Reprint from

RECENT ADVANCES IN DOPING ANALYSIS (12)

W. Schänzer
H. Geyer
A. Gotzmann
U. Mareck
(Editors)

Sport und Buch Strauß, Köln, 2004

D. KWIATKOWSKA, K. CHROSTOWSKI, A. POKRYWKA, E. PARTYKA, B. WÓJCIKOWSKA – WÓJCIK, E. TUREK-LEPA, D. STANCZYK, D. MICHALAK, R. GRUCZA:

Aldosterone Concentrations in the Blood Plasma and in the Urine Samples as the Biological Marker of Anabolic Adrogenic Steroids (AAS) Abuse

In: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck (eds.) Recent advances in doping analysis (12). Sport und Buch Strauß, Köln (2004) 395-401

D. Kwiatkowska, K. Chrostowski, A. Pokrywka, E. Partyka, B. Wójcikowska-Wójcik, E. Turek-Lepa, D. Stańczyk, D. Michalak, R. Grucza

Aldosterone Concentrations in Blood Plasma and in Urine as a Biological Marker of Anabolic Androgenic Steroids (AAS) Abuse

Department of Anti-Doping Research, Institute of Sport, Warsaw, Poland

Abstract

Eighteen body builders and 18 sportsmen (12 oarsmen and 6 weight-lifters) - control group, participated in the study. The group of body builders was split in two subgroups: the first (1) "positive" subgroup of 9 body builders, in which metabolites of steroids were detected; and the second (2) "negative" subgroup of 9 body builders, who declared that they did not use any drugs and their urine sample found to be negative. Aldosterone concentrations in blood plasma and in urine were measured by radioimmunoassay RIA.

It was found that concentrations of aldosterone in blood plasma and in 24h urine excretion rate in the group of "positive" body builders were significantly greater than in "negative" body builder ($p<0.01$ and $p<0.00005$, respectively) as well as in the control group ($p<0.0002$ and $p<0.00001$, respectively). There was no statistical difference of aldosterone plasma concentrations and aldosterone urine excretion rates within the subgroup of "positive" body builders between "on cycle" and "off cycle". Significant correlation coefficients were found in body builders between aldosterone in plasma (ng/ml) and in urine and in some biochemical parameters.

The results suggest that long lasting application of supraphysiological doses of anabolic steroids induces activation of Renin-Angiotensin-Aldosterone System which increases synthesis and actions of tissue aldosterone in body builders. Concentration of aldosterone in ng/mg of creatinine in ml of urine sample would serve, therefore, as a biological marker of AAS abuse.

Introduction

In recent years the knowledge concerning functions of aldosterone in an organism has been significantly enlarged (5,6,7). Aldosterone, discovered over 50 years ago, is known primarily

as an important mineralocorticoid hormone, produced in zona glomerulosa of the outer layer of adrenal cortex, (regulated mainly by angiotensin II) which promotes retention of sodium and water, influence excretion of potassium through the renal tubules and contribute to blood pressure regulation (6,7).

Recent clinical and experimental studies indicated that aldosterone may be synthesised, beyond the adrenal cortex in other nonepithelial cells as in: heart, brain, vasculature (endothelium), kidney and other tissues where may exhibited autocrine, paracrine and intracrine action which may affect the function and structure of the cells and organs (6,7). The tissue production of aldosterone is potentate in pathological hypertrophic cells (5,7).

Animal studies and clinical trails suggested that tissue aldosterone plays a major pathological role in heart hypertrophy, vascular injury, development of interstitial fibrosis and diffuse inflammation lesions (5,6,7). It could be postulated, therefore, that side health effects which have been observed in AAS abusers such as heart hypertrophy and failure, arrhythmias and heart thrombosis as well as disturbance of lipid metabolism (6) would be related to the degree of activation of Renin-Angiotensin System associated with increased local aldosterone production (7). In 1963, Campbell et al. published results of an autopsy of suddenly died 21 years old man (who took the anabolic steroids) which were similar to that observed in the animal model with mineralocorticoids excess (1). Our previous study also indicated an elevated plasma aldosterone levels in body builders during "on cycle" of AAS voluntary application (3).

Aim of the study

The aim of the study was, therefore, to verify hypothesis that long lasting application of supraphysiological doses of anabolic androgenic steroids could additionally stimulate activation of Renin-Angiotensin-Aldosterone System. In result, the concentrations of aldosterone both in blood samples and in 24h urine collection might be increased in AAS abusers comparing to normal subjects.

Material and methods of the study

This study was approved by the Ethical Commission of the Institute of Sport.

Eighteen body builders and 18 sportsmen (12 oarsmen and 6 weight-lifters) - being a control group, participated in the study. The group of body builders was split in two subgroups: the first (1) "positive" subgroup of 9 body builders, in which metabolites of steroids were

detected; and the second (2) "negative" subgroup of 9 body builders, who declared that they did not use any drugs and their urine sample found to be negative.

Anabolic androgenic steroids self-administered by the "positive" body builders are presented in Table 1.

Aldosterone concentrations in blood plasma and in urine were measured by radioimmunoassay RIA according to the method of Ignatowska-Świtalska (3). Cross-reactivity of the antibody with cortisol, corticosterone and dezoxycorticosterone was less than 0.001%. The intra- and inter-assay coefficient of variation at the level of 8% and 12% was, respectively, estimated. Normal values of range 3-12.0 ng/dl, with mean 7.8 ±2.4 ng/dl, of plasma aldosterone was determined by this method. All tests were performed in duplicate with LKB1209 RACK BETA instrument (3). Plasma Renin Activity (PRA) was measured using Immunotech RIA kit (CAT# 3518). Intra- and inter-assay coefficient of variation 5.2% and 10.6% was estimated, respectively. The normal range of values 0.3-3.5 ng/ml/h, with mean 1.7 ±1.1 ng/ml/h, was found for this method. Angiotensin-converting enzyme (ACE) activity was determined by the Liberman's spectrophotometric method (4).

Results

The concentrations of aldosterone in blood plasma as well as in 24h urine excretion found in the group of 9 "positive" body builders (with presence of metabolites of anabolic androgenic steroids in urine samples) were significantly greater than in 9 "negative" body builders (with absence of metabolites in urine samples) (18.3±2.7 vs. 9.2±1.5; $p<0.01$; and 31.0±6.6 vs. 5.2±1.3; $p<0.0002$, respectively) and in the control group (18.3±2.7 vs. 8.2±0.5; $p<0.00005$ and 31.0±6.6 vs. 5.2±0.8; $p<0.00001$, respectively) (Table 2). No statistical difference in aldosterone plasma concentration and in aldosterone urine excretion was found within subgroup of "positive" body builders between "on cycle" and "off cycle" of AAS self-administration (Table 3). This result might suggest rather long lasting effect of anabolic steroids abuse.

Statistical analyses of some biochemical and haematological results obtained in all tested subjects are presented in Table 2.

Pearson's correlation coefficients for plasma aldosterone (ng/dl), aldosterone (μg/24h) excretion rate in urine and for some biochemical and haematological tests results obtained in the control group are presented in Table 4.

Conclusions

- Results of the present study suggest that long lasting application of supraphysiological doses of AAS may induce a strong activation of RAAS, with increased synthesis of aldosterone (beyond the zona glomerulosa of adrenal cortex) in other hypertrophic tissues including skeletal muscles and heart muscle.
- The strong statistical correlation between concentrations of both aldosterone in blood plasma and in 24h urine excretion rate and results of analytical tests could, indirectly, suggest that aldosterone would play a role in pathogenesis of health side effects of AAS abuse in body builders.
- Concentration of aldosterone in ng/mg of creatinine in ml of urine sample may serve as a biological marker of supraphysiological doses of AAS abuse. Results of such test could help in differentiation between accidentally present metabolites of AAS in urine samples and the conscious doping application. The validation of the test in diagnosis of AAS doping in sports requires further studies.

Table 1. Anabolic androgenic steroids used by "positive" body builders

No	Code of user	Age (years)	Declaration of dosage of self-administered drugs	Years of abuse	Amount of cycles	Duration of cycle	Substance and metabolites detected in urine samples
1	V1	23	Metandienone 1000 mg Nandrolone 1600 mg Omnandren 3500 mg	3	4	"on cycle" 4 weeks	19-NA = 222 ng/ml
2	V2	30	Oxandrolone 859 mg Boldenone 800 mg Nandrolone 1600 mg Vinstrol 1600 mg	11	39	"on cycle" 4 weeks	19-NA =227.5 ng/ml Boldenone Metandienone Stanozolol Clenbuterol
3	V3	29	Metandiennone 1000 mg Nandrolone 1000mg Omnandren 3000 mg	8	12	"on cycle" 6 weeks	19-NA=227 ng/ml 17-epimetendiol Methyltestosterone 3'OH stanozolol T/Et ratio = 75
4	V4	31	Testosterone 4000 mg Primabolone 3000 mg	10	12	"on cycle" 3 weeks	19-NA=7 ng/ml Metenolone I and II DHT, T/Et ratio = 41
5	V5	30	No drugs declaration	11	39	"off cycle" 2 months	19-NA=50 mg/ml 3'OH stanozolol
6	V6	26	No drugs declaration	7	13	"off cycle" 1 months	19-NA=56.5 ng/ml
7	V7	32	No drugs declaration	6	7	"off cycle" 4 months	Metenolone I and II Boldenone II
8	V8	22	No drugs declaration	2	4	"off cycle" 12 months	19-NA=4.2 ng/ml THC=83.4 ng/ml
9	V9	23	No drugs declaration	3	4	"off cycle" 2 months	19-NA=4.1 ng/ml

Table 2. Anthropometric characteristic and values of RAAS markers: Plasma Renin Activity (PRA), ACE (angiotensin converting enzyme) activity and aldosterone (ALDO) in plasma and 24h urine excretion as well as selected analyses in two groups of "positive" (with present) and "negative" (absent) AAS metabolites of the body builders and in the control group (mean±SE)

Groups	(1)"positive"	(2)"negative"	(3) control	t - Test (p<)*		
No. Subjects	9	9	18	(1)vs(2)	(1)vs(3)	(2)vs(3)
Age (years)	28.5±1.1	29.4±2.8	18.9±0.7	NS	**0.000001**	**0.00008**
Height (cm)	176.5±1.,8	176.3±1.8	185.6±2.1	NS	**0.01**	**0.008**
Body mass (kg)	108.2±1.5	86.6±4.8	85.7±2.9	**0.001**	**0.00006**	NS
PRA (ng/ml/h)	1.8±0.5	1.4±0.4	0.7±0.07	NS	**0.007**	**0.008**
ACE (mU/ml)	30.9±3	25.2±3.6	30.8±2	NS	NS	NS
Aldosterone in plasma (ng/dl)	18.3±2.7	9.2±1.5	8.2±0.5	**0.01**	**0.00005**	NS
ALDO/PRA ratio	28.4±17.3	8.4±1.7	13.7±1.3	NS	NS	**0.03**
24h Urine collection (ml)	2864±291	1633±133	1605±140	**0.0008**	**0.005**	**NS**
Creatinine mg/dl in urine	166.0±10	166.1±16	196±15	**NS**	**NS**	**NS**
ALDO excretion rate μg/24h	31.0±6.6	5.2±1.3	5.2±0.8	**0.0002**	**0.00001**	**NS**
ALDO ng/mg creatinine/ml(urine)	7.6±1.8	6.4±2.6	1.8±0.3	**0.006**	**0.0002**	**NS**
LH (mIU.1^{-1})	1.17±0.4	5.5±1.1	3.9±0,4	**0.002**	**0.0005**	NS
HDL-cholesterol (mg/dl)	22.8±3.1	32.8±3.2	45.9±2.8	**0.04**	**0.00004**	**0.01**
Creatine Kinase (U/L)	1294.1±426	257.6±53	305±83	**0.028**	**0.004**	NS
AspAT (U/L)	781±12	38.4±2.4	35.3±2.9	**0.005**	**0.0001**	NS
AlAT (U/L)	112.7±23	50±5.6	31.8±3.18	**0.02**	**0.00006**	**0.006**
K mEq/24h excretion	152.8±13.6	89.5±11.7	51.5±6.9	**0.002**	**0.000001**	**0.01**
Na/K in urine	1.7±0.2	3.3±0.6	6.4±0.9	NS	**0.001**	**0.03**
RBC (T/L)	5.6±0.1	5.0±0.1	5.0±0.06	0.003	**0.0003**	NS
HGB (G/L)	169.2±3.4	156.0±3.1	156.4±2.1	**0.01**	**0.003**	NS
HT (%)	49.9±0.8	46.7±0.9	46.4±0.6	**0.03**	**0.002**	NS

**Student's test t for unpaired variables was applied. Bold indicated p<0.05*
NS –statistically insignificant
Aldosterone in ng/dl x 27.74 = Aldosterone in pmol/l

Table 3. Comparison between values of aldosterone urine excretion rate (μg/24h),aldosterone in urine ng/mg creatinine/ml and levels of aldosterone in plasma (ng/dl) in three tested groups: (1) "positive ON" cycle, (2)"positive OFF" cycle and the negative group of body builders (means ± SE)

Groups	(1)"positive ON" cycle	(2) "positive OFF" cycle	(3) " negative"	Statistical analysis t - Test (p<) *		
No. Subjects	4	5	9	(1) vs (2)	(1) vs (3)	(2) vs (3)
Aldosterone in urine excretion rate (μg/24h)	41.8±12.3	22.6±5.3	5.18±1.3	NS	**0.0008**	**0.001**
Aldosterone in urine ng/mg creatinine/ml	9.02±3.1	6.45±2.6	1.8±0.3	NS	**0.01**	**0.003**
Aldosterone in plasma (ng/dl)	17.02±2.2	17.9±5.4	8.2±0.5	NS	**0.0006**	**0.03**

**Student's test t for unpaired variables was applied. Bold indicated p<0.05*
NS – statistically insignificant
Aldosterone in ng/dl x 27.74 =Aldosterone in pmol/l

Table 4. Comparison of Pearson's correlation coefficients between aldosterone in plasma concentrations and aldosterone 24h excretions in urine and values of same anthropometric parameters, biochemical and haematological tests in the body builders and in the control group

Parameters	Body builders N=18		Control group N=18	
Correlation*	Aldosterone In plasma (ng/dl)	Aldosterone Excretion (μg/24h)	Aldosterone In plasma (ng/dl)	Aldosterone Excretion (μg/24h)
Age (in years)	0.04	-0.27	0.03	**0.50**
Body mass (kg)	**0.53**	**0.73**	-0.12	0.38
BMI (g/m^2)	**0.62**	**0.68**	0.02	0.27
PRA(ng/ml/h)	0.29	0.23	0.40	0.01
ACE(mU/ml)	**0.49**	0.34	0,23	0.12
LH (mIU/ml^{-1})	**-0.64**	**-0.51**	-0.02	-0.06
HDL- cholesterol	-0.34	-0.41	-0.05	-0.11
CK U/L	**0.56**	**0.75**	0.21	0.07
AspAT (U/L)	**0.53**	**0.74**	0.26	-0.07
AlAT (U/L)	0.35	**0.69**	0.35	-0.06
RBC (T/L)	**0.48**	**0.64**	-0.27	0.38
HT (%)	**0.52**	**0.48**	-0.18	0.27
HGB (G/L)	**0.58**	0.46	-0.20	0.34
24h urine collection	**0.58**	**0.66**	-0.02	0.04
K mEq/ 24h excretion	0.09	**0.68**	-0.01	-0.17
ALDO 24h/urine	**0.51**	X	-0.29	X

X – non applicable
**the all results to log10 values have been transformed*

References

1. Cambell S.E.,A. Farb, K.T. Weber Pathological remodeling of the Myocardium in a Weightlifter Taking Anabolic Steroids. Blood Pressure. 1993; 2:213-216
2. Chrostowski K.,J. Kozera, R. Grucza Medical Consequences of Anabolic steroids Abuse. Biology of Sport. 2000;17;2:133-162
3. Ignatowska-Świtalska H. Radioimmunologiczna metoda oznaczania aldosteronu w moczu. Pol. Arch. .Med. Wew. 1976; 56;23-34
4. Liberman J. Elevation of serum angiotensin converting enzyme (ACE) level in sarcoidosis. AM.J.Med. 1975; 59:365-372
5. Pärissnen M., T. Seppälä Steroid Use and Long-Term Health Risks in Former Athletes. Sports Medicine. 2002; 32(2):83-94
6. Stowasser M. New Perspectives on the Role of Aldosterone Excess in Cardiovascular Disease. Clinical and Experimental Pharmacology and Physiology. 2001; 28:783-791
7. White P.C. Aldosterone: Direct Effects on and Production by the Heart J. Clin Endocrynol. Metab. 2003; 88 (6): 2376-2383