Avicenna J Neuro Psych Physio. 2015 August; 2(3): e34440. doi: 10.17795/ajnpp-34440

Published online 2015 August 29. **Research Article**

The Effect of Different Training Modes on Serum Apelin and Pain Threshold in Morphine-Dependent Rats

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Received 2015 June 1; **Revised** 2015 July 15; **Accepted** 2015 August 15.

Abstract

Background: Apelin has recently been identified as an analgesic agent and a novel neuropeptide. On the other hand, it has been shown that exercise can lead to reduced pain in morphine-dependent patients.

Objectives: Therefore, the aim of this study was to evaluate apelin and pain threshold changes in healthy and morphine-dependent rats in response to two exercise paradigms.

Materials and Methods: In this study, 30 healthy and 30 morphine-dependent rats were used. Morphine-dependent and healthy rats were divided into six groups: 1, a control (healthy) group; 2, a healthy endurance group; 3, a healthy strength-training group; 4, an addicted control group; 5, an addicted endurance group; 6, an addicted strength-training group. Then, the training groups performed aerobic and strength training for eight weeks. After the training program, the tail flick and formalin tests were used to assess pain. Apelin was also measured by ELISA.

Results: Regardless of the type of exercise, exercise significantly increased the apelin serum levels in healthy rats. The apelin levels significantly increased in the morphine-dependent rats compared with the healthy control group. Endurance, unlike strength training, significantly increased apelin in the serum compared to the addicted control group. The training led to pain relief in the morphine-dependent rats and returned it to the healthy control group level. The Pearson correlation showed a reverse significant correlation between the serum apelin level and the tail flick test in the morphine-dependent rats.

Conclusions: The results showed that endurance training reduced pain by increasing apelin in morphine-dependent rats. Therefore, it is suggested that this type of training be considered for the morphine-dependent patients for pain relief.

Keywords: Exercise Training, Analgesics, Pain Threshold, Morphine, Apelin

1. Background

Drug abuse is one of the most important health, social, and cultural issues. Over 90% of people have expressed serious concerns about drug abuse as a worldwide problem. According to the latest data from the united nations office on drugs and crime (UNODC), drug abuse is rising at a gentle slope; in 2012 – 2013, an average of 226 million (5%) of people between the ages of 15 and 64 years old had used drugs at least once per year (1). Addiction is now being introduced as a disease associated with molecular and physiological changes in which various factors are involved, including genetic, environmental, and neurobiological factors; therefore, therapy methods are very different and complicated and no satisfactory results have yet been achieved (2). Exercise has been reported to be effective in the treatment and even prevention of many disorders, including depression, memory impairment, Alzheimer disease, and addiction (3, 4). It has been shown that the release of several neurotransmitters like dopamine, glutamate, acetylcholine, serotonin, and androgen opioids in the brain can be altered by exercise (5). Exercise can also compensate for the reduced production of dopamine, serotonin, and norepinephrine resulting from drug abuse. Therefore, exercise can be considered a valuable factor in the treatment of addiction and can improve the lives of addicted individuals (6). In this regard, it has also been shown that exercise can lead to the release of the certain neurotransmitters in the brain to reduce mental and physical pain (3). Despite significant progress, the molecular mechanism of pain relief in addiction patient following exercise is still unclear. In this regard, Xu et al. (2009) showed that apelin plays an important role in the reduction of pain. Apelin is a multifunctional 36 amino acid peptide derived from a 77 amino acid precursor (Prepro Apelin). It has four isoforms, including apelin 12, 13, 17, and 36. Among these four isoforms, apelin 13 has 13 amino acids at the C-terminal of the pre pro-peptide, which has the most frequent biological activity, and its sequence is fully protected in all species (7, 8). The wide distribution of apelin receptors (APJs) in the amygdala, hippocampus, and spinal cord indicate apelin's crucial role in the reduction of pain (8). APJs can be seen in several areas of the brain associated

with the descending pain transmission system, such as the amygdala, hypothalamus, and dorsal raphe nucleus (9). Xu et al. (2009) showed that apelin can demonstrate its analgesic effect through the hair and receptors in the hippocampus, which these APJ play a key role in drug withdrawal syndrome by increasing the levels of opioids. They showed that the apelin levels are very high at the areas of brain in which the highest level of opioids can be found, indicating interrelationships between apelin and analgesic effects (7).

2. Objectives

Most studies regarding the reduction of pain are focused on aerobic activity; however, the effect of strength training is not clear. This study aimed to investigate the relationship between the serum apelin levels and analgesic effects in morphine-dependent rats following two types of exercise (aerobic and strength-training) for the first time.

3. Materials and Methods

In this experimental study, male Wistar rats aged 6 – 8 weeks weighing 180 – 200 g (Razi Pasteur institute, Iran) were used. Animals were kept in the rodent's standard laboratory (12 hours light-dark cycle at 2 ± 22 C°). They had free access to food and water.

3.1. Dependency Induction Method

In this study, morphine dependency was induced orally with morphine at continuous concentrations of 1.0, 2.0, and 3.0 mg/mL for 48 hours, then 4.0 mg/mL in the next 15 days was poured in the drinking water. Sucrose sulfate (3%) was added to the drinking water due to the bitter taste of morphine. The water and morphine were covered by thin aluminum sheets to prevent the degradation of morphine by light. The rats were addicted to morphine. Naloxone (3 mg/kg body weight) was injected intraperitoneally to one or two rats in each group randomly to examine their morphine dependency. After confirming rats' addiction to morphine, morphine-dependent and healthy rats were divided into the following six groups: 1, a control (healthy) group; 2, a healthy endurance group; 3, a healthy strength-training group; 4, an addicted control group; 5, an addicted endurance group; and 6, an addicted strength-training group.

3.2. The Training Protocols

3.2.1. Endurance Training

The rats were trained for 8 weeks, 5 days per week. The training period was divided to 2 stages: overload and load intensity stabilization stages. During the first week, the rats ran every day for 10 minutes at a speed of 17 m/min on a treadmill. Gradually, from the second to the fifth week (overload stage), the training length was increased to 55 minutes per session. From the sixth to the eighth week (the length stabilization stage), the rats ran on treadmill for 55 minutes at 30 m/min (75% VO_{2max}), (Table 1) (10). The incline of the treadmill was 0° in all stages.

3.2.2. Strength Training

The rats were placed 3 days per week for 15 minutes on a 36-step ladder (Iran) with no weights for adaptation and reducing their stress. Then, the regular exercise protocol was performed 5 days per week in three sets, which included 4 trainings with 3-minute intervals between the sets and 15 seconds between iterations, for 8 weeks. The rats were trained in the first 3 weeks with weights weighing 20, 40, and 60% of their body weight; with weights weighing 80, 100, and 120% of their body weight in the following 3 weeks; and finally with weights weighing of 140 and 160% of their body weight in the final 2 weeks. The ladder was located perpendicular to the ground next to the wall (Table 2). All the rats had 2 days of rest.

3.3. Tail Flick Test

After training, a tail flick test was used to measure the pain threshold in the studied groups. The tail flick apparatus consisted of two parts: a restrainer and a control system. The rats were placed in the restrainer while their tail was out and the beginning of the tail was on a light-sensitive sensor. In this study, the light intensity was adjusted to achieve a basic average responsiveness time of 4 to 5 seconds to obtain approximately 50°C, which was appropriate for testing; 12 seconds was considered the cut-off time to the middle of the tail. For adaption with the restrainer, the animals were trained 3 days before the experiment, 1 hour per day to reduce the stress caused by the apparatus immobilization. After putting a rat in the restrainer, the tail was fixed and the start button was pressed for radiation. The mean withdrawal time was measured 3 times in 1-minute intervals. To prevent tissue damage, the rats were removed from the light when there was no reaction for 30 seconds (11).

3.4. Formalin Test

The formalin test was used to assess the rats' pain threshold. For adaptation, before the test, the rats were placed inside the glass chamber for at least an hour. Then, formalin (25 μL, 2.5%) was injected subcutaneously into their hind leg in the restrainer. Following the formalin injection, the rats' first signs of pain and behavioral responses, including keeping up, pulling, limping, licking, biting, and shaking their injected legs were evaluated within 60 minutes. In this study, a score of 1 was considered for pulling and limping behaviors, 2 for keeping up, 3 for licking, biting, and shaking their legs. In 15-second intervals for one hour, the time taken to achieve each score was measured. The rats' reactions to the painful stimuli were divided into an acute phase (0 – 10 minutes) and chronic phase (15 – 60 minutes); the time taken to exhibit limping, keeping up, and licking behaviors was measured in each phase (11).

3.5. Apelin Measurement Method

The blood samples (3 cc) were taken from the rats' eyes 48 hours after the last training session. They were centrifuged at 10,000 rpm for 10 minutes. The serum samples were stored at -80°C before the measurements. Apelin was measured using ELISA kits (Apelin ELISA kit, RAB0018, Sigma Aldrich USA) and the ELISA method according to the manufacturer's instructions by Elisa reader (ELX800, USA).

3.6. Statistical Analysis

Data were analyzed using SPSS 18. The normality of the data was confirmed using the Shapiro-Wilk test. The one-way ANOVA and Tukey's post-hoc tests were used to evaluate the differences between the groups. Data are presented as mean ± SEM, and $\alpha < 0.05$ was considered significant.

4. Results

Apelin: The one-way ANOVA results showed that the apelin levels were significantly different between groups (P = 0.0001, F = 34.59). Tukey's post-hoc test results showed a significant difference between the control group and the other addicted groups. There was a significant difference between the healthy groups and the addicted strength-training group. Tukey's test results showed a significant difference between the endurance group and the control and the healthy strength-training groups. There was significant difference between strength-training group and control (healthy) group (Figure 1).

4.1. The Pain Threshold

The tail flick test results showed that there is a significant difference between the groups' pain threshold (P = 0.02, F = 7.04). Tukey's post-hoc test results showed that the pain threshold in the addicted control group was significantly higher than the other groups. The results showed no significant difference between the trained groups and the control (healthy) group. According to Tukey's post-hoc test, there were no significant differences between the trained groups (Figure 2).

The one-way ANOVA results showed a significant difference between the groups in the pain threshold in the formalin test (P = 0.04, F = 5.73). Tukey's post-hoc test results showed that the pain threshold in the addicted control group is significantly higher than other groups. Moreover, the results showed no significant difference between the trained groups and the healthy control group. According to Tukey's post-hoc test, there was no significant difference between the trained groups as well (Figure 3).

4.2. Pearson Correlations Between Parameters

There was no significant correlation between apelin and the tail flick test in healthy rats ($\alpha = 0.072$, R = -0.34) (Figure 4). There was also no significant correlation between apelin and the formalin test in the healthy rats ($\alpha = 0.68$, R = -0.01) (Figure 5).

There was a significant inverse correlation between apelin and the tail flick test in morphine-dependent rats ($\alpha = 0.0001$, R = -0.79) (Figure 6). However, there was no significant correlation between apelin and the formalin test in the morphine-dependent rats ($\alpha = 0.47$, R = -0.14) (Figure 7).

Table 1. Endurance Exercise Training Program[a]

Weeks	Duration, min	Speed, m/min	Intensity (VO_{2max}), %
0	15	5	45
1	20	10	50
2	25	15	60
3	30	20	65
4	35	25	70
5	40	30	75
6	45	30	75
7	50	30	75
8	55	30	75

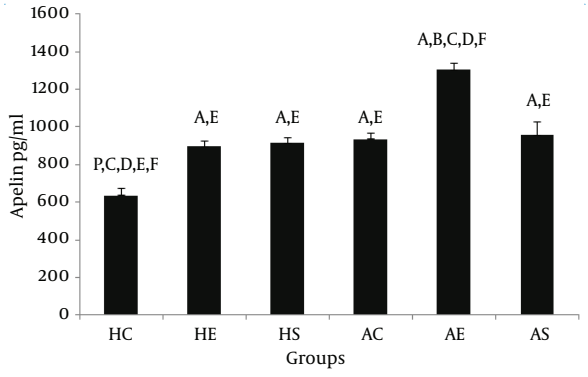
[a]The speed and duration were gradually increased to 30 m/min and 55 min.VO

Table 2. Resistance Exercise Training Program[a]

Weeks	Weight of Body, %	Weight, g
Familiarity	NA	NA
1	20	50
2	40	100
3	60	150
4	80	200
5	100	250
6	120	300
7	140	350
8	16	400

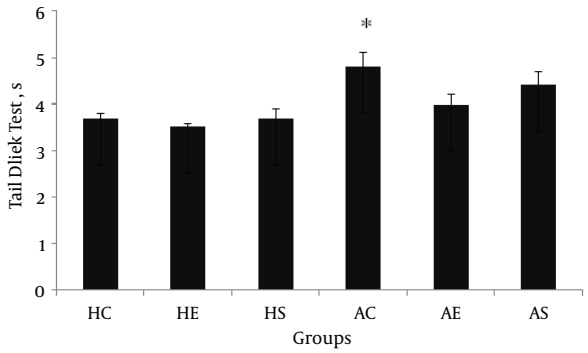
Abbreviation: not available.
[a]The resistance was gradually increased to 400 gram.

Figure 1. Effect of Different Exercise Training Modes on Serum Apelin in all Experimental Groups



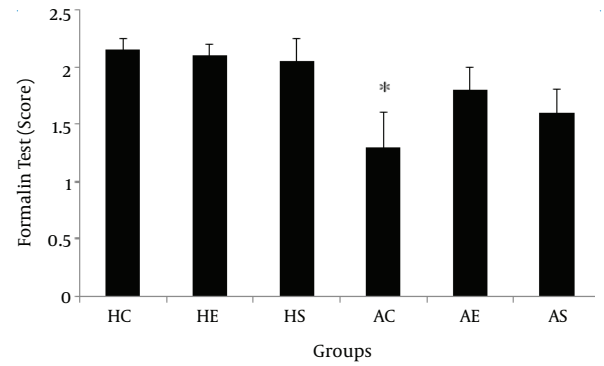
Each column and bar represents mean ± S.E.M: A, significant difference from the HC group at the level of P < 0.05; B, significant difference from the HE group at the level of P < 0.05; C, significant difference from the HS group at the level of P < 0.05; D, significant difference from the AC group at the level of P < 0.05; E, significant difference from the HE group at the level of P < 0.05; F, significant difference from the HE group at the level of P < 0.05.

Figure 2. Effect of Different Exercise Training Modes on Tail flick test in all Experimental Groups



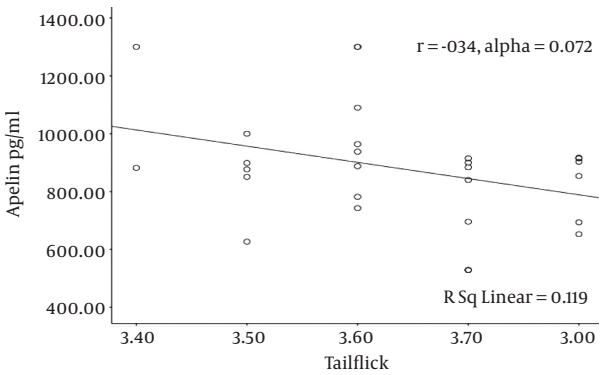
Each column and bar represents mean ± S.E.M significant difference from the other experimental groups at the level of P < 0.05.

Figure 3. Effect of Different Exercise Training Modes on Formalin Test in all Experimental Groups



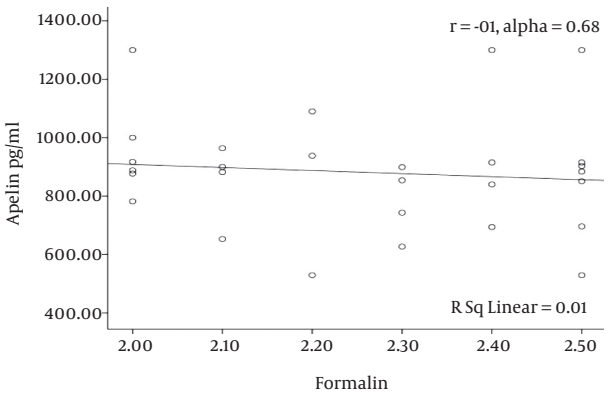
Values are mean ± SEM; Significant difference from the other experimental groups at the level of P < 0.05.

Figure 4. There are No Correlation Between Apelin and Tail Flick Test in Healthy Rats



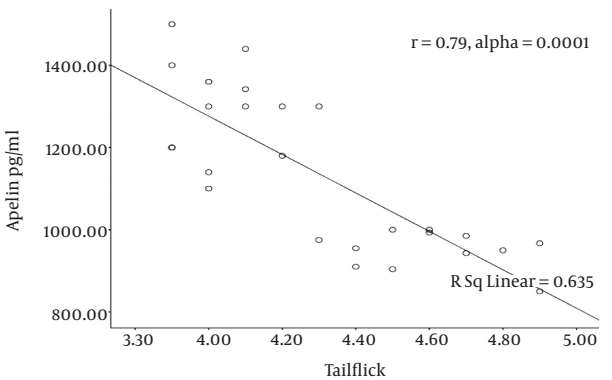
r = -0.34; P = 0.072.

Figure 5. There are no Correlation Between Apelin and Formalin Test in Healthy Rats



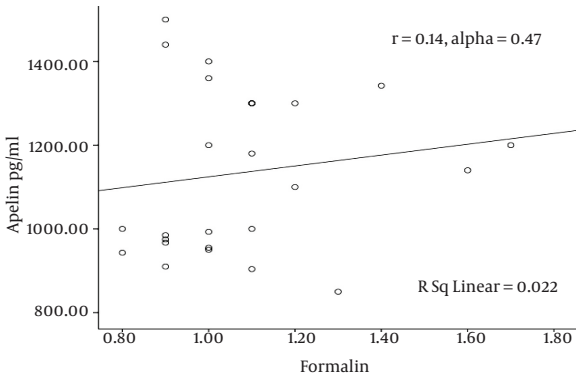
r = -0.1; P = 0.68.

Figure 6. Significant Inverse Correlation Between Serum Apelin and Tail Flick Test in Morphine-Dependent Rats



r = -0.79; P = 0.0001.

Figure 7. There are No Correlation Between Apelin and Formalin Test in Morphine-Dependent Rats



r = -0.14; P = 0.47.

5. Discussion

Apelin is a multi-functional neuropeptide that plays an important role in energy homeostasis regulation, immune system function, gastrointestinal function, and pain relief regulation (7, 8). Morphine is used extensively to relieve acute pain, but morphine injections to relieve chronic pain are associated with side effects, including addiction (2). This study was the first to show that strength training can provide relief in morphine-dependent rats by increasing the serum apelin neuropeptide. Regardless of the type of exercise, morphine led to a significant increase in the serum apelin levels in healthy rats. Moreover, no significant differences were observed between the trained groups and the healthy subjects. On the other hand, the serum apelin levels significantly increased in the addicted groups compared with the control (healthy) group. The results showed that strength training unlike endurance training significantly increased the serum apelin levels compared with the addicted control group. On the other hand, endurance training can lead to a reduced pain threshold in morphine-dependent rats and change it to the control (health) group's level. The Pearson correlation results showed a reverse correlation between the serum apelin levels and the tail flick test in morphine-dependent rats. This may be due to the wide distribution of the apelin receptors in the pain control centers like the spinal cord, hypothalamus, and medulla oblongata (12-14). The distribution of apelin receptors in the brain pain control centers can imply the physiological function of apelin in pain relief. The results of this study are consistent with those of Yang et al., indicating that 0.3 to 3 g intraventricular injections of the apelin-13 using water immersion tests in healthy rats reduced pain by activating apelin-specific receptors and hair receptors. They also showed that these changes do not mean that apelin is able to reduce all types of pain (15). The results demonstrated that there is a significant correlation between apelin and the tail flick test, while there is no significant correlation with the formalin test. This may be due to the different receptors activated by apelin and the complex regulatory mechanisms in the different types of pain (8). The exact mechanism of exercise-induced pain relief caused by increased apelin is unclear. Previous studies have shown that opioid receptors play an important role in pain transmission. Apelin is quite effective in opioid signaling (15-17). apelin can release opioids by binding to the apelin receptors. Then, it can relieve the pain by binding opioids to their specific receptors (8). Anatomical evidence has indicated that apelin can be found in areas rich in opioids, such as the hypothalamus, demonstrating the interrelationships between apelin and opioids for pain relief. Regarding the increased pain threshold in morphine-dependent rats and the training role on reducing the pain threshold, Befort et al. have reported that the apelin duplication in the hypothalamus of morphine-dependent rats is reduced due to the stimulation of opioid hair receptors by morphine (16). Fujie et al. have shown that apelin gene expression increases as a result of training (17). They indicated that aerobic training for eight weeks can increase plasma apelin through apelin release by the skeletal muscles. The present findings indicated that aerobic training has the greatest impact on the increased serum apelin, which is consistent with Kadoglou et al.'s results, indicating apelin serum levels will increase after aerobic training in patients with type II diabetes, but this was not observed after strength training (18). Kadoglou et al. showed that apelin serum levels elevated after aerobic training for 6 months, including walking, running on a treadmill, and cycling (60% – 70% VO_{2max}), while strength-training for 8 weeks (60% – 80% one repetition maximum) did not change the serum apelin levels of type II diabetic patients (18). The reason for the increased plasma apelin after training exercises is unclear (17). Zhan et al. demonstrated that apelin gene expression in the aorta and heart will be increased after training. Thus, the aorta and heart can be considered resources for releasing apelin into the plasma after training (19). In addition, researchers have demonstrated that other tissues including adipose and kidney tissues can be regarded as the resources of apelin after training (20, 21). HIF-α, TNF-α, insulin, and mechanical stress induced by training have been shown to be the main causes of the increased apelin after training (17-21). O'carroll et al. also showed that apelin gene expression in the hypothalamus is affected by glucocorticoids (22). However, it is unclear which factors involved in apelin production are affected by the different types of training, and more studies are needed to explore this in greater depth. In summary, this study demonstrated for the first time that endurance training can lead to pain relief by increasing apelin in morphine-dependent rats. Therefore, endurance training is suggested for pain relief in morphine-dependent patients.

Footnote

Authors' Contribution: Ali Heidarianpour wrote the manuscript; Ebrahim Zarrinkalam executed the study protocols.

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