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The effects of high intensity cycle exercise on  
sympatho-adrenal-medullary response patterns

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William J. Kraemer, John F. Patton, Howard G. Knuttgen,  
Charles J. Hannan, Thomas Kettler, Scott E. Gordon,  
Joseph E. Dziados, Andrew C. Fry, Peter N. Frykman, and  
Everett A. Harman

Exercise Physiology Division, U.S. Army Research Institute of  
Environmental Medicine, Natick, MA 01760-5007, Department of  
Clinical Investigation, Madigan Army Medical Center, Tacoma,  
WA 98431 and Center for Sports Medicine, The Pennsylvania State  
University, University Park, PA 16802 U.S.A.

Running Head: Peptide F responses to high intensity cycle  
exercise

Address Correspondence to: William J. Kraemer, Ph.D.

Center for Sports Medicine

Greenberg Sports Complex

The Pennsylvania State University

University Park, PA 16802

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## ABSTRACT

Plasma proenkephalin Peptide F immunoreactivity (ir) and catecholamines were examined before and after maximal exercise to exhaustion at four intensities [36, 55, 73 and 100% maximal leg power (MLP)] by means of a high resistance cycle ergometer. All intensities were greater than those eliciting peak  $\dot{V}O_2$  uptake for the individual subjects. Blood samples were obtained immediately after exercise and at 5 and 15 min post-exercise. Significant ( $p < 0.05$ ) increases in plasma Peptide F ir were observed immediately following exercise at 36% MLP. Significant increases in plasma epinephrine were observed immediately following exercise at 36% and 55% MLP and at 15 min following exercise at 100% MLP. Significant increases for plasma norepinephrine were observed immediately following exercise (36, 55, 73, and 100% MLP), 5 min after exercise (36, 55, and 73% MLP) and 15 min following exercise (36% MLP). Increases in whole blood lactate were observed at all points post-exercise for 36, 55, and 73% MLP and at 5 min post-exercise for 100% MLP exercise intensity. These data show that brief, high intensity exercise results in differential response patterns of catecholamines and proenkephalin Peptide F ir. These data suggest that exercise-induced increases may be the result of non-colinear storage and release mechanisms in the adrenal medulla.

Key Words: proenkephalins, opioid peptides, epinephrine, norepinephrine, anaerobic exercise, skeletal muscle morphology

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Many studies have demonstrated that endurance exercise at intensities ranging from submaximal to peak  $O_2$  uptake stimulates the sympatho-adrenal-medullary system as evidenced by increases in plasma catecholamines (10,13,17,19,21,28,29,31,33,36). Less clear, however, are the exercise-induced responses of preproenkephalin peptides found in the adrenal-medullary chromaffin cells which have been shown to be responsive to the same stimuli that induce epinephrine release (18,31,40). Studies examining the smallest preproenkephalin biosynthetic end-products (i.e. methionine-enkephalin and leucine-enkephalin) have typically demonstrated no significant increases above rest after endurance exercise stress (10,23). Conversely, increases above rest have been observed for larger preproenkephalin fragments (e.g. Peptide F) consequent to graded exercise (24,25,27).

While receiving greater attention, only limited data concerning the adrenal-medullary responses to short-term, high intensity exercise (greater than peak  $O_2$  uptake) are available. Previous studies have demonstrated significant exercise-induced increases in plasma epinephrine and norepinephrine following short-term exercise tasks ranging from a 20 sec sprint to repetitive interval runs (6,12,19,39). Still, to our knowledge, no attempt has ever been made to examine catecholamine and preproenkephalin peptide (i.e. Peptide F) responses to a wide range of exercise intensities at very high power outputs. Since enkephalin-containing polypeptides (ECPs) are responsive to the same stimuli

that induce epinephrine secretion, it was hypothesized that ECPs would be responsive to high intensity exercise stress. Thus, the primary purpose of this study was to examine the plasma alterations of catecholamines and Peptide F [preproenkephalin-(107-140)] at maximal power production and at various percentages of maximum. These data will enhance the understanding of adrenal-medullary responses to high-intensity exercise.

## METHODS

Subjects. Nine normally active, healthy men provided written informed consent prior to their participation. All were medically screened by a physician, and none were taking any medications or had a history of endocrine disorders. The subjects' physical characteristics were (mean  $\pm$  1 SD): age, 23.9  $\pm$  4.0 yr; height, 178.5  $\pm$  5.0 cm; weight, 78.9  $\pm$  7.3 kg; percent body fat, 14.8  $\pm$  4.9; maximal O<sub>2</sub> uptake ( $\dot{V}O_{2\max}$ ), 47.3  $\pm$  7.7 ml·kg<sup>-1</sup>·min<sup>-1</sup>.

Subjects were thoroughly familiarized with all testing equipment and procedures prior to taking part <sup>in the study.</sup> Measurements of body density (11,41) and  $\dot{V}O_{2\max}$  (7,32) were made approximately two weeks before the determination of maximal power production. These methods have been previously described in detail (26).

Muscle biopsy procedures and analyses. To examine the relationships between skeletal muscle morphological characteristics and peripheral blood hormonal concentrations, muscle biopsy samples were obtained approximately 10 days prior

to maximal power testing. Muscle tissue samples were obtained from the superficial portion of the vastus lateralis muscle of the dominant leg utilizing the percutaneous needle biopsy technique of Bergstrom (3) as modified by Evans et al. (9). Special care was taken to approximate the same biopsy location in all subjects using a depth of approximately 2 cm. {Data from repeat biopsies (randomly performed) demonstrated nonsystematic and insignificant interbiopsy variations in fiber-type distributions.} <sup>ref?</sup>

Muscle tissue samples were oriented, placed in embedding medium, frozen in isopentane cooled to  $-160^{\circ}\text{C}$  with liquid  $\text{N}_2$  and stored at  $-120^{\circ}\text{C}$  until analyzed. Serial cross-sections ( $12\text{ }\mu\text{m}$  thick) were cut on a cryostat (American Optical, Buffalo, NY) at  $-20^{\circ}\text{C}$  for histochemical analysis.

Histochemical analyses used for fiber typing consisted of assaying for myofibrillar adenosinetriphosphatase (ATPase) activity at pH 4.3, 4.6, and 10.3 (5,37). Muscle fiber types were divided into three groups (Types I, IIA, and IIB) based on the stability of their ATPase activity in the preincubation medium (37).

Fiber type percentages were calculated from muscle tissue sections containing an average of 953 fibers. Calculations of the various fiber type percentages were performed according to methods previously described (26). Fiber type percentages were computed by a Zeiss Interactive Digital Analysis System (ZIDAS) from projections at a constant magnification with a Zeiss

microscope (standard 16 drawing tube) onto a digitizing tablet with self-contained computer containing appropriate morphometric programs. This was interfaced with a mainframe computer (VAX 11/780, Digital Equipment Corporation, Maynard, MA) system which allowed immediate data storage and analysis.

Muscle fiber areas were determined using NADH-tetrazolium reductase stained fibers (34). The perimeter of all intact fibers of each muscle fiber type was measured. Cross-sections were projected at a constant magnification with a Zeiss microscope onto the digitizing tablet. Fiber areas were determined by tracing the perimeter of all fibers of each type on the digitizing tablet with the areas calculated by the ZIDAS system. The relative muscle area occupied by type II muscle fibers was calculated using a formula described elsewhere (38).

Capillary density ( $\text{cap}\cdot\text{mm}^{-2}$ ) and capillaries per fiber ( $\text{cap}\cdot\text{fib}^{-1}$ ) were determined from amylase-periodic acid-Schiff-stained fibers and analyzed by methods previously described (1,2).

#### Maximal leg power (MLP) and exercise intensity determinations.

MLP was determined by using a specially constructed cycle ergometer and computerized data collection-processing system and test protocol previously described (15,16,22). Subjects were tested for maximal power for one revolution (i.e. highest score of 5 revolutions) on the cycle ergometer at 60 rpm. Three consecutive tests with a minimum of 20 min rest between tests were performed. MLP was operationally defined as the mean of the

highest two scores of the three tests to avoid the influence of an aberrant result. Subjects were seated in a rigid metal armchair behind the crank to provide back support. The distance from the chair to pedal crank was established for each subject according to leg length and kept constant for all testing.

In addition to the MLP (100%), subjects were tested at exercise intensities of 36, 55, and 73% of MLP. These intensities were approximately 115, 175, and 230% of those which elicited  $\dot{V}O_{2\max}$ , respectively, while MLP was at an intensity of 318% of  $\dot{V}O_{2\max}$ . These exercise intensities and the test protocol utilized in our laboratory have been described in detail (26).

Blood collection procedures. Each subject performed the four exercise intensities in random order. Testing was conducted between 0800 and 1000 hrs, with each subject being tested at the same time to reduce the influence of diurnal variation. Prior to testing, subjects refrained from food for 8 hr and exercise and caffeine for 24 hr. None of the subjects used tobacco products. A 20-gauge Teflon cannula was placed into an antecubital arm vein and kept patent with a continuous flow of isotonic saline (approx  $30 \text{ ml} \cdot \text{hr}^{-1}$ ) prior to exercise. After the cannula was inserted, subjects rested in the seated position and two pre-exercise resting blood samples ( $R_1$  and  $R_2$ ) were obtained 20 min apart. Blood samples were also taken immediately after each exercise bout to exhaustion and at 5 and 15 min post-exercise. Blood for catecholamines was collected in pre-cooled ( $4^\circ\text{C}$ ) plastic monovettes (Sarstedt Inc., Princeton, NJ) containing sodium

heparin, immediately transferred into pre-cooled glass vacutainers containing appropriate preservatives (i.e. EGTA and reduced Glutathione), mixed gently and centrifuged at 1500 x g at 4°C for fifteen minutes. Blood to be used for subsequent radioimmunoassay (RIA) for proenkephalin Peptide F was collected into pre-cooled plastic monovettes (Sarstedt Inc., Princeton, NJ) containing sodium heparin and 25  $\mu\text{l}\cdot\text{ml}^{-1}$  whole blood of aprotinin (Sigma Chemical Co., St. Louis, MO). Plasma samples were stored at -70°C until analyzed. Samples were thawed only once for analysis.

Biochemical analyses. The methods used to purify the samples, conduct the RIA, identify the immunoreactivity (ir), and show cross-reactivities have been previously described in detail (23,24,30). Briefly, Peptide F ir was measured by RIA in duplicate using commercially available  $^{125}\text{I}$  ligand and antisera (Peninsula Laboratories, Belmont, CA). The plasma ir showed parallel displacement to Peptide F. The inter-assay coefficient of variation was 4.9% and the intra-assay coefficient of variation was 3.5%. Determinations of plasma ir values were accomplished with the use of a Beckman 5500 gamma counter and on-line data reduction system.

Plasma catecholamines were determined using high performance liquid chromatography (HPLC). Preliminary sample preparation involved transferring one ml plasma aliquots to 1.5 ml polypropylene tubes and adding 10  $\mu\text{l}$  internal standard (160 ng/ml 3,4-dihydroxybenzylamine HBr in 0.1 M perchloric acid)



and approximately 20 mg acid washed aluminum oxide. Four control samples were prepared by mixing 1 ml of buffer (0.05 M HEPES, pH 7.2, in Ringers solution with 1 % bovine serum albumin) with 5, 10, or 15  $\mu$ l of catecholamine standard solution (75 ng/ml norepinephrine and dopamine and 25 ng/ml epinephrine in 0.1 M perchloric acid) and were processed with each group of 10 unknowns. Samples and controls had 400  $\mu$ l of Tris/EDTA buffer (1.5 M Tris, 2% EDTA, pH 8.6) added, and tubes were capped and mixed for exactly 10 min. The alumina quickly settled, allowing aspiration and discard of the supernatant. The alumina was washed twice with approximately 1.5 ml of alkaline water (water brought to pH 11 with 10 N NaOH). The alumina was then quantitatively transferred in alkaline water to centrifugal filtration tubes (spin-X filters, 0.22  $\mu$ m) and centrifuged for 2 min at 10,000 rpm. Catecholamines were removed from the alumina by adding 250  $\mu$ l of 0.1 M perchloric acid to the upper reservoir of the centrifugal filter and vortexing twice with a 10 sec delay between. This filtrate was injected (150  $\mu$ l) onto the reverse phase column (Altex ultrasphere-ODS, 5  $\mu$ m). Mobile phase (50 mM sodium acetate, 20 mM citric acid, 0.2 mM sodium acetyl sulfate, 0.135 mM sodium EDTA and 5% methanol) was pumped at 1 ml $\cdot$ min<sup>-1</sup> by a Waters model 6000 pump. Detection was accomplished with an ESA Coulochem electrochemical detector. Data were accumulated and calculated on an IBM system 9000 computer.

Hemoglobin was analyzed in triplicate by using the cyanmethemoglobin method (Sigma Chemical, St. Louis, MO) and

hematocrit was determined in triplicate by microcapillary technique. Changes in plasma volume, pre- to post-exercise, were calculated from changes in hematocrit and hemoglobin (8). Blood lactate was analyzed in triplicate with a micro blood lactate analyzer (model 640, Wolverine Medical, Alto, MI).

Statistical analyses. Statistical evaluation of the data was accomplished by using an analysis of variance with repeated measures and subsequent Tukey's post hoc tests. For analysis of the blood and plasma responses, the baseline was quantified as the mean response of the pre-exercise values. Pearson product-moment correlation coefficients were calculated for the entire data set. Statistical significance was chosen as  $p < 0.05$ .

## RESULTS

A description of the physiological responses at each exercise intensity (% MLP) and at  $\dot{V}O_{2\max}$  is presented in Table 1. The results of the muscle fiber analysis are presented in Table 2.

The whole blood lactate responses are presented in Table 3. Significant increases were observed at all measurement time points for exercise intensities of 36, 55, and 73% MLP. At 100% MLP, a slight but significant increase was observed at 5 min post-exercise.

The plasma responses of norepinephrine are presented in Figure 1. Significant increases above resting baseline values were observed immediately after and at 5 min after exercise for exercise intensities of 36, 55, and 73% MLP. A significant

increase was observed immediately following exercise at 100% MLP.

The plasma responses of epinephrine at the various exercise intensities are presented in Figure 2. Significant increases above resting baseline were observed immediately following exercise at 36 and 55% MLP. A significant increase above rest was also observed 15 min after exercise at 100% MLP. Additionally,  $R_2$  plasma epinephrine values at 100% MLP were significantly greater than  $R_1$  values. This was the only difference in resting baseline values observed for any of the blood measures.

In Figure 3 the responses of plasma Peptide F are shown. The only significant increase observed above resting baseline was immediately after exercise at 36% MLP. Since epinephrine and Peptide F are found in the adrenal chromaffin cells, for purposes of comparing concomitant responses of epinephrine and proenkephalin Peptide F, Figure 4 shows the simultaneous responses of these hormones.

Changes in mean plasma volume pre- to post-exercise were as follows (mean  $\pm$  1 SD): 36% MLP,  $-10.9\% \pm 8.27$ ; 55% MLP,  $-5.3\% \pm 7.87$ ; 73% MLP,  $-0.2\% \pm 5.97$ ; and 100% MLP,  $-2.6\% \pm 7.32$ .

Significant relationships observed between muscle fiber morphological variables and peripheral blood concentrations of hormones were: Type II fiber area and epinephrine at 100% MLP ( $r = 0.86$ ) and Type II area and norepinephrine at 73 and 100% MLP ( $r = 0.72$  and  $r = 0.63$  respectively).

Proenkephalin peptide F showed significant inverse correlations

with epinephrine at rest for 55% MLP ( $r = -0.91$ ) and 100% MLP ( $r = -0.67$ ) and at 15 min following exercise at 100% MLP ( $r = -0.99$ ); with norepinephrine at rest for 100% MLP ( $r = -0.70$ ) and immediately after exercise at 73% MLP ( $r = -0.77$ ).

## DISCUSSION

While previous studies have examined the effects of various short-term exercise protocols on catecholamine responses<sup>7</sup>, limited data are available concerning the concomitant responses of preproenkephalin derived peptides (10,23,24,27). Furthermore, no previous studies have examined these responses over a wide range of high intensity exercise. Catecholamines and proenkephalin peptides are found in the same chromaffin cells and are sensitive to similar stimuli (18,31,40). Still, previous studies have demonstrated differential responses to exercise, suggesting that preproenkephalin peptides and catecholamines may not be found in the same secretory vesicles or in equal molar ratios in the secretory cell (10,24).

Plasma Peptide F ir concentrations increased above resting values immediately following exercise only at 36% MLP while increases were observed for epinephrine immediately following exercise at both 36 and 55% MLP and 15 min following exercise at 100% MLP. These data support previous hypotheses related to the existence of non-colinear release mechanisms (24,40). Based upon present results, these mechanisms appear also to be operative following exercise at very high power outputs. Because the

magnitude of increases were far greater than could be accounted for by changes in plasma volume shifts, other physiological mechanisms may influence these responses. Kjaer et al. (20) demonstrated that, for long-term exercise at submaximal intensities (i.e. 30-76%  $\dot{V}O_{2\max}$ ), epinephrine concentrations increased more than could be accounted for by changes in clearance. Still, the influence of clearance rates on responses to short-term high intensity exercise remains unknown.

Parabolic functions have typically been shown for untrained subjects when plasma catecholamines and Peptide F ir response patterns are plotted against exercise intensity (24). Our data demonstrate that these functions do not describe the plasma response as the exercise intensities are increased to 100% MLP. These data also indicate that an upper response limit may exist (i.e. more intense exercise does not stimulate increases in the peripheral plasma concentrations of these hormones).

While it has been suggested that other endogenous opioid peptides are influenced by or related to "anaerobic factors" such as blood lactate, no such relationships were observed between Peptide F ir and blood lactate in this investigation. This lack of a relationship between endogenous opioid peptides and blood lactate at very high power outputs was observed for beta-endorphin in our previous work (26). Thus, due to the short exercise times and large distribution space, anaerobic feedback mechanisms may not be fully operational.

It is interesting to observe the time period prior to exercise

at 100% MLP, when a significant increase in epinephrine was observed from  $R_1$  to  $R_2$ . Previous studies have not completely documented resting baselines prior to brief high intensity exercise. Our data show that, while a mean increase in plasma epinephrine values was observed prior to each high intensity exercise bout, it became significant only prior to 100% MLP. No concomitant increases in norepinephrine values were observed, which suggests that this phenomenon was primarily directed toward the adrenal-medullary axis, where epinephrine is released in greater molar quantities (4). Our previous work examining such high intensity exercise demonstrated that cortisol did not respond in this manner prior to maximal exercise (26). Thus, physiological mechanisms involved with preparation for short-term (i.e. < 10 sec) maximal power outputs appear to be related to adrenal-medullary function. It is interesting to note that, concomitant with the changes in epinephrine, a trend of a mean decrease in <sup>value</sup> Peptide F <sub>A</sub> ir was also observed prior to exercise. While no significant decreases in Peptide F ir occurred, significant inverse correlations between Peptide F and epinephrine were observed prior to exercise. This suggests that the magnitude of concomitant changes in secretory rates via differential release mechanisms could influence the absolute change in epinephrine concentrations observed prior to high intensity exercise.

The mechanisms involved with the increase observed in epinephrine at 15 min post-exercise at 100% MLP are unclear.

Previous studies have not shown this type of "rebound" effect. Still, no study has examined the extended recovery pattern following such brief, high intensity exercise. A significant correlation ( $r = -0.99$ ) was observed between epinephrine and Peptide F ir, suggesting a close interaction between the differential release of these two hormones. The stimulatory mechanisms which could account for such a response remain highly speculative and are possibly related to different recovery stimuli. Previous data have shown increases in Peptide F ir 5 min into recovery (24,25). This recovery pattern, however, was not observed in the present investigation.

The influence of muscle fiber type on hormonal responses to exercise remains unclear. In our investigation, no consistent relationships were observed between skeletal muscle fiber morphological variables and blood variables. Correlations between Type II fiber area and epinephrine at 100% MLP ( $r = 0.86$ ) and Type II area and norepinephrine at 73 and 100% MLP ( $r = 0.72$  and  $r = 0.63$  respectively) were the only significant relationships observed. While highly speculative, these data suggest that the total area occupied by Type II fibers may be more important than percentages when examining such relationships consequent to short duration high intensity exercise, especially in non-athletic heterogeneous sample populations.

## DISCLAIMERS

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. *signature*

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.

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TABLE 1. Cardiorespiratory responses and performance characteristics for each exercise intensity

|                                          | MAXIMAL LEG POWER |                 |                 |                | $\dot{V}O_{2\max}$ |
|------------------------------------------|-------------------|-----------------|-----------------|----------------|--------------------|
|                                          | 100%              | 73%             | 55%             | 36%            |                    |
| Power, W                                 | 834<br>(128)      | 605<br>(93)     | 454<br>(69)     | 303<br>(46)    | 267<br>(39)        |
| $\dot{V}O_2$ , L $\cdot$ min $^{-1}$     | 1.16<br>(0.11)    | 1.70<br>(0.51)  | 2.58<br>(0.61)  | 3.48<br>(0.58) | 3.72<br>(0.60)     |
| Heart Rate,<br>beats $\cdot$ min $^{-1}$ | 133<br>(17)       | 157<br>(14)     | 164<br>(11)     | 175<br>(6)     | 179<br>(9)         |
| Time, min                                | 0.10*<br>(0)      | 0.270<br>(0.12) | 0.781<br>(0.30) | 3.31<br>(2.35) | 3.53<br>(0.44)     |

Values are means ( $\pm$  1SD). \*All subjects performed 5 revolutions at 60 rpm. Maximal leg power was the highest of the 5 revolutions in this test.

N=9

TABLE 2. Muscle fiber characteristics of <sup>study</sup> ~~experimental~~ subjects

|                                           |              |
|-------------------------------------------|--------------|
| % Type II fibers                          | 51.5 ± 8.8   |
| % Type IIA fibers                         | 41.2 ± 6.9   |
| % Type IIB fibers                         | 10.4 ± 8.9   |
| Type I area, $\mu\text{m}^2 \times 100$   | 54.2 ± 8.6   |
| Type I-I area, $\mu\text{m}^2 \times 100$ | 63.0 ± 14.0  |
| % Type II area                            | 54.8 ± 8.1   |
| Capillary, $\text{mm}^{-2}$               | 282.4 ± 62.1 |
| Capillary, fiber <sup>-1</sup>            | 2.64 ± 0.47  |

Values are means ± 1SD; n=9.



TABLE 3. Whole blood lactate responses ( $\text{mmol}\cdot\text{L}^{-1}$ ) for each level of exercise intensity

| % MAXIMAL<br>LEG POWER | PRE EXERCISE   |                | POST EXERCISE (min) |                  |                 |
|------------------------|----------------|----------------|---------------------|------------------|-----------------|
|                        | R1             | R2             | 0                   | 5                | 15              |
| 36                     | 1.23<br>(0.32) | 1.16<br>(0.22) | 8.45*<br>(3.92)     | 10.57*<br>(1.52) | 8.57*<br>(1.92) |
| 55                     | 1.40<br>(0.35) | 1.09<br>(0.21) | 4.20*<br>(2.74)     | 7.83*<br>(2.29)  | 6.33*<br>(1.93) |
| 73                     | 1.25<br>(0.27) | 1.12<br>(0.18) | 2.18*<br>(1.15)     | 5.38*<br>(0.89)  | 3.75*<br>(0.41) |
| 100                    | 1.21<br>(0.32) | 1.18<br>(0.29) | 1.52<br>(0.40)      | 2.25*<br>(0.76)  | 1.72<br>(0.45)  |

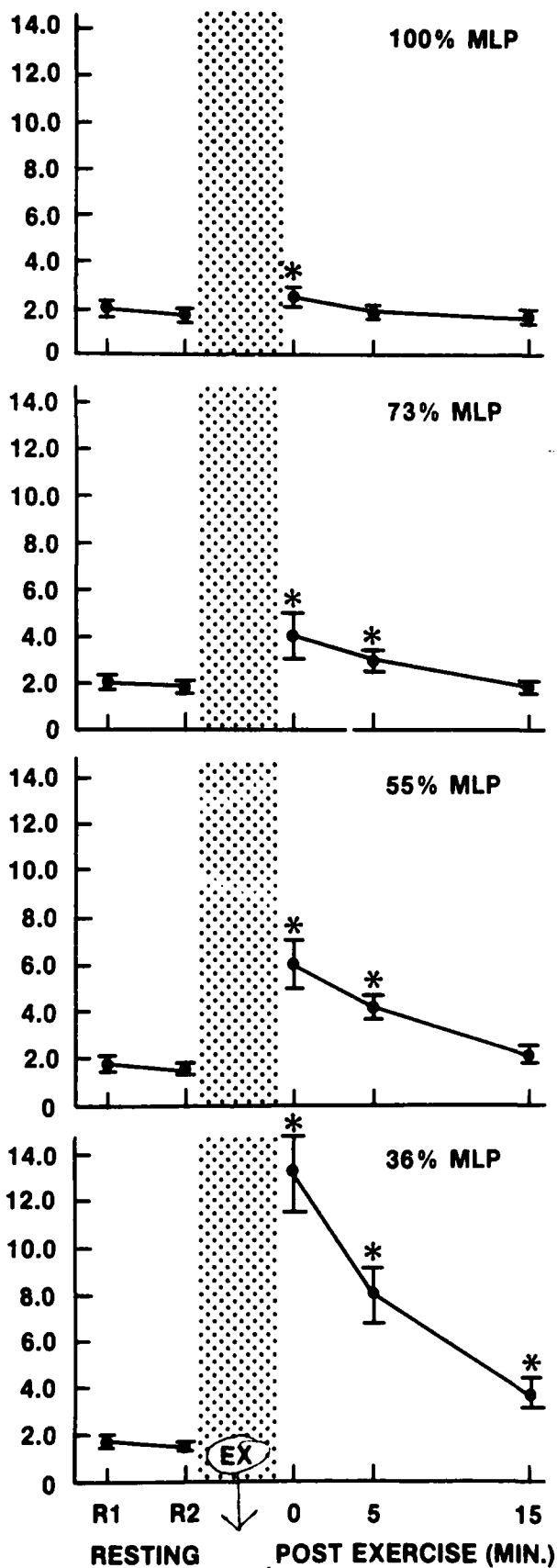
Values are means ( $\pm$  1SD). \*  $p < 0.05$  from corresponding mean resting baseline.

*n=9?*

Figure 1. Responses of plasma norepinephrine to four high intensity exercise bouts (means  $\pm$  SE). \*  $p < 0.05$  from corresponding resting baseline.

$n =$        $EX = \text{exercise}$   
 $n =$

PLASMA NOREPINEPHRINE (nmol•L<sup>-1</sup>)



Why not use  
different symbols  
# - doing as  
single figure?

Figure 2. Responses of plasma epinephrine to four high intensity exercise bouts (means  $\pm$  SE). \*  $p < 0.05$  from corresponding resting baseline.

Ex = exercise

n =

PLASMA EPINEPHRINE (pmol·mL<sup>-1</sup>)

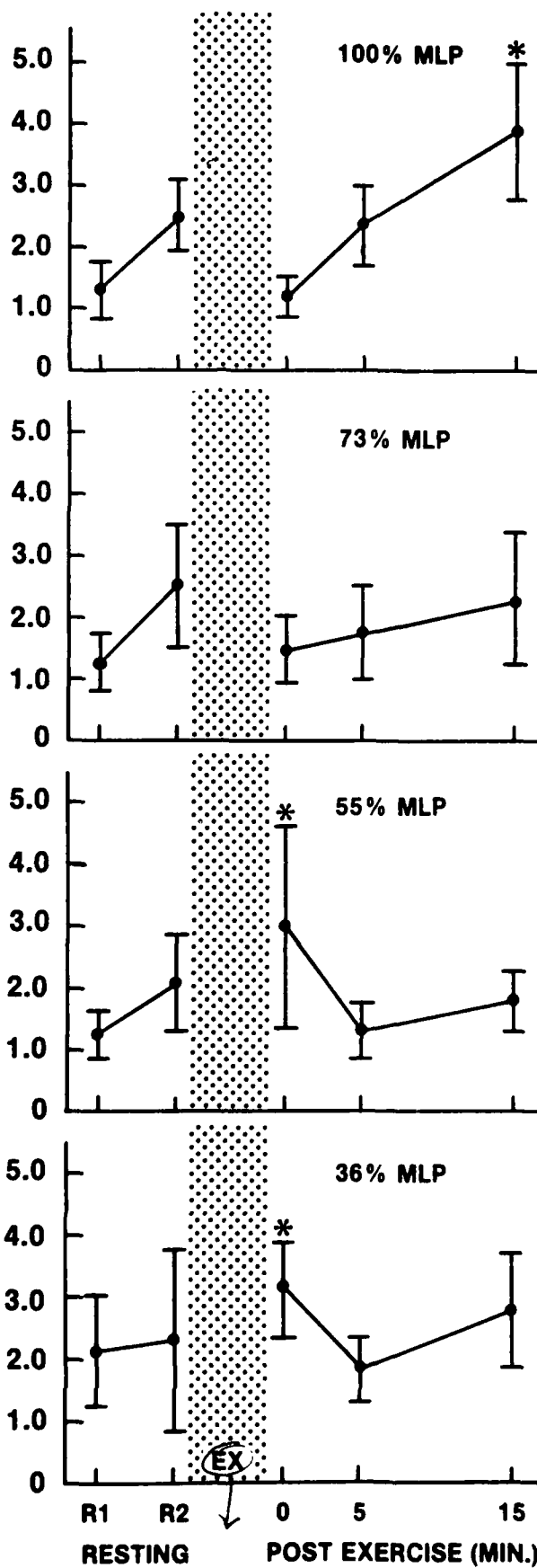


Figure 3. Responses of plasma Peptide F to four high intensity exercise bouts (means  $\pm$  SE). \*  $p < 0.05$  from corresponding resting baseline.

E4

m-?

PLASMA PEPTIDE F ( $\text{pmol} \cdot \text{mL}^{-1}$ )

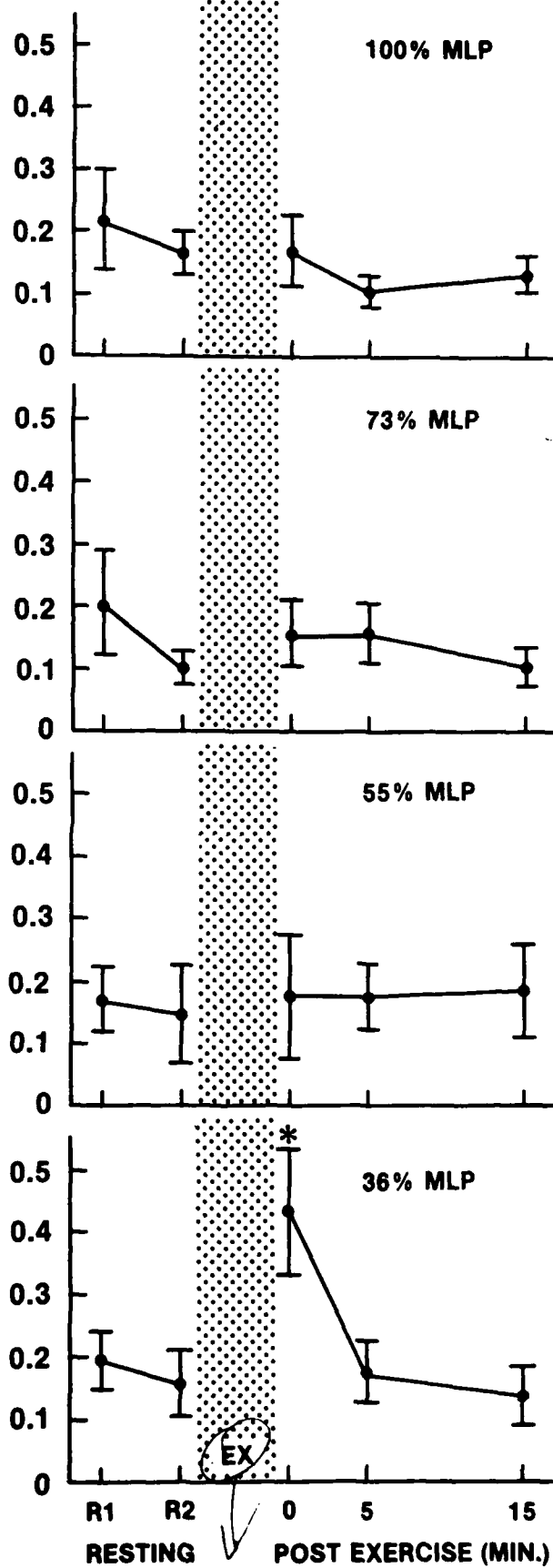


Figure 4. Comparison of mean responses of plasma Peptide F ir and plasma epinephrine to the four high intensity exercise bouts.  
(epinephrine = ..... ) (Peptide F = \_\_\_\_\_ )

?

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Et?



