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Assessment of C-reactive protein, IL-6 and TNF-α Levels in Performance Enhancing-Substance (PES) Users Among Gym-Goers in Iraq

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Abstract:
The use of performance-enhancing substances (PESs) across gyms and fitness communities is a growing public health concern, particularly the evidence of their potential consequences for health in both the short and long term; Objectives: The current cross-sectional study was carried out between 1st of May 2024 – 4th of January 2025 to assess the levels of CRP, IL-6 as well as TNF-α among the population of gym-goers in Dyala City/Iraq. Those using PES presented significantly higher levels of CRP (5.30 ± 0.51 mg/L) when compared to non-users (1.64 ± 0.22 mg/L) (P < 0.001), showing an increased inflammatory response. Moreover, IL-6and TNF-αconcentrations were significantly increased among PES users than those non-users (55.67 ± 9.91 pg/ml vs. 40.82 ± 2.28 pg/ml, P<0.001 and 87.16 ± 10.39 pg/ml vs. 75.11 ± 2.88 pg/ml, P<0.001, respectively),and may represent an immunological change induced with PES usage.

Keywords:
Performance-enhancing substances (PESs); Androgenic–anabolic steroids (AAS); CRP; IL-6; TNF-α.

Introduction

Performance-enhancing substances (PESs) refer to any compounds taken in non-pharmacologic doses to boost athletic performance and improve physical fitness [1]. These substances include stimulants, anabolic-androgenic steroids (AAS), erythropoietin, human growth hormone, and diuretics which can influence behavior, alertness and pain perception [1]. In recent years, the growing use of PES, especially AAS, in fitness and strength training settings has raised significant public health concerns [2, 3]. While the short-term health risks of PES use have long been recognized, their long-term effects are only now becoming clearer [4]. Additionally many individuals consume PES without medical or nutritional supervision, highlighting a lack of awareness regarding the potential dangers of using unregulated or untested substances [5].

Androgenic-anabolic steroids (AAS) are synthetic versions of testosterone, the male sex hormone [6].They have two main effects: anabolic and androgenic [7]. The anabolic impact reduces body fat, enhances bone density and skeletal muscle mass, and promotes erythropoiesis

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[8]. Androgenic effects are connected with the development of male sexual traits [9]. They have a considerable impact on athletic performance [6].

A high level of AAS in human body can cause a variety of psychological and physical issues. It includes as myocardial infarction, high blood pressure, cardiac hypertrophy, atherosclerosis, fluid retention, jaundice, hepatic tumors, as well as acne. Psychiatric complications such as euphoria, irritability, aggressiveness, and mood disturbance can occur [6,10]. Furthermore, Piacentino et al. [11] found that AAS can indeed cause , shrinking of the testicles, decreased sperm count, infertility, a as well as an increased risk of developing prostate and breast cancers.

Elevated levels of inflammatory markers such as CRP, IL-6, and TNF-α are indicative of the body's response to stressors, including the introduction of PES. CRP is a well-established marker for systemic inflammation and has been linked to various health conditions [12]. IL-6 plays a dual role in inflammation; while it can promote healing [13], its chronic elevation is associated with adverse outcomes, including muscle wasting and metabolic disorders [14]. TNF-α is also a pro-inflammatory cytokine that promotes the inflammatory response and plays a role in several chronic diseases. Nevertheless, an abnormal or overwhelming activation of TNF-α signaling is related to chronic inflammation and can finally result in pathological disorders like autoimmune diseases [15]. And knowing how these markers interact in PES users can provide insights into the users' health status and risks associated with their substance use.

The concept of gym culture is still relatively new in Iraq, but the use of PES has slowly spread among people who go to the gym. The growing trend requires a detailed study on the health impact of these practices. In this population, the biological effects of PES consumption can be clarified by comparing the levels of CRP, IL-6, and TNF-α between PES users and non-users. This study not only serves to sketch the physiological changing environment surrounding PES use, but also to gather attention to the possible long-term health risks.

Material And Methods

1. Comparative Analysis of Performance-Enhancing Substances (PESs) Users and Non-Users Among Gym-Goers

Between May 1, 2024 and January 4, 2025, this study was conducted on users and non-users of performance-enhancing substances (PESs) among gym-goers, in various gyms in Diyala city. It consisted of 60 cumulative 1-year PES users aged 18 to 30 years. A secondary control group of 30 healthy adults (nonusers), aged 17 to 32 years, was recruited.

2. Exclusion criteria

Performance-Enhancing Substances (PESs) users and non-users were selected based on two criteria: First, they did not suffer from any inflammatory disorder (e.g., COVID-19, bacterial diseases). Second, they did not have any other chronic diseases (e.g., diabetes, malignancy, autoimmune diseases, and others).

3. Performance-Enhancing Substances (PESs) Users Characteristics

A questionnaire was used to collect data from each PES users, which included age, gender, duration of using, chronic disease, and type of PES used.

4. Blood collection and preparation

Under strict aseptic circumstances, approximately 4 mL of blood was collected from PES users and the same volume from healthy controls (non-users). The blood was subsequently gathered in

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gel tubes and left to coagulate at room temperature. Samples were centrifuged at 3000 rpm for fifteen minutes. The serum was carefully split into four Eppendorf tubes and refrigerated at -20°C for analysis. IL-6 and TNF-α levels were measured using ELISA kits, while serum C-reactive protein (CRP) levels were quantified using the cobas® c 311 analyzer. All participants provided thair consent before being included in the study.

5. Determination of CRP by cobas® c 311 analyzer

Serum CRP levels were measured using the cobas® c 311 analyzer (Roche Diagnostics, Germany) following the manufacturer's protocol.

6. Determination of Cytokine by Enzyme linked Immunosorbent assay (ELISA)

Inflammatory biomarkers (IL-6 andTNF-α) were evaluated using very sensitive ELISA kits (Company–Bioassay Technology Laboratory,England) according to the manufacturer's instructions.

7. Statistical Analysis

The data were analyzed with SPSS version 26 software. The results were presented as mean ± standard error (M ± SE). The independent-samples T test was employed to examine if the values of research parameters differed significantly between PES users and non-users. Statistically significant differences were identified with a P-value < 0.05.

Results

1. Characteristics related to the demographics of the study groups

In this study, a total of 90 individuals participated, comprising 60 PES Users and 30 Non-Users (controls). The mean ages for the PES Users and controls were 25.1 ± 1.39 and 24.4 ± 1.23 years, respectively. There was no significant difference observed between the two groups in terms of age (P = 0.614) (Table 1).

Table 1: The Mean Age of Study Groups.

Study Group		Number	Age (Mean ± Standard Error)
1.	**PES Users**	60	25.1 ± 1.39
2.	**Non-Users**	30	24.4 ± 1.23
P-value <0.05		0.614	

2. Patterns of Performance-Enhancing Substances (PESs) Use Among Gym-Goers

In this study, a variety of PES were reported among gym-goers. The most commonly used PES was Anabolic-Androgenic Steroids (AAS), with 35% incorporating it into their fitness regimen. Protein powders were the second most frequently used supplement, reported by 25%, highlighting their role in muscle recovery and growth. Additionally, Creatine was used by 20%, indicating that a significant portion of gym-goers relied on these substances to enhance muscle mass and performance. Human Growth Hormone (hGH), known for its role in muscle development and fat metabolism, was used by 11.67%. Finally, BCAAs were consumed at 8.33%, indicating their use for muscle recovery and endurance. These discoveries propose that whereas some gym-goers prefer authorized and extensively recognized supplements for example creatine and protein powders, others turn to hormonal boosters and anabolic steroids which might pose health dangers (Table 2).

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Table 2: Types and Prevalence of Performance-Enhancing Substances (PESs) Use Among Gym-Goers.

Characteristics			Number (n = 60)	Percentage (%)
Performance-Enhancing Substances (PESs)	1.	Anabolic-Androgenic Steroids (AAS)	21	35%
	2.	Protein Powders	15	25%
	3.	Creatine	12	20%
	4.	Human Growth Hormone (hGH)	7	11.67%
	5.	Branched-Chain Amino Acids (BCAAs)	5	8.33%

3. C-reactive Protein (CRP) Levels Among PES Users and Non-Users

The results show the levels of CRP between PES users and non-users. PES users had a mean CRP level of 5.3007 ± 0.51372 mg/L (mean ± SEM), significantly higher than the 1.6440 ± 0.21802 mg/L seen in non-PES users (P = < 0.001) (Fig. 1). The line graph illustrates the mean CRP levels among users of different Performance Enhancing Substances (PES). The data suggest a progressive decreasing pattern of the C-reactive protein (CRP) levels among the groups, with AAS users presenting with the highest mean CRP, followed by users of hGH, and finally creatine users who have the lowest levels of CRP. This would imply that AAS use results in a greater inflammatory response as compared to hGH as well as creatine (Fig. 2).

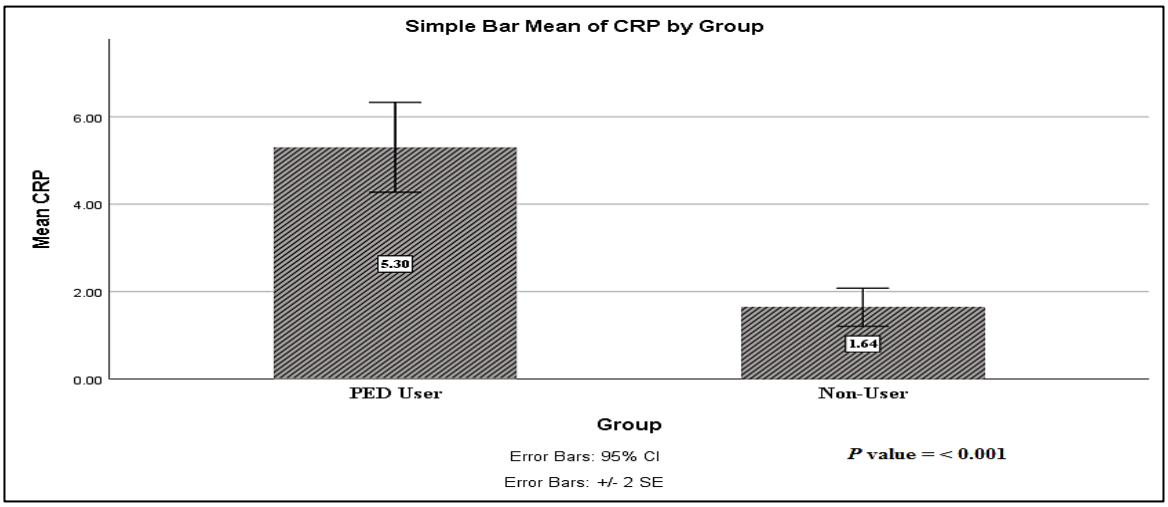

Figure 1: C-Reactive Protein (CRP) Levels in PES Users and Non-Users.

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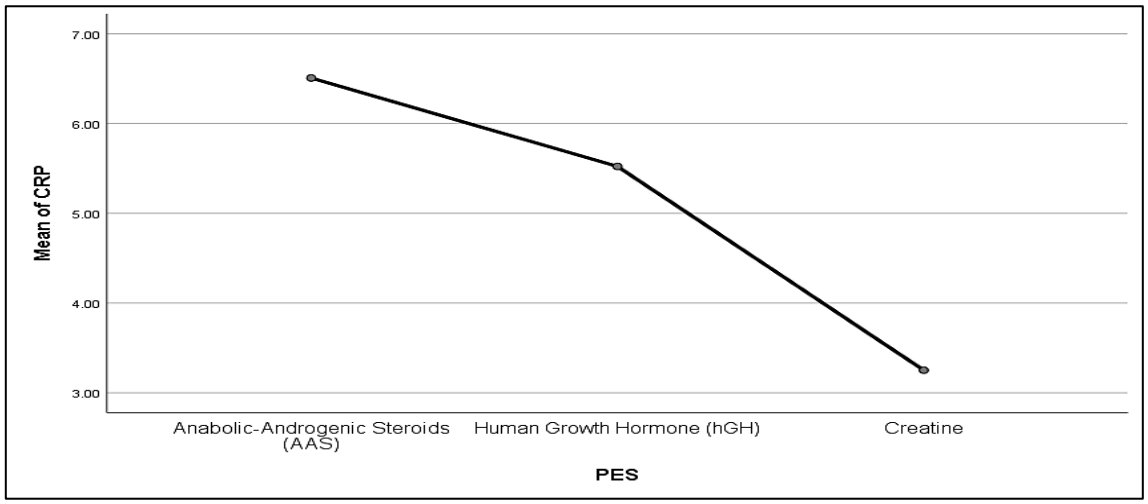

Figure 2: Correlation Between PES Use and Mean CRP Levels.

4. Cytokine Levels Among PES Users and Non-Users

This study shows that the serum levels of interleukin -6 (IL-6) and tumor necrosis factor-alpha (TNF-α) are significantly different between PES users and non-users. PES users had a mean IL-6 value of 55.67 ± 9.91 pg/ml which was higher than non-users, who had a mean IL-6 level of 40.82 ± 2.28 pg/ml ($p < 0.001$). Similarly, PES users exhibited higher TNF-α levels, with a mean of 87.16 ± 10.39 pg/ml, compared to 75.11 ± 2.88 pg/ml in non-users ($P = < 0.001$) (Table 3). These results show that the consumption of PES is associated with higher levels of these inflammatory markers suggesting a dysregulated immune status in performance-enhancing chemical substances user.

Table 3: The serum IL-6 and TNF-α levels among PES Users and Non-Users.

Groups		N (90)	IL-6 (*pg/ml*) (*Mean ± SE*) *	TNF-α (*pg/ml*) (*Mean ± SE*) *
1.	**PES Users**	60	55.67 ± 9.91	87.16 ± 10.39
2.	**PES Non-Users**	30	40.82 ± 2.28	75.11 ± 2.88
P-Value*			0.001	0.001
P value < 0.05 * *Values are presented as mean±standard error (SE).*				* *Independent-samples T test*

5. Correlation Between DifferentPES Use and Mean CRP Levels

We found a significant correlation between IL-6 levels and PES use at 0.03, and a significant relationship between TNF-α levels and PES at 0.02. The IL-6 levels were significantly elevated in AAS users (60.77 ± 7.82) following the hGH users (50.23 ± 5.43) and lastly the Creatine user (43.33 ± 3.34). Likewise, the TNF-α levels found in AAS users were the highest (89.28 ± 9.46), followed by hGH use (80.60 ± 8.53), and the lowest levels were observed in Creatine users(72.51 ±

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3.82). As presented in Table 4, these findings suggest that the use of PES, specifically AAS, might correlate with elevated inflammation markers.

Table 4: Correlation BetweenPES Use and Mean of IL-6 and TNF-α Levels.

PES		N	Mean	Std. Error	P-value
IL-6	**Anabolic-Androgenic Steroids (AAS)**	21	60.77	7.82	0.03
	Human Growth Hormone (hGH)	7	50.23	5.43	
	Creatine	12	43.33	3.34	
TNF-α	**Anabolic-Androgenic Steroids (AAS)**	21	89.28	9.46	0.02
	Human Growth Hormone (hGH)	7	80.60	8.53	
	Creatine	12	72.51	3.82	
	P value <0.05*			*One-way ANOVA*	

DISCUSSION

Assessment of inflammatory biomarkers including, CRP, IL-6,and TNF-α levels in Performance-Enhancing Substances (PESs) users in gym-goers in Iraq is a multidimensional aspect of research connecting health, sport science, and public health. Such an increase in the use of PESs, in particular anabolic steroids and other performance enhancing substances, raises serious questions about its physiological and psychological effects on users. These substances are often perceived as shortcuts to improved athletic performance and physique, yet they carry substantial risks that can lead to chronic health issues, including cardiovascular diseases, liver dysfunction, and psychological disorders [6, 16]. Recent study conducted in Saudi Arabia by Albaker et al. [2] reported that AAS use is related to adverse effects affecting 77% of users, such as psychiatric problems (47%), acne (32.7%), hair loss (14.2%), and sexual dysfunction (10.7%).

In another recent study conducted in Italy, Di Girolamo et al. [17] found that hormonal abuse, especially of anabolic steroids, growth hormone, and insulin is common among bodybuilders and is associated with significant metabolic derangements. Some of those changes, include modified lipid profiles, increased levels of liver enzymes, and shift in fatty acid metabolism. Such results highlight the possible effects of hormone doping in bodybuilding.

The C-reactive protein (CRP) is an important inflammatory biomarker whose concentration can reflect acute or chronic inflammatory states. This study showed that PES users had significantly higher CRP level as compared to non-users ($P < 0.001$). This marked difference highlights the relationship between PES usage and a more vigorous inflammatory reaction as measured by CRP levels. Such findings align with previous research indicating that substance use, including anabolic androgenic steroids (AAS), correlates with increased inflammatory markers like CRP [18, 19]. According to Arazi et al. [20], AAS use is associated with oxidative stress and cell damage, which can trigger inflammatory pathways.

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Conversely, some recent studies suggest that regular physical activity can reduce CRP levels; however, this effect may not apply to individuals engaging in excessive exercise or Performance-Enhancing Substances (PESs)use. The contrasting outcomes highlight the complexity of inflammation regulation in different contexts of physical activity and substance use [21.22].

Through our review of the literature on the potential role of creatine in triggering inflammatory reactions in the body, as well as its negative effects on the heart, kidneys, and liver, we did not find any conclusive studies confirming such adverse effects. However, based on the available evidence, creatine appears to be safe for healthy individuals. Nevertheless, idiosyncratic reactions may occur, particularly when large amounts of an exogenous amino-containing compound are consumed, potentially increasing the metabolic burden on the liver and kidneys.

In addition to CRP, this study found significant differences in cytokine (IL-6 and TNF-α) levels between PES users and non-users ($P < 0.001$). This elevation in pro-inflammatory cytokines further supports the hypothesis that PES use alters immune responses and promotes systemic inflammation. In study occur in Germany, Bernecker et al. [23] found that acute vigorous exercise leads to a significant increase in circulating IL-6 and TNF-α. This pro-inflammatory response is likely due to local production or release from exercised tissues rather than blood mononuclear cells.

In addition, a recent review on the adverse effects of anabolic-androgenic steroids by Albano et al. [24] demonstrating that AAS abuse is associated with increased levels of inflammatory cytokines (IL-6, TNF-α, IL-1β) and elevated WBC counts and causes systemic inflammation and organ damage among AAs users. The increase in inflammatory cytokines and WBC counts associated with AAS usage can largely be attributed to the release of inflammatory cytokines, immune cell alterations, such as neutrophilia, monocytosis, and macrophage activation mediated by oxidative stress-induced NF-κB activation, as well as the induction of endothelial dysfunction, aiding propagating vascular inflammation and immune cell infiltration. These mechanisms collectively heighten the risk of cardiovascular disease, immune dysregulation, and organ damage in chronic AAS users. However, further research is needed to clarify the long-term immunological consequences of AAS use [24].

Several underlying mechanisms may explain the increase of CRP and cytokines in PES users. First, the direct effects of performance-enhancing substances (PES), which illicit many physiological stress responses that increase the eicosanoid production of inflammatory markers [25]. Second, altered immune response, the use of PES may disrupt normal immune function, resulting in heightened inflammatory states [26]. Finally, lifestyle factors; PES users may undertake vigorous training regimens that can lead to muscle damage and subsequent inflammation [27].

CONCLUSION

Performance-enhancing substances (PESs) are extensively used and abused in the gym in Iraq, and the lack of medical supervision and care continues to exert pressure on the latest health trends without a definitive conclusion. The current study specifically shows that PES use is positively correlated with increased inflammation, noting significant elevations in CRP, IL-6, and TNF-α in PES users, suggesting PES users have an augmented inflammatory response. The verdict

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highlights the negative impact of PES overuse on the immune system, inflammatory response, and health in general.

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