

## FROM TRAINING CAMP TO LAB: BIOCHEMICAL PROFILING IN PROFESSIONAL BOXING

**Dr. Mehmet Akif Yılmaz, Prof. Ayşe Seda Demir and Mr. Mustafa Cemal Oğuz**

Ataturk University, Faculty of Medicine, Department of Medical Biochemistry, Erzurum, Turkey

Ataturk University, Physical Education and Sports High School, Erzurum, Turkey

Ataturk University, Kazim Karabekir Education Faculty, Department of Physical Training and Sports, Erzurum, Turkey

### ABSTRACT

Boxing, one of the oldest and most contentious sports, involves short yet intense bursts of activity, often leading to injuries in the head, neck, face, and hands. Neurological dysfunction stemming from brain trauma is prevalent among boxers. High-intensity exercise and its traumatic effects have prompted extensive research, delving into enzyme activities, lipid profiles, stress markers, brain injury biomarkers, pituitary functions, and more. This study, building on prior research, sought to assess specific biochemical parameters in boxers before and after training sessions.

**Keywords:** Boxing, High-intensity exercise, Traumatic brain injury, Biochemical parameters, Neurological dysfunction

### 1. Introduction

Boxing is one of the oldest sports known to mankind and it is controversial. This sport is characterized by short duration, high intensity bursts of activity. Since it is a combat sport, serious injuries may occur on the head, neck, face, and hands during fighting. Additionally, neurological dysfunction depending on brain trauma is very common in boxers. On the subject of high intensity exercise and its traumatic effects, various studies were performed by researchers to investigate a number of issues including enzyme activities, lipid and lipoprotein profiles, stress and cellular brain injury biomarkers, pituitary functions, endocrine and neuroendocrine dysfunctions, calcium, phosphorus and trace elements. (Saengsirisuwan et al., 1998; Khanna and Manna, 2006; Chatterjee et al., 2007; Karakucuk et al., 2013; Lukaski, 1995; Jordan, 2000; Tanriverdi et al., 2008; Nindl and Pierce, 2010) The aim of this study was to measure some biochemical parameters in boxing sportsmen before and after training.

### 1. Methods

#### 1.1. Participants

Twenty professional healthy male boxing sportsmen (age range: from 16 to 30 years and BMI range: from 19.0 to 26.8) were included in the study, who were students in Ataturk University. A detailed boxing and self medical history were obtained for each participant. The selected subjects had no pituitary, neurological or psychiatric disorders, and no medications. Sporters had taken regular exercise

for at least 3 years at frequency of at least three days per week. Mean duration of boxing in sporters was  $3, 42 \pm 1,5$  years (range 1-10). There was no significant difference between BMI indexes and ages of sporters ( $p > 0.05$  for both parameters).

### **1.2. Measurement of body composition**

The body composition measurements were performed with a Bio Impedance Analysis system device (Tanita TBF-300). Body mass (BM), body mass index (BMI), basal metabolism rate (BMR), impedance (IMPD), body fat percentage (BFAT %), body fat mass (BFMASS), lean body mass (LBM) and total body fluid (TBF) were measured by this device. Measurements were taken before and after exercise with the same device and method.

### **1.3. Measurement of biochemical parameters**

Two blood samples were taken from sporters: before training and 6 minutes after subjected to 30-minutes fighting training. All samples were taken in the morning hours. After serum separation, the following parameters were analyzed: aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), total cholesterol (CHOL), HDLcholesterol (HDL-C), LDL- cholesterol (LDL-C), triglycerides (TG), adrenocortico tropic hormone (ACTH), human growth hormone (HGH), insulin-like growth factor1 (IGF-1) and zinc (Zn). Serum ALP, AST, ALT, and GGT activities and total cholesterol, HDL-C LDL-C and TG levels were analyzed in Beckman Coulter AU5800 analyzer by the respective methods. GH, ACTH, and IGF-1 were analyzed at Siemens Immulite 2000 analyzer by the chemiluminescence method. Serum zinc levels were determined by atomic absorption spectrometry (Perkin Elmer AA850).

### **1.4. Statistical analysis**

Statistical analysis was performed using the SPSS 20.0 program (SPSS Inc., Chicago, IL, USA). Data were presented as mean and standard deviation ( $\text{mean} \pm \text{SD}$ ). Kolmogorov-Smirnov test was used for testing normality of data. The differences between the groups were compared by paired samples t-test. A difference was considered as significant when p value was less than 0.05 in 95 % confidence of interval.

### **1.5. Ethical Statement**

An institutional review board for human experimentation approved the protocol (Ataturk University Faculty of Medicine Ethics Committee, authorization number B.30.2.ATA.0.01.00/9). Informed consent was obtained from each participant and the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 1983.

### **1.6. Conflict of Interest**

The authors declare that there is no conflict of interest.

### **1.7. Previous Presentation**

This study was presented at the IFCC World Lab Congress in Istanbul, June 2014.

## **2. Results**

The mean age of sporters was  $20,35 \pm 3,32$  years (range 16-30) and the mean duration of boxing was  $3,42 \pm 1,5$  years (range 1-10). According to Kolmogorov-

Smirnov normality test all parameters were normally distributed ( $p > 0.05$ ). To analyze the differences between the groups, paired samples t-test was used. There were significant differences between pre-training and post-training levels of ACTH, AST, GGT, ALT, HGH-1 and IGF-1 ( $p < 0.05$  for each parameter). But there were no significant differences between pre-training and post-training levels of IMPD, cholesterol, HDL-C, TG, ALP, LDL-C and zinc ( $p > 0.05$  for each parameter).

Descriptive statistics of sporters' demographical characteristics before and after training and the significance of changes are given in Table 1.

**Table 1: Descriptive statistics of sporters' demographical characteristics and test statistics p values**

Demographical characteristics	Before training	After training	p value
BM	72.77 ± 11.79	72.16 ± 11.76	0.000
BMI	22,57±2,08	22,40±2,12	0.001
BMR	1796,40±157,56	1789,05±159,38	0.000
IMPD	479,30±54,33	475,70±51,09	0.221
BFAT %	8,05±3,52	7,60±3,73	0.006
BFMASS	5,84±3,19	5,52±3,33	0.005
LBM	64,20±6,94	63,96±6,81	0.039
TBF	47,00±5,08	46,82±4,99	0.017

(BM: body mass, BMI: body mass index, BMR: basal metabolism rate, IMPD:

impedance, BFAT %: body fat percentage, BFMASS: body fat mass, LBM: lean body mass, TBF: total body fluid) Descriptive statistics of sporters' biochemical parameters before and after training and the significance of changes are given in Table 2.

**Table 2: Descriptive statistics of biochemical parameters before and after training and test statistics p values**

Biochemical parameter	Before training		After training		p value
	Mean ± SD	Range	Mean ± SD	Range	
AST(U/L)	27,45±3,18	23,00-36,00	30,30±5,12	24,00-45,00	0,001
ALT (U/L H)	28,55±10,94	17,00-47,00	32,65±13,33	16,00-55,00	0,001
ALP(U/L)	115,60±38,72	69,00-221,00	119,10±25,25	83,00-171,00	0,674
GGT (U/L H)	24,45±10,45	11,00-55,00	27,05±12,54	14,00-64,00	0,004
CHOL (mg/dL)	169,85±48,05	29,00-273,00	185,05±37,12	143,00-283,00	0,121
HDL-C (mg/dL)	48,55±9,73	32,00-61,00	50,75±9,69	32,00-68,00	0,103
LDL-C (mg/dL)	122,45±30,19	77,00-202,00	126,80±31,79	87,00-212,00	0,237

TG (mg/dL)	180,90±77,62	43,00-352,00	186,70±83,71	52,00-420,00	0,536
ACTH ((pg/mL)	18,41±8,71	5,57-43,30	31,12±15,41	6,54-65,20	0,002
HGH (ng/ml)	1,42±1,94	0,05-6,17	8,03±8,41	0,05-36,79	0,002
IGF-1(ng/mL)	392,13±90,42	201,37-580,92	99,00±108,89	2,63-399,93	0,000
Zn (µmol/L)	112,05±28,38	59,00-166,00	118,75±28,99	77,00-118,75	0,191

(AST: aspartate amino transferase, ALT: alanine amino transferase, ALP: alkaline phosphates, GGT: gamma glutamyl transferase, CHOL: total cholesterol, HDL-C: HDL-cholesterol, LDL-C: LDL-cholesterol, TG: triglycerides, ACTH: adrenocorticotrophic hormone, HGH: human growth hormone, IGF-1: insulin-like growth factor-1, Zn: zinc).

### **3. Discussion**

Since there were no significant differences between demographical characteristics of sportsmen, the discussion of biochemical parameters becomes more reliable. The main findings of this study were as follows.

#### **3.1. AST, ALT, GGT**

Although ALT and AST tests may not be specific for the liver, plasma levels of these enzymes have generally been used to evaluate hepatocellular damage (Apple and McGue, 1983).

The serum levels of these enzymes may increase in some diseases and toxins affecting liver and in muscle degeneration (Haralambie 1973) or lipid peroxidation (Nikolaidis et al., 2008). Many studies have indicated that plasma enzyme activities increase after acute and chronic exercise. In a study conducted on twenty Thai boxing sportsmen, Saengsirisuwan et al. reported that serum AST and ALT levels of boxing sportsmen was significantly higher than those of age-matched healthy controls even during the period of normal training (Saengsirisuwan et al., 1998). These results point the effect of chronic exercise on plasma enzyme activities. Additionally, they reported increases in both amino transferases after a boxing match and consequently concluded that the reason of this increment was more likely to muscle injury, rather than liver cell damage.

GGT is a membrane-bound enzyme that has a critical role in glutathione homeostasis by initiating extracellular glutathione breakdown and it acts as a critical cellular antioxidant defense (Karp et al., 2001). Ayca et al. measured urinary GGT levels in 12 female and 12 male volleyball players before and 1 hour after the exercise. They reported insignificant exercise-induced increases in urinary GGT levels and considered this result were due to the relatively short-course of the exercise and the timing of post-exercise urine collection (Ayca et al., 2006).

As far as we are concerned, no studies have been done on serum GGT levels of boxing athletes, but there are few studies conducted on some other athletes investigating serum (Diaz et al., 2010) and urinary GGT levels (Ayca et al., 2006; Shavandi et al., 2012). In a study conducted by Lowlor et al., researchers asked a physical activity questionnaire to a random sample of 3789 British women and measured serum GGT levels of participants. Researchers found an inversely linearly association between physical activity and serum GGT levels and they concluded that physical activity was independently associated with

higher levels of GGT (Lawlor et al., 2005). Normally, lower GGT levels are expected in sporters, but in our study, similar to Ayca and co-workers study, we found that acute exercise did not affect serum GGT levels. However, we suppose that if we had compared serum GGT levels of sporters with healthy sedentary controls, we could find lower serum GGT levels in sporters. This is a limitation of our study. Additionally, post exercise proteinuria and increased urinary GGT levels can be indicatives of exercise-induced renal damage (Shavandi et al., 2012). Further studies should include post-exercise urinary proteinuria parameters in order to investigate possible renal damage. In our study serum AST, ALT and GGT levels were significantly increased by training ( $p < 0.005$ ), whereas there was no significant difference between before and after training ALP serum levels ( $p > 0.05$ ). Taken together serum AST, ALT and GGT results in our study, similar to Saengsirisuwan's conclusion, we suppose that our results point muscle injury. Additionally, one should consider the increased GGT levels to be due to increased bile duct stasis, and the increased GGT is not necessarily be caused by direct cell damage.

### **3.2. Lipid profile**

It is commonly suggested that the regular exercise positively affects plasma lipid profiles (Criqui, 1986; Cullinane et al., 1981). However, any change in the plasma lipid profile differs related to the type and level of exercise. Furthermore, there are some studies suggesting that acute exercise changes lipid parameters, and, according to common opinion, a positive change of plasma lipid profile is dependent on longterm and regular exercise. Additionally, physically fit and active individuals tend to have lower levels of lipids than less active individuals (Enger et al., 1997; Marti, 1991).

In a study conducted by Cardoso et al, it was reported that serum levels of HDL-C and the ratio of HDL-C to total cholesterol were increased in those players having more aerobic exercise in their training program (Cardoso Saldana et al., 1995). In another study, sporters exposed to more aerobic exercise are reported to have higher levels total cholesterol, triglycerides and LDL-C cholesterol (Khanna and Manna, 2006). In a study conducted by Chatterjee et al, plasma lipid profiles of 45 women boxers were measured at the beginning and end of the six weeks training camp. Researchers reported significant difference ( $p < 0.01$ ) in levels of total cholesterol, LDL-cholesterol, and HDL-cholesterol in the post-test. Researchers concluded that women who practice sport of boxing on regular basis had a favorable lipid profile (Chatterjee et al., 2007). In another study, plasma lipid profiles were examined in 127 elite athletes who were regularly training for over 3 years in different specialties of nine olympic sports including boxing. In this study researchers found that regular training was positively affected plasma lipid profiles in almost all sports types. Boxing athletes showed lower VLDL, TG and total cholesterol levels compared to sedentary controls (Tsopanakis et al., 1986).

In our study, there were no significant differences in serum levels of cholesterol, HDL-cholesterol, LDL-cholesterol and TG of boxing sporters before and after training. We can conclude that regular exercise positively affects plasma lipid profiles. Our study provides additional evidence to the conclusion that plasma lipid profile does not change by an acute exercise. Considering that aerobic exercise positively affects plasma lipid profile, we can conclude that the sportsmen in our study were exposed to anaerobic training at a higher rate.



### **3.3. Zn**

Zinc is an essential micronutrient for human health and has numerous structural and biochemical roles. Since zinc is required for the structure and activity of more than 300 enzymes in human organism, numerous important events, including regulatory actions, nucleic acid synthesis, glycolysis and carbon dioxide removal, may be impaired in its deficiency (Vallee and Falchuk, 1993).

Despite zinc has the potential for affecting physical performance with its numerous biochemical functions, there are limited data reporting direct relationship between zinc and physical performance. There are few studies reporting low circulating zinc concentrations in physically active adults (Lukaski, 2004).

In a study conducted by Karakukcu et al, significant decreases in serum zinc levels were reported after both acute (1-hour boxing training program) and chronic (4 weeks of regular boxing training program) exercise in young amateur boxing sporters<sup>4</sup>. In a study conducted on ten male volunteers who had taken regular exercise for at least six months on at least three days per week, the subjects were enrolled in two different acute training sessions. No significant differences in zinc values were observed after any of the sessions compared to control group (Rosa et al., 2011). According to Koury et al, zinc changes after physical exercise are directly related to intensity and length of exercise (Koury and Donangelo, 2003). Studies have reported significant decreases in zinc concentration after long-term (2-24 hours) exercise (Lukaski, 1995; Cordova and Alvarez-Mon et al., 1995). In our study no significant alterations were observed in the serum zinc levels probably due to the short duration of exercise.

### **3.4. HGH, ACTH:**

Traumatic brain injury (TBI) is a common result of combative sports, particularly of boxing, caused by repetitive blows on head and it is an important cause of hypopituitarism (Jordan, 2000). Twenty five - 50% of patients with TBI have been reported to have some degree of hypopituitarism (Schneider et al., 2007). In a study conducted in 44 competing and 17 retired boxers, growth hormone (GH) deficiency was reported in 9 of 61 boxers (15%) and in 8 of 17 retired boxers (47%), and ACTH deficiency was reported in 5 of 61 boxers (8%). In addition, significantly lower pituitary volume was measured in retired boxers with GH deficiency, compared with retired boxers with normal GH.

Researchers suggested that retired boxers have a high rate of pituitary dysfunction. Therefore, they recommended investigation of pituitary function in boxers, particularly in retired ones (Tanriverdi et al., 2008). In another study conducted on twenty-two amateur kick boxers and twenty-two age-matched healthy controls, two (9,1%) of the 22 kick boxers had ACTH deficiency when mean basal hormone levels were compared between kick boxers and the controls (Tanriverdi et al., 2007). In a study conducted by Tanriverdi et al., GH deficiency was reported in 21.9% of forty-one actively competing or retired male boxers (n: 27) and kick boxers (n: 14) (Tanriverdi et al., 2013).

ACTH levels significantly decreased by training in our study. As far as we investigated, our study is the first one reporting decreased ACTH levels by exercise in boxing sporters.

Sports related to TBI are important public health concern, with consequences ranging from physical disabilities to long-term cognitive, behavioral, psychological, and social defects. However, it is known that many TBI cases are unrecognized and untreated (Richmond and Rogol et al., 2014). Therefore, we

join the recommendation to investigate pituitary function of competing and retired boxers. It is well known that a greater public health benefit would be achieved by preventing potential consequences of TBI, in particular pituitary dysfunctions. Czirjak et al., recommend performing a complete hormonal investigation after one year of the trauma (Czirják et al., 2012). Patients who experience TBI like boxing athletes should be followed up carefully by physicians.

Repeated muscular exercise is known to have positive effects on endocrine system by several different mechanisms. Therefore, sportsmen and sport women are usually not expected to have abnormal endocrine functions (Duclos, 2001). Similar to common opinion, in our study none of the sporters showed GH deficiency before or after training. Nevertheless, we conclude that boxing sporters should be regularly followed up with respect of pituitary functions, due to exposing of repetitive trauma. Luger et al. reported increased plasma GH concentrations induced by acute exercise in three groups of healthy male volunteers (Luger et al., 1992). In accordance with this report, GH levels were significantly increased by training in our study. It is an expected result that exercise alters pituitary functions. It is considered that, this increase is associated with response to exercise stress.

### **3.5. IGF-1:**

The insulin-like growth factors (IGFs) are synthesized by almost all tissues and involved in many critical physiological processes (Le Roith, 1997). Because of their numerous biologic effects, the IGFs have been researched in many situations. Since sedentary lifestyle is a risk factor of insulin resistance and development of type 2 diabetes, exercise is theoretically thought to have a positive effect on IGF levels. However, intensive training in athletes may be related to unfavorable changes in secretion of adipose tissue hormones and may cause pathogenesis of hormonal disturbances observed in sporters (Plinta, 2013). Hereby, different results are reported in studies researching the effect of exercise on IGFs.

In a study conducted on nine trained adolescents having high physical activity and seven sedentary male adolescents, statistically increased IGF-1 levels was reported by exercise (Pareja-Galeano, 2013). The studies reported increased (Bang et al., 1990; Cappon et al., 1994), decreased (Eliakim et al., 1998; Smith et al., 1987), or unaltered (Fontana et al., 2006; Vitiello et al., 1997) IGF-1 levels after endurance or resistance exercises. These different responses of IGF axis hormones are attributed to the intensity, duration and type of exercise (Nishida et al., 2010). In a study conducted by Barnard et al. researchers reported decreased IGF-1 levels after acute exercise (Barnard et al., 2003). Since increased IGF-1 levels after exercise were observed in growth hormone-deficient subjects (Bang et al., 1990), increased IGF-1 levels after acute exercise are not considered to be related to increased levels of exercise-induced growth hormone (Nindl and Pierce, 2010). Serum IGF-1 level is generally reported to be increased in exercise, mostly in aerobic one. In our study, decreased serum IGF-1 levels support our conclusion that the boxing sportmen were exposed to anaerobic training at a higher rate.

### **4. Conclusion**

The results of this study suggest that boxing sport affects some substantial biochemical parameters, and all biochemical parameters must be carefully considered when evaluating physically active patients' laboratory testing results.

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