes in hematocrit and hemoglobin (5). Whole blood lactate was analyzed in duplicate with a micro blood analyzer (Model 640, Wolverine Medical, Alto, MI).

Three milliliters of blood for measurement of plasma catecholamines were collected into pre-cooled (4°C) plastic syringes containing sodium heparin, and immediately transferred into pre-cooled (4°C) glass vacutainers containing appropriate perservatives (i.e. EGTA and reduced Glutathione), mixed gently and centrifuged at 1500 x g at 4°C for fifteen minutes. Catecholamines were determined from a one milliliter plasma sample using a preliminary aluminum oxide extraction and Waters High Performance Liquid Chromatography System (Division of Millipore Corp., Milford, MA) which utilized an M-45 solvent delivery system and the 460 electrochemical detector (31). Data ~~was~~ were accumulated, chromatographs digitized, and plasma values calculated with the use of a computerized system and main frame computer (VAX 11/780, Digital Equipment Corp., Maynard, MA).

Three milliliters of blood for measurement of plasma Peptide F ir [preproenkephalin-(107-140)] were collected into pre-cooled (4^0C) plastic syringes containing sodium heparin and 25 µl/ml whole blood of aprotinin (Sigma Chemical Co., St. Louis, MO), gently mixed, and centrifuged at 1500 x g, 4^0C for fifteen minutes. A one ml volume of plasma was used in an extraction procedure to avoid non-specific displacement in the radioimmunoassay (RIA). Each sample was partially purified using "HPLC-type minicolumns" (i.e. C_{18} extraction columns, J.T. Baker Co.). The methods used to purify the samples, conduct the RIA, as well as the identified cross-reactivities have been previously described in detail (16,18). Briefly, Peptide F ir was measured by RIA in duplicate using commercially available ^{125}I ligand and antisera (Peninsula Laboratories, Belmont, CA). The mean recovery of the radioactively labeled Peptide F was 86%. The partially purified samples were then stored at -120^0C until analyzed. Further identification of the peptide showed that no substantial degradation of Peptide F was seen with these methods. This was demonstrated by the readioactivity (> 93%) eluted with the authentic labeled peptide (see Figure 1). Using the HPLC elution time as the measurement, the partially purified Peptide F ir (215 fmol) was isocratically eluted from an Altex C_8 column with 0.5 acetic acid/0.2 pyridine, pH 4.0, containing 22.8% (vol/vol) 1-propanol. Recovery was 200 fmol. The use of this method was sensitive enough to separate the iodinated peptide from the native peptide. This has also been previously demonstrated for these methods (18). The plasma ir showed parallel displacement to Peptide F, the inter-assay coefficient of variation was 5.1%, and the intra-assay coefficient of variation was 3.9%. Determinations of plasma ir values were accomplished with the use of a Beckman 5500 gamma counter and on-line data reduction system.

Statistical evaluation of the data was accomplished by using an anlysis of variance with repeated measures and Tukey's post hoc test. Pearson product-moment correlation coefficients were calculated to examine selected bivariate relationships. Statistical significance in this study was chosen as $p < 0.05$.

RESULTS

The the responses of plasma Peptide F ir, epinephrine, norepinephrine, and whole blood lactate are all shown in Figure 2. Plasma Peptide F ir and norepinephrine values increased significantly above rest at 75% and 100% $\dot{V}O_{2max}$ and at five minutes into recovery. Significant plasma epinephrine increases above rest were observed only at the 75% and 100% of

$\dot{V}O_{2max}$. Increases in whole blood lactate were observed at 50, 75. and 100 % of $\dot{V}O_{2max}$ and at 5 minutes into recovery. A mean decrease of -13.09±5.43% in plasma volume was observed pre-to-post-exercise. No significant bivariate correlations were observed at any exercise or recovery time points between Peptide F ir and epinephrine, norepinephrine or whole blood lactate.

DISCUSSION

The data from this investigation demonstrate that plasma Peptide F ir concentrations significantly increase in response to graded exercise at sea level. The increases were observed at the higher exercise intensities (i.e. 75 and 100 % of the $\dot{V}O_{2max}$) and at 5 minutes into recovery as previously reported for untrained subjects at moderate altitude (18,19). These exercise-induced increases were greater than could be explained by changes in plasma volume shifts. Concomitantly, epinephrine, norepinephrine, and whole blood lactate demonstrated typical responses to graded exercise (8,14,15,21,22,25,29).

The lack of a significant increase in plasma Peptide F ir consequent to exercise in a previous study by Kraemer et al. (20) at sea level remains unclear. With the use of a larger subject sample size and a more homogenous exercise training background in this investigation, a reduction in experimental variances from various extraneous variables (e.g. fitness levels) probably occurred. Conflicting evidence concerning the exercise responses of enkephalins and enkephalin fragments have been previously observed (6,11,16). Differences in receptor interactions, posttranslational processing, and degradation in the peripheral plasma might explain such conflicts (10,13). The effects of clearance rates on proenkephalin peptides is less clear. Kjaer et al.(14) have previously demonstrated that exercise-induced increases in epinephrine concentrations in the blood consequent to submaximal endurance exercise (30-76% $\dot{V}O_{2max}$), reflect changes in secretion rather than clearance.

Studies by Kjaer et al. (14,15) have also demonstrated that training increases maximal adrenal medullary secretory capacity. This is consistent with previous findings by Kraemer et al.(18) showing that trained endurance athletes demonstrate higher epinephrine values than untrained subjects at a maximal exercise intensity (i.e. 100% $\dot{V}O_{2max}$). The higher maximal exercise response of epinephrine in athletes may possibly be due to the simultaneous reductions observed in Peptide F and/or other proenkephalin peptide secretion rates (18).

In the present study, no significant relationships were observed between plasma Peptide F ir and epinephrine values. Previously, untrained subjects had demonstrated significant positive bivariate correlations between plasma Peptide F ir and epinephrine values (18). Additionally, trained endurance athletes in the same study had demonstrated significant negative bivariate correlations between plasma Peptide F ir and epinephrine values suggesting non co-secretion from the adrenal medulla in endurance athletes. The data from the present study add additional support to previous suggestions that proenkephalin peptides and catecholamines may not always be found in the same secretory vesicles or in equal molar ratios in the secretory cell (18,20,24). Furthermore, the release mechanisms needed to stimulate such responses would appear to require different regulatory control mechanisms. Our data on physically active but not highly trained subjects fits into this continuum of responses between untrained and highly trained subjects. This is demonstrated, in part, by the shifting of the correlational relationships between plasma Peptide F ir and epinephrine as the training level increases (i.e. untrained subjects show positive correlations, active but not highly trained subjects show no relationships, and highly trained endurance athletes show negative correlations). This again supports previous studies that demonstrate exercise training may be an important influence on adrenal chromaffin cell storage and release mechanisms (14,15,18,21). Further direct longitudinal training studies are needed to examine the adaptational transition in secretion of epinephrine and proenkephlin peptides from the adrenal medulla.

Hypoxia induces adrenal medullary secretion in proportion to the fall in P_{O2} (2). Conversely, alkalosis has been observed to result in reduced plasma catecholamine concentrations (1). Blood lactate concentrations consequent to maximal exercise have been shown to be related to exercise-induced levels of Beta-endorphin and other proopiomelanocortin peptides (17). In this study, Peptide F ir was not correlated to blood lactate responses. This suggests that the stimulatory influence of "anaerobic factors" on

Peptide F ir secretion is minimal. A lack of a relationship between Peptide F ir and blood lactate production has been previously observed (18,20). Different from epinephrine, Peptide F ir levels declined as blood lactate production increased in response to higher exercise intensities (18). While the physiological role of Peptide F is unknown, these data would suggest a differential role from epinephrine. Catecholamines, particularly epinephrine, have been suggested to play a major role in muscle glycogen breakdown during intense exercise (26,27,28). A possible modulatory role for proenkephalin peptides in the adrenal medulla in response to the physiological demands of strenous exercise remains to be studied.

In summary, this study has demonstrated that graded exercise increases plasma levels of Peptide F ir at sea level and the response patterns are similar to responses observed at moderate altitude for untrained subjects. The physiological role(s) of proenkephalin Peptide F and other proenkephalin peptides in stress responses and adaptations of the adrenal medulla remain to be examined but present many possible new hypotheses.

REFERENCES

1. Bouissou, P.G.; Defer, G.; Guezennec, C.Y.; Estrade, P.Y.; Serrurier, B. Metabolic and blood catecholamine responses to exercise during alkalosis. Med. Sci. Sports. Exerc. 20:228-232; 1988.

2. Brenner, G.M. Adrenal medulla. In: Handbook of Endocrinology, edited by Gass, G.H.; Kaplan, H.M., Boca Raton, Fl: CRC Press Inc. 1982; pp. 229-240.

3. Costill, D.L.; Fox, E.L. Energetics of marathon running. Med. Sci. Sports 1: 105-115; 1969.

4. Cote, M.G.; White, D.M.; Mello, R.P.; Sharp, D.S.; Patton, J.F. Development and assessment of an on-line aerobic measurement system. USARIEM Technical Report, T-1/79; 1978.

5. Dill, D.B.;Costill, D.L. Calculation of percentage changes in volume of blood, plasma and red cells in dehydration. J. Appl. Physiol. 37: 247-248; 1974.

6. Farrell, P.A.; Gustafson, A.B.; Morgan, W.P.; Pert, C.B. Enkephalins, catecholamines, and psychological alterations: Effects of prolonged exercise. Med. Sci. Sports Exerc. 19: 347-353; 1987.

7. Fitzgerald, P.I.; Vogel, J.A.; Miletti, J.; Foster, J.M. An improved portable hydrostatic weighing system for body composition. USARIEM Technical Report, T-4/88; 1987.

8. Galbo, H. Hormonal and Metabolic Adaptation to Exercise. New York: Thieme-Stratton, 1983.

9. Galbo, H.; Holst, J.; Christensen, N.J. Glucagon and plasma catecholamine responses to graded and prolonged exercise in man. J. Appl. Physiol. 38: 70-76; 1975.

10. Goodman, R.R.; Fricker, L.D.; Snyder, S.H. Enkephalins. In: Brain Peptides, edited by Krieger, D.T.; Browstein M.J.; Martin, J.B. New York: John Wiely and Sons, 1983, pp. 827-849.

11. Howlett, T.A.; Tomlin, S.; Ngahfoong, L.; Rees, L.H.; Bullen, B.A.; Skrinar, G. A.; McArthur, J.W.; Release of B-endorphin and met-enkephalin during exercise in normal women: response to training. Br. Med. J. 288: 1950-1952; 1984.

12. Jezova, D.; Vigas, M.; Tatar, P.; Kvetnansky, R.; Nazar, K.; Kaciuba-Uscilko, H.; Kozlowski, S. Plasma testosterone and catecholamine responses to physical exercise of different intensities in men. Eur. J. Appl. Physiol. 54: 62-66; 1985.

13. Kilpactrick, D.L.; Lewis, R.V.; Stein, S.; Undenfriend, S. Release of enkephalins and enkephalin-containing polypeptides from perfused beef adrenal glands. Proc. Natl. Acad. Sci. USA 77: 7473-7475; 1981.

14. Kjaer, M.; Christensen, N.J.; Sonne, B.; Richter, A. E. and H. Galbo. Effect of exercise on epinephrine turnover in trained and untrained male subjects. J. Appl. Physiol. 59: 1061-1067: 1985.

15. Kjaer, M.; Galbo, H. Effect of physical training on the capacity to secrete epinephrine. J. Appl. Physiol. 64: 11-16; 1988.

16. Kraemer, W.J.; Armstrong, L.E.; Marchitelli, L.J.; Hubbard. R.W.;Leva N. Plasma opioid peptide responses during heat acclimation in humans. Peptides 8: 715-719; 1987.

17. Kraemer, W.J.; Fleck, S.J.; Callister, R.; Shealy, M.; Dudley, G.; Maresh, C.M.; Marchitelli, L.; Cruthirds, C.; Murray. T.; Falkel, J.E. Training responses of plasma beta-endorphin. adrenocorticotropin and cortisol. Med. Sci. Sports and Exerc. 21: 146-153, 1989.

18. Kraemer, W.J.; Noble, B.; Culver, B.; Lewis, R.V. Changes in plasma proenkephalin Peptide F and catecholamine levels during graded exercise in men. Proc Natl. Acad. Sci. USA 82:6349-6351; 1985.

19. Kraemer, W.J.; B. Noble; K. Robertson; R.V. Lewis. Response of plasma proenkephalin peptide F to exercise. Peptides 6:Suppl 2, 167-169; 1985.

20. Kraemer, W.J.; Rock, P.B.; Fulco, C.S.; Gordon, S.E.; Bonner, J.P.; Cruthirds, C.D.; Marchitelli, L.J.; Trad, L.; Cymerman, A. Influence of altitude and caffeine during rest and exercise on plasma levels of proenkephalin Peptide F. Peptides 9: 1115-1119; 1988.

21. Lehmann, M.; Keul, J.; Huber, G.; Da Prada, M. Plasma catecholamines in trained and untrained volunteers during graduated exercise. Int. J. Sports Med. 2: 143-147; 1981.

22. Lehmann, M.; Schmid, P.; Keul, J. Plasma catecholamine and blood lactate cumulation during incremental exhaustive exercise. Int. J. Sports Med. 6: 78-81; 1985.

23. Lewis, R.V. Endephalin biosynthesis in the adrenal medulla. In: Costa, E.; Trabucchi, M. eds. Advances in Biochemical Psychopharmacology. vol 33. New York: Raven Press; 1982: 167-174.

24. Livett, A.R.; Dean, D.M.; Whelan, L.G.; Undenfriend S.; Rossier, J. Co-release of enkephalin and catecholamines from cultrued adrenal chromaffin cells. Nature 289: 317-318; 1981.

25. McMurray, R.G.; Forsythe, W.A.; Mar, M.H.; Hardy, C.J. Exercise intensity-related responses of B-endorphin and catecholamines. Med. Sci. Sports Exer. 19: 570-574; 1987.

26. Richter, E.A.; Ruderman, N.B.; Gavras, H.; Belur, E.R.; Galbo, H. Muscle glycogenolysis during exercise: dual control by epinephrine and contractions. Am. J. Physiol. 242: E25-E32; 1982.

27. Stainsby, W.N.; Sumners, C.; Andrew, G.M. Plasma catecholamines and their effect on blood lactate and muscle lactate output. J. Appl. Physiol. 57: 321-325,; 1984.

28. Stainsby, W.N.; Sumners, C.; Eitzman, P.D. Effects of catecholamines on lactic acid output during progressive working contractions. J. Appl. Physiol. 59: 1809-1814; 1985.

29. Viru, A. Hormones in Muscular Activity, Vol. 1, Hormonal Ensemble in Exercise, Boca Raton: CRC Press, 1985. pp. 7-24.

30. Viveros, O.H.; Dilberto, E.J.; Hazum, E.; Chang, K.J. Opiate-like materials in the adrenal medulla: Evidence for storage and secretion with catecholamines. Mol. Pharmacol. 16: 1101-1108; 1979.

31. Waters Plasma Catecholamine Manual, Part No. 040530, Waters Chromatography Division, Millipore Corp., Milford, MA, Edition: 6.2, 1986.

32. Wilmore, J.H.; Vodak, P.A.; Parr, R.B.; Girondolf, R.N.; Behirg, J.E. Further simplification of a method for determination of residual lung volume. Med. Sci. Sports Exerc. 12: 216-218; 1980.

Figure 1. Isocratic HPLC of Peptide F ir from plasma sample. The solid trace is fluorescence of snythetic Peptide F detected after treatment with flourescamine in a run immeadiately following the sample and the hatched bars are the immunoreactivity from the sample.

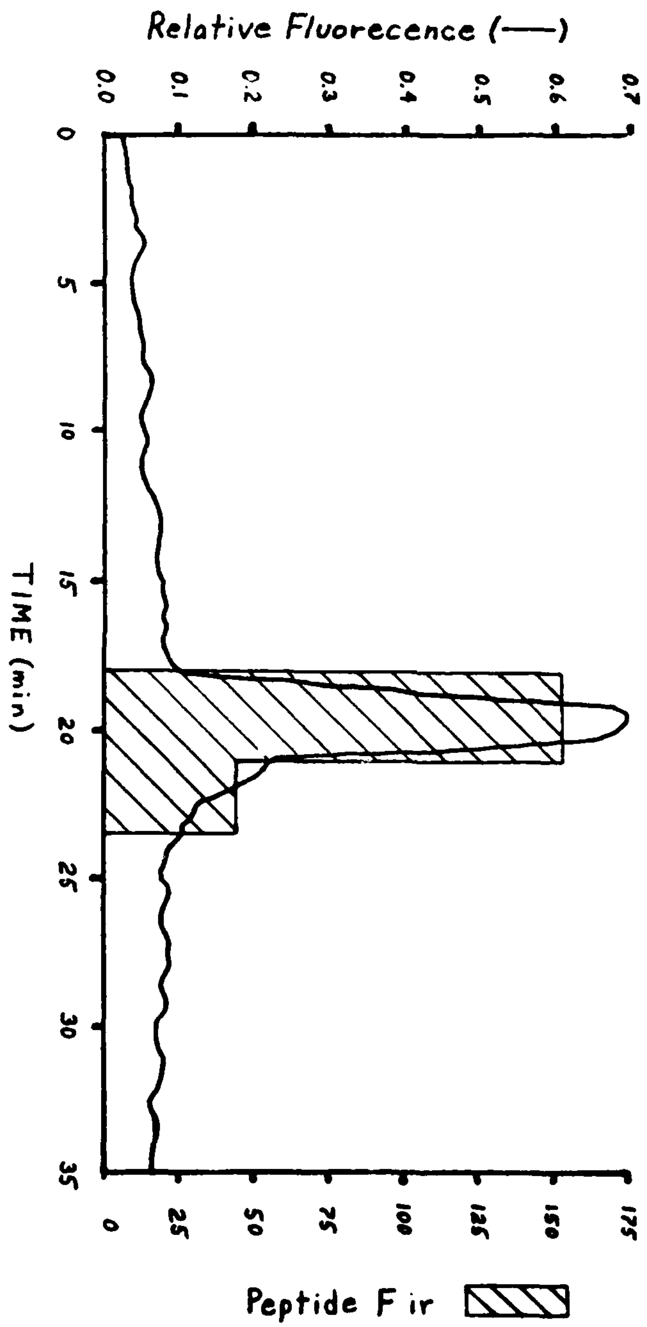
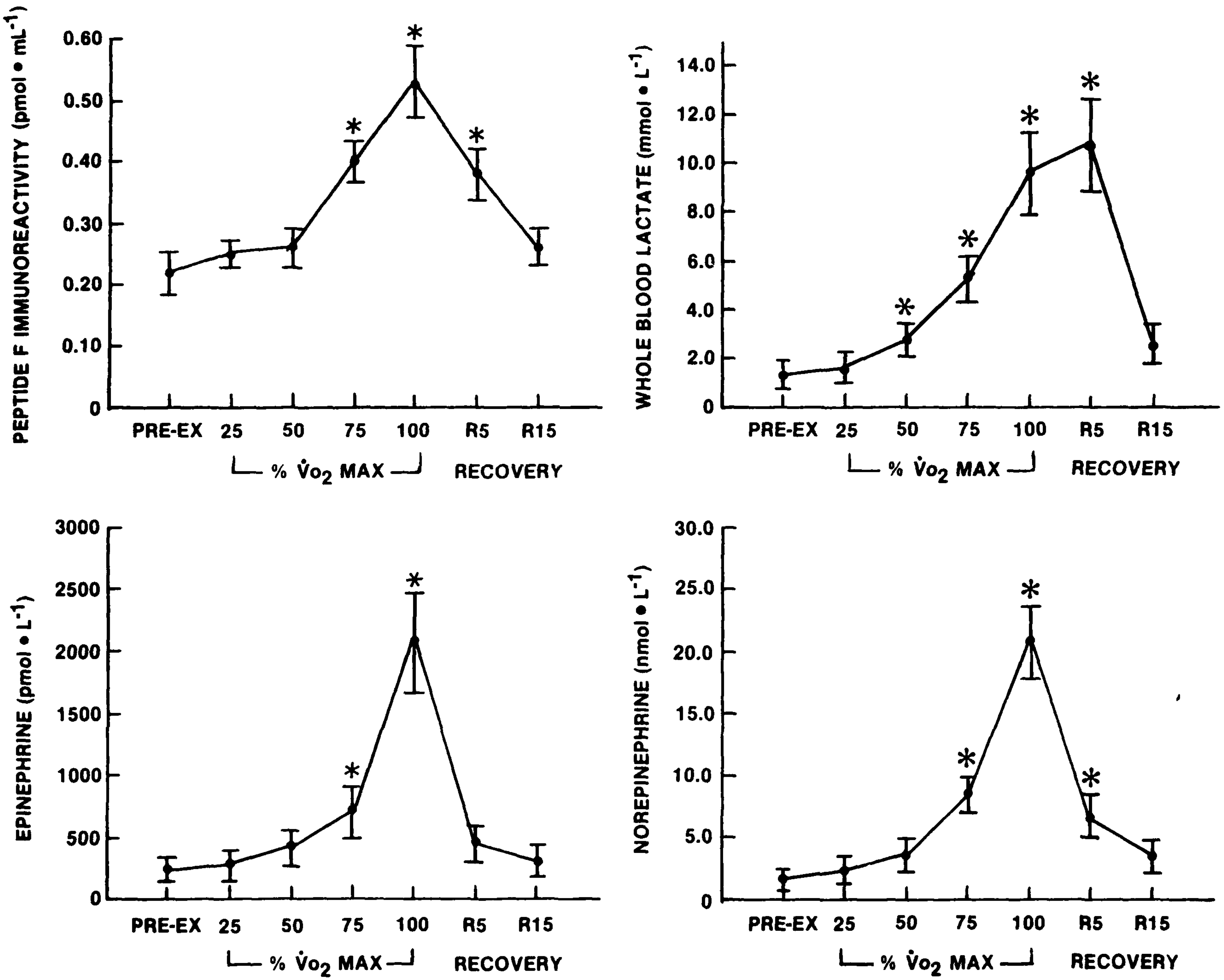

Relative Fluorecence (—)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
TIME (min)
0
5
10
15
20
25
30
35
0
25
50
75
100
125
150
175
Peptide F ir

Figure 2. The exercise and recovery responses of plasma Peptide F ir, whole blood lactate, plasma epinephrine and plasma norepinephrine are presented. * = $p < 0.05$ from corresponding pre-exercise values.



PEPTIDE F IMMUNOREACTIVITY (pmol • mL-1)
0.60
0.50
0.40
0.30
0.20
0.10
0
PRE-EX 25 50 75 100 R5 R15
% V̇o2 MAX
RECOVERY
WHOLE BLOOD LACTATE (mmol • L-1)
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0
PRE-EX 25 50 75 100 R5 R15
% V̇o2 MAX
RECOVERY
EPINEPHRINE (pmol • L-1)
3000
2500
2000
1500
1000
500
0
PRE-EX 25 50 75 100 R5 R15
% V̇o2 MAX
RECOVERY
NOREPINEPHRINE (nmol • L-1)
30.0
25.0
20.0
15.0
10.0
5.0
0
PRE-EX 25 50 75 100 R5 R15
% V̇o2 MAX
RECOVERY

Acknowledgements

The authors would like to thank Louis J. Marchitelli, Robert P. Mello, Chris Butkovich and Al Vela for their expert help in the collection and analyses of these data. We would also like to thank a dedicated group of test subjects who made this project possible.

1. Human subjects participated in these studies after giving their free and informed voluntary consent. Investigators adhered to AR 70-25 and USAMRDC regulation 70-25 on Use of Volunteers in Research.

2. The views, opinion, and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy, or decision. unless so designated by other official documentation.