3-3-2017

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Peptides 91 (2017) 26–32

Contents lists available at ScienceDirect

Peptides

journal homepage: www.elsevier.com/locate/peptides

# The effects of different exercise training modalities on plasma proenkephalin Peptide F in women

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ARTICLE INFO

*Article history:*
Received 25 November 2016
Received in revised form 13 February 2017
Accepted 16 February 2017
Available online 3 March 2017

*Keywords:*
Opioid peptides
Physical training
Endurance exercise
Strength

ABSTRACT

Due to the important interactions of proenkephalin fragments (e.g., proenkephalin [107–140] Peptide F) to enhance activation of immune cells and potentially combat pain associated with exercise-induced muscle tissue damage, we examined the differential plasma responses of Peptide F to different exercise training programs. Participants were tested pre-training (T1), and after 8 weeks (T2) of training. Fifty-nine healthy women were matched and then randomly assigned to one of four groups: heavy resistance strength training (STR, $n = 18$), high intensity endurance training (END, $n = 14$), combined strength and endurance training (CMB, $n = 17$), or control (CON, $n = 10$). Blood was collected using a cannula inserted into a superficial vein in the antecubital fossa with samples collected at rest and immediately after an acute bout of 6 X 10 RM in a squat resistance exercise before training and after training. Prior to any training, no significant differences were observed for any of the groups before or after acute exercise. With training, significant ($P \leq 0.95$) elevations were observed with acute exercise in each of the exercise training groups and this effect was significantly greater in the CMB group. These data indicate that in untrained women exercise training will not change resting of plasma Peptide F concentrations unless both forms of exercise are performed but will result in significant increases in the immediate post-exercise responses. Such findings appear to indicate adrenal medullary adaptations opioid production significantly altered with exercise training.



## 1. Introduction

We have been interested in Peptide F since 1985 as this opioid peptide is the only proenkephalin fragment (e.g., E, F, I, 8.6 kDa) that has been studied over the years in humans [16]. It also has been shown to be responsive to exercise stress, yet the effects of physical training remain speculative, especially in women. The importance of elevations in plasma concentrations of Peptide F resides in its roles in immune modulation in the circulation, most notably B-cell activation, and potential influence on pain sensations due to exercise-induced muscle tissue damage [3,8,23]. At present the receptor for the proenkephalin Peptide F has not been specifically determined in peripheral target cells yet it is thought that mu and kappa binding sites may be viable due to their interactions with such proenkephalin fragments in the brain [21]. It is not clear what molar quantities of Peptide F clear the blood–brain barrier from the peripheral circulation as so much is tied up in the buffy coat [3]. Original work on sequencing the proenkephalin opioid peptide family found great difficulty in finding enough fragments in the brain for such sequence uses [6,19]. Due to the high molar quantities that are found in the buffy coat it was hypothesized that meaningful interactions of the proenkephalin fragments would occur in the peripheral circulation with immune cells or pain receptors in tissues [3,8,10,23]. Thus, our working hypothesis was based on our prior finding that if an exercise stress elicited

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http://dx.doi.org/10.1016/j.peptides.2017.02.006
0196-9781/

an increase in plasma Peptide F concentrations, it was highly predictive of enhanced B-cell activation [23], Therefore, elevations in the plasma were considered meaningful [23]. Secondarily, we then want to observe if physical training might affect this response in untrained women.

To add more context for this investigation, it is important to understand that few data exist as to the influence of exercise training on the release of these opioid peptide fragments. Thus far, most of our understanding has been primarily derived from cross-sectional comparisons between untrained and trained individuals [4,16,17]. This is primarily due to the costly nature of conducting training studies in humans. To date, the only exercise training study in the literature was performed in men [13]. We had demonstrated that the plasma concentrations of Peptide F are increased with exercise training in response to a maximal treadmill exercise test. Furthermore different types of training (i.e., resistance or endurance) were shown to lead to potentially different plasma response patterns. We therefore also hypothesized that in women, a similar training effect might be observed. Additionally, from this study it was suggested that combined modes of training may further augment this post-exercise release and we hypothesized that this may be true for women as well. From a larger study we had the unique opportunity to gain some initial insights into such questions [18,20]. Therefore, the overall purpose of this study was to examine the effects of acute resistance exercise and then different exercise training programs on the response of plasma concentrations of Peptide F in women.

## 2. Methods

### 2.1. Experimental approach

We examined a sub-set of participants from our prior studies [18,20]. We provide the methods in brief where duplicate so that a reader can see what was done without having to go back to the prior studies. This will allow for a full view of the experiment as it relates to Peptide F and its response to acute resistance exercise and 8 weeks of training.

### 2.2. Participants

All participants were fully informed of all study procedures, protocols, risks, and benefits before signing a consent form that was approved by the Institutional Review Boards of the US Army Research Institute of Environmental Medicine and the University of Connecticut before participation in the investigation. All participants were healthy untrained women with no known conditions that would prevent or limit full participation in all testing and training protocols and procedures. All women were tested on approximately the same day of their menstrual cycles (±1 day) before and after the 8 weeks of training in order to help reduce any potential confounding influence of reproductive hormones on the expression of plasma proenkephalin peptide F. However, little or no effects of the menstrual cycle have been observed for plasma Peptide F at rest or in response to intense exercise [15]. Again, the data presented here were part of a larger investigation examining other distinct hypotheses related to high-intensity strength and endurance training on bone and other hormonal and skeletal muscle adaptations not linked to opioid peptides [18,20].

In this study, we sampled fifty-nine of the healthy recreationally active women that were recruited for the study where plasma samples were available for analysis. In our original design groups of 4 subjects were matched for age, body mass, height, strength, and peak $O_2$ consumption ($VO_{2peak}$). Each subject was then randomized into one of the four experimental groups using a randomized block procedure. The four experimental groups consisted of a control group and three training groups that were composed of the following programs: combined high intensity strength and endurance training (CMB, $n = 17$); or heavy resistance strength training only (STR, $n = 18$); or high intensity endurance training only (END, $n = 14$). The control group did not complete any exercise training programs but maintained normal activity and were tested at the same time points as the other groups (CON, $n = 10$). Participant characteristics are shown in Table 1. We again with the matching of the subjects in the groups showed no significant differences among any of the variables at the start of the investigation.

Participants were fully familiarized with all of the study procedures, testing, and exercise protocols. They were also allowed to practice any of the testing or training protocols prior to the start of the study. This was done in the attempt to reduce any learning effects. After the formal familiarization period, participants in the training groups completed 8 weeks of physical training on three alternating days per week while the control group participants continued their everyday normal daily routines.

### 2.3. Anthropometrics

Participants' height (cm) was measured using a standard clinical stadiometer. Participants' body mass (kg) was measured using a calibrated digital scale. Per our dietetic monitoring, body mass was measured each week on the same day at approximately the same time to confirm participants were consuming the appropriate amount of calories to remain weight stable. Total body percent body fat and non-bone fat-free was estimated using previously described techniques (Total Body Analysis, version 3.6, Lunar, Madison, WI) for the measurements of tissue mass (kg), lean body mass (kg), and fat mass (kg) obtained from a fan-beam densitometer (Prodigy, GE Medical Systems, USA) [18].

### 2.4. Strength assessment

One repetition maximum strength (1 RM) was measured pre and post-training using the Max Rack (Max Rack, Columbus, OH, USA) a sliding cam system that allows the Olympic bar to move both vertically and longitudinally while maintaining the side-to-side lateral stability of a traditional Smith machine as previously noted [18]. Proper form and technique was monitored and spotters were stationed on both sides of the machine to help catch the weight if a lift failed.

### 2.5. Peak oxygen consumption ($VO_{2peak}$) assessment

Peak oxygen consumption was measured pre and post-training. The peak volume of oxygen consumption ($VO_{2peak}$) for each subject was determined using a running protocol on a treadmill. Each subject completed a 4-min warm up period at 8 km/h. If the subject's heart rate was less than 140 beats per minute (bpm) following 3-mins of running on the treadmill, then the speed was increased to 8.9 or 9.7 km/h in order to elicit a heart rate greater than 150 bpm. After the warm up period, the treadmill grade was increased to 5% and then increased an additional 2.5% every 2-mins thereafter. The subject's expired air was measured continuously using a calibrated metabolic measurement system (ParvoMedics, Salt Lake City, UT, USA). Measurements were averaged over 20-s periods. We used a standard criteria to validate subject's $VO_{2peak}$ reflected by an increase of less than 2.0 mm per kilogram of oxygen uptake, an increase in the exercise intensity and a respiratory exchange ratio greater than or equal to 1.10. Or, obviously with volitional fatigue when the subject could no longer maintain the current pace.

**Table 1**
Characteristics of experimental subjects (Mean ± SD).

| Group | *N* | Age (yrs) | Height (cm) | Body Mass (kg) |
|---|---|---|---|---|
| *Characteristics of participants (mean ± SD)* | | | | |
| Combined | 10 | 20.2 ± 0.4 | 167.6 ± 1.4 | 65.8 ± 6.8 |
| Strength | 17 | 20.5 ± 0.3 | 166.7 ± 1.2 | 66.1 ± 7.3 |
| Endurance | 14 | 20.1 ± 0.4 | 167.2 ± 1.4 | 65.9 ± 6.9 |
| Control | 10 | 20.8 ± 0.3 | 167.1 ± 1.4 | 66.2 ± 8.3 |

### 2.6. Dietary intake

To assess dietary patterns and to ensure that dietary intakes met caloric needs of the training for each individual, participants completed a food frequency, nutrition, and body weight history questionnaire. In addition, participants completed a five-day food record prior to the pre-training testing session. The five-day food records were photocopied and returned to the subjects so that they could replicate their dietary intake 5 days prior to the post-training testing session. The five-day diet records were analyzed using Nutritionist Pro (NUTRITIONIST PRO, version 1.1, First Databank, The Hearst, San Bruno, CA, USA) computer software to monitor energy, macronutrient, and micronutrient intake for each subject. Registered dieticians in the project assisted participants to complete all dietary assessment records correctly and accurately.

### 2.7. Exercise training protocols

#### 2.7.1. Endurance exercise only (END, n = 14)

An outline of the endurance training program is listed in Table 2. Participants in the END group endurance workouts on 3 alternating days/wk. The endurance workouts were periodized alternating between a continuous running program and a sprint-type interval training program on different days.

Endurance training sessions began with a 5–10 min light jogging warm up followed by dynamic range of motion exercises followed by a 5–10 min cool down. Weekly training was comprised of 20–30 min of continuous running at a prescribed target heart rate of 70–85% maximum heart rate, or interval running consisting of 400-, 800-, 1200-, and 1600-min runs conducted with maximal intensity using a 1:1 exercise-to-rest ratio.

#### 2.7.2. Resistance exercise training only (STR, n = 18)

An outline of the resistance training program is listed in Tables 3 and 4. A nonlinear periodized model was used in which loads and repetitions were varied on a daily basis over each week. Each training session lasted approximately 40–60 min. Participants trained on 3 alternating days/wk. During weeks 3–5, "light" days used 12-repetition maximum (RM) load, "moderate" days used 8- to 10-RM loads, and "heavy" days used 6- to 8-RM loads. During training weeks 6–8, "light" days used 12-RM loads, "moderate" days used 6–8-RM loads, and "heavy" days utilized 3–5-RM loads.

#### 2.7.3. Combined endurance and resistance exercise training group (CMB, n = 17)

Participants in the CMB group exercised 3 alternating days/wk using the same periodization plan as the STR and END groups. Participants performed both endurance and the resistance exercise programs on the same day, during the same session. Resistance training workouts were performed first followed immediately by the endurance training session to help ensure that lower body resistance exercises were performed in a no fatigued state with high quality loading conditions. Individuals in the CMB performed the exact same prescribed workouts as subjects in the END and STR groups including the warm up protocol for each respective group. In addition, participants in this group trained on the same days and at the same time as the respective END and STR groups to ensure that they received the same encouragement and coaching.

#### 2.7.4. Control group (CON, n = 10)

This group was not formally trained, but instead maintained their current activity levels. However, they completed all testing protocols and procedures that the experimental groups performed for comparison purposes.

### 2.8. Exercise testing protocol

Before and after 8 weeks of training, participants reported to our lab for testing. All post training testing was completed at least 48 h after the last training session. Participants arrived between 0600 and 1100 h after fasting, no food or fluids except water, for at least 8 h. In addition, each testing session was performed at approximately the same time of day (±1 h) and during the same phase of the menstrual cycle as few differences have been observed across the cycle of women [15].

Upon arrival participants were queried on questions to confirm that they were in compliance with the study's controls. Once compliance was confirmed, urine specific gravity was tested to determine if they met a minimum level of hydration (USG ≤ 1.025). If participants' USG > 1.025, they were instructed to sip water and retested until USG ≤ 1.025. After minimum hydration status was confirmed, participants sat ~15 min to allow equilibration back to rest.

After resting ~15 min a trained phlebotomist inserted an indwelling venous catheter into a superficial vein in the cubital fossa and a pre exercise (Pre-Ex) blood draw was taken. As with prior studies in our laboratory over the years in this part of the study we used a 6 × 10 resistance exercise protocol to elicit in this case and adrenal stress response [9]. A heavy resistance exercise protocol (ARET) consisting of six sets of ten-repetition back squats at 75% of 1RM with a two-minute rest period in between each set using the MaxRack squat machine If a subject experienced fatigue and was unable to successfully complete ten repetitions, the spotters gave the minimum assistance needed to complete the set. The load for the subsequent set was reduced so that the subject could complete ten repetitions without assistance. Immediately after the ARET, a trained phlebotomist obtained an immediate post exercise (IP-Ex) blood sample.

### 2.9. Blood collection and blood processing

Blood samples (″3 mL) for Peptide F analysis were obtained using plastic syringes which contained sodium heparin and aprotinin (25 μL. 1 $mL^{-1}$ of whole blood) (Sigma–Aldrich Co. LLC, St. Louis, MO, USA). Blood was centrifuged at 4 °C at 2000 × *g* (3161 rpm) for fifteen minutes. We estimated from hematocrit values the plasma volume shift was <10% for post-exercise samples. Plasma samples were then aliquoted in appropriate amount into micro centrifuge tubes with and immediately stored at −80 °C for analysis later.

**Table 2**
Endurance training program characteristics.

| Week | Monday (easy SS, longer) | Wednesday (interval and TT) | Friday (SS, threshold) |
|---|---|---|---|
| *Program variables for the endurance exercise only training program* | | | |
| 1–2 | Pre training testing and familiarization | | |
| 3 | 20–30 min SS or jog/walk (100 m jog/100 m walk) | 20–30 min SS | 20–30 min SS |
| 4 | 30 min total SS, 75% HRmax | 30 min total (1 mi alternating straights fast; jog/walk corners) | 30 min SS, 80–85% HRmax |
| 5 | 30 min total SS, 75% HRmax | (1 × 800 m, 1 × 400 m, 2 × 200 m) | 30 min SS, 80–85% HRmax |
| 6 | 30 min total SS, 75% HRmax | (3 × 800 m) | 30 min SS, 80–85% HRmax |
| 7 | 30 min total SS, 75% HRmax | (8 × 400 m) | 30 min SS, 80–85% HRmax |
| 8 | 30 min total SS, 75% HRmax | (1 × 1 mi, 1 × 800 m, 2 × 400 m) | |
| 9 | 30 min total SS, 75% HRmax | 2 × 1 mi (5 min rest in between) | 30 min SS, 80–85% HRmax |
| 10 | 30 min total SS, 75% HRmax | 3 mi SS | 2mi TT |
| 11–12 | Post training testing | | |

SS, steady state, TT, time trial, mi, mile, min, minutes, HRmax intensity of exercise relative to maximal heart rate.
All interval workouts used 2.0 mi TT intensity and had 1:1 work-to-rest ratios between multiple bouts.

**Table 3**
Characteristics of resistance training program.

| Pr-Tr (weeks 1–2) | Pre-training testing and familiarization | | |
|---|---|---|---|
| *Acute program variables for the non-linear periodized resistance exercise training program* | | | |
| *Weeks 3–5* | Light | Moderate | Heavy |
| Sets | 3 | 3 | 3 |
| Reps | 12 | 8–10 | 6–7 |
| Rest (s) | 90 | 120 | 120 |
| Total time (min) | 40 | 48 | 47 |
| *Weeks 6–9* | Light | Moderate | Heavy |
| Sets | 3 | 3 | 3 |
| Reps | 12 | 6–8 | 3–5 |
| Rest (s) | 90 | 150 | 180 |
| Total time (min) | 40 | 57 | 63 |
| *Weeks 10–11* | Light | Moderate | Heavy |
| Sets | 3 | 3 | 3 |
| Reps | 12 | 6–8 | 3–5 |
| Rest (s) | 90 | 150 | 180 |
| Total time (min) | 40 | 57 | 63 |
| *Week 11–12* | Post training testing | | |

**Table 4**
Loading variations for resistance training.

| | Monday | Wednesday | Friday |
|---|---|---|---|
| *Program exercises for the non-linear periodized resistance exercise training program* | | | |
| Wks 1–2 | Pre-training testing and familiarization | | |
| 3 | Light | Moderate | Heavy |
| 4 | Moderate | Light | Moderate |
| 5 | Heavy | Moderate | Light |
| 6 | Moderate | Heavy | Moderate |
| 7 | Heavy | Light | Heavy |
| 8 | Moderate | Heavy | Moderate |
| 9 | Light | Heavy | Moderate |
| 10 | Heavy | Moderate | Light |
| 11–12 | Post-Training testing | | |
| Exercises used | Squat, stiff-leg deadlift, week bench, lat pulldown, upright row*, calf exercises, abdominal work | Leg press*, stiff-leg deadlift, incline bench press, seated row, shoulder press*, calf exercises, abdominal work | Squat, stiff-leg deadlift, bench press, lat pulldown, upright row*, calf exercises, abdominal work |

* At week 7,the following exercises were changed: leg press to deadlift; upright row to high pull; shoulder press to push press.

### 2.10. Biochemical analysis

Frozen plasma samples were thawed once and analyzed. 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 mini-columns" (i.e., 3 mL $C_{18}$ extraction columns, J.T. Baker Co.). The methods used to purify the samples, conduct the radioimmunoassay (RIA), as well as the identified cross-reactivities have been previously described in detail [16,18]. Peptide $F_{ir}$ was measured by RIA in duplicate using commercially available $^{125}$I ligand and antisera (Peninsula Laboratories, Belmont, CA, USA). The mean recovery of the radioactive labeled Peptide F following extraction was 89%. The partially purified samples were then stored at −80 °C until analyzed. Further identification of the peptide showed that no substantial degradation of Peptide F was seen with these methods as demonstrated by the radioactivity (>94%) eluded with the authentic labeled peptide. Using HPLC elution time as the measurement and previously described methods [11], the plasma ir showed parallel displacement of Peptide F, the inter-assay coefficient of variation was 3.2%.

Determinations of plasma ir values were accomplished with the use of a gamma counter and on-line data reduction system.

### 2.11. Statistical analyses

Data are presented as means ± standard deviations. A four (group) × two (T1, T2) block analysis of variance (ANOVA) with repeated measures was used for statistical analyses. Tukey post hoc tests were used when appropriate to determine pairwise differences. The assumptions for linear statistics (e.g. normality, sphericity, homogeneity of variance etc.) were tested and if they were not met a $\log_{10}$ transformation of the data were used and the data set was reanalyzed to demonstrate that assumptions were met. Significance for this investigation was set at $P \leq 0.05$.

## 3. Results

### 3.1. Training related changes

Consistent with other study analyses, the training changes in strength and maximum oxygen consumption were vital to the understanding of the effectiveness of the associated training programs. The bench press demonstrated significant pre to post-training increases of +9.2 ± 2.9 kg for the STR group; +8.3 ± 4.4 kg for the CMB training group; and no significant changes in the CON or END training groups. For the squat exercise it was demonstrated that a significant pre- to post-training increases were +27.2 ± 7.9 kg for the STR group; +26.3 ± 6.4 kg for the CMB training group; and no significant changes in the CON or END training groups. The changes in maximal oxygen consumption (mL kg $min^{-1}$) for the END training group was +3.7 ± 1.2 mL kg $min^{-1}$; for the CMB group 3.9 ± 1.5 mL kg $min^{-1}$ with no significant changes observed for the STR or CON Group.

### 3.2. Pre-training Peptide F

Before training there were no significant differences in the concentrations of plasma proenkephalin Peptide F either pre-exercise or immediately post-exercise in the squat exercise protocol for any of the experimental groups.

### 3.3. Control group

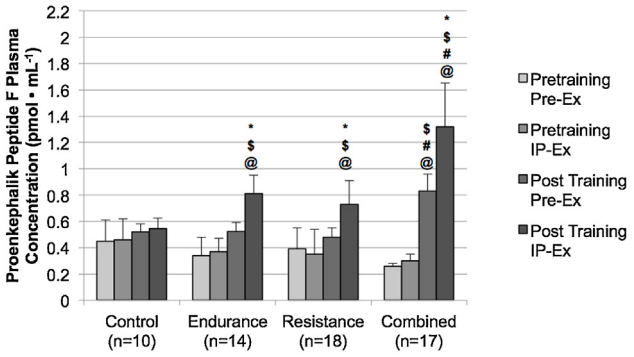
The control group demonstrated no significant changes in Peptide F concentrations in any of the time points measured in this study. Thus, no acute exercise or training related changes were observed.

### 3.4. Endurance training

After the endurance training program there was no difference observed for the resting concentration of Peptide F compared to pre-training values. However, with the training program a significant increase in Peptide F was observed after the squat exercise protocol and this value was also significantly higher than the pre-training value.

### 3.5. Resistance training

Similar to the endurance training group, the resistance training program resulted in no significant differences for the resting concentration of Peptide F compared to pre-training values. However, with the training program a significant increase in Peptide F was observed after the squat exercise protocol and this value was also significantly higher than the pre-training value.




(*) Significant (P ≤ 0.05) difference found between corresponding Pre-Exercise values.
($) Significant (P ≤ 0.05) difference found between corresponding before training values.
(#) Significant (P ≤ 0.05) difference found between CMB and corresponding END, STR, and CON values
(@) Significant (P ≤ 0.05) difference found between the value and the corresponding CON values.

**Fig. 1.** The responses of plasma Peptide F to the acute resistance exercise test before and after 8 weeks of training are presented for control, endurance, resistance and combined exercise groups. Significance was defined as $P \leq 0.05$.

### 3.6. Combined training

When participants performed both types of training programs, there was a significant increase in Peptide F concentrations pre-exercise when compared to pre-training values and also the other experimental groups' resting values. This was also observed with significant elevations post-exercise and these concentrations of Peptide F were significantly higher than any of the corresponding values for the other groups.

## 4. Discussion

The primary finding from this investigation was that in untrained women exercise training results in acute plasma Peptide F concentrations immediately post-exercise. The modality of training does not appear to create any differential response after training. However, if both exercise modalities are included in the exercise training program the plasma Peptide F response post-exercise is almost doubled in response to the resistance exercise protocol (see Fig. 1). Additionally, there was an increase in resting values which may indicate an additional need for even more immunomodulation due to the dramatic increase in exercise demands and tissue damage [3,14,23].

Such data may reflect training-induced adaptations in the adrenal glands' chromaffin granules leading increased secretion of Peptide F mediating the elevated plasma concentrations beyond the saturation of the white blood cell biocompartment in the blood [3].

With the prior discovery of Peptide F in each of the blood biocompartments (i.e., plasma, buffy coat, red blood cells) the transitory trafficking relationships between cell tissues remains unknown [3,12]. Changes in the buffy coat remain relatively stable suggesting that white blood cell opioid receptors are immediately available for binding of proenkephalin fragments upon release [3,8]. The increase in plasma concentrations indicates that the white blood cell biocompartment is saturated and thus the plasma element has the ability to target other receptors [4,10,12]. Thus, no changes in the plasma concentrations even with exercise stress may well present a new paradigm of thought indicating the buffy coat biocompartment has the highest priority for proenkephalin fragment binding even more than previously thought [10].

In an untrained physical fitness status, normal release of opioid peptides from the adrenal medulla may well be used immedi-

ately to saturate the immune cell receptors in the white blood cell fraction of blood. With training, changes in the adrenal medullary release patterns previously observed may in fact allow for greater synthesis and quanta of release of these proenkephalin fragments (e.g., Peptide F) thus resulting in significant plasma increases [12,13]. The importance of this role in the immune function has been hypothesized for some time in animal models [8]. In our prior study we underscored this importance in women showing the augmentation of B cell activity with exercise induced concentrations of Peptide F and the importance of exercise training [23]. Alternatively, the time line we used in this study may have missed the eventual increase in the plasma concentrations in response to the exercise stressor after saturation of the white blood cell tissue component was completed temporally. Yet other investigations have observed immediate post-excise increases in Peptide F reflecting potential unknown factors in rate and movement of Peptide F in the cell trafficking in and out of circulation and receptor binding dynamics. Nevertheless, the importance of exercise training for health and fitness is reflected in our data with the eventual exercise training-induced responses to the resistance exercise stress test [23]. This study again will present a fertile area for further immunological and opioid peptide research [8,23].

The amount of exercise or the integrated diversity of the exercise stimuli seems to provide a potent combination for an augmentation of both resting and exercise-induced plasma elevations of Peptide F in young women. The women in the combined group exercised 20–30 min more each training session than the resistance exercise only group and 40–63 min more each training session than the endurance exercise only group. Essentially the CMB group did more total work and had no doubt greater energy expenditures over the training program. However, with our dietetic monitoring no deficit was observed in nutritional support needed to maintain body mass and energy expenditures [18]. In contrast, a combined training program in men who had started with much high fitness levels as enlisted soldiers did not show any augmentation of Peptide F responses [13]. However, the exercise stimuli was a maximal treadmill test and it may not have the same damage and repair relationships as a resistance exercise protocol which has been indirectly shown to influence opioid peptide responses [14]. Thus, future studies will need to examine identical testing protocols in order to directly determine any sexual dimorphism in the response patterns of proenkephalin fragments to exercise stress and training.

No significant differences were observed between pre-exercise and post exercise concentrations for any of the groups before training in these untrained women. This was consistent with our prior study which showed that untrained women showed no changes in Peptide F plasma concentrations to cycle exercise at 60% and 80% of $VO_{2peak}$ intensities [23]. However consistent with our findings, fit women did show an elevation in plasma Peptide F concentrations at the 80% of $VO_{2peak}$ cycle intensity [23]. As such the elevations in Peptide F in the prior study were related to the number of antibody-producing B cells in the fit group of women accounting for 49% of the shared variance between the two variables [23]. Thus, in women going from an untrained to physically trained state, exercise training appears to have a positive influence on immune modulation when elevations in Peptide F occur in the plasma. It is also possible that Peptide F may have increased later in the recovery time frame as seen in a prior study in men [2]. It may also be speculated that in untrained women the potential for immune enhancement provides a greater “sink” for newly released peptide F to be bound rapidly to immune cells thereby masking the actual increase in synthesis, release, and secretion and elevations in plasma concentrations [3,12,14].

## 5. Conclusions

In conclusion, this was just an initial study into physical exercise training in women. We showed that adaptations can occur in the early phase of training and may well impact to a degree the adrenal medulla and the release patterns of proenkephalin peptide F into the blood. This investigation is the first to present data in women on the simultaneous temporal changes in proenkephalin peptide F to different types of training modalities. While previous studies have examined the effects of various short-term training programs on catecholamine responses [5,7,22,24], this study provides data on the concomitant responses of proenkephalin-derived peptides. Training did not impact resting concentrations in the two programs that performed a single exercise program but did result in significantly elevated the post-exercise responses. Combined programs may require even more immune support and thus the more dramatic changes in Peptide F in the plasma at rest and with exercise stress may reflect this greater need. How volume of training impacts adrenal function and the potential future of our understanding of adrenal exhaustion may well have an answer in studying the proenkephalin family of peptides [1].

## Acknowledgements

The authors thank the volunteers from the University of Connecticut for their time and dedication. The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the Army or the Department of Defense. Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations. This research was supported by a grant from the US Army Medical Research and Material Command Bone Health and Military Medical Readiness Research Program to BCN and to WJK at the University of Connecticut. The authors have no other conflict of interests to report.

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