ORIGINAL ARTICLE

Effect of semi-professional boxing training on selected inflammatory indicators and anaerobic performance

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C Statistical Analysis
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Abstract

Background and Study Aim: Boxing is a popular combat sport which requires many years of physical preparation and a high level of physical fitness. However, there are limited data about the influence of boxing training induced adaptation on blood indicators specially in case of post-exercise inflammatory response. The aim of the study was to verify hypotheses: long lasting boxing training induces adaptation changes in biochemical response during anaerobic exercises.

Material and Methods: Total 17 male boxers with more than 5 years' experience in boxing and 11 physical active man voluntarily participated in this study. Venous blood samples were taken before, immediately after, 60 min and 24 h after Wingate anaerobic test.

Results: Significantly higher mechanical indicators after two-time lower body WAnT was observed in semi-professional boxing groups, for mean power, peak power and mean power normalized to body mass. Furthermore, boxing training causes changes in selected serum inflammatory markers interleukin-10 (IL-10), interleukin-15 (IL-15) and tumour necrosis factor alpha (TNF-α). Moreover, boxing training is associated with the higher pre-WAnT creatine kinase (CK) status and its lover post exercises changes.

Conclusions: Boxing training is associated changes in serum concentration of interleukin 10, interleukin 15, tumour necrosis factor alpha induced by anaerobic exercise indicate that boxing training may be associated with attenuation of post-exercises pro-inflammatory response and induce anti-inflammatory cytokines secretion.

Key words: inflammation process • interleukin-6 • interleukin-10 • interleukin-15 • tumour necrosis factor alpha

Conflict of interest: Authors have declared that no competing interest exists

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INTRODUCTION

Boxing – combat sport involving direct fist fighting between two competitors which seek to punch each other without receiving a counter hit; much is won when a number of clean punches lands successfully on an opponent's head and body or if an opponent is knocked out [25].

Combat sports – competitive contact sports where two combatants fight against each other using certain rules of engagement [26].

Combat sport – ***noun*** a sport in which one-person fights another, e.g. wrestling, boxing and the martial arts [27].

Martial arts – ***plural noun*** any of various systems of combat and self-defence, e.g. judo or karate, developed especially in Japan and Korea and now usually practiced as a sport [27].

Inflammation process – (from Latin: ***inflammatio***) is part of the complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells, or other [28, 29].

Boxing is a one of the oldest cooperative combat sports, involving direct fist fighting between two competitors which seek to punch each other without receiving a counter hit. A full boxing fight is performed according to specific rules, with a limited striking field and time. A modern-day boxing match consists of a determined number of 3 min rounds, divided by a 1-min rest [1].

Historical boxing is one of the oldest sports in the world and probably was known by the Sumerians around 5000 BC. Just like in the past, today's boxing is still very demanding sport discipline characterized by short duration, high intensity, and intermittent activity with very high technical, tactical and psychological level [2]. It has been proven that same like other combat sports boxing induce many biochemical changes, mostly associated with physiological response during the fight (ex. cortisol, testosterone, secretory IgA, α-amylase and many other [3].

There is still lack of knowledge how long-lasting boxing training induces physiological adaptations for the exercises, especially in the context of induced inflammation process. It has been proven that sports rivalry induces changes in the cellular redox state [4, 5]. In turn of these changes, many pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) are being released to the blood flow and induces inflammation process. It contributes to tissue peroxidation, lipid oxidation and many other free radical dependent reactions between tissues and reactive oxygen species. On the other hand, this process is followed by the release of anti-inflammatory cytokines (IL-10, IL-13, Il-15 and many other) which one of the roles is attenuation of the primary inflammation response [6]. Such type of activity in many situations may lead to better exercise efficiency and less likelihood of injury (many of the injuries are inflammation-dependent). Nowadays, it is known that changes in the pro- and anti-inflammatory cytokine secretion can be modulated by the physiological adaptation during specific sport training [7]. In some situations, it may lead not only to a better sport performance but many other pro-health body systems functioning changes.

The aim of the study was to verify hypotheses: long lasting boxing training induces adaptation changes in biochemical response during anaerobic exercises.

MATERIAL AND METHODS

Experimental Overview

This observational research includes biochemical tests performed on serum samples from 17 male boxers (aged 23.82 **±3.55 years)** with more than 5 years' experience in boxing and 11 physical active man (aged 21.55 **±3.26** years). All the participants were apparently healthy, non-smokers, and had refrained from drugs and herbal remedies categorized in WADA prohibited list. The participants were informed of the nature and possible inconveniences associated with the experiment. Both samples from the population did not differ statistically significantly in somatic terms (Table 1).

The study was undertaken in compliance with the Declaration of Helsinki and approved by the Bioethics Committee for Clinical Research at the Regional Medical Chamber in Gdańsk (decision no. KB-24/16).

Table 1. Somatic characteristics in semi-professional boxers (n = 17) and physical active man (n = 14).

Variable	Boxers Mean & SD	Physical active man Mean & SD	t	p
Body height (cm)	180.00 ±5.30	177.73 ±3.25	1.270	0.2154
Body mass (kg)	71.63 ±5.29	69.82 ±4.28	0.949	0.3514
BMI (kg · m^2)	21.06 ±1.79	21.91 ±0.81	-1.473	0.1528
Percent body fat (%)	11.39 ±1.47	10.40 ± 2.52	1.307	0.2027

Experimental protocol

Experimental protocol begins from the measurement of anaerobic components of fitness. We used double Wingate Anaerobic Test (WAnT) in adaptation on lower limbs with the load adjusted individually.

Furthermore, assessment of post-exercises inflammation process was done basing on serum measurement of interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-15 (IL-15) and tumour necrosis factor alpha (TNF-α). Moreover, pre- and post-exercises measurement of creatine kinase was done to show the effect of Wingate anaerobic Training on muscle damage. Samples were collected before, 5 and 60 min and 24h after two-time Wingate anaerobic testing.

All participants attended an orientation session to ensure that they were familiar with the testing equipment and procedures. Two days before the experiment all participants were asked to refrain from extensive exercise, hydrate them self and maintain their normal dietary habits, excluding any drugs and stimulants.

Double WAnT protocol

Double WAnT testing procedure was conducted on a cycle ergometer Monark 894E (Sweden) in Laboratory of Gdańsk University of Physical Education and Sport. Saddle position was oriented individually so that the knee remained slightly flexed after the completion of the downward stroke (with final knee angle approximately 170–175°). Participants' feet were stiffened and held firmly in place and in contact with the pedals. Before WAnT procedure each participant completed a standardized warm-up on the cycle ergometer (5 minutes at 60 rpm, 1.0 $W \cdot kg^{-1}$).

During the testing each of the participant was required to pedal with maximum effort for a 30 second against a fixed resistive load of 0.075 $kg \cdot kg^{-1}$ of total body mass [8]. Participants were instructed to cycle as fast as possible and were given a 3 second countdown before the set resistance was applied. Verbal encouragement was given to all participants to maintain their highest possible cadence throughout WAnT. After the first Want test 30s rest started and second WAnT procedure was performed immediately. Data obtained from the cycle ergometers were recorded via the MCE 5.1 software. The average power output during the 30 second test was measured relative to body mass ($W \cdot kg^{-1}$).

Samples collection, and inflammation markers measurements

The blood samples were collected in four time points by a medical diagnostic professional, according to the experimental protocol, i.e.: before the test, immediately after (to 5 min post testing), 60 min after and 24h after the test. The blood was collected into 5 mL BD Vacutainer Clot Activator Tubes. The serum was separated by centrifugation 4000 r.p.m × 10 min) and aliquoted into 500 µL portions. All prepared samples were frozen and stored at −80 °C until further analysis.

Levels of the following inflammatory response cytokines was messured: IL-6, IL-10, Il-15 and tumour necrosis factor alpha (TNF-α) using Luminex assays kits [Luminex Corp.; Luminex Human Magnetic Assay].

Blood creatine kinase (CK) concentrations were measured in tree time points: before the test, 60 min after and 24h after the WAnT on a spectrophotometrically (Vario II Photometer, Diaglobal, Germany) using commercially available detecting kits (CK 321, diaglobal, Germany).

Statistical analysis

To assess statistical significance of the changes in inflammation status before and after exercise (2×30sWAnT), a repeated measures analysis of variance (ANOVA) was used. To calculate differences between groups, two-way ANOVA of repeated measures (2 groups × 4 measures) was applied. Post-hoc analyses were implemented when appropriate with Tukey's post hoc test. All calculations and graphics were performed using GraphPad Prism 6.0 (ftx.pl/program/graphpad-prism).

Power analysis for the interactions between effects, to determine the appropriate sample size, was performed in GPower ver. 3.1.9.2 (Franz Faul et al. [9], Universität Kiel, Kiel, Germany).

Significant interaction between factors was subsequently analysed using Tukey's post-hoc test. Descriptive statistics included mean, standard deviation (± or SD) for all measured variables and: result of the analysis of variance (*F*); degrees of freedom (*df);* effect size (η^2); significance level, probability (*p*). The reliability of assumptions of this statistical test was checked using the Shapiro–Wilk test for normality, and

Levene's test for the homogeneity of variance. Statistical analysis was performed using All calculations were done in Statistica 12 (StatSoft, Tulsa, OK, USA). Differences were considered statistically significant at p≤0.05.

RESULTS

Obtained mechanical indicators during 2x30s WAnT showed significant effect of long term boxing training (Table 2). A significant repeated measurement effect was observed in all presented WAnT indicators. Regardless of the group division, a significant decrease in all tested indicators was observed in the second WAnT. There was also a significant group factor effect and a significant interaction of group factor and repeated measurement. Post hoc analysis only in the first WAnT showed significantly higher mean (17.5%, p<0.01), peak power (12.9%, p<0.01) and mean (14.1%, p<0.01) and peak power normalized to body mass (10.2 % p<0.05) in boxers compared to controls (Figure 1).

Two-way repeated measure analysis (two-way ANOVA) showed a significant repeated measure effect – before and 24 hours after maximal anaerobic exercise (Table 3). Regardless of the group division, a significant increase in CK (creatine kinase) was observed 24 hours after 2 ×30sWAnT test (36.6%, p<0.01).

A significant effect of the 2×30sWAnT was detected on all analysed biochemical inflammation markers (IL-10, Il-15 and TNF-α) except of IL-6. Indicating their highest concentration 60 min after WAnT (Table 4). Regardless of the point of blood collection, post hoc results showed significantly higher levels of IL10 (29.1%, p<0.01) and IL-15 in (10.9%, p<0.01) boxers compared to the physical active man group. An inverse relationship was shown by TNF-α whose concentration values were significantly lower in semi-professional boxers (44.2%, p<0.01). The two-way ANOVA also showed a significant interaction of group factor and repeated measure for Il-10, Il-15 and TNF-α.

Post hoc results revealed that while IL10 and IL15 concentrations did increase immediately (20.6%, p<0.01, IL-10; 9.4%, p<0.01, IL-15) and 60 min after (28.3%, p<0.01, IL-10; 15.7%, p<0.01, IL-15) 2×30sWAnT in the

Table 2. Two-way (two groups × two repeated measurements) ANOVA tests for the 2 × anaerobic exercise in boxers (n = 17) and control group (n = 14).

Variable	Effect	F	df	p	Effect size (η^2)	Post hoc outcome
Mean power	GR RM GR × RM	14.53 204.66 12.57	(1. 29) (1. 29) (1. 29)	<0.01** <0.01** <0.01**	0.35 0.88 0.32	B > C 1-WAnT > 2-WAnT $B^{1\text{-WAnT}} > C^{1\text{-WAnT}}$
Peak power	GR RM GR × RM	7.39 511.01 7.89	(1. 29) (1. 29) (1. 29)	0.01* <0.01** <0.01**	0.22 0.95 0.23	B > C 1-WAnT > 2-WAnT $B^{1\text{-WAnT}} > C^{1\text{-WAnT}}$
Mean power body mass normalized	GR RM GR × RM	5.32 204.01 11.70	(1. 29) (1. 29) (1. 29)	0.02* <0.01** <0.01**	0.16 0.88 0.31	B > C 1-WAnT > 2-WAnT $B^{1\text{-WAnT}} > C^{1\text{-WAnT}}$
Peak power body mass normalized	GR RM GR × RM	2.83 562.42 6.92	(1. 29) (1. 29) (1. 29)	0.10 <0.01** 0.01*	0.09 0.78 0.21	1-WAnT > 2-WAnT $B^{1\text{-WAnT}} > C^{1\text{-WAnT}}$

GR group, **RM** repeat measure, **1-WAnT** first 30s Wingate Anaerobic Test, **2-WAnT** second 30s Wingate Anaerobic Test, **B** boxers, **C** control group, *significant differences at p<0.05, **significant differences at p<0.01

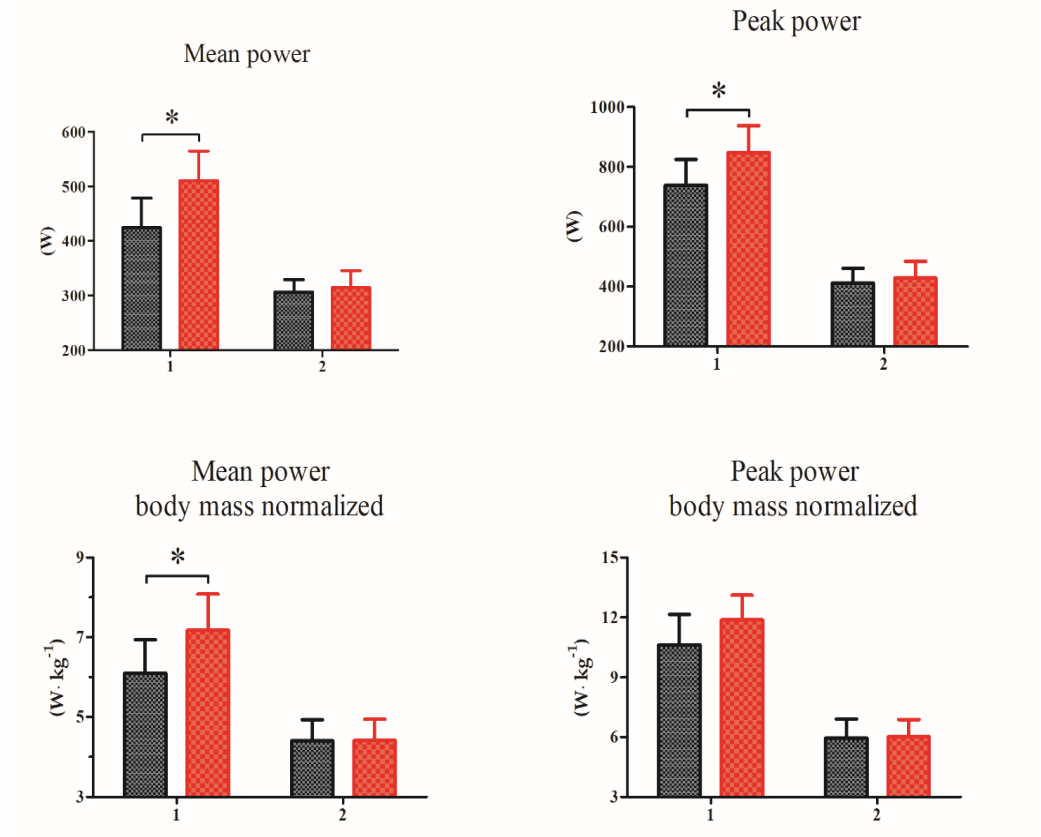

Figure 1. Characteristics of anaerobic capacity in 17 semi-professional boxers (red colour) and 14 physical active man (black colour): vertical bars inform about mean and standard deviation, while *p<0.01 difference between boxers and control group; 1 – first 30s Wingate Anaerobic Test, 2 – second 30s Wingate Anaerobic Test

Table 3. Two-way (two groups × two repeated measurements) ANOVA tests for the creatine kinase induced anaerobic exercise in boxers (n = 17) and control group (n = 14).

Variable	2×30sWAnT			
	before (baseline) mean & SD		24 hours after mean & SD	
Creatine Kinase	91.58 ±24.24	76.35 ±16.45	128.29 ±9.95†	133.71 ±29.92†

WAnT Wingate Anaerobic Test, † significant difference vs. baseline at p<0.01

semi-professional boxers group, these changes were not significant in the physical active man – control group (Figure 2). In contrast to IL10 and IL15, TNF-α concentrations showed a significantly greater concentration immediately after (26.6%, p<0.01) and 60 min after (44.8%, p<0.01) 2×30sWAnT in the control group compared to the boxers (Figure 2).

Table 4. Two-way (two groups × four repeated measurements) ANOVA tests for the inflammation markers induced lower body anaerobic exercise in boxers (n = 17) and control group (n = 14).

Variable	Effect	F	df	p	Effect size (η^2)	Post hoc outcome
IL-6	GR RM GR × RM	3.16 90.03 3.54	(1. 29) (1. 29) (3. 87)	0.09 <0.01** 0.02*	0.09 0.75 0.11	III > I, II, IV; I > II
IL-10	GR RM GR × RM	24.27 16.23 6.11	(1. 29) (1. 29) (3. 87)	<0.01** <0.01** <0.01**	0.45 0.35 0.17	B > C III > I, II, IV; I > II $B^{II, III, IV} > C^{II, III, IV}$
IL-15	GR RM GR × RM	7.87 14.74 7.48	(1. 29) (1. 29) (3. 87)	<0.01** <0.01** <0.01**	0.21 0.33 0.20	B > C II, III > I, IV; II > IV $B^{III,} > C^{III}$; $B^{IV} > C^{IV}$
TNF-α	GR RM GR × RM	16.69 108.24 28.67	(1. 29) (1. 29) (3. 87)	<0.01** < 0.01** <0.01**	0.36 0.78 0.49	B < C II, III > I, IV; II < III; I < IV $B^{II, III} < C^{II, III}$

IL-6 interleukin 6, **IL-10** interleukin 10, **IL-15** interleukin 15, **TNF-α** tumour necrosis factor alpha, **GR** group, **RM** repeat measure, **B** boxers, **C** control group, **I** rest value, **II** 5 minutes after the 2×30sWAnT (Wingate Anaerobic Test), **III** 60 minutes after the 2×WAnT, **IV** 24 hours after 2×WAnT, *significant differences at p<0.05, **significant differences at p<0.01

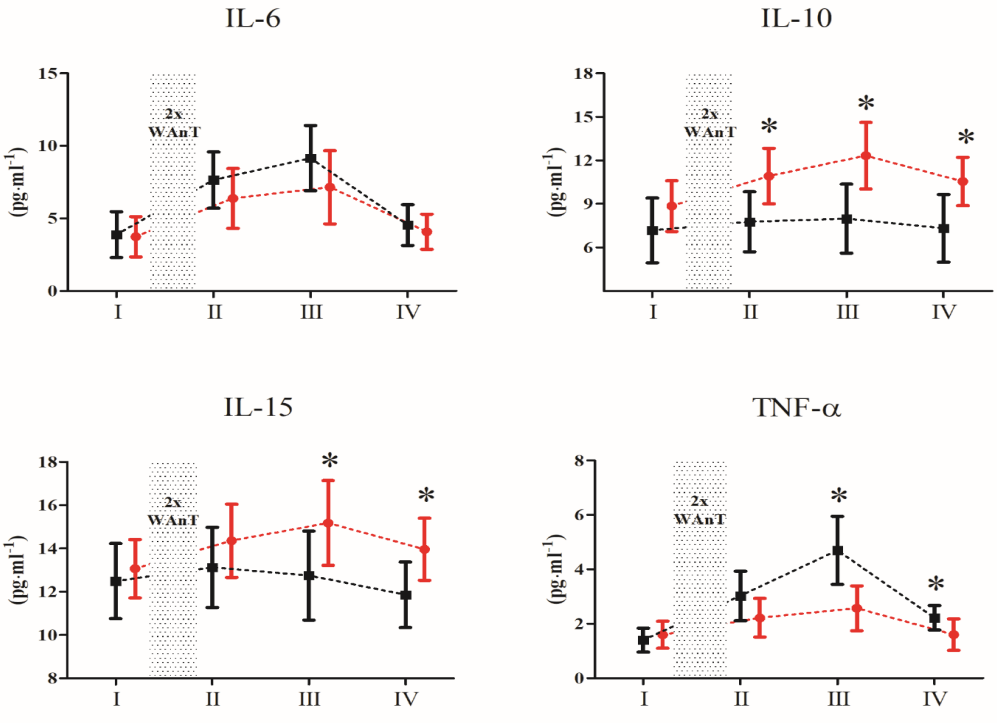

Figure 2. Changes in serum concentration of inflammation markers induced anaerobic exercise in boxers (red coulor) and control (black coulor): vertical bars inform about mean and standard deviation, **IL-6** interleukin 6, **IL-10** interleukin 10, **IL-15** interleukin 15, **TNF-α** tumour necrosis factor alpha, **I** rest value, **II** 5 minutes after the 2×30sWAnT (Wingate Anaerobic Test), **III** 60 minutes after the 2×WAnT, **IV** 24 hours after 2×WAnT, *significant differences at p<0.05, **significant differences at p<0.01

DISCUSSION

Sport training is associated with many positive effects on body functioning [10-12]. On the other hand, long-lasting professional training may lead to some potential threats for the athlete's health. It is mostly due to the training discipline specificity, the conditions in which training sessions and sport matches are being performed and the physiological and biochemical changes that occur during sport training and rivalry [13]. A high-level of a boxing match requires well-built technical, tactical, and physical skills but also many years of adaptations to training process [2]. It can give opportunity not only to perform according to the technique but also to resist the physiological demands during sport rivalry.

In the literature there is still limited data on the effect of various combat sport training on physiological response and biochemical status, especially in context of a redox homeostasis, organ functioning and gene expression [1, 14-16].

In the presented work, we hypothesized that WAnT results would be higher after double lower body exercise for all indicators in semi-professional boxers, both after a single and multiple performance of the anaerobic testing. This hypothesis was not fully correct. One of the results (peak power normalized to body mass) did not show statistically significant differences between populations after first WAnT (Table 2). Despite this, semi-professional boxers comparing to physical active men (controls) had significantly greater peak and mean power and relative mean power in the lower body double. Such WAnT assessment, indicated specific adaptation of anaerobic capabilities that developed during boxing training. Similar sport specific anaerobic adaptations were also observed in populations of athletes training in judo [17] and wrestling [18]. In contrast, anaerobic capabilities of the second lower body WAnT appeared in both populations very similar. Semi-professional boxers and physical active man recorded a clear decrease in all tested exercise indicators, which may indicate a very difficult course of the test and high fatigue in both populations. Furthermore, their results were not significantly different from each other. But it is worth to notice that observed in first WAnT test results in population of boxers was statistically much higher, so there is a possibility that their physiological fatigue was much greater, therefore they were unable to maintain exercise intensity at a higher level during second testing.

Delving further into the complexities of adaptations induced by boxing training, we find out that even less data we can find about the effect of long-lasting boxing training on a biochemical response to a demanding physical exercise. Herein, our results indicated that many years of semi-professional boxing training caused significant changes in post exercises inflammatory indicators. We have observed that there is a direct link between semi-professional boxing training adaptation and post exercises cytokines secretion, in particular IL-10, IL-15 and, TNF-a [19, 20].

We hypothesized that other cytokines will show changes in the relation of boxing training adaptation but IL-6, that play a central role in the activation of cytokine cascade and regulation of energy metabolism during exercise [21, 22] reacted only on the type of the exercises, not showing statistically significant changes in both populations. It may be associated with the fact that both populations were physical active and the role of IL-6 in human body is very specific and affects many biological systems (ex. regulation of energy use, anti-inflammatory activity and many other) so even the amount of physical activity that was realized by the controls was enough for its adaptation.

Strenuous exercise during 2×30sWAnT induced an increase in the pro-inflammatory cytokines TNF-a in both populations, but the increase in physical active man was much higher, comparing to the boxer's population. Furthermore, we observed as well a statistically significant rise in anti-inflammatory cytokine IL-10, IL-15, which may restrict the magnitude of the inflammatory response to exercise [23, 24] .

Consistently, in the present study, serum levels of both pro-inflammatory cytokines (TNF-a) and anti-inflammatory cytokine (IL-6, IL-10, IL-15) were significantly increased after the anaerobic exercises.

On the other hand, we also showed that muscle damage biomarkers such as creatine kinase was a bit higher before 2×30sWAnT testing in semi-professional boxing population. It may be associated with the amount of the physical activity during boxing sport training. On the other hand, we have to have in mind that this difference did not achieve statistical significance so we shouldn't take far-reaching conclusions from it.

Post exercise increase in creatine kinases concentration in both populations can be considered as an indicator of muscle and tissue damage induced by 2x30sWAnT. Observed change was similar in semi-professional boxers and physical active man so we can conclude that this result indicates the effectiveness of used testing method as a post exercises tissue damage tool. It is worth to notice that during such type of testing in many studies it is quite difficult to force people who do not train professionally to maximum effort that is needed during maximal testing. In our case we observed changes in most of the measured indicators that were associated with the physical effort during WAnT testing so we can conclude that participants performed testing correctly.

Summing up the whole analysis, we can conclude that even in case of few redox homeostasis indicators, we can see that semi-professional boxers show much higher adaptation status in inflammation process regulation associated with the physical activity.

The study had some limitation; although we presented acute effects of 2×30sWAnT exercises on a boxing training adaptation in field of post exercises induced inflammation process it should be noted that we selected only few of well-known indicators which may not fully show the complexity of the adaptation process induced by many years of training.

It would be necessary to extend the research both in terms of the analysed population size and the examined variables, illustrating both the positive and negative aspects of many years of sports training.

CONCLUSIONS

We showed that boxing training has significant, positive effects on the physiological and biochemical variables under research. This impact may be the result of participating in a boxing training program regularly, which declares that boxing exercises induce changes in various physiological and biochemical indicators. Observed post exercises changes may point out high-energy turnover and show that during long-lasting sport training, body of a sportsmen adapts to repeated situations inducing inflammation process. It's increasing all mechanisms associated with post exercise inflammation in field of homeostasis protection, so despite the fact that intensive physical activity induces inflammation process by its self-there are many positive aspects of this situation.

Furthermore, the present findings suggest that a counter regulation for biological adaptation during a boxing performance may occur through a pro- and anti-inflammatory cytokine production., essential for the body homeostasis.

In conclusion, detecting relationships associated with the effects of training adaptations on physiological and biochemical aspects adding new dimensions that can assist in evaluating, directing and developing boxing training programs not only for the professional sport but also for the amateur boxing training.

Our findings on boxing training induced adaptations could be valuable information about health-promoting aspects of this sport discipline training.

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