Ethno Med, 10(1): 1-5 (2016)
PRINT: ISSN 0973-5070 ONLINE: 2456-6772
DOI: 10.31901/24566322.2016/10.01.01

Effects of Acute Exercise on Serum Cytokine Composition in Elite Boxers: Th1/Th2/Th17 Balance

Numan Alpay

Schools of Sports and Education, Gazi University, Ankara, Turkey
Telephone: 0905322379098, E-mail: numanalpay@gmail.com

KEYWORDS Exercise. Boxers. Cytokine. Immune Balance

ABSTRACT The purpose of this study is to investigate effects of heavy exercise such as a boxing match on serum cytokine composition of elite boxers. Blood samples were collected from boxers before and after a 3-round of boxing match. Cytokines were assessed by high sensitive ELISA. Acute traumatic exercise increased serum levels of IL-6, TNFa, and IL-10 whereas no significant change was observed for serum levels of IFN-g, IL-4, and IL-17A. To the best of the researcher's knowledge, this is the first report to investigate Th1/Th2/Th17 balance in heavily trained boxers. These findings suggested that strenuous exercise did not affect IL-17 secretion during exercise. In conclusion, alterations in serum cytokine composition of elite boxers could be important to have a properly working immune system against to infectious agents.

INTRODUCTION

Immune system is the main defense system of human body against infectious agents (Parkin and Cohen 2001; Alam1998). Cytokines are hormone-like glycoproteins that play very important roles in regulation of the immune system (Tsokos et al. 1997; Rankin 2004). T helper (Th) cells have a crucial role in the immune response. Plasticity of Th cells is known to regulate cellular and humoral immunity. As reported, Th1 cells regulate cellular immunity via production of interleukin (IL)-2 and interferon (IFN)-g, whereas Th2 cells regulate humoral immunity via production of IL-4, IL-5 and IL-13 (Abbas et al.1996; Zhang et al. 2014). Th17 cells, a third Th cell lineage, with their capacity to secrete IL-17 but not IFN-g or IL-4 regulate inflammation by their production of distinct cytokines such as interleukin (IL)-17 (Harrington et al. 2005; Park et al. 2005).

Pro-inflammatory cytokines such as interleukin -6, tumor necrosis factor alpha (TNFa) take place in inflammatory processes during initiation of inflammatory reactions. Interleukin-10 (IL-10) and tumor growth factor beta (TGFb), as anti-inflammatory cytokines, on the other hand, inhibit inflammatory reactions (Cavaillon 2001; Dinarello 1997). Such regulations are particularly crucial factors for the immune system of athletes, because it has been suggested by many studies that athletes are more susceptible to minor infections (Peake 2005). It seems that immune cell functions are temporarily impaired due to prolong continuous heavy exercise (Nieman 1994; Pedersen 1995), therefore, sore throats and flu-like symptoms are more common in athletes when compared to general population (Gleeson 2007).

Boxing is an intermittent sport characterized by high intensity short duration, and bursts of activity. Therefore, it is an intense and strenuous sportive activity. Boxing is determined as 20-30 percent aerobic and 70-80 percent anaerobic (Smith 2006; Khanna 2006). Based on these characteristics, boxers should be more susceptible to minor infections. Although there are many studies regarding negative effects of heavy exercise on the immune system of athletes such as, swimmers, long distance runners, and soccer players, almost no study about boxers' immune system exist in the literature (Malm 2004; Mackinnon 1994; Alpay 2014). As to cytokines produced by Th17 cells, there have been a limited number of studies so far. Perry et al. reported that exercise induced an elevation in Th17 cell counts, while Tregs levels diminished, both in healthy athletes and in patients with chronic lymphocytic leukemia (CLL) (Perry et al. 2012; Perry et al. 2013).

The purpose of this study is to investigate for serum cytokine composition, in particular for

Address for correspondence:
Dr. Numan Alpay
Gazi University, School of Sports and Education, Ankara, Turkey
Telephone: 0905322379098
E-mail: numanalpay@gmail.com

IL-17 levels, of elite boxers before and after a boxing match. Results of this study were discussed with comparison of other sports activities.

METHODOLOGY

Participants and Experimental Protocol

A total of 20 elite male boxers (n= 20) participated in this study. These participants were from Turkish National Boxer Team. Physical features of the participating athletes were shown in Table 1. The participating athletes were informed about the aim and any possible risk of the study before they signed a written consent. Certification of the study protocol was obtained from The Local Ethics Committee and experiments were performed according to the Declaration of Helsinki. Participating elite boxers were asked to complete a three-round of boxing match during the National Championship in 2014. Each round was 3 minutes. Boxers spent a minute rest period between each round.

Collection of Blood Samples

Blood specimens were collected from the participating boxers before and immediately after a three-round of boxing match in vacutainer tubes (BectonDickinson, Franklin Lakes, NJ, USA). Each sample was centrifuged at 2000 rpm for 15 minutes in an Allegra® X-15R centrifuge (Beckman & Coulter, USA). Serum was separated and placed in 15 ml tubes. Serum samples were stored in a deep freezer (Sanyo, Japan) at -85 ^{o}C until use.

Enzyme-linked Immuno Sorbent Assay (ELISA)

Cytokines, interleukin-4 (IL-4), IL-6, IL-10, IL-17A, interferon gamma (IFN-g), and tumor necrosis factor alpha (TNFa) were quantified by using commercially available high sensitive ELISA kits. Tests were performed according to the manufacturer's recommendations (eBioscience, CA, USA). Briefly, serum samples were placed in wells of ELISA plates, and incubated in the presence of biotin-labeled conjugate for 2 hours at room temperature followed by a 1-hr incubation with HRP-conjugated streptavidin. Measurements were performed at 450 nm by using a microplate reader (BioTek Instruments Inc. Winooski, VT, USA). All samples were tested in duplicates.

Statistics

Statistics were performed by using two-way analysis of variance (ANOVA). Student's t-test was applied for paired samples. $P<0.05$ was considered statistically significant. Data were expressed as mean ± SD.

RESULTS

Twenty elite boxers were included in the present study because of their physical characteristics and experiences as elite boxers. The boxers were 49-110 kg and 20-30 years of age. They had at least 3-year experience in boxing. The mean values for the physical characteristics of those boxers were shown in Table 1.

Table 1: Mean physical characteristics of participating elite boxers

Age	*Weight (Kg)*	*Height (cm)*
24.35 ± 4.74* (20-30)	71.3 ± 14.93 (49 - 110)	173.7 ± 8 (162 - 190)

n = 20, *: SD

Circulating cytokine composition was determined before and after exposing to a physical activity and trauma by a 3-round of boxing match. Pro-inflammatory cytokines, IL-6 and TNF-a increased dramatically in circulation of boxers after boxing games (Fig. 1).

IL-10, an anti-inflammatory cytokine increased significantly whereas another anti-inflammatory cytokine, IL-4 levels were not altered (Fig. 2).

Likewise, there was no significant change in the Th1 cytokine, IFN-g and Th17 cytokine, IL-17A after the boxing games (Fig. 3).

DISCUSSION

In this study, effects of heavy exercise and trauma on serum cytokine levels in elite boxers were investigated. Cytokine levels that were measured before and after a 3-round of boxing match were compared.

A large number of investigators have reported that athletes under influence of prolonged, continuous heavy exercise are more susceptible to minor infections (Peake et al. 2005; Gleeson

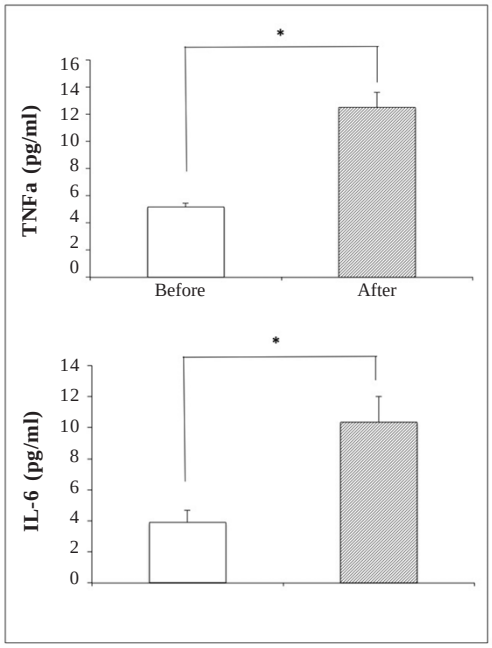

Fig. 1. Serum levels of proinflammatory cytokines, IL-6 and TNFa
Cytokine levels ware tested by ELISA before and after 3-round of boxing match *p<0.05

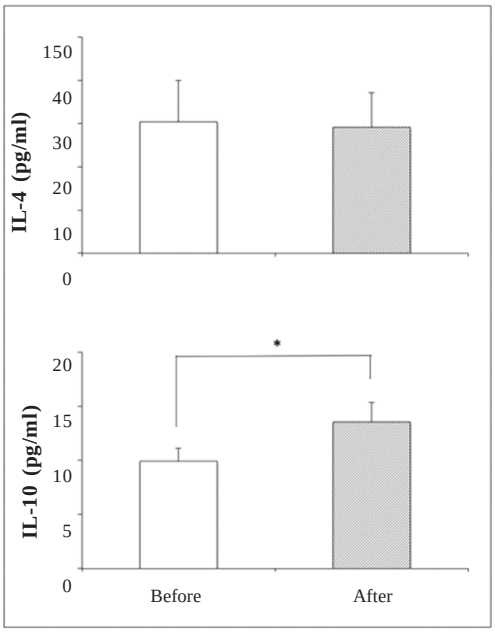

Fig. 2. Serum levels of anti-inflammatory cytokines, IL-4 and IL-10
Cytokine levels were tested by ELISA before and after 3-round of boxing match *p<0.05

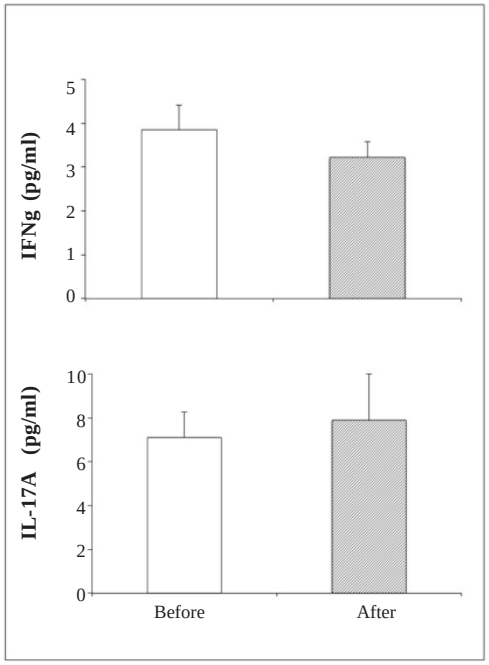

Fig. 3. Serum levels of Th1 cytokines, IFN-g and IL-17A
Cytokine level was tested by ELISA before and after 3-round of boxing match *p<0.05

2007). For example, some minor infections such as sore throats and flu-like symptoms are more common in athletes than in the general population and these infections may be prolonged in athletes compared with normal sedentary people (Gleeson 2007). Most of the studies regarding anti-inflammatory effects of exercise have been achieved in elite athletes such as swimmers, marathon runners, and soccer players. Boxers are exposed to heavy training conditions as well as trauma (Smith 2006; Khanna 2006; Malm 2004; Mackinnon 1994; Alpay 2014). Very few studies investigated negative influences of exercise on the immune system of boxers.

After boxing game, IL-6 increased in boxer's serum. The researcher's results agree with other studies formerly done. Serum IL-6 levels increase dramatically in people who are influence of trauma (Northoff et al. 1998). Serum IL-6 levels increase in athletes as a result of muscle injury during acute endurance exercise (Starkie et al. 2001; Steensberg et al. 2000). Boxers receive many hits to various parts of their body during a boxing match. These hits plus physical activity contribute muscle injury that may cause secretion of IL-6 from tissue cells such as muscle cells

and fibroblasts, and from immune system cells (Alpay 2014; Sacheck et al. 2006). Most probably, accumulation of those effects resulted in increase of serum levels of IL-6.

TNFa increased in circulation of boxers. It has been reported that TNFa might increase in human blood during exercise (Bernecker et al. 2013; Moldoveanu et al. 2000). Moldeveanu et al. (2000) reported that TNFa at protein level increased in blood during prolonged endurance exercise. However, they also found that mRNA of TNFa did not change in PBMC of those athletes during prolonged endurance exercise. Therefore, they speculated that leucocytes in blood circulation were not the source of elevated TNFa during the exercise.

In the present study, IL-10 increased in circulation of boxers. IL-10 is an anti-inflammatory cytokine. It usually increases during inflammatory responses such as tissue injury or infections to regulate local immune responses. Therefore, in this case, IL-10 increase would be a part of normal regulatory processes in response to elevation of pro-inflammatory cytokines (Dinarello 1997; Peake et al. 2005).

Function of IL-17 has been published in the last decade. Therefore, studies regarding IL-17 production during exercise have not been established completely. The present study showed that heavy exercise did not change IL-17 levels in circulation of elite boxers. In a recent comparative study, Tofighee et al. (2014) showed that IL-17 did not increase after intense exercise in different groups of athletes. Therefore, results of that study supported the results found in the present investigation. In another study reported recently, Kakanis et al. (2014) suggested that endurance exercise in ten highly trained road cyclists resulted in post-exercise changes in concentrations of plasma cortisol, IL-2, TNF, IL-4, IL-6, IL-10, and IL-17A compared with pre-exercise values. However, these changes were observed at 4-24 hrs post-exercise measurements. The present data represents results of circulating cytokine levels right after the boxing match (probably in 30 minutes). Therefore, results of the present study regarding IL-17A production may not be similar due to the time difference. In fact, Kakanis et al. (Kakanis et al. 2014) concluded that changes in concentrations of serum cytokines begun to appear at the 4th hour after endurance exercise.

CONCLUSION

In conclusion, it seemed that Th1/Th2/Th17 balance was not affected by the traumatic exercise in elite boxers. On the other hand, acute traumatic exercise led to increased serum levels of IL-6, TNF-a, and IL-10. However, these results may not be applied all the other boxers or athletes in other sports branches. Additional experiments are required to have a definitive final conclusion.

REFERENCES

Abbas AK, Murphy KM, Sher A 1996. Functional diversity of helper T lymphocytes. *Nature*, 383: 787–793.

Alam R 1998. A brief review of the immune system. *Prim Care*, 25(4): 727-738.

Alpay N 2014a. Comparison of the results of some hematological parameters in wrestling coaches. *International Journal of Sport Studies*, 4(9): 1131-1136.

Alpay N 2014b. Effects of vitamin E application on IFN-γ, TNF-α, IL-2 and IL-6 levels in elite taekwondo athletes. *Journal of Basic and Applied Scientific Research (JBASR)*, 4(9): 90-93.

Bernecker C, Scherr J, Schinner S, Braun S, Scherbaum WA, Halle M 2013. Evidence for an exercise induced increase of TNF-α and IL-6 in marathon runners. *Scand J Med Sci Sports*, 23(2): 207-214.

Cavaillon JM 2001. Pro-versus anti-inflammatory cytokines: Myth or reality. *Cell Mol Biol (Noisy-le-grand)*, 47(4): 695-702.

Dinarello CA 1997. Role of pro- and anti-inflammatory cytokines during inflammation: Experimental and clinical findings. *J Biol Regul Homeost Agents*, 11(3): 91-103.

Gleeson M 2007. Immune function in sport and exercise. *J Appl Physiol*,103: 693–699.

Harrington LE, Hatton RD, Mangan PR, Turne H, Murphy TL, Murphy KM, Weaver CT 2005. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat Immunol*, 6: 1123–1132.

Kakanis MW, Peake J, Brenu EW, Simmonds M, Gray B, Marshall-Gradisnik SM 2014. T helper cell cytokine profiles after endurance exercise. *J Interferon Cytokine Res*, 34(9): 699-706.

Khanna GL 2006. Manna I. Study of physiological profile of Indian boxers. *J Sports Sci Med*, 5: 90-98.

Mackinnon LT 1994. Current challenges and future expectations in exercise immunology: Back to the future. *Med Sci Sports Exerc*, 26(2): 191-194.

Malm C 2004. Exercise immunology: The current state of man and mouse. *Sports Med*, 34(9): 555-566.

Moldoveanu AI, Shephard RJ, Shek PN 2000. Exercise elevates plasma levels but not gene expression of IL-1beta, IL-6, and TNF-alpha in blood mononuclear cells. *J Appl Physiol*, 89(4): 1499-1504.

Nieman DC 1994. Exercise, infection and immunity. *Int J Sports Med*, 15: S.131–141.

Northoff H, Berg A, Weinstock C 1998. Similarities and differences of the immune response to exercise

and trauma: The IFN-g concept. *Can J Physiol Pharmacol,* 76: 497–504.
Park H, Li Z, Yang XO, Chang SH, Nurieva R, Wang YH, Wang Y, Hood L, Zhu Z, Tian Q, Dong C 2005. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat Immunol,* 6: 1133–1141.
Parkin J, Cohen B 2001. An overview of the immune system. *Lancet,* 357(9270): 1777-1789.
Peake JM, Suzuki K, Hordern M, Wilson G, Nosaka K, Coombes JS 2005. Plasma cytokine changes in relation to exercise intensity and muscle damage. *Eur J Appl Physiol,* 95: 514-521.
Pedersen BK 1995. Bruunsgaard H. How physical exercise influences the establishment of infections. *Sports Med,* 19: 393–400.
Perry C, Pick M, Bdolach N, Hazan-Halevy I, Kay S, Berrl I, Reches A, Herishanu Y, Grisaru D 2013. Endurance exercise diverts the balance between Th17 cells and regulatory T cells. *Plos One,* 8(10): e74722.
Perry C, Herishanu Y, Hazan-Halevy I, Kay S, Bdolach N, Naparstek E, Polliack A, Grisaru D 2012. Reciprocal changes in regulatory T cells and Th17 helper cells induced by exercise in patients with chronic lymphocytic leukemia, *Informa Healthcare, Leukemia and Lymphoma,* 53(9): 1807–1810.
Rankin JA 2004. Biological mediators of acute inflammation. *AACN Clin Issues,* 15(1): 3-17.
Sacheck JM, Cannon JG, Hamada K, Vannier E, Blumberg JB, Roubenoff R 2006.Age-related loss of associations between acute exercise-induced IL-6 and oxidative stress. *Am J Physiol Endocrinol Metab,* 291(2): E340-349.
Smith MS 2006. Physiological profile of senior and junior England international amateur boxers. *J Sports Sci Med,* (CSSI): 74-89.
Starkie RL, Rolland J, Angus DJ, Anderson MJ, Febbraio M 2001. Circulating monocytes are not the source of elevations in plasma IL-6 and TNF-a levels after prolonged running. *Am J Physiol Cell Physiol,* 280: C769–C774.
Steensberg A, Van HG, Osada T, Sacchetti M, Saltin B, Pedersen BK 2000.Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6. *J Physiol,* 529: 237–242.
Tofighee A, Khazaei HA, Jalili A 2014. Comparison of effect of one course of intense exercise (Wingate test) on serum levels of Interleukin-17 in different groups of athletes. *Asian J Sports Med,* 5(4): e22769.
Tsokos GC, Kovacs B, Liossis SN 1997. Lymphocytes, cytokines, inflammation, and immune trafficking. *Curr Opin Rheumatol,* 9(5): 380-386.
Zhang Y, Zhang Y, Gu W, He L, Sun B 2014. Th1/Th2 cell's function in immune system. *Adv Exp Med Biol,* 841: 45-65.

Paper received for publication on July 2015
Paper accepted for publication on November 2015