The Effect of Testosterone and Anabolic Steroids on the Skin Surface Lipids and the Population of Propionibacteria acnes in Young Postpubertal Men

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The effect of testosterone and anabolic steroids on skin surface lipids and the population of Propionibacteria acnes (P. acnes) was studied in power athletes. The subjects used self-administered high doses of testosterone and anabolic steroids during a 12-week strength training period. After 8 weeks' use of hormones the amount of dissolved skin surface lipids (SSL), and the Colony Forming Units/cm^2 (CFU/cm^2) of P. acnes had increased ($p<0.01$). The percentage values of dissolved SSL constituents changed. The cholesterol (CHO) and also the relative values of free fatty acids (FFA) increased. SSL constituents obtained by collection on absorbent paper likewise changed the dissolved constituents. It was concluded that high doses of testosterone and anabolic steroids may increase the SSL, the P. acnes population, and the percentage of the CHO and FFA of the skin surface lipids in healthy young men. *Key words: Androgenic hormones; Skin lipids' constituents; Sports medicine.*
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In early acne investigations it has been suggested that free fatty acids (FFA) and Propionibacteria acnes (P. acnes) play an important role in the aetiology of acne vulgaris (1, 2) (see also a review 3). A positive correlation between the prevalence of P. acnes and the presence of acne vulgaris has in fact been found in young men (4). McGinley et al. (5) demonstrated an age-related association between the frequency of P. acnes and sebaceous gland activity. It has also been suggested that the number of the P. acnes population is proportional to the amount of skin free fatty acids (6).

However, the hypothesis of free fatty acids being the prime irritants in acne vulgaris has come to be questioned. In some studies the quantity of FFA does not differ in acne patients from that in controls (7, 8). It has also been suggested that the number of P. acnes does not differ between the acne patients and the non-acne controls (9). Many studies have demonstrated high serum androgen levels in men and women with severe acne (for a rewiew see 10). Also constitutionally tall, pubescent boys who received testosterone to stop their growth developed acne (11).

However, no attention has been paid to the effect of exogenous androgens causing acne, changes in the skin propionibacteria, and in surface lipids, probably because of the dominant hypothesis of maximal stimulation of the sebaceous gland by endogenous androgens (for a review see 10). Contrary to this maximal stimulation hypothesis, we have shown that high doses of exogenous androgens increased the sebaceous gland activity in young postpubertal men, and increased gland size (12, 13).

Our aim was to study the effect of exogenous testosterone and anabolic steroids on the

Table I. *Individual daily doses (mg) of self-administered testosterone and anabolic steroids*

The values indicate means for the 4-week-periods (I = 0–4 wk, II = 5–8 wk, III = 9–12 wk) between the skin lipid and bacterial collections. The mean ± SE for the experimental group is also given

		Subjects							
		1	2	3	4	5	6	7	$\bar{x}$ ± SE
Methandienone									
I	(0–4 wk)	21	–	13	13	15	10	10	14±1.5
II	(5–8 wk)	30	–	20	20	20	10	20	20±2.4
III	(9–12 wk)	30	–	20	20	20	10	18	20±2.4
Nandrolone									
I	(0–4 wk)	19	7	7	7	5	5	6	8±1.8
II	(5–8 wk)	26	14	7	7	3	5	8	10±3.0
III	(9–12 wk)	17	4	8	8	9	6	6	8±1.5
Stanozolol									
I	(0–4 wk)	–	19	2	2	–	–	–	8±3.9
II	(5–8 wk)	–	13	2	2	–	–	7	6±1.8
III	(9–12 wk)	2	25	5	5	–	–	3	8±3.6
Testosterone									
I	(0–4 wk)	23	7	19	19	26	23	15	19±2.4
II	(5–8 wk)	27	9	23	23	17	24	25	21±2.3
III	(9–12 wk)	12	6	12	12	19	31	9	14±3.2

Trivial and systemic names: methandienone: 17 α-methyl-17β-hydroxy-1,4-androstadien-3-one; nandrolone phenylpropionate, 17β-hydroxy-4-estren-3-one phenylpronionate; stanozolol, 17α-methyl-5α-androstano (3,2-c)-pyrazol-17-βol; testosterone: 17β-hydroxy-4-androsten-3-one.

appearance of acne, on surface lipids and on skin P. acnes population in healthy young postpubertal men, who used self-administered high doses of these hormones.

MATERIAL AND METHODS

Seven power athletes (mean age 29 years, range 24–34 years), who had previously used androgenic and anabolic steroids in their strength training, volunteered for this study as an experimental group (EG). Three had a history of atopic dermatitis, but none a history of acne. All these athletes had been without any hormones for a period of 12 weeks prior to this study. The experimental subjects were included in the study from the moment they began the self-administration of non-medically prescribed testosterone and anabolic steroids. The steroids were obtained on the black market and were thus used outside of medical control. The self-administration of hormones was followed by means of medical diaries. Methandienone was taken orally (5 mg–20 mg) (n=6) by most of the subjects daily. Nandrolone (50 mg) (n=7) and stanozolol (50 mg) (n=4) were usually injected once a week. Testosterone (250 mg, consisting of 30 mg testosterone propionate, 60 mg testosterone phenylpropionate, 60 mg testosterone isocaprionate and 100 mg testosterone decanoate) (n=7) was self-injected 1–2 times per month. The doses of the drugs are shown in Table I. The body weight, the amount of body fat of the subjects (14) and the testicular volume (15) were examined before and after the study. The body weight increased from 90.9±3.4 kg to 97.1±3.1 kg during the course of the study (p<0.001) (12). The amount of body fat did not change during the course of the study (11.1±1.0% at the start and 11.0±2.1% at the end). A decrease (p<0.001) in testicular volume was noticed between the start and the end of the study (from 21.4±1.4 ml to 13.0±2.3 ml, respectively) as a consequence of the use of testosterone and anabolic steroids (16).

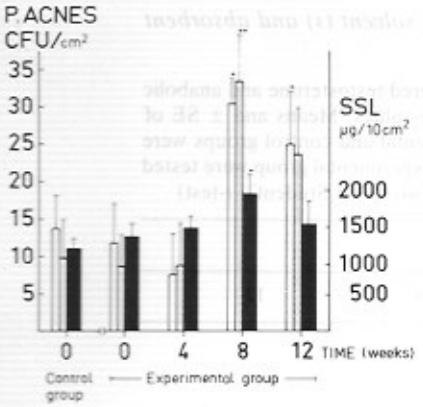

Fig. 1. The figure shows the bacterial density of Propionibacteria acnes (P. acnes) and the amount of the dissolved skin surface lipid (SSL) of the forehead skin in the groups studied. The experimental group (n=7) self-administered testosterone and anabolic steroids during weeks 1–12 (see Table I). The light columns indicate the single colony forming units/cm^2 (CFU/cm^2) of P. acnes in dilutions 1 to 100 (white columns) and 1 to 1000 (gray columns), and the SSL, µg/10 cm^2 (black columns) before (0-week) and during (4–12 weeks) the use of hormones. The control group (n=8) was examined once, at the beginning of the study (0-week). Means and ± SE of means are given. * $p<0.05$, ** $p<0.01$. Differences between the mean values of the experimental and control groups were tested by unpaired t-test and differences between the values inside the experimental group were tested by paired t-test for significance.

Eight power athletes (mean age 31 years, range 24–34 years) served as control subjects (CG). They had no experience in the use of androgenic hormones. Four had a history of acne vulgaris.

METHODS

In order to determine the gravimetrical values (µg/10 cm^2) of skin surface lipid (SSL) of the forehead a direct application of solvent (penthane) using a glass cylinder was employed (17). Briefly, 5 ml of penthane was poured onto the skin through a glass cylinder which was firmly held on the skin of each lateral forehead skin area. The solvent was gently stirred with a plastic spatula for 1 min and removed with a glass pipette and pooled. In addition to the above SSL, dissolved SSL specimens were collected on absorbent paper sheets, as also skin surface lipid. Briefly, the first paper sheets were collected until a spotty lipid pattern appeared on the papers (12). On all of these lipids thin-layer-chromatography (TLC) was performed immediately using a method described elsewhere (12).

Bacteria were obtained from forehead skin by the surface "scrub-technique" of Williamson & Kligman (18). Two successive samples were taken symmetrically from the lateral forehead and pooled. Propionibacteria were enumerated by plating out serial 10 fold dilutions onto Brain-Heart-Infusion (BHI) Agar which was incubated for 7 days anaerobically at 37°C. Propionibacteria were differentiated from staphylococci on the basis of colony morphology and Gram stain and by testing for reduction ability of nitrate, for production of indole, and for ability of casein hydrolysation (19). Counts were expressed as a single colony forming unit/cm^2 (CFU/cm^2) of forehead skin. The indole production test was carried out after four days' anaerobic subculture on Tryptic Soy Agar (Gibco) and using the Rosco Diagnostic Tablets (Rosco Diagnostica, Taastrup, Denmark) following the manufacturer's instructions. For the casein hydrolase test the strains had to be bred for 13–14 days anaerobically on casein agar plates, after which casein hydrolase will appear as a clearing zone around the growth. In order to confirm that the clearing rings were casein hydrolysis the plates are rinsed with 10% hydrochloric acid, after which the clearing zone remains if it is caused by casein hydrolysis.

For subcultures the Blood Agar Base No. 2 (Oxoid) with 8.0 g/100 ml distilled water and Skim Milk Powder (Oxoid) 10% solution were used. Both the agar and the milk were sterilized at 121°C for 15 min. The cooled solutions were mixed in a 1 to 1 relation and the mixtures poured onto the plates.

The SSL collections, TLC analyses, and the enumerating of bacterial population were performed in weeks 0, 4, 8 and 12 in the experimentals (EG) and in week 0 in the controls (CG).

Means and ± SE of means were calculated. Differences between the mean values of the experimen-

Table II. *The skin lipid fractions of forehead skin obtained by solvent (s) and absorbent paper (p) in the group studied*

CG=control group (n=8). The experimental group (n=7) self-administered testosterone and anabolic steroids during weeks 0–12 (see Table I). The figures indicate relative values. Means and ± SE of means are given. Differences between the mean values of the experimental and control groups were tested by unpaired t-test and differences between the values inside the experimental group were tested by paired t-test for significance. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ (two-tailed Student's t-test)

	Time (weeks)				
	0	0	4	8	12
Free fatty acids (FFA)	*CG*	*Experimental group*			
s:	9.0±2.3	12.5±0.6	17.7±3.1***	16.8±2.4	19.3±3.1***
p:	7.9±1.3	10.5±2.5	11.2±2.7**	11.9±2.3**	12.1±2.8**
Squalen (SQ)					
s:	14.3±1.2	12.5±0.6	11.7±1.0	11.0±0.8	11.1±0.5
p:	13.4±0.6	12.9±0.6	12.8±0.6	12.3±0.8	15.3±1.1
Triglycerides (TG)					
s:	26.4±1.3	21.1±1.6*	19.8±2.4	17.5±1.6	16.9±2.6***
p:	28.1±1.3	26.1±2.1	28.1±2.5	25.9±2.2	25.7±2.2
Wax esters (WE)					
s:	29.4±1.0	27.7±1.1	27.9±1.0	27.5±1.4	26.0±1.2
p:	29.7±0.9	30.3±1.2	29.4±0.6	30.4±0.9	30.9±0.9
Cholesterol (CHO)					
s:	3.3±0.7	3.3±0.3	5.6±1.7**	5.5±1.0	6.0±1.5**
p:	3.7±0.7	2.6±1.9	4.3±0.7***	4.3±1.1	4.1±1.3*

tal and control groups were tested by unpaired t-test and differences between the values inside the experimental group were tested by paired t-test for significance.

RESULTS

There were no differences in skin surface lipid (SSL) constituents at the beginning between experimentals (EG) and controls (CG), except in the triglyceride values (TG) (Table II) of samples obtained by dissolving (Fig. 1 and Table II).

The skin surface lipid (SSL) increased from 1277.4±155 μg/10 cm^2 to 1942.8±245 μg/10 cm^2 ($p<0.05$) after 8 weeks' use of steroids (Fig. 1). The constituents of these dissolved SSL changed as follows: the free fatty acids (FFA) increased ($p<0.001$) from 12.5±0.6 to 19.3±3.1% after 12 weeks' self-administration of hormones and the CHO% values increased from 3.3±0.3 to 6.0±1.5 ($p<0.01$) (Table II). The squalen (SQ) and the wax esters (WE) constituents did not change. Unexpectedly, the triglycerides decreased ($p<0.001$) during the course of the study (Table II).

The tendencies towards change in paper-collected SSL constituents were similar to dissolved samples. The FFA rose from 10.5±2.5% to 12.1±2.8 ($p<0.01$) and the CHO from 2.6±1.9% to 4.1±1.3% ($p<0.05$) (Table II).

The Propionibacteria population did not differ at the beginning between EG and CG.

After 8 weeks' use of hormones the CFU number increased from 9.7±5.1 CFU/cm^2 to 33.6±6.6 CFU/cm^2 ($p<0.01$) in samples with a dilution of 1 to 1000 (Fig. 1). The increase of the CFU number in a weaker dilution (1 to 100) was also statistically significant ($p<0.05$) (Fig. 1).

Mild acne was observed. After 8 weeks' use of the steroids four out of seven subjects developed 35–50 papulopustulotic lesions on the upper trunk and face.

DISCUSSION

Interestingly, during the 12-week-period of testosterone and anabolic steroid self-administration a number of salient effects seemed to occur: there was an increase in skin surface lipids and their constituents. The population density of P. acnes increased and when the values of the single CFU/cm^2 were at their highest they were approximately 3.8 times those seen at the beginning of the study. The statistical increase of SSL ($p<0.05$) is thought to be a direct effect of exogenous androgens, because the forehead SSL consists of about 96% sebum (20), and we have shown in our earlier study (12) that the sebum excretion rate had increased after testosterone and anabolic steroids. An interesting observation was also that the Propionibacteria density and the amount of skin surface lipid were at a lower level in the 12-week-analysis, probably due to lowered mean doses of testosterone and anabolic steroids used during weeks 9–12.

Contrary to the earlier hypothesis (for a review see 10), the sebaceous gland in young postpubertal males can be stimulated beyond the basic physiological level, as our observation suggested regarding the skin surface lipid level.

Interestingly, the increase in cholesterol (CHO) was revealed in the samples collected in both ways, i.e. in dissolved and paper collected samples. The CHO tendency to increase was also similar to that noted in sebum lipid samples in our previous work performed under the same conditions (12).

Our group has shown that the use of testosterone and anabolic steroids leads to a decrease in serum HDL-cholesterol (21). In hirsute women the levels of plasma cholesterol, triglycerides and total lipids were significantly higher in the work of Dimiliscu & Bartoc (22) than in the controls. Further, Melico-Silvestre et al. (23) observed higher free skin cholesterol lipids in subjects with hyperlipidaemia. Regarding the above suggestions, the increased cholesterol level in our work may reflect an effect of androgens on the sebaceous glands. However, it is necessary to remember that sebaceous glands are halocrine structures and the skin lipids are almost entirely produced within the sebaceous glands (24). The two different sampling techniques, absorbent paper and dissolvent collection, may affect the level of percentages obtained. On the one hand, dissolved samples may contain more CHO and FFA and on the other hand samples collected by absorbent paper may contain less FFA (25). However, the changes in the tendencies of the constituents were parallel during the course of the study.

The increase of free fatty acids and the decrease of triglycerides during the course of the study are probably due to the suggested hypothesis that there may be hydrolysis of triglycerides to free fatty acids (26). However, between the controls and the experimentals there was a statistical difference in the triglyceride values at the beginning of the study in dissolved samples. Thus, it is not possible to suggest conclusions.

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