Effects of Dietary Supplements and Anabolic Hormones on Hematological and Biochemical Parameters among Young Bodybuilders in Kurdistan Region of Iraq

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ABSTRACT
Athletes and bodybuilders frequently utilize vitamins and anabolic hormones. Utilizing these substances, gymgoers can achieve their goals faster by consuming a combination of supplements and hormones boosts benefits. The current study was conducted to determine the possible adverse effects that several commonly used hormones and supplements may have on hematological and biochemical markers. Ninety people who participated in the research were split into three groups. The first group consisted of healthy people who were not athletes (control), the second group consisted of bodybuilders who did not use hormones and supplements (non-supplementary athletes), and bodybuilders who took hormones and supplements made up the third group (supplementary athletes). Here, hematological, liver function, and renal function parameters were investigated. Hematological parameters such as white blood cells (WBC), Lymphocytes (LYM), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), and haematocrit (HCT) percentage change significantly between the groups. Similarly, the statistical change in serum alkaline phosphatase (ALP) and total serum bilirubin (TSB) level indicates the influence these substances have on the liver function. Serum urea and serum creatinine values reveal decreased renal function in group I. Mixed usage of supplements/hormones has more damaging effects on consumers' health.
Introduction

Dietary supplements, including pro-hormones, creatine, amino acids, whey protein, and fat burners, are some of the dietary supplements that athletes utilize to enhance their physical capabilities and obtain a competitive edge [1]. Some athletes have been adhering to an ever-expanding list of banned drugs for quite a long time [2]. Researchers have recently revealed that bodybuilders take supplements generally based on personal recommendations rather than accumulating trustworthy information about the chemicals. They frequently purchase supplements from merchants and online websites [3, 4]. Numerous dietary supplements, including caffeine, ephedrine, creatine, whey protein, antioxidants, ginseng, hormones, multivitamins, vitamin C, iron, and magnesium supplements, have been mistakenly or appropriately connected with the increase of performance and health maintenance [5]. Consuming nutritional supplements in excessive amounts might result in potentially harmful side effects as well as serious health problems [6]. For instance, the usage of iron supplements for an extended period of time by competitive bodybuilders can lead to hemochromatosis in some athletes. In a similar vein, taking in an excessive amount of vitamin C can be detrimental, particularly when combined with iron [7, 8]. This combination can damage the gastrointestinal tract (GI) and exacerbate the symptoms associated with persistent GI diseases [9]. In addition, the long-term consequences of creatine are not yet understood. Nevertheless, specific short-term adverse effects, such as cramping and dehydration, have been observed. For this reason, it has been suggested that creatine usage be carried out under the supervision of a medical professional [10]. Therefore, supplements should not be utilized as a long-term solution to complete the diet, but rather as a short-term solution [11].

It is common for bodybuilders to utilize nutritional supplements and hormones in both their training and competitions without fully comprehending its possible adverse effects. Except for supplements that have been proven to improve performance based on evidence, such as creatine, research has shown that random usage of supplements can have both short-term and long-term negative impacts on the body [12]. Furthermore, sports supplements in the gym setting appear to have a quantitative nature, focusing on predictors and correlated variables. However, they lack a deeper analysis and explanations of why these side effects manifest themselves. As a result, there has not been much research done to develop a more in-depth grasp of the logic behind using sports supplements as part of a regular diet.
Therefore, the purpose of this study is to investigate the influence that a variety of nutritional supplements have on the physiological and biochemical characteristics of bodybuilders.

**Martials and Methods**

**Design and setting**

Three male groups of 30 individuals were included in the investigation. The first group as a control group contains persons who are not involved in the sport of bodybuilding, the second group is comprised of bodybuilders who do not use any supplements or hormones, and the third group are bodybuilders and gym-goers that use dietary supplements and hormones. A variety of nutritional supplements, such as whey protein, whey protein isolate, mass gainer, Big mass, Big fit, creatine monohydrate, and branched-chain amino acids (BCAA), have been utilized by the bodybuilders. In addition, the third group uses a variety of anabolic hormones, including testosterone, methandienone (Dianabol), Boldenone, growth hormone, and stanozolol (Winstrol). These supplements and hormones have been taken by the participants in group III for a period of at least one year. The age was self-reported by the participants. To assess the height and weight of our patients, we resorted to the traditional methods. The body mass index (BMI) was computed by taking participants' height and weight into account. The reference ranges were established according to what the World Health Organization (WHO) classifies as underweight, normal weight, overweight, and obese [13]. The body mass index (BMI=weight (kg)/height (m²) remains the most widely used indicator of weight status, with a BMI of 18.525.9 kg/m² indicating normal weight, 25.029.9 kg/m² indicating overweight, and 30.0 kg/m² indicating obesity.

**Blood collection and analysis**

The venous blood from all of the individuals was taken into sterilized tubes. To separate the plasma, each sample was run through a centrifuge. Using a fully automated blood analyzer (Swelab Alpha Plus, Sweden), a complete blood count (CBC) was carried out on each blood sample immediately following its collection. The serum was then subjected to further testing to determine liver function, including estimations of TSB, ALP, serum alanine aminotransferase (ALT), and serum aspartate aminotransferase (AST). In addition, renal function tests were measured; namely, blood urea and serum creatinine. Commercial kits (Biolab, France) were used to measure the levels of liver and kidney function tests using the Chemistry Analyzer Smart-150 apparatus (GenoTEK, USA).

**Ethical consideration**

This study was approved by the scientific committee of Medical Laboratory Science Department, College of Science, University of Raparin. Permissions have been taken from participants in the study.

**Statistical analysis**

All data are expressed as mean ± standard error (mean ± SE) and the statistical analysis was carried out using (SPSS version 25). Statistical differences were determined by the Duncan test for multiple comparisons and analysis of variance (ANOVA). P-values of $P \leq 0.01$ and $P \leq 0.05$ were considered statistically significant.

**Results and Discussion**

Athletes and bodybuilders often take supplements, hormones, or their combination to improve their performance and get results more quickly. Nevertheless, this time-saving method can result in significant and ongoing health issues. Some known scientific data showed significant cardiovascular adverse effects following the use of performance-enhancing medications, including sudden death, cardiac arrhythmia, an increase in blood pressure, and other conditions [14, 15]. In spite of the fact that there is a wealth of data originating from various regions of the world concerning the use of supplement and hormone, there is a scarcity of research from the Kurdistan region of Iraq and Iraq itself.

The age range of 18-35 was consistent across all of the groups. The body mass index (BMI) of
people in groups control, non-supplementary athletes, and supplementary athletes was 24.2 kg/m², 22.1 kg/m², and 26.8 kg/m², respectively. According to the findings, the body mass index of group I bodybuilders is slightly higher than the usual BMI. The NIH Consensus Development Conference on the Health Implications of Obesity determined that athletes and bodybuilders are considered overweight if they have a BMI that is greater than 27.8 kg/m² [16].

It has been known for a long time that participating in endurance sports can result in haematological and biochemical shifts [17]. Statistical analysis revealed that the number of WBCs increased considerably (P < 0.05) in both groups of bodybuilders compared to the control group. In line with the findings of this investigation, previously, researchers found that the injection of 200 milligrams of testosterone once a week led to a slight rise in the number of white blood cells [18]. The subsequent results showed that the number of lymphocytes considerably increased (P < 0.01) in both groups of bodybuilders as compared to the control group. In a similar manner, the MCHC levels (P < 0.01) rose in both groups of athletes. On the other hand, the decrease in HCT percentage (P < 0.01) and MCV level (P < 0.05) was statistically significant in both gym-practicing groups, as indicated in Table 1. Similar to our study, significant changes in MCHC and MCV levels have been found in Pakistani athletes, but the HCT percentage was found unchanged [19]. Red blood cells (RBC), mean corpuscular haemoglobin (MCH), haemoglobin (HGB), and platelets (PLT), did not differ substantially among three groups (data not shown).

Table 1: Hematological parameters from blood samples of participants

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Non-supplementary athletes</th>
<th>Supplementary athletes</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC * (10⁹/µL)</td>
<td>6.680 ± 0.301 a</td>
<td>7.801 ± 0.403 ab</td>
<td>8.270 ± 0.467 b</td>
</tr>
<tr>
<td>LYM ** (10⁹/µL)</td>
<td>2.193 ± 0.119 a</td>
<td>3.625 ± 0.274 b</td>
<td>3.529 ± 0.235 b</td>
</tr>
<tr>
<td>MCHC ** (g/dL)</td>
<td>32.34 ± 0.322 a</td>
<td>35.84 ± 0.847 b</td>
<td>35.63 ± 0.696 b</td>
</tr>
<tr>
<td>MCV ** (fl)</td>
<td>87.48 ± 1.619 b</td>
<td>80.80 ± 2.309 a</td>
<td>80.58 ± 2.012 a</td>
</tr>
<tr>
<td>HCT ** (%)</td>
<td>49.55 ± 0.965 b</td>
<td>44.53 ± 1.253 a</td>
<td>43.51 ± 1.001 a</td>
</tr>
</tbody>
</table>

n= 30 in each group; data are presented as mean and standard error; for a given raw, the same letters mean no statistical differences, different letters mean statistical differences (**: P-value <0.01 and *: P-value <0.05).

Abbreviation: WBC: White blood cells, LYM: Lymphocytes, MCHC: Mean corpuscular haemoglobin concentration, MCV: Mean corpuscular volume, and HCT: Haematocrit.

Table 2: Biochemical parameters from blood samples of participants

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Non-supplementary athletes</th>
<th>Supplementary athletes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver function parameters</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>ALP ** (U/L)</td>
<td>171.4 ± 1.821 a</td>
<td>196.2 ± 1.897 ab</td>
<td>266.5 ± 2.247 b</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>25.10 ± 4.746 a</td>
<td>21.18 ± 2.522 a</td>
<td>23.18 ± 1.824 a</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>26.62 ± 2.943 a</td>
<td>27.67 ± 2.613 a</td>
<td>31.16 ± 2.211 a</td>
</tr>
<tr>
<td>TSB ** (mg/dL)</td>
<td>0.70 ± 0.086 ab</td>
<td>0.480 ± 0.041 a</td>
<td>0.770 ± 0.093 b</td>
</tr>
<tr>
<td>Kidney function parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea ** (mg/dL)</td>
<td>29.71 ± 1.562 a</td>
<td>30.09 ± 1.931 a</td>
<td>39.69 ± 1.945 b</td>
</tr>
<tr>
<td>Creatinine ** (mg/dL)</td>
<td>0.628 ± 0.055 a</td>
<td>0.703 ± 0.069 a</td>
<td>0.951 ± 0.041 b</td>
</tr>
</tbody>
</table>

n= 30 in each group; data are presented as mean and standard error; for a given raw, the same letters mean no statistical differences, different letters mean statistical differences (**: P-value <0.01 and *: P-value <0.05).


When it comes to bodybuilding, anabolic hormones and creatine pre-workout supplements are two of the essential products that are administered. These substances are typically more hepatotoxic than other supplements because the liver is the organ that is responsible for metabolizing them. ALP and TSB level considerably altered among the liver function...
tests that were carried out. In both groups of bodybuilders, the serum ALP level indicates a statistically significant rise when compared to the control group with a p-value of less than 0.01, indicating that the increase is statistically significant. The change was also significant in TSB levels between the group of supplement/hormone users and supplement/hormone-free bodybuilders but increased in the former and decreased in the latter (P < 0.05). A study obtained a similar ALP result but with a different TSB outcome [20]. The higher level of blood ALP and TSB in those who take supplements or hormones suggests hepatocellular injury. On the other hand, serum ALT and AST levels did not change significantly across all three groups, as presented in Table 2. In spite of the fact that researchers discovered an important correlation between the consumption of anabolic hormones and elevated serum AST and ALT levels, our data showed non-significant changes in both mentioned tests [21]. These contradicting results might be due to the mixed usage of anabolic hormones and dietary supplements.

Tests that evaluate renal function, including blood urea and serum creatinine levels, also reached statistically significant levels. According to the findings, the levels of serum urea and serum creatinine rose considerably (P < 0.01) in bodybuilders who used nutritional supplements and hormones compared to the levels seen in the other two groups. The change in group non-supplementary bodybuilders, on the other hand, did not approach the significant level as shown in Table 2. Creatine is the pre-workout substance used by the vast majority of athletes. The liver, pancreas, and kidney are indeed the organs that are responsible for the natural synthesis of creatine [22]. However, it is also possible to obtain creatine exogenously by consuming its primary sources. Creatinine is a characteristic marker of kidney function and is produced in the body as a by-product of the breakdown of creatine phosphate in the muscle [22, 23]. There is an increase in blood urea and serum creatinine level in both groups of bodybuilders. Nevertheless, there is a marginal increase in both measures in the group that did not take any supplements or hormones and this change was not statistically significant. On the other hand, the levels of blood urea and serum creatinine were dramatically altered in the third group of bodybuilders. These findings are consistent with earlier researches conducted and published [24]. Likewise, studies found that mice that were given creatine supplements had higher protein deposition and structural alterations in their livers [25, 26].

Consuming an excessive amount of nutritional supplements and hormones might cause health problems for bodybuilders, especially if they are not utilized under the appropriate supervision. There is no question that combining the use of these substances poses a threat to both liver and kidneys. Once these supplements and hormones are no longer being taken, the majority of these parameters frequently return to the normal range. However, a comprehensive investigation into the impact that these medications have over the long term is required. However, additional criteria have to be taken into consideration to expose the unfavourable effects of supplements. A few publications are available regarding the impact of consuming a variety of dietary supplements/hormones on health. Therefore, additional research needs to be carried out so that these supplements' potentially harmful effect has to be confirmed. In addition, the majority of the people who were evaluated were of the male gender. As a result, it is advised that research be conducted to determine the effect that these chemicals have on female counterparts.

**Conclusion**

It should come as no surprise that liver and kidneys are the two organs impacted by the use of sports nutrition supplements and hormones. It is without a doubt that high quantities of supplements, vitamins, and hormones will create an excessive strain on the liver to detoxify, as well as on the renal system to remove. The combined usage of these materials presents an additional danger to the health of individual consumers.
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Authors' Contributions
All authors contributed to data analysis, drafting, and revising of the article and agreed to be responsible for all the aspects of this work.

Conflict of interest
There are no conflicts of interest in this study.

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