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The Cutaneous Bacterial Microflora of the Bodybuilders Using Anabolic-Androgenic Steroids

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Background: Anabolic-androgenic steroids (AAS) abuse by the athletes has dramatically increased during the recent decades. These substances might increase the skin lipids and enhance the cutaneous microbial proliferation.
Objectives: The current study aimed to investigate the potential side effects of AAS on the bacterial microflora colonization of the bodybuilders` skin.
Patients and Methods: The skin samples of 94 male bodybuilders (71 AAS users, 23 non-AAS users) and 46 subjects of the control group, with similar gender and age, were cultured and incubated in both aerobic condition to isolate *Staphylococcus aureus* and anaerobic condition for *Propionibacterium acnes*. The isolated bacteria were identified by standard microbiological techniques.
Results: The skin lesions were more frequent in the body builders than the controls. Moreover, statistically significant differences were also observed in skin lesions among the AAS users and the non-AAS user athletes. The prevalence of *S. aureus* and *P. acnes* in the athletes was higher than that of the control group. In addition, there was a significant difference in distribution of *P. acnes* between the bodybuilders who used AAS and those who did not.
Conclusions: A higher number of bacterial flora was found in the bodybuilders particularly those using AAS in comparison to the controls, which might be due to the influence of these AAS on the skin microflora and transmission of the bacteria through the direct contact of the naked skin with the exercise instruments.

Keywords: Athletes; cutaneous; *Staphylococcus aureus*; Drug Abusers

1. Background

Abuse of anabolic-androgenic steroids (AAS) by the members of fitness centers and others has reached alarming dimensions. Today, it is estimated that one to three million people has abused AAS in the United States (1-3). AAS mainly mimics the effects of the male sex hormones. They induce the protein synthesis in cellular tissues, which results in the buildup of cellular generations, especially in muscles, and in return can increase the strength and body weight in the athletes (1, 4). Several side effects are reported following the abuse of the AAS. Elevation of blood pressure, depression of serum high-density lipoprotein (HDL)-cholesterol levels and altering fasting blood sugar following the consumption of AAS drugs are reported to increase the risk of cardiovascular diseases (1, 5, 6).

Consumption of high doses of AAS may also cause liver damage by steroids metabolites (1, 7). Reduced sexual function and temporary infertility can occur in males as well (8, 9). In addition, it is reported that skin diseases like acne vulgaris and folliculitis are more common in AAS users (1-3, 10). These AAS increase the activation of sebaceous glands and consequently cholesterol and free fatty acids of the skin surface lipids which in turn may provide a better condition for colonization of some lipophilic bacteria such as *Propionibacterium acnes* and *Staphylococcus aureus* (10-12). Moreover, secretion of lipase by these bacteria, known to be resistant to antimicrobial activities of the fatty acids, provides a suitable environment for colonization in sebaceous follicles which in turn may present as sebaceous follicles comedones and inflamed lesions such as papules, pustules, and cysts (13).

2. Objectives

The effect of these supplements on the skin microbial flora is not clearly determined. The current study aimed to investigate the colonization of some bacterial flora among the male bodybuilders both in AAS and non-AAS users.

3. Patients and Methods

Seventy one male bodybuilders using AAS were studied. In addition 23 male bodybuilders AAS non-users and 46 non-athlete students, with similar gender and age range, were selected as the control group. The average and median ages of the bodybuilders were 24.7 and 24 years, respectively. All the participants completed a questionnaire including bathing habits, using AAS and other supplements, administration of antimicrobial drugs for at least four weeks, and general medical conditions. The study was approved by the ethics committee of Shiraz University of Medical Sciences. All the participants willingly provided written informed consent approved by the ethics committee of Shiraz University of Medical Sciences. The skin of the examinees was checked for the presence of the lesions. Specimens were obtained from the skin surface by swabbing 1 cm^2 of the back and chest areas by two wet swabs. Those received systemic or topical antibiotic drugs during the month before sampling were excluded from the study.

The swab was directly spread on Tryptone Soy Agar (Merck, Germany) supplemented with 5% v/v defibrinated sheep blood under an anaerobic condition at 37°C to isolate *P. acnes* and under aerobic condition for *S. aureus* species. The isolated strains of *S. aureus* were identified by standard methods including colony morphology, gram stain, catalase test, mannitol fermentation and coagulase test on aerobic overnight cultured samples. To isolate *P. acnes*, following seven days of anaerobic incubation, the plates were examined to detect bacterial colonies that were not present in the aerobic conditions. Suspected anaerobic colonies were then subcultured in the aerotolerance plates. *P. acnes* were identified by lack of growth in aero tolerance plate, colony morphology, Gram stain, and indole test (S+) (14). The number of isolated bacteria from each swab was counted and recorded for each bacterium per anaerobic and aerobic plates. The quantity of both isolated *P. acnes* and *S. aureus* from each sample site was clustered into four groups (0, 1-9, 10-49, > 50) based on the corresponding colony forming unit/cm_2 (CFU/cm_2). The $\chi 2$ test was used to examine the bacterial colonization at the examined sites between the AAS users and the others. Values of $P < 0.05$ were considered statistically significant.

4. Results

Of the 94 bodybuilders, 75.5% (n = 71) used the complements. It is Mean (average) and Median which are 24.7±5.1 and 24 years, respectively. Distribution of the lesions is shown in Table 1. According to this table, lesional skins were higher in the athletes than in the non-athlete controls and this difference was statistically significant ($P < 0.05$). Moreover, statistically significant differences were also observed in the distribution of the lesions between the complement users and the rest of the bodybuilders ($P < 0.05$).

In the examined groups, only nine persons (6.4%) reported smoking cigarette including three AAS-user athletes, two AAS non-user athletes and four non-athletes. No significant differences were found in smoking habit between AAS-users and the control groups ($P > 0.05$). Of the AAS-user athletes, 40 (56.3%) reported to take bath 2-5 times per week and the rest (n = 31, 43.7%) took bath daily or more. Similarly, 12 (52.2%) and 11 (47.8%) of AAS non-user athletes took bath 2-5 times per week and on daily basis, respectively. No significant difference in the number of taking bath per week was found between the above mentioned groups of athletes (AAS-user vs. AAS non-user athletes).

The results of the specimen cultures are shown in Table 2. The anaerobic cultures of 24.2% of the athletes were positive; among them 94.1% were from the AAS users whereas the aerobic cultures of the skin yielded positive results in 92.6% of the athletes among whom 75.9% were AAS users. Actual data based on colony counts and in the athletes and control groups are shown in Table 3. There were statistically significant differences regarding bacterial flora including *S. aureus* and *P. acnes* between athletic (AAS-user and AAS non-user) and non-athletic groups. In addition, there was a significant difference in distribution of *P. acnes* was found between bodybuilders who used AAS and those who did not ($P < 0.001$). Additionally, in 88.7% (n = 63) of the AAS-user and 87.0 % (n = 20) of AAS non-user athletes, *S. epidermidis* was isolated from their skin which was not statistically significant ($P > 0.05$).

Table 1. Distribution of the Types of Lesions in Bodybuilder and Control Groups [a,b]

Types of lesions	AAS (+) athl	AAS (-) athl	AAS (-) non-athl	Total
Papula	15 (21.1)	2 (8.7)	7 (15.2)	24 (17.2)
Pustula	25 (35.2)	2 (8.7)	5 (10.9)	32 (22.8)
Negative	31 (43.7)	19 (82.6)	34 (73.9)	84 (60.0)
Total	71 (100.0)	23 (100)	46 (100)	140 (100.0)

[a] all of values are present as No. (%).
[b] Abbreviations: AAS (+): Anabolic-Androgenic Steroids consumer, athl: athletes, AAS(-): Anabolic-Androgenic Steroids non-users, Non-athl: non- athletes, N: Number, %: Percent.

Table 2. Skin Culture Swabs of the Athletes and Control Groups [a]

	AAS (+) athl	AAS (-) athl	AAS (-) non-athl	Statistics [b]
P. acnes				P < 0.001
+	32 (45)	4 (17.4)	2 (4.3)	
-	39 (55)	19 (82.6)	44 (95.6)	
S. aureus				P < 0.001
+	24 (33.8)	9 (39.1)	2 (4.3)	
-	47 (66.2)	14 (60.9)	44 (95.6)	

[a] Abbreviations: AAS (+): Anabolic-Androgenic Steroids consumer, athl: athletes, AAS (-): Anabolic-Androgenic Steroids non-users, non-athl: non-Athletics, N: Number, %: Percent .
[b] Between AAS (+) athl and non-athl.

Table 3. Colony Counts of the Athlete and Control Groups [a, b]

Number of Colonies	*P. Acnes*			*S. aureus*		
	AAS (+) athl	AAS (-) athl	AAS (-) non-athl	AAS (+) athl	AAS (-) athl	AAS (-) non-athl
0	39 (55)	19 (82.6)	44 (95.6)	47 (66.2)	14 (60.9)	44 (95.6)
1-9	2 (2.8)	0 (0.0)	2 (4.3)	1 (1.4)	1 (4.3)	2 (4.3)
10-49	15 (21.1)	3 (13.0)	0 (0.0)	2 (2.8)	2 (8.7)	0 (0.0)
> 50	15 (21.1)	1 (4.4)	0 (0.0)	21 (29.6)	6 (26.1)	0 (0.0)
Total	71 (100.0)	23 (100.0)	46 (100.0)	71 (100.0)	23 (100.0)	46 (100.0)

[a] AAS (+): Anabolic-Androgenic Steroids consumer, athl: athletes, AAS (-): Anabolic-Androgenic Steroids non-users, non-athl: non- athletes, N: Number, %: Percent.
[b] all of values are present as No. (%) .

5. Discussion

Utilizing performance enhancing drugs has dramatically increased among the young athletes to rebuild body mass and strength, increase delivery of oxygen to muscles and treat pain without any concern about their serious side effects. On the other hand, all adverse effects of these drugs are not clearly investigated (1, 2, 15). The occurrence of the cutaneous striae, oily skin, alopecia and male patter hair loss is commonly reported as a result of AAS abuse in athletes (10, 16). In addition, the frequency of acnes in AAS users was 40%-54% (2, 17, 18). The current study reported statistically significant higher skin lesions in AAS abusers compared to the others.

Previously, it was indicated that methyl testosterone and its metabolites may enhance the bacterial proliferation and their enzymatic activities (19). In another study, Kiraly showed that mild acne induced following eight weeks of self-administration of high dose testosterone and anabolic steroids (16). Kiraly also found that high doses of testosterone and AAS may increase the skin surface lipids which in turn may provide a suitable condition for the growth of *P. acnes* (16). On the other hand, supra-physiologic doses of AAS may or can affect the immune system (20, 21). Several common AAS alter the immune reaction by adversely influencing lymphocyte differentiation and proliferation, antibody production, natural killer cytotoxic activity and the production of certain cytokines, and thereby enhancing bacterial proliferation (20, 21).

In the present study, skin cultures yielded positive results for *P. acnes* in 45% of the AAS-user bodybuilders which was about four times as much as those of non-AAS user bodybuilders and 18 times as much as the controls. These results were in agreement with those of the study by Kiraly who showed that the population of *P. acnes* increased significantly in the self-administrated AAS young individuals following eight weeks of using AAS (16). In addition, the current study also found more frequent positive cultures (odds ratio = 3.8) in the non-AAS user bodybuilders in comparison with those of the non-athletic control group; this might be caused by higher rates of bacterial transmission among athletes through direct skin contact with exercise instruments in the gym. However, there were no differences in the lesional skin between the groups.

Staphylococcus aureus is the most common cause of primary and secondary human skin infections (22, 23), though it is not considered as an inhabitant normal flora of the skin. It is commonly found on the skin of the normal population with frequency of 18%-40% (24, 25). The present study found a higher rate of *S. aureus* positive cultures in the athletes compared to the controls. However, there was no statistically significant difference in the culture results between the two studied athletic groups; it may suggest that using AAS has no role in *S. aureus* colonization. It was previously shown that environmental sources such as shared equipment and towels may play a role in community acquired *S. aureus* in the athletes (26, 27). The higher colonization of *S. aureus* in the athletes in comparison with the controls may be caused by the bacterial transient skin flora passing through the exposure of bare skin to the exercise instruments. A significant association was previously reported by Singh et al. between high colonization of *S. aureus* and the possibility of the skin infection (28). Therefore, as shown previously (28, 29), the high number of colonies (> 50) isolated in about one third of the bodybuilders may predispose them to skin infections.

To investigate the side effect of AAS further, studies should include other sports and both sexes. In addition, expanding the investigation to a larger cohort could provide more valuable information about the impact of AAS on the bacterial skin colonization.

Authors' Contributions

Study concept and design: Kamiar Zomorodian. Sample collection and laboratory examinations: Mohammad Javad Rahimi, Mohammad Taheri, Ali GhanbariAsad, Soghra Khani and Iman Ahrari; data interpretation: Keyvan Pakshir, Kamiar Zomorodian, Reza Khashei; manuscript drafting: Kamiar Zomorodian, Keyvan Pakshir; manuscript revision: all the Authors. Statistical analysis: Kamiar Zomorodian and Mohammad Taheri.

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