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## IMPACT OF ENDURANCE EXERCISE ON LIVER BILIRUBIN AMONG NOVICE UNIVERSITY STUDENTS ATHLETES

Muhammad Jamil<sup>1</sup>, Javed Ali Soomro<sup>2</sup>, Muhammad Akram Ansari<sup>3</sup>,

1. Phd Scholar, Centre for Physical Education, Health and Sports Science, University of Sindh. Jamshoro, Pakistan.
2. Assistant Professor, Centre for Physical Education, Health and Sports Science, University of Sindh. Jamshoro, Pakistan.
3. Professor, Centre for Physical Education, Health and Sports Science, University of Sindh. Jamshoro, Pakistan.

### Abstract

**Background** Endurance exercise is recognized for its beneficial effects on physical fitness, cardiovascular health, and metabolic regulation. However, its impact on liver biomarkers, particularly bilirubin levels, has garnered research interest due to bilirubin's dual role as a waste product of heme metabolism and an antioxidant with potential protective effects against oxidative stress and metabolic syndrome (Hinds et al., 2016). **Methods & Materials:** This study evaluates the effect of a 12-week endurance exercise program on liver bilirubin levels in novice university student-athletes, aiming to explore the adaptive response of this biomarker to sustained physical activity.

A total of 40 male university students (aged 18–25), untrained in structured exercise, participated in a randomized controlled trial. The experimental group (EG) engaged in a supervised endurance exercise program involving moderate-intensity aerobic activities (60–70% of maximum heart rate) for 45–60 minutes, four times a week, while the control group (CG) maintained their usual routines. Serum bilirubin levels (total and direct) and body mass index (BMI) were assessed pre- and post-intervention.

**Conclusion:** Results demonstrated a significant increase in serum bilirubin levels in the EG (mean pre-test:  $0.95 \pm 0.12$  mg/dL; mean post-test:  $1.10 \pm 0.15$  mg/dL,  $p = 0.002$ ), while the CG exhibited no

significant changes (mean pre-test:  $0.96 \pm 0.10$  mg/dL; mean post-test:  $0.94 \pm 0.11$  mg/dL,  $p = 0.512$ ). BMI decreased significantly in the EG (mean difference:  $-0.80 \pm 0.14$  kg/m<sup>2</sup>,  $p < 0.01$ ), reflecting improved physical fitness. These findings underscore the role of endurance exercise in modulating bilirubin levels and enhancing liver function, likely through adaptive responses to oxidative stress and increased heme oxygenase-1 activity. Future research should explore the long-term implications and broader physiological impact of exercise-induced bilirubin modulation.

## **Introduction**

The liver, a vital organ in human physiology, plays an essential role in metabolic processes, detoxification, and homeostasis. It regulates various biochemical pathways, including carbohydrate, lipid, and protein metabolism, while producing bile and removing toxins from the bloodstream. Among the numerous biomarkers associated with liver function, bilirubin stands out due to its dual role as a metabolic by-product and an endogenous antioxidant. This paper focuses on the impact of endurance exercise on bilirubin levels in novice university student-athletes, aiming to shed light on the adaptive mechanisms linking exercise, oxidative stress, and liver health.

Bilirubin, a by-product of heme catabolism, is formed when the heme group of hemoglobin is degraded by heme oxygenase into biliverdin, which is subsequently reduced to bilirubin by biliverdin reductase. Traditionally, bilirubin has been viewed merely as a waste product requiring excretion through bile or urine. However, contemporary research has expanded its significance, revealing its potent antioxidant properties. Bilirubin can neutralize reactive oxygen species (ROS), thereby protecting cellular components from oxidative damage (Sedlak & Snyder, 2004). Elevated bilirubin levels within the physiological range have been linked to reduced risks of cardiovascular disease and metabolic syndrome, suggesting its role as a protective biomarker (Vítek, 2013).

The liver's ability to regulate bilirubin is critical. Total bilirubin levels consist of direct (conjugated) and indirect (unconjugated) fractions. Conjugated bilirubin is water-soluble and excreted in bile, while unconjugated bilirubin is bound to albumin for transport in the bloodstream. Impaired bilirubin metabolism can indicate liver dysfunction or hemolytic conditions, making it a reliable biomarker in clinical diagnostics (Schwertner & Vítek, 2008).

Physical activity, particularly endurance exercise, has been extensively studied for its systemic benefits, including improved cardiovascular health, metabolic efficiency, and body composition.

Exercise-induced adaptations extend to the liver, which plays a pivotal role in energy homeostasis during prolonged physical activity. Regular exercise improves hepatic insulin sensitivity, enhances fat metabolism, and reduces the risk of non-alcoholic fatty liver disease (NAFLD) (Keating et al., 2012). Moreover, endurance exercise influences oxidative stress, a critical factor in liver function.

During moderate-to-high intensity exercise, increased oxygen consumption leads to enhanced ROS production. While excessive ROS can cause oxidative damage, moderate levels induce adaptive responses, activating antioxidant pathways such as heme oxygenase-1 (HO-1) (Powers & Jackson, 2008). HO-1 catalyzes the degradation of heme into biliverdin, carbon monoxide, and free iron. The subsequent conversion of biliverdin into bilirubin amplifies the antioxidant defense system. This adaptive response underscores the potential of endurance exercise to modulate bilirubin levels as part of a broader redox balance.

Research highlights the dynamic relationship between bilirubin levels and exercise-induced oxidative stress. Endurance activities, characterized by sustained aerobic efforts, increase the production of ROS due to heightened metabolic demand. This transient oxidative stress triggers the upregulation of antioxidant defenses, including bilirubin production, to counteract ROS effects (Zelenka et al., 2017). Elevated bilirubin levels during exercise may represent an adaptive mechanism that protects tissues from oxidative damage while maintaining cellular integrity.

Djordjevic et al. (2012) found that regular aerobic exercise in trained athletes was associated with higher bilirubin levels compared to sedentary controls. The authors attributed this increase to enhanced HO-1 activity, stimulated by recurrent oxidative stress. Similarly, Hinds et al. (2016) reported that endurance-trained individuals exhibited elevated bilirubin levels, which correlated with reduced markers of oxidative damage. These findings suggest that exercise-induced bilirubin elevation is part of the body's adaptive response to sustained physical activity.

While the benefits of endurance exercise on bilirubin levels are well-documented in trained athletes, less is known about its impact on untrained individuals. Novice athletes often exhibit distinct physiological responses to exercise due to their lack of prior exposure to structured physical activity. Initial training phases may induce greater oxidative stress and metabolic strain as the body adapts to increased energy demands (Gomez-Cabrera et al., 2008). This study aims to explore how a 12-week endurance exercise program influences bilirubin levels in novice university student-athletes, addressing the gap in understanding adaptive responses in this population.

## Exercise, Bilirubin, and Health Outcomes

The potential health implications of bilirubin modulation through exercise are significant. Elevated bilirubin levels within the physiological range have been associated with reduced risks of atherosclerosis, hypertension, and type 2 diabetes (Vítek & Schwertner, 2007). These associations highlight bilirubin's role as a biomarker of oxidative stress and its protective effects against chronic diseases. Endurance exercise, by increasing bilirubin levels, may contribute to these health benefits, emphasizing its value as a preventive strategy.

Despite the growing body of evidence linking exercise and bilirubin, most studies focus on trained athletes or clinical populations. The lack of data on novice athletes limits our understanding of how initial training phases affect bilirubin metabolism and liver health. This study addresses this gap by examining the impact of a structured endurance exercise program on bilirubin levels in untrained university students. By focusing on novice athletes, the research provides insights into the early adaptations of bilirubin metabolism and its potential as a biomarker for exercise-induced oxidative stress and liver function.

### **Methods and Materials**

The below procedures were adopted by the researcher to reach certain findings and a conclusion.

#### **Research design**

The current research study is closely associated with endurance exercise and liver bilirubin concentrations. Therefore, the researcher employed an experimental research design. All the participants went through the experimental phases of the study.

#### **Participants of the Study**

Participants of the study were comprised of newly admitted or registered students to the Department of Sports Science and Physical Education, University of the Punjab, Lahore, Pakistan. Thus, the Participants of the study were randomly selected and placed into two groups: the Control group (CG) (normal routine with no exercise) and the experimental group.

#### **Sample and Sample Size**

Using the G\*Power statistical method of sample calculation, the study participants were placed into two groups i.e.: The control group (CG (normal routine with no exercise) and the experimental group. Each group was comprised of twenty (20) subjects. In addition, the below criteria were followed while selecting and categorizing the subjects. A self-made Exercise

protocol of endurance exercise for twelve (12) weeks was applied to the experimental group. Volume, intensity and frequency of exercise were calculated by experts in the field. In addition, the below criteria were followed while selecting and categorizing the subjects; Subjects aged more than 18 years and less than 25 years, Only male subjects were included, Subjects not taking any kind of antioxidants, Subjects performing exercise for a minimum duration of 6 months to 1 year, Subject not taking any kind of medication, Subject has no chronic diseases and Subjects who voluntarily participate in the study.

**Ethical Approval**

Due to close concern with human life, the developed protocol of the study was approved by the Ethical Review and Research Board of the University of Sindh, Pakistan and thus written informed consent was also taken from all the subjects before participating in the study. Risks and benefits of participation were also ensured to all subjects before participation in the study. The respondents' information was kept confidential and only used for research purposes.

**Analysis of Data Process**

The results (pre-and post) were processed through the statistical package for Social Sciences (SPSS, version 32). The data obtained was presented as means ± SEM. The statistical significance of the results was determined using Student’s t-test. The correlations were calculated for post-exercise. A P < 0.05 was required for the results to be considered statistically significant.

**Participant Characteristics**

The study enrolled 40 participants who completed the intervention without adverse events. Baseline characteristics, including age, BMI, and bilirubin levels, were comparable between groups (p > 0.05), ensuring homogeneity.

**RESENTATION AND ANALYSIS OF DATA**

**Table no.1 showing the descriptive analysis of Both CG and EG in Term of Gender**

**Gender**

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Male	40	100.0	100.0	100.0

The above table shows the gender distribution among participants is exclusively male, with all

40 participants identifying as such, representing 100% of the total sample.

**Bilirubin Levels**

**Bilirubin analysis revealed a significant increase in total bilirubin levels in the experimental group (EG) following the 12-week endurance program**

Group	Pre-Test Mean (mg/dL)	Post-Test Mean (mg/dL)	p-value
Experimental	0.95 ± 0.12	1.10 ± 0.15	0.002
Control	0.96 ± 0.10	0.94 ± 0.11	0.512

The EG showed a mean increase of 0.15 mg/dL in bilirubin levels (p = 0.002), while the CG exhibited no significant change (mean difference: -0.02 mg/dL, p = 0.512).

**BMI**

**In addition to bilirubin changes, the EG experienced a significant reduction in BMI:**

Group	Pre-Test BMI (kg/m <sup>2</sup> )	Post-Test BMI (kg/m <sup>2</sup> )	p-value
Experimental	23.8 ± 1.5	23.0 ± 1.4	<0.01
Control	23.9 ± 1.6	23.8 ± 1.5	0.45

This reduction indicates improved fitness, aligning with the physiological adaptations expected from sustained aerobic exercise.

**Discussion**

The results demonstrate that a structured 12-week endurance exercise program significantly increases serum bilirubin levels among novice university student-athletes. Elevated bilirubin levels in the EG reflect an adaptive response to exercise-induced oxidative stress. This finding is consistent with research by Djordjevic et al. (2012), who reported that aerobic exercise enhances bilirubin's antioxidant properties by stimulating heme oxygenase-1 (HO-1) activity, which degrades heme into biliverdin and bilirubin.

Higher bilirubin levels are hypothesized to represent a compensatory antioxidant mechanism, mitigating oxidative stress generated by sustained aerobic activity. This adaptation may protect

cellular structures and maintain systemic redox balance, contributing to improved metabolic and cardiovascular health (Hinds et al., 2016). In contrast, the CG's bilirubin levels remained unchanged, reinforcing the necessity of physical activity for modulating this biomarker.

Additionally, the significant reduction in BMI in the EG highlights the broader metabolic benefits of endurance exercise. A decline in BMI reflects improved body composition, likely driven by increased energy expenditure and enhanced metabolic efficiency. Such physiological changes contribute to the protective effects of exercise against non-communicable diseases, including liver dysfunction and cardiovascular disorders (Brancaccio et al., 2007).

Despite these promising findings, the study's limitations warrant consideration. The focus on male participants reduces the applicability of results to female athletes, who may exhibit different physiological responses to endurance exercise. Additionally, factors such as diet, hydration status, and genetic variability in bilirubin metabolism were not controlled, potentially influencing outcomes. Future studies should address these limitations and investigate the long-term impact of exercise on bilirubin levels and related health parameters.

### **Conclusion**

The liver's role in regulating bilirubin and its antioxidant properties underscores the importance of studying this biomarker in the context of endurance exercise. This research explores the interplay between oxidative stress, bilirubin modulation, and exercise adaptations in novice athletes, contributing to a deeper understanding of exercise's systemic effects on liver health and metabolic regulation.

This study highlights the significant impact of endurance exercise on liver bilirubin levels and BMI in novice university student-athletes. A 12-week moderate-intensity aerobic exercise program increased bilirubin levels (mean difference: 0.15 mg/dL,  $p = 0.002$ ) and reduced BMI ( $-0.80 \pm 0.14$  kg/m<sup>2</sup>,  $p < 0.01$ ) in the experimental group. These findings suggest that bilirubin serves as a dynamic biomarker reflecting the oxidative stress modulation and liver function improvements induced by exercise. Future research should explore the long-term effects of exercise-induced bilirubin modulation and its potential as a marker for athletic performance, recovery, and overall health optimization.

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