ORIGINAL ARTICLE

Anabolic androgenic steroids induce micronuclei in buccal mucosa cells of bodybuilders

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Objective: To evaluate genotoxicity of anabolic androgenic steroids (AAS) in male bodybuilders by a micronucleus assay in buccal mucosa cells.
Methods: 11 male bodybuilders volunteered to participate in this study and two groups were formed: group 1 (n = 6), without AAS consumption and group 2 (n = 5), with AAS consumption. A sample of buccal epithelium was taken from each participant once a week for 6 weeks. Samples were fixed, stained and analysed by a light microscope, and 2000 cells were counted from each slide. Results are expressed as micronucleated cells (MNC) per 1000 cells and were analysed by the Mann–Whitney U test and Wilcoxon's test.
Results: A marked increased in MNC was seen in bodybuilders with AAS consumption compared with those without AAS consumption (mean (SD) 4.1 (2.4) MNC/1000 cells vs 0.4 (0.4) MNC/1000 cells, respectively; $p<0.004$). Intragroup comparisons showed no differences in the MNC frequencies during the sampling time in group 1, whereas the MNC frequency in group 2 varied significantly, reaching the highest MNC frequencies in the third and fourth week of sampling (5.9 (2.4) MNC/1000 cells; 5.8 (1.8) MNC/1000 cells, respectively); frequency in the first sampled week was 1.1 (0.1) MNC/1000 cells. Significant differences in all sampled weeks were found between the two groups.
Conclusion: AAS consumption increased the frequency of MNC from buccal mucosa in bodybuilders.

Ergogenic drugs are used to enhance athletic performance. These drugs include illicit substances as well as compounds that are marketed as nutritional supplements. Many such drugs have been widely used by professional and elite athletes for several decades. However, in recent years, some reports indicate that younger athletes are increasingly experimenting with these drugs to improve both their appearance and athletic abilities.[1 2] Ergogenic drugs that are commonly used by youths today include anabolic androgenic steroids (AAS), steroid precursors (androstenedione and dehydroepiandrosterone), and growth hormone, creatine, and ephedra alkaloids. Anabolic steroids and creatine do offer potential gains in body mass and strength but can cause adverse effects to multiple organ systems.[3–5] Steroid precursors, growth hormone, and ephedra alkaloids have not been proved to enhance any athletic measures, but they do impart many risks to their users.[1–5]

In some cases ergogenic substances cause physiological dependence when they are mixed with other drugs such as cocaine, marihuana and alcohol.[6] The main group of ergogenic substances refers to the anabolic steroids, such as testosterone and its derivatives. Information suggests that the use of hormonal anabolic substances causes collateral effects such as reproductive toxicity, behavioural changes, hepatic and renal disorders, among others.[7 8] Therefore, it is necessary to conduct integrative studies to describe and quantify objectively the genotoxic consequences of these substances.

A low-cost, quick and direct alternative technique for evaluating such consequences is the micronucleus (MN) test. The MN is formed during transition between the metaphase and the anaphase in cellular mitosis. The chromosome left over (aneuploid event) leads to the loss of complete chromosomes, and the chromosomal fracture produces acentric fragments (clastogenic event) which are unable to become part of the nucleus of cell daughters, thereby creating an MN.[9–14] The MN assay as a measure of genotoxicity, detects clastogenic agents (that fractured the chromosomes), as well as aneugenic agents (that affect the mitotic apparatus). These events are spontaneous, but in the presence of certain endogenous and exogenous agents such phenomena are more common.[9–15]

The presence of an MN is an indicator of the effect of mutagenic or genotoxic agents, especially micronucleogenic agents. An MN can be found in any tissue, whether it be plant,[12] laboratory animal,[9 12–14] wild animal[14–16] and even in human tissue.[11 12 16–21] The following tissues are useful for the biomonitoring of human populations: lymphocytes,[11] reticulocytes[9] and erythrocytes.[16–18] Exfoliated epithelial cells are also biomarkers of genotoxicity, with the advantage that they do not need to be kept in vivo.[19–21] The MN seen in exfoliated cells are formed in the basal layer of the epithelium, after which they migrate to the surface of the epithelium. Therefore, monitoring of genotoxic exposition in this tissue can reflect the damage occurring during the course of 3 weeks.[19 20] This study aimed at evaluating the genotoxicity of AAS in bodybuilders by an MN assay in buccal mucosa cells.

SUBJECTS AND METHODS

Source population

A total of 11 male bodybuilders (aged 20–40 years) volunteered to participate in the study. They had training experience of at least 3 years, comprising at least four strength training workouts a week or 8 h of strength training a week. They were recruited in regional (Guadalajara, Jalisco, Mexico) gym clubs. After being informed about the detailed consent procedure, participants completed an extensive questionnaire about health status, history, training habits and the use of AAS and had a full physical examination to exclude any diseases such as hypertension, diabetes mellitus, liver disease, hereditary hypercholesterolaemia, infertility, smoking and drug addiction.

Abbreviations: AAS, anabolic androgenic steroids; MN, micronucleus; MNC, micronucleated cells

Table 1 Regimens, doses and consumption scheme of anabolic hormonal compounds

Cases	Consumption of anabolic hormonal compounds (g/week)											
	1st Week						2nd Week					
	I	II	III	IV	V	VI	I	II	III	IV	V	VI
1	0.4	0.3	0.06	–	0.5	0.25	0.4	0.4	0.06	–	0.5	0.25
2	0.3	0.5	–	1.0	–	–	0.3	0.5	–	1.0	–	–
3	0.3	0.5	–	1.0	–	–	0.3	0.5	–	1.0	–	–
4	0.1	0.3	0.04	–	–	–	0.1	0.3	0.04	–	–	–
5	0.2	–	–	–	–	–	0.2	–	–	–	–	–
	3rd Week						4th Week					
1	0.4	0.4	0.6	–	0.5	0.25	0.5	0.4	0.5	–	–	–
2	0.3	0.4	–	1.0	–	–	0.3	0.4	–	1.0	–	–
3	0.3	0.4	–	1.0	–	–	0.3	0.4	–	1.0	–	–
4	–	–	0.04	–	–	–	–	–	0.04	–	–	–
5	0.2	–	–	–	–	–	0.2	–	–	–	–	–

Anabolic hormonal compounds: I, decadurabulin (nandrolone ester), II, equipoise (boldenone undecylnate); III, diabole (metrandostendine); IV, testosterone entanate; V, testosterone cypionate; VI, Sostenon 250 (propionate, phenylpropionate, isocapoate and testosterone decanoate.
In the 5th and 6th week no AAS were consumed by any of the subjects.

The study was approved by the medical ethical review committee at Universidad Autonoma de Guadalajara, and all subjects gave their written approval before participating, based on the Ley General de Salud and the NOM-008-SSA2-1993, norm.

Sixty-six samples of buccal mucosa were collected: 36 from bodybuilders without AAS consumption before or during the experiment (group 1; n = 6), and 30 from bodybuilders who consumed hormonal anabolic substances (group 2; n = 5; table 1) such as: equipoise (boldenone undecylnate), decadurabulin (nandrolone ester), diabole (metadostendine), testosterone cypionate, testosterone entanate, and Sostenon 250 (propionate, phenylpropionate, isocapoate and testosterone decanoate). Such anabolic hormones were taken mixed or alone for 4 weeks during the experiment.

From each group, two samples of buccal epithelium cells were taken consecutively once a week for 6 weeks, and for group 2, samples were additionally taken weekly before and after this period.

Sample preparation

Participants were asked to rinse their mouths with water, then two smears from the buccal mucosa of the right and left cheeks were taken onto clean slides. The smears were allowed to dry, were fixed with 80% ethanol for 48 h, then stained with orcein for 2 h and fast green for 10 min (fig 1). All slides were coded before microscopic analysis.

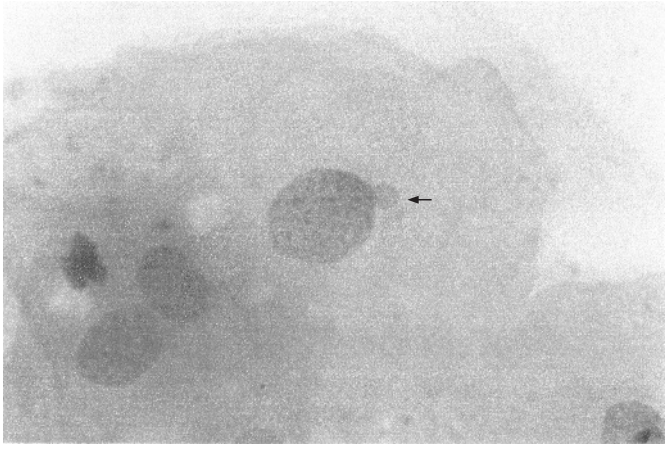

Figure 1 Micronucleated cell from buccal mucosa stained with orcein and fast green. The arrow indicates a micronucleus from buccal mucosa cells.

Sample analysis

The samples were examined by two readers who counted the micronucleated cells (MNC) blindly, using a light microscope. The scoring was done according to the criterion established by Torres-Bugarín *et al*.[19] Each reader counted 2000 cells from each slide, and the results are presented as MNC per 1000 cells.

Statistical analysis

All data were tested for normality using the Shapiro–Wilks test. Because the results did not show a normal distribution, the frequency of the MNC was evaluated by Wilcoxon's test for intragroup comparisons and by the Mann–Whitney U test for intergroup comparisons using the Statistical Program for the Social Sciences (SPSS v 11.0) for Windows medical pack (SPSS Chicago, IL, USA). A p value of <0.05 was considered significant.

RESULTS

Table 1 shows the AAS compounds and the doses consumed weekly by the bodybuilders (group 2; n = 5); these were consumed singly or combined and the doses were variable according to the hormonal mixture.

Figure 1 presents an MNC in the buccal mucosa of bodybuilders. Table 2 and fig 2 provide the individual values and mean frequency of MNC per week for groups 1 and 2. The mean MNC values were significantly different between groups ($p<0.001$), being much higher for the group with AAS consumption (4.1 (2.4) MNC/1000 cells) than for those without AAS consumption (0.4 (0.4) MNC/1000 cells).

Intragroup comparisons showed no differences in the frequencies of MNC during sampling in the group without AAS consumption, whereas in the bodybuilders with AAS consumption, MNC frequencies increased significantly from the second until the fifth weeks of sampling, reaching the highest MNC values at the third and fourth sampling weeks (5.9 (2.4) MNC/1000 cells; 5.8 (1.8) MNC/1000 cells, respectively); however, in the sixth sampling week the MNC frequency fell to 3.0 (2.3) MNC/1000 cells (table 2 and fig 2).

In intergroup comparisons, the MNC frequencies were significant increased in all sampling weeks of the AAS consumption group compared with the corresponding control group sampled week (table 2).

Individual values of MNC in group 2 showed variation among them, but in all cases, the MNC frequency increased in the third sampling week (fig 3) independently of AAS regimens and doses.

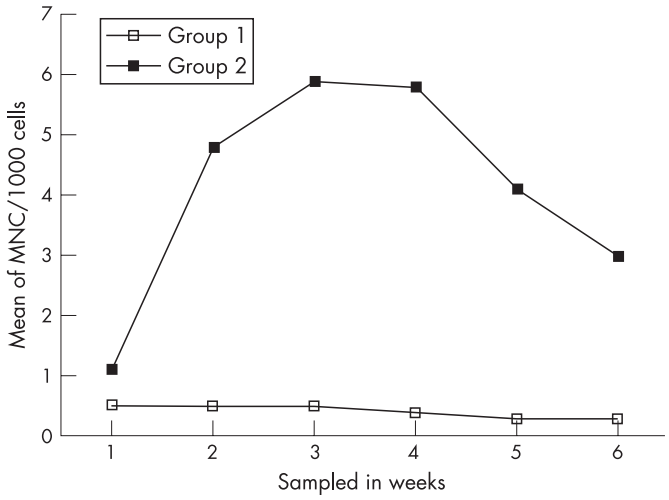

Figure 2 Micronucleogenic effect of anabolic androgenic steroids in bodybuilders. MNC, micronucleated cells

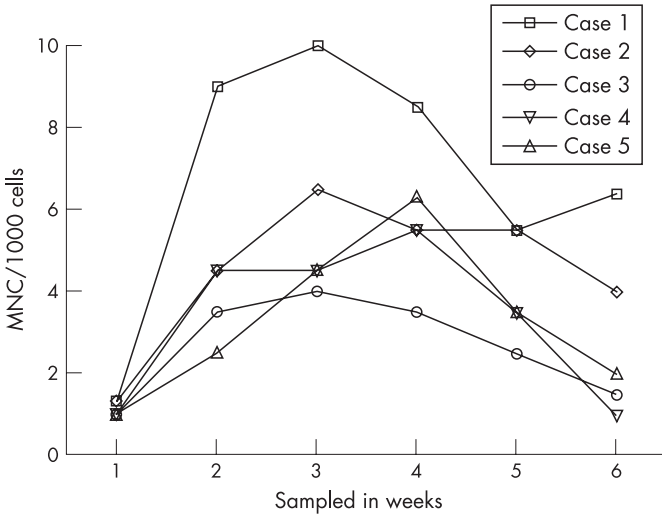

Figure 3 Individual micronucleus values from bodybuilders with anabolic androgenic steroid consumption. MNC, micronucleated cells.

DISCUSSION

The results of the present work indicate that the consumption of AAS compounds, regardless of the testosterone derivative, promotes the presence of MN in buccal epithelium cells, as can be seen in fig 2. From an ethical point of view, research into the genotoxic effects of AAS in buccal mucosa is complicated because there are some factors which preclude the investigation of drug administration. First, researchers have to be sure that participants have self-administered AAS. Second, participants may not admit the use of AAS, because they feel they are being judged.

As far as we know, this study is the first to demonstrate a genotoxic effect of AAS in athletes, showing that testosterone is essential for the function of the hypothalamus–hypophysis axis, having a negative control over gonadotrophic secretions.[22] It is suggested that the testosterone administration at high doses (in the case of AAS users) may saturate the cellular receptors. The testosterone derivatives could become aromatic and convert to 17-β-oestradiol, which is a potent genotoxic, mutagenic and cancer-forming compound.[23–25] This molecule also induces tumour proliferation in various organs and multiple species, including humans.[26] This suggests that the aromatic reaction of testosterone and conversion to 17-β-oestradiol might be one possible mechanism by which the bodybuilders in this work registered greater MN values.[27]

It is important to point out that all the compounds that the bodybuilders had self-administered were chemically modified testosterone hormones, which indicates that during the metabolic process some metabolites linked to the cancer process

Table 2 Consumption of anabolic androgenic steroids consumption increased the frequency of micronucleated cells from buccal mucosa in bodybuilders

Cases	Sampling week 1st	2nd	3rd	4th	5th	6th
Group 1 (with no AAS consumption)						
1	0.5	1.5	1.0	0.5	0.5	0
2	0.5	0	0.5	0	0	1.0
3	0.5	0	0	0	0	0
4	1.0	0.5	0.5	0.5	1.0	1.0
5	0.5	1.0	1.0	0.5	0	0
6	0	0	0	1.0	0	0
Mean (SD)/week	0.5 (0.3)	0.5 (0.6)	0.5 (0.4)	0.4 (0.3	0.2 (0.4)	0.3 (0.5)
Mean (SD)/group	0.4 (0.4)					
p Value*		NS	NS	NS	NS	NS
Group 2 (with AAS consumption)						
1	1.3	9.0	10	8.5	5.5	6.8
2	1.3	4.5	6.5	5.5	5.5	4.0
3	1.0	3.5	4.0	3.5	2.5	1.5
4	1.0	4.5	4.5	5.5	3.5	1.0
5	1.0	2.5	4.5	6.3	3.5	2.0
Mean (SD)/week	1.1 (0.1)	4.8 (2.4)	5.9 (2.4)	5.8 (1.8)	4.1 (1.3)	3.0 (2.3)
Mean (SD)/group	4.1 (2.4)					
p Value†		0.043	0.042	0.043	0.041	NS
p Value‡	0.030	0.004	0.004	0.004	0.004	0.009
p Value§	0.001					

Results are presented as micronucleated cells per 1000 cells.
AAS, anabolic androgenic steroids.
Comparisons were made per week: intragroup (1st sampled week vs weeks 2–6; *group 1; †group 2) and ‡intergroup (treated group weeks vs the corresponding control group week). §Comparison between MNC mean per group (group 1 vs group 2).

What is already known on this topic?

- Ergogenic drugs are substances used to enhance athletic performance to improve both appearance and athletic abilities.
- The use of hormonal anabolic substances causes collateral effects like reproductive toxicity, behavioural changes, hepatic and renal disorders.
- Little is known about the effect of hormonal anabolic substances on genetic material.

What this study adds

- This study investigated the genotoxic risk associated with the use of anabolic androgenic steroids in bodybuilders by a micronuclei assay in buccal mucosa cells.
- The frequency of micronucleated cells is increased in bodybuilders by anabolic androgenic steroids, leading to in situ or systemic loss or DNA damage.

may be formed, together with high concentrations of 17-β-oestradiol. Another possible explanation of the results is that the formation and insertion of free radicals may create such a modification. These chemical radicals belong to the alkyl groups, which are unstable and highly reactive because they easily lose a hydrogen atom and form covalent bonds with cellular constituents.[28 29] Most alkylates are bifunctional—that is, they have two alkyl groups that allow fixation to two separate helixes of separate DNA chains, creating a bridge or a union between the two chains that prevents their separation for the duplication process, and thus inhibits cellular growth. This bond can be produced with separate DNA chains, between the bases of the same DNA molecule, or between the DNA and RNA, forming MN during cell division.[30]

The collateral effects of alkyl agent treatment include toxicity and cancer due to genetic damage. The mutagenic potential of these compounds can be expressed only after its metabolic activation, mainly by the cytochrome P450,[30] which can be accentuated by other substances or chemicals (alcohol, caffeine, cocaine, tobacco, drugs, etc).[20]

Toxicity, mutagenicity, genotoxicity and cancerogenesis of sexual hormones are the result of a combination of genetic and epigenetic factors. Moreover, the genotoxic activity of steroids is also due to metabolic activators and to an indirect process that takes place in the redox cycle, and the production of oxygen reactive types.[31] In this way, the metabolic activation of the testosterone derivatives leads to the formation of free radicals and, consequently, the formation of DNA adducts, which induce immediate alteration in this molecule.[26] Similarly, steroids can induce the activation of repair systems, indicating that some form of extensive DNA damage might be provoked.[26] The above-mentioned facts explain the results obtained for the presence of MN in exfoliated buccal epithelial cells in bodybuilders who use AAS. For a better evaluation of the effect of AAS on MNC formation in buccal epithelium cells, it is recommended that further research should be conducted with a greater number of participants. The correlation between the use of anabolic steroids and other factors like behaviour and daily habits should also be considered.

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Conflict of interest: None declared.

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COMMENTARY

This paper emphasises the precautions that need to be taken with steroids, especially by those people who want to enhance their atheletic perfomance and who ignore the deleterious consequences.

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