Changes in Health-related Parameters Associated with Sports Performance Enhancement Drugs

Authors
Magdalena Wiacek[1], Bartosza Trąbka[2], Ryszard Tomasiuk[1], Igor Z Zubrzycki[3]

Affiliations
1 Faculty of Medical and Health Sciences, Kazimierz Pulaski University of Technology and Humanities in Radom, Radom, Poland
2 Faculty of Physical Culture, Gdansk University of Physical Education and Sport, Gdansk, Poland
3 Biometry and Mathematics, Botswana University of Agriculture and Natural Resources, Gaborone, Botswana

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Georg Thieme Verlag KG, Rüdigerstraße 14,
70469 Stuttgart, Germany

Correspondence
Prof. Igor Z Zubrzycki
Botswana University of Agriculture and Natural Resources
Biometry and Mathematics
Gaborone
P/B 0027
Gaborone
Botswana
Tel.: +26772493886
igorzubrzycki@gmail.com

ABSTRACT

The purpose of this study was to evaluate changes in health-related parameters caused by the administration of anabolic-androgenic steroids and "fat-burning drugs" during a 6-month competition preparation period. The physiological, biochemical, and anthropometric parameters studied included serum cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, aspartate aminotransferase, alanine transaminase, bilirubin, body mass, and percentage of total body fat. Changes in the parameters studied were analyzed at monthly intervals during six months of preparation for competition. The study revealed a continuous increase in body mass, accompanied by a decrease in body fat percentage to the physiologically essential level. Total cholesterol levels remined in the *desirable* concentration range. The mean levels of triglycerides fluctuated between *borderline high* and *high*. Mean high-density lipoprotein cholesterol levels remained within the *low* range, while low-density lipoprotein cholesterol fluctuated between *near-optimal* / above-optimal, *borderline high*, and *high* levels. Serum levels of aspartate aminotransferase and alanine transaminase remained within the high concentration. The bilirubin concentration remained in the desirable range. The blood nitrogen urea concentration fluctuated between *normal* and *elevated* levels. Sports-enhancing drugs analyzed in this study do not have an immediate detrimental impact on the selected biochemical, physiological, and anthropometric parameters that define health.

Introduction

A PubMed search for studies reporting on sports performance enhancement drugs (PED) revealed ~3,000 documents, almost half of which focused on anabolic androgenic steroids (AAS). AASs are derivatives of the human hormone testosterone and are responsible for stimulating the biosynthesis of cellular proteins [1]. Their effectiveness depends on the number of metabolically available nutrients [2, 3] and the genetic control of muscle growth [4].

Despite its illegality [5] and possible health-related risks [6], AAS helps people achieve increased lean body mass and strength [7] for various recreational and professional sports. In particular, most competitors, in addition to Olympic and well-paid professionals, use AAS on a trial-and-error basis [8].

To date, some scientific reports suggest that AAS are responsible for adverse health effects [9–13]; however, the previous review of the literature did not confirm this [14]. Furthermore, Hargens et al. [15], in a study on the effects of AAS on strength-training athletes, pointed to the practical shortcomings of many study methods, indicating that most focus on athletes using only one or two types of AAS. Although such a method is justified from a clinical perspective, it has shortcomings from a practical point of view and is irrelevant in professional sports practice.

It has been shown [14] that competitive bodybuilders experiment with various AAS, which, in their judgment, allow them to achieve better musculature and vascularization and concomitantly decrease their level of adipose tissue to a physiological minimum. Therefore, a simplistic approach does not provide an accurate picture of the influence of AAS on human physiology in contemporary sports, especially in sports such as bodybuilding, weightlifting, and wrestling.

This study is an explicit analysis of the health-defining parameters of world-class bodybuilders as a function of self-administration of PED, including AAS [testosterone propionate, drostanolone propionate (Masteron), trenbolone acetate, oxandrolone (Anavar), stanozolol (Winstrol) and boldenone undecylenate], human growth hormone (HGH) and fat-burning drugs [triiodothyronine (t3), clenbuterol, mesterlone (Proviron), tamoxifen citrate (Nolvadex), and 2, 4-Dinitrophenol (2,4-DNP)]. The study was conducted with a group of eight top amateur/professional bodybuilders during the competition preparation period [16]. It is also an attempt to establish to what extent AAS are used in competitive sports. Due to the lack of readily available scientific reports on the topic, most people use YouTube and other unsaturated Internet sources to learn about the topic. Furthermore, some scientific reports also provide misleading information on the physiological implications of AAS without specific evidence. Some also duplicate specific health-related comments without proper substantiation of the given statements.

We want to stress that the data presented in this report should not be associated with any particular bodybuilding organization or sports event. Furthermore, we were unable to check the quality of the drugs used by the competitors, and we did not require information on the origin of the drugs.

The null hypothesis of this study states that extensive use of PED results in immediate detrimental changes in the parameters that define human health. To our knowledge, this study is the first to analyze the competitive use of PED and its relationship with the basic physiological parameters that define the health of competitors in a real-life scenario.

Materials and Methods

The study was carried out by gaining access to competitor notes and results of the medical examination that included the time, amount, and brand of specific drugs used in self-administered form during six months of preparation for international competition.

Study subjects

All experiments and methods were performed in accordance with the relevant guidelines and regulations. All experimental protocols were approved by the Medical Chamber Licensing Committee: KB-20/14. Informed consent was obtained from all subjects. The study was carried out with the funding of the participants and the authors. The study was carried out in a group (N = 8) of the best European (Caucasian ethnicity) male bodybuilders of different countries and nationalities in Europe who used PED during the preparation period for the contest. Due to the extreme difficulty in collecting the data, the presented data were collected with the underlying objective of studying a group of at least five competitors for a comparable competition preparation period. The ages of the study subjects ranged from 30 to 35 years (M = 32.49, SD = 1.47). The BM of the competitors was between 97.2 and 110.4 (M = 104,4, SD = 4.28).

Information on self-administered amounts of PED is presented as ranges (minimum-maximum) and is shown in ▶ **Table 1**. After six months of drug administration, some competitors apply a period of 1–2 months of drug-free training before competition.

An analysis of the competitor's notes exposed a specific diet for the competition preparation period consisting of specific protein-fat-carbohydrate ratios. Therefore, until the fifth month of preparation, the diet consisted of six meals between 7:00 h and 23:00 h, containing ~40 % protein, ~20 % fat, and ~40 % carbohydrates. As a result, competitors consumed 3.8–4.2 g of protein/kg of body mass/day, 1–1.2 g of fat/kg of body mass / day and 3–4 g of carbohydrates / day / kg of body mass. This ratio provided ~200 % Cal of each competitor's calculated basal metabolic rate (BMR). During the last 5 and 6 months of the preparation period, the levels of carbohydrates and fat gradually decreased to zero. Such an approach led to a decrease of total caloric consumption to ~150 and ~100 % of BMR for the 5th and 6th months of the preparation period, respectively.

Study protocols

In this study, the following physiological, biochemical, and anthropometric parameters were analyzed: serum lipids (serum cholesterol levels [total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL-C) and low-density lipoprotein (LDL-C)]), aspartate aminotransferase (AST), alanine transaminase (ALT), Bilirubin, blood urea nitrogen levels (BUN), bone mineral density (BMD, and percentage of total body fat (tissue) (%BF).

To avoid direct contact between study subjects and the research team, all subjects were asked to perform a blood biochemical analysis in an accredited laboratory. All laboratory measurements were made in the morning after a fast of 12 hours (no food or drink, except water). The analytical procedure provided in the following is derived by analyzing laboratory-accredited protocols.

4.5 ml of blood was collected in Mint Green Top (Lithium Heparin Gel) and centrifuged within 2 hours of sample collection for 10 minutes at 2800 rpm. Plasma TC level was measured enzymatically [17, 18] HDL-C cholesterol levels were analyzed using four reaction procedures, resulting in quinone-imine dye, whose concentration is directly proportional to HDL-C levels and measured at λ = 600 nm. Serum TG levels are measured enzymatically via a series of coupled reactions in which TGs are hydrolyzed to produce

▶ **Table 1** Drugs used by competitors during the competition preparation cycle. *q.wk.* – once a week, *q.o.d.* – every other day, *q.d.* – every day, *d. in p. æ.* – divided into equal parts, *b.d.s.* – twice daily, NaN lack of the data.

Name	Lit. Reference	Month: 1–5	Month: 6
Testosterone propionate	[52–53]	100–150 mg q.o.d.	100 mg q.o.d.
Drostanolone propionate (Masteron)	–	100–150 mg q.o.d.	X
Trenbolone acetate	–	75–100 mg q.o.d.	X
Oxandrolone (Anavar)	[89–91]	50–75 mg q.d.	X
Stanozolol (Winstrol)	[55]	50–100 mg q.d	X
Boldenone undecylenate	–	500–600 mg q.wk.	X
Triiodothyronine (t3)	[66]	x	10–25 mg q.d.
Clenbuterol	[71–72]	0.08–0.04 mg b.d.s.	0.02 mg d. in p. æ.
Mesterolone (Proviron)	[73]	20–25 mg q.d.	X
Tamoxifen citrate (Nolvadex)	[77]	20–30 mg b.d.s.	20 mg q.d.
Human growth hormone (HGH)	[80]	2 x 3UI–4IU q.d.	2 x 2UI–3IU q.d.
2,4-dinitrophenol (2,4-DNP)	[92]	x	100–200 mg q.d. (7th day off)

glycerol. LDL-C levels were calculated using the following formula: LDL-C = TC - HDL-C-(TG/5). AST and ALT activities were evaluated using the kinetics of a set of enzymatic reactions, in which the final step (i. e. oxidation of NADH to NAD^+), directly proportional to the activity of ALT or AST, can be measured colorimetrically at = 340 nm.

The concentration of blood urea nitrogen (BUN) was measured enzymatically.

The total concentration of bilirubin was measured photometrically at λ = 548 nm.

Standing height was measured using a stadiometer with a fixed vertical backboard and an adjustable headpiece with an accuracy of 0.1 cm. Body mass was determined using a digital weight with an accuracy of 0.1 kg.

For the analysis of bone mineral density (BMD) and body fat percentage (%BF), dual energy X-ray absorptiometry (DXA), a standard for clinical diagnosis, was used [19]. All BMD and %BF measurements were performed using the Lunar Prodigy Primo PE + 303599 (GE Healthcare).

Reference sample

Data for the reference sample were collected from the National Health and Nutrition Examination Surveys (NHANES). The purpose of NHANES is to gather information and to descriptively and quantitatively monitor the physical state, disease, and interrelations of physiological and psychical conditions and nutrition [20, 21] in the population of the U.S.A. The survey allows sample stratification by sex, age, race, and income.

For the purpose of direct comparison between professional bodybuilders and the random population, 100 Caucasians between 30 and 35 years of age (M = 34, SD = 3.3) were randomly selected from the NHANES database. Such an approach was shaped by the financial and logistic constraints of the study. Therefore, we have decided to use a database that is employed worldwide to elucidate the reference values for the study's physiological/biochemical parameters.

Nine outcome variables were analyzed: total cholesterol, triglyceride, high-density lipoprotein, low-density lipoprotein, aspartate aminotransferase, alanine transaminase, bilirubin, body mass, and percentage of total body fat. The selection of a specific ethnic group was based on racial disparities in cardiovascular health [22].

Body mass of the reference sample was measured according to the specifications provided in the NHANES manual [23]. Serum lipid levels (SLL), including TC, TG, and HDL-C, were measured according to published procedure [24]: Concentrations of aspartate aminotransferase and alanine transaminase were evaluated using Beckman Synchron LX20 following the NHANES-provided procedure [25]. Bilirubin concentration was evaluated using the Beckman Coulter UniCel DxC 800 Synchron clinical system, according to the procedure manual [26]. Blood urea nitrogen was evaluated using the Beckman Synchron LX20 [27]. Body fat percentage was evaluated using the Hologic QDR 4500 A scanner [28] .

Statistical analysis

The normality of the distribution of the samples was verified using the Shapiro-Wilk test [29]. The equality variances for the response variables as a sampling function were analyzed using Levene's test [30]. If Levene's test showed variance heterogeneity across all measured parameters, the Welch procedure (Welch ANOVA) was used for a time-dependent analysis [31, 32]. Post-hoc analysis was performed using the Games-Howell test for multiple pairwise comparisons with unequal variances [33]. The null hypothesis for the statistical tests was verified at $P < 0.05$.

Results

▶ **Table 2** collects data on the differences in the mean values of the studied parameters between professional bodybuilders and the reference sample. Although there are statistically significant differences at $P < 0.05$ in the level of TC, HDL, AST, ALT, bilirubin, BUM and % BF, all the parameters studied (besides LDL-C) reside in normal physiological ranges for both study groups.

Changes in body mass (BM) and percentage of total body fat (%BF)

BM continuously increases during the training period from a mean value of 105 kg to 109 kg (▶ **Fig. 1a**: the color codes follow the weight division guidelines of the International Federation of Bodybuilding and Fitness [IFBB]). Simultaneously, %BF decreases from the percentage defining athletes (5 ≤ %BF < 11) to the optimal level of body fat for bodybuilders, that is, essential body fat (%BF < 5) [34, 35], ▶ **Fig. 1b**. Although discussing monthly changes in bone mineral density (BMD) over such a short period is meaningless, we examined the mean BMD values among professional bodybuilders in the first and final months of the competition preparation period.

▶ **Table 2** Differences in health-defining parameters between world-class bodybuilders and a randomly selected sample of white Caucasians. *Triglyceride – TG (mmol / L); Total Cholesterol – TC (mmol / L); High-density Lipoprotein levels – HDL-C (mmol / L); Low-density Lipoprotein levels – LDL-C (mmol / L); Aspartate Aminotransferase – AST (µkat / L); Alanine Transaminase – ALT (µkat / L); Bilirubin (µmol / L); Blood Urea Nitrogen - BUN (mmol / L); Total Body Fat Percentage – %BF; Testosterone (nm/L).*

Parameter	Descriptive statistics	Month: 1 (N = 8)	$P < 0.05$	N = 100
TC	min	3.83		2.56
	max	4.53		13.94
	Mean (SD)	4.15 (0.22)	*	5.17 (1.17)
TG	min	2.11		0.45
	max	2.42		8.19
	Mean (SD)	2.22 (0.11)		1.67 (1.17)
HDL	min	0.49		0.61
	max	0.56		2.28
	Mean (SD)	0.52 (0.02)	*	1.18(0.30)
LDL	min	3.39		1.06
	max	3.74		5.61
	Mean (SD)	3.59 (0.12)		3.32(0.91)
AST	min	1.38		0.20
	max	1.73		2.74
	Mean (SD)	1.60 (0.12)	*	0.45(0.23)
ALT	min	1.82		0.15
	max	2.1		3.17
	Mean (SD)	1.99 (0.09)	*	0.53(0.35)
Bilirubin	min	6.27		3.4
	max	7.49		39.33
	Mean (SD)	6.83 (0.44)	*	4.63(1.29)
BUN	min	7.18		2.14
	max	8.94		8.57
	Mean (SD)	8.29 (0.57)	*	4.8(1.25)
BF %	min	6.81		11.6
	max	8.39		43.4
	Mean (SD)	7.62 (0.48)	*	26.56(6.15)

In the first month, the BMD is equal to 1.34 ± 0.11 g/cm2, while in the sixth month, it equals 1.44 ± 0.5 g / cm^2. Furthermore, following information on the relations between BMD and testosterone levels [36], we measured the average testosterone level in all study periods; it was equal to 52.05 nm/L. ▶ **Table 2** revealed a statistically significant difference between the normal sample and professional bodybuilders, indicating a three-fold lower %BF in the competitor group than observed in the normal sample. Furthermore, professional bodybuilders are defined by an android fat percentage equal on average 6.19 ± 1.28 % (data not shown)

Changes in serum total cholesterol

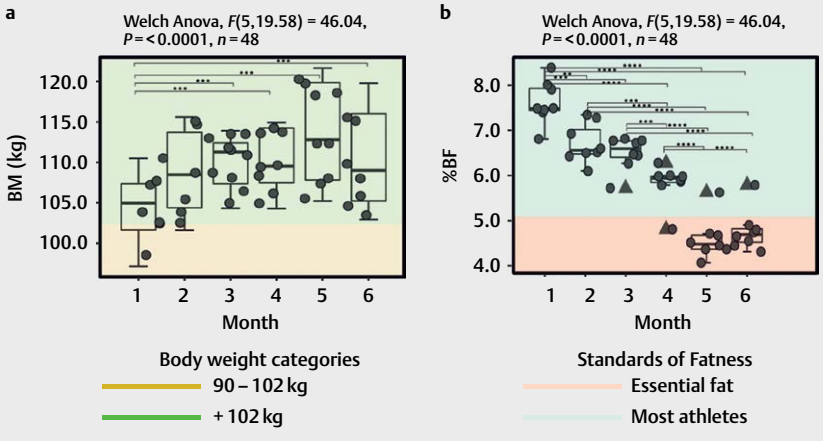
Serum TC levels of professional bodybuilders vary significantly within the desirable range (▶ **Fig. 2a**), reaching a minimum in the fourth month and a maximum in the sixth month. In the sixth month of the preparation period, only three of the eight competitors were found to have borderline high levels of TC (5.14 mmol/L ≤ TC < 6.20 mmol/L) [37]. On the contrary, the values for five competitors fell within the desirable range (TC < 5.14 mmol/L) [37]. Although there is a statistically significant difference between professional competitors and 'laymen', TC levels in both groups, ▶ **Table 2**, are within the physiologically desirable range.

Changes in serum triglycerides

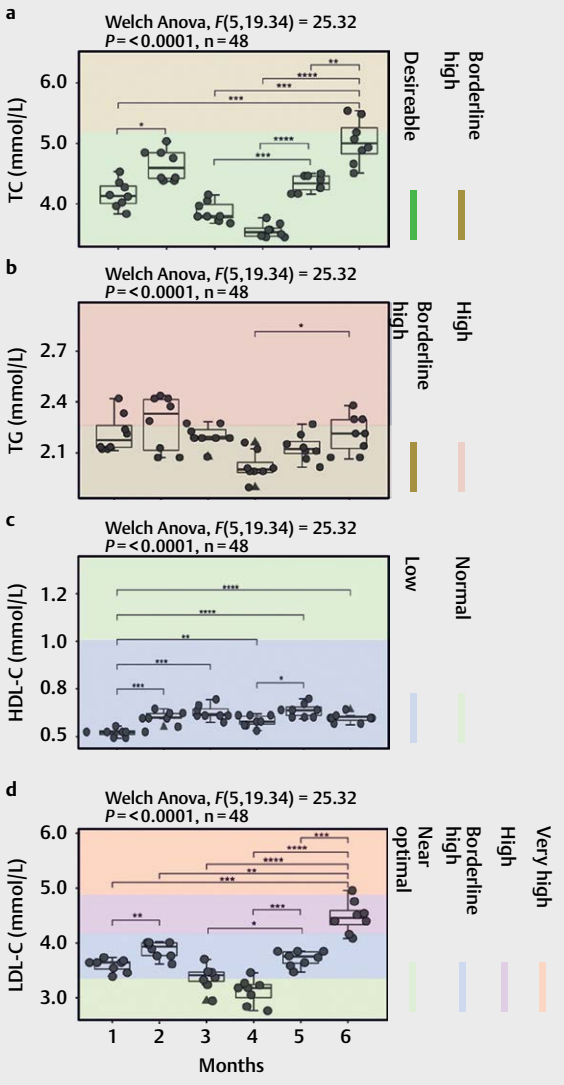
The serum TG concentration in professional bodybuilders fluctuates on the border of borderline high (1.69 mmol/L TG < 2.26 mmol /L) and high values (2.26 ≤ TG < 5.64) [37] (▶ **Fig. 2b**). There are no statistically significant differences in TC levels between professional bodybuilders and the general population, ▶ **Table 2**.

Changes in serum high-density lipoprotein cholesterol

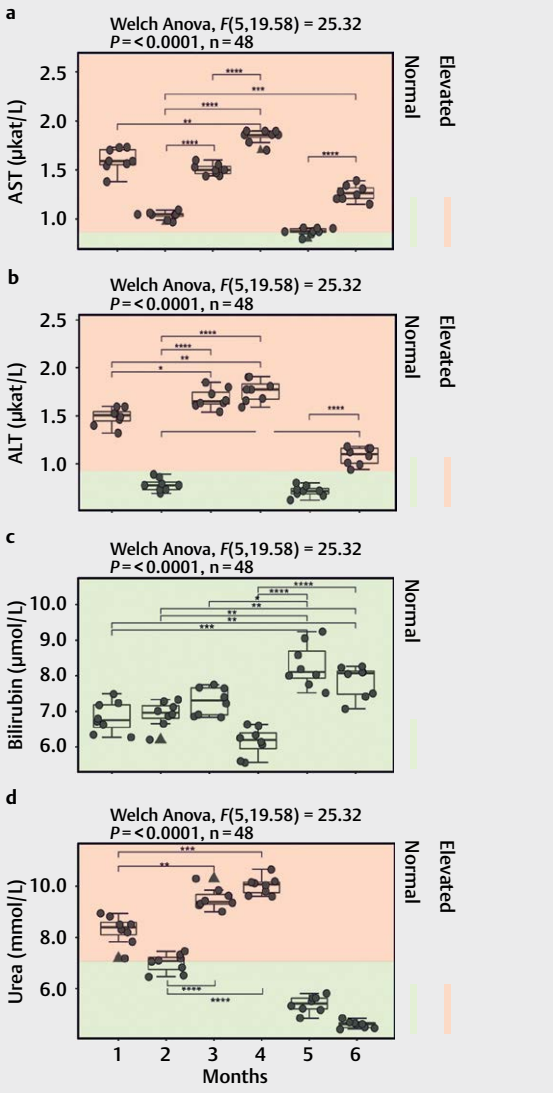
HDL-C levels in competitors vary significantly during the preparation period (▶ **Fig. 2c**), with three distinct motifs, i. e. an increase during months one to two and four to five and a decrease between months five and six. Although HDL-C levels fluctuate substantially, their values remain within the low concentration range (HDL-



▶ **Fig. 1** Changes in body mass (BM) and total body fat percentage (BF %), resulting from the administration of sports doping drugs as a function of a competitive preparation period.



▶ **Fig. 2** Changes in serum levels of **a**) Total Cholesterol (TC), **b**) Triglyceride (TG), **c**) High-density Lipo-protein Cholesterol (HDL-C), and **d**) Low-density Lipoprotein Cholesterol (LDL-C), rendered by administration of sport-doping drugs as a function of a competition preparation period. *p<0.5, **p<0.01, ***p<0.001, ****p<0.0001.



▶ **Fig. 3** Changes in serum levels of **a**) Aspartate Aminotransferase (AST), **b**) Alanine Aminotransferase (ALT), **c**) Bilirubin, and **d**) Urea, rendered by administration of sport-doping drugs as a function of the competition preparation period. *p<0.5, **p<0.01, ***p<0.001, ****p<0.0001.

C < 1.01 mmol/L) [37]. ▶ **Table 2** revealed that the HDL-C level was statistically higher in the general population than in professional bodybuilders: 1.18 vs. 0.52, respectively. Thus, the general population is defined by a normal HDL-C concentration, whereas a low HDL-C concentration defines professional bodybuilders.

Changes in serum low-density lipoprotein cholesterol

Bodybuilders LDL-C levels fluctuate within the near optimal/above optimal (2.58 mmol/L ≤ LDL-C < 3.36 mmol/L), borderline high (3.36 mmol/L ≤ LDL-C < 4.13 mmol/L), and high (4.13 mmol/L ≤ LDL-C < 4.91 mmol/L) [37] levels (▶ **Fig. 2d**). It achieves a minimum in the fourth month and a maximum in the sixth month of preparation for competition preparation. Although ▶ **Table 2** revealed that there are no differences between competitors and subjects that belong to general human populations in LCL-C levels: 3.59 vs 3.32 mmol/L, the mean level of LDL-C of competitors is included in the *borderline high* reference range, while the mean levels of LDL-C of the general population are encompassed by a near optimal range.

Changes in aspartate aminotransferase

The AST levels of professional competitors fluctuate between month one and six of the preparation period in the high-concen-

tration region (AST > 0.58 μkat/L) [38]. It reaches its minima in the second and fifth months, ▶ **Fig. 3a**. Furthermore, AST activity decreases significantly between the first and second and fourth and fifth months, and increases between the second to fourth months and from the fifth to the sixth months. There is a statistically significant difference between professional bodybuilders and subjects belonging to the 'normal' population: 1.60 vs. 0.45 μkat/L, respectively, ▶ **Table 2**. Therefore, bodybuilder AST levels are nearly four times higher than those observed in a 'normal' population. Furthermore, the 'normal' population is defined by normal levels of AST.

Changes in alanine aminotransferase

In bodybuilders, the pattern of changes in ALT levels is analogous, although more pronounced, to that observed for AST levels. ALT concentration fluctuates during the competition preparation period, adopting values encompassed by the high concentration region (ALT > 0.91 μkat/L) [38]. They reach a minimum in the second and sixth months, ▶ **Fig. 3b**. Additionally, there is a substantial decrease in ALT activity during the first and second months and the fourth and fifth months. An increase in ALT activity is observed between the 2nd and 4th, and 5th to 6th months of the contest preparation period. There is a nearly four-fold increase in the mean AST level between competitors and 'normal' subjects: 1.99 vs. 0.53 μkat /L, respectively, ▶ **Table 2**. Thus, normal subjects are defined by AST levels in the normal region, whereas competitors are defined by levels twice the value of a high concentration threshold.

Changes in bilirubin

In professional bodybuilders, the concentration of bilirubin varies considerably during the preparation period. However, all changes occur within the desirable range (12.7 ± 4.3) [39], ▶ **Fig. 3c**. Bilirubin concentration reaches a minimum in the fourth month and a maximum between the fifth and sixth months of the preparation period. A minor increase in bilirubin concentration spanning months one to three is followed by a substantial decrease between the third and fourth months and a considerable increase between the fourth and fifth months. There is a statistically significant difference in bilirubin concentration between professional bodybuilders and subjects in the general population: 6.83 vs. 4.63 μmol/L, ▶ **Table 2**. However, the mean levels that define both groups are within the desirable range.

Changes in blood urea nitrogen

Professional bodybuilders are defined by significant changes in BUN throughout the entire competition preparation period between the desirable (2.1 mmol/L ≤ BUN < 7.1 mmol/L) and high values (BUN ≥ 7.1 mmol/L) [40]. BUN levels reach a maximum in the fourth month and a minimum in the fifth and sixth months, ▶ **Fig. 3d**. There is a nearly two-fold difference in BUN levels between competitors and 'normal' subjects: 8.29 vs. 4.8 mmol/L, ▶ **Table 2**. Therefore, competitors are, on average, defined by high values of BUN, whereas 'normal' subjects are defined by desirable BUN concentration.

Discussion

The limitations of the presented study are the small study sample and the lack of clinical control over the quality of drugs used by competitors. However, to our knowledge, it is the only report, apart from two general reviews [41, 42], that discloses the extent of PED employment in preparation for a bodybuilding competition. The results of this study do not reveal changes in selected physiological, biochemical, and anthropometric parameters that could cause immediate health-related problems.

This study shows that professional bodybuilders are defined by unfavorable changes in the blood biochemistry profile and serum lipid levels. There is also a statistically significant decrease in body fat percentage between the 'normal' population and professional bodybuilders, which unfolds in the latter body fat percentage in the physiologically essential fat area [34, 35].

The results of this study on the relations between PED and HDL-C levels with the report on changes in HDL-C [43] indirectly confirms the presence of low-levels of HDL-C among AAS users. They also partially support (referring to the results obtained in the sixth month of competition preparation) the results of the study on changes in LDL-C profile as a function of AAS abuse [44]. Nevertheless, this study does not confirm the previous observation regarding cross-correlations between AAS employment and a decrease in TG levels [41] This study also confirmed the previous results indicating that administration of AAS results in increase in total lean body mass, and a decrease of body fat percentage [42].

The study also shows that self-administration of AAS increases serum testosterone levels to concentrations greater than 52.05 nm /L, that is, triple that of the analogous 'normal' male age group (17.29 nm/L) [45–47].

This study reveals that AAS administration leads to an increase in BMD. Since the majority of PED employed by the competitors are derivatives of testosterone, this study indirectly confirms the previous findings, that there is an increase in BMD in response to testosterone administration [48, 49]. The reported BMD values are also significantly higher than those observed for similarly aged European men [50]. %BF decreases in response to drug administration. However, the analysis of %BF using the DXA technique is viable for methodological variability and standardization of procedures [51]. Thus, when reviewing the DXA data, one may expect increased variability of the distribution of the data in and between time frames.

An analysis of the literature on the intake of testosterone propionate reveals that it can cause mild myocyte hypertrophy of the heart muscle [52]. Furthermore, some reports indicate that it can be accompanied by myocardial dysfunction and accelerated coronary atherosclerosis [53]. However, this study could not confirm or disprove these observations. Nevertheless, the cross-correlation of our observations with the study on correlations between coronary heart disease and serum lipid levels [54] indicates that the development of coronary heart disease is probable. We also cannot confirm the previous observations that indicate Winstrol [55], Anavar, and other AAS [56, 57] as potent hepatotoxic agents. Although the observed changes in AST, ALT, and bilirubin levels may indicate a hepatotoxic response, the observed elevation of AST and ALT may also be the product of micromuscular injuries [58] that occur during heavy-load training. Since, at the time of the survey, all com-

petitors had been using PED for more than five years, the expected changes in AST, ALT, and bilirubin levels should be greater in magnitude and should fall into the adversity brackets defined by the 'Common Toxicity Criteria for Adverse Events' [59], that is, between 1 × ULN and 1.5 × ULN to 3 × ULN to 8 × ULN, for AST and ALT, respectively.

We cannot confirm or disprove health-related adversity [60, 61] of boldenone undecylenate, a veterinary steroid known for its propensity to increase body mass and appetite [62], as well as reproductive function [60]. Furthermore, this study does not confirm the adverse correlations between T3 levels and TC, HDL-C, and LDL-C concentrations [63–65]. Although indirectly, through a decrease in body fat tissue, we confirm the previous report that indicated an increase in lipid metabolism [66] attributed to T3 intake. The study also confirms previously reported alterations in AST and ALT levels [67] as a function of T3 administration.

An animal model study shows that clenbuterol decreases TC levels and leads to fluctuations in TG levels [68]. When administered orally, clenbuterol can also affect kidney [69] and liver [70] functions. Although our study does not confirm these observations, there are fluctuations in serum lipid levels, indicating possible impairment of lipid metabolism. Furthermore, we were unable to confirm the reported correlations between AST, ALT, and bilirubin levels in response to oral administration of clenbuterol [70, 71]. However, this report confirms a moderate increase in AST and ALT levels in response to AAS intake, previously described by Abdulredha [70, 72]. In particular, the magnitude of the increase in AST and ALT levels can be masked by the physiological properties of clenbuterol, which decreases AST, ALT and bilirubin levels [71].

This study indirectly confirmed reports indicating Proviron as a prominent body mass increasing agent [73, 74].

Nolvadex is a hepatotoxic [75] estrogen receptor modulator [76], and a potent drug against gynecomastia [77]. However, this study did not confirm Nolvadex-induced hepatotoxicity [78] and hyperlipidemia [79]. It is probably due to the action of clenbuterol, resulting in decreased levels of hepatotoxicity markers.

This report also indirectly confirms previous findings that HGH increases lean muscle mass and decreases body fat tissue by approximately 2 kg and 0.9 kg [80], respectively, in a few weeks. However, it has to be stressed that the changes observed in lean body mass are also the results of other drugs including clenbuterol and 2.4-DNP.

Nevertheless, we could not confirm reports that indicate that HGH use/abuse could cause health problems [81, 82].

A previous study reported that extreme abuse of 2,4-DNP by bodybuilders for fat burning purposes resulted in death when taken in excessive amounts, that is, 3–46 mg of 2,4-DNP per kg of body weight per day [83]. This report shows that competitive bodybuilders consume, on average, between 0.9 mg and 1.9 mg of 2,4-DNP per day/kg of body mass, which amounts to ~40 % of the lowest lethal dose [84].

Conclusions

Some reports indicated that AAS and other sport enhancement drugs, such as HGH, clenbuterol, tamoxifen-citrate, and 2,4-DNP, are serious health risk factors [85–88]. In contrast to this study, however, they did not reflect real-life scenarios, such as administering of PED during preparation for competition. Furthermore, this study showed that the observed changes in the levels of parameters studied induced by performance enhancing drugs are within physiologically acceptable ranges. This report also showed that use of PED does not cause immediate health breakdown, which leads us to reject the null hypothesis. Nevertheless, long-term influence of performance enhancing drugs on human health has not yet been established.

Authors' Contributions

Significant manuscript writers: IZZ and MW. Concept and design: IZZ, MW, BT, RT. Data Analysis and Interpretation: IZZ and MW. Statistical expertise: IZZ. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

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