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Norandrosterone and Noretiocholanolone Concentration Before and After Submaximal Standardized Exercise

Abstract

19-Norandrosterone (19-NA) and 19-noretiocholanolone (19-NE) are the two main urinary indicators used to detect illegal use of nandrolone. Recent studies showed that 19-NA and 19-NE can be endogenously produced in non-treated humans. The concentrations were close to the threshold of the International Olympic Committee (IOC), i.e. 2 ng/ml for men and seem to increase after prolonged intense effort. Androgens are involved in the biosynthesis of estrogens and estrogen has a protective effect against skeletal muscle damage following eccentric exercise. Furthermore, the testicular tissue can synthesize 19-norandrogens from androgens, we hypothetisize that the 19-norandrogen production might be influenced by muscle damage following eccentric exercise. Therefore the purpose of this study is to examine if three different exercise methods will influence the urinary concentration of 19-NA and 19-NE in healthy young subjects. Fifteen amateur hockey players undertook a 30 min submaximal standardized exercise protocol. They were randomised for three different types of exercise, namely a cycle ergometer test (cyclic muscle activity), a treadmill test (concentric muscle activity), or a bench-steptest (eccentric muscle activity) at a target heart rate corresponding to 65% (± 5%) of Karvonen heart rate. Urine samples were obtained before the test and 60 min and 120 min after the test. Subjects completed a Likert scale of muscle soreness before and 12 h after exercise. 19-NA and 19-NE were determined by gas chromatography-tandem mass spectrometry (GC-MS-MS). Baseline urinary 19-NA and 19-NE concentrations were under limit of detection of 0.05 ng/ml, except for one sample (0.13 ng/ml). No 19-NA or 19-NE could be detected post exercise. In one pre-exercise sample 19-NA rose above detection limit (0.13 ng/ml). In our experimental conditions, the exercise mode (eccentric or concentric) had no impact on 19-NA or 19-NE excretion. Our findings confirm that the current International Olympic Committee threshold level for nandrolone metabolites is sufficiently high to avoid false positive cases.

Key words
Nandrolone · antidoping control · human urine · submaximal standardized exercise · eccentric · hockey

Introduction

Anabolic-androgenic steroids (AAS) have been used since WO II to promote/stimulate wound healing and recovery [15]. Today they are used in human medicine in cases of protein deficiency diseases [22], osteoporosis [11] and burns [3].

The use of AASs as a doping agent to improve muscular strength and performance [13] was rapidly extended in sport because of its androgenic activity [13]. Physicians and athletes first used testosterone. Testosterone is quickly metabolized by the liver and so the molecular structure of testosterone was changed in order to make it more efficacious. The first synthetic AAS was 19-nortestosterone (19-NT; 17β-hydroxyestr-4-en-3-one) also named nandrolone.

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The control of the illegal nandrolone administration is mainly based on the urinary detection of 19-norandrosterone (19-NA; 3α-hydroxy-5α-estran-17-one) and 19-noretiocholanolone (19-NE; 3α-hydroxy-5β-estran-17-one) [26].

The endogenous origin of nandrolone is a controversial topic in doping control: firstly, because its urine detection is based on the presence of the two main metabolites, 19-NA and 19-NE [6,20], secondly, because low-dose oral administration of 19-norandrostenedione can produce similar urinary metabolites [6], and thirdly, because endogenous production of nandrolone by aromatase-rich tissues has been reported [5].

The International Olympic Committee (IOC) puts the urinary concentration limit for 19-NA and 19-NE on 2 ng/ml for men and on 5 ng/ml for women.

Recently, controversy has arisen among some sports administrations concerning the presence or the absence of nandrolone metabolites in the urine of untreated sedentary men [4,10,13,19,20] and women [19,30], male athletes [10,19,20], and female athletes [10,19] submitted to current screening procedures for anabolic steroids.

Dehennin et al. [7] stated that the effect of possible, but yet unknown, physiological stimuli of 19-norsteroid biosynthesis *in vivo* will have to be investigated in order to find out if the decision limit for positive nandrolone doping can be exceeded in a non-surreptitious way.

Since the late 1990s, 19-norandrostenedione, 19-norandrostenediol, DHEA, and other steroids have been sold legally over the counter (OTC), in the United States and via the Internet as food supplements [2]. After the use of 19-norandrostenedione and 19-norandrostenediol, two precursors of the potent anabolic nandrolone [7], high concentrations of 19-norandrosterone and 19-noretiocholanolone, the two main metabolites from nandrolone [26,27] appear in the urine, similar to nandrolone. These two metabolites are detectable in urine for 7 – 10 days after a single oral dose of 19-norandrostenedione [29].

The dietary supplement industry is completely unregulated in the United States; as a consequence, an abundance of supplement products of dubious value, content, and quality are now available around the world [24].

Therefore, athletes needing supplements should be very careful as some supplements may contain prohormones not declared on the label [9,16]. In addition, many sport organisations prohibit OTC steroids; thus athletes who use these products are at risk for positive urine test results [12]. Moreover following international rules athletes remain responsible for what is found in their bodyfluids, irrespective of the origin [9].

Theoretically, the 19-NA and/or 19-NE apparent concentration in urine can increase under certain conditions, i.e. very long and intense exercise (marathon run, football game, cycling race, etc.) or dehydration periods before some specific competitions (weight loss in some sports due to weight categories [judo, boxing, wrestling, etc]). Hence, in individuals whose 19-NA baseline level would be slightly lower than 1 ng/ml, there might be some circumstances where (i.e. pronounced dehydration) this value reaches 2 ng/ml or even exceed this value [20]. Furthermore, there are some reports [16,19] showing that after a prolonged non-standardized intense effort, the 19-NA concentration can be increased, by a factor varying between 2 and 4 [20].

It has been shown that androgens are involved in the biosynthesis of estrogens [1], and stipulated that estrogens have a protective effect against skeletal muscle damage following eccentric exercise [18]. Taking into account that the testicular tissue can synthesize 19-norandrogens from androgens [25] 19-norandrogen production is hypothetically influenced by muscle damage following eccentric exercise.

However, no study has been conducted on the effect of eccentric muscle contractions on the 19-NA or 19-NE excretion.

Therefore the purpose of the present study is to examine if different forms of exercise could stimulate a hypothetical endogenous production of nandrolone and/or his metabolites in amateur field hockey players. To investigate the above mentioned hypothesis our athletes performed a 30 min standardized concentric or eccentric physical effort. Urine samples were collected before and after a submaximal physical effort.

Materials and Methods

Prior to participation, all subjects underwent a full medical examination by a physician for evaluation of their health status. All subjects were expected to be free of AAS administration. This was checked by information provided by each subject during the medical examination. They were given an explanation of the purpose, procedure, and potential risks, and all gave their written informed consent. The study and methods were approved by the local ethical committee. A total of 15 healthy male amateur field hockey players, aged 20 – 33 yr (25.6 yr ± 3.6) volunteered to participate in the study. They all trained regularly for at least 6 months before the experiment. Care was taken to keep notice of the players' diet and training habits.

Anthropometric data are summarized in Table **1**.

All tests were performed between 4 and 9 pm, on Tuesday or Thursday. After registering resting heart rate and blood pressure a pre-exercise urine sample (40 ml) was collected for 19-NA and 19-NE determination and blood sample (20 µl) from the earlobe for blood lactate determination. All subjects performed a 30 min exercise session with a 5 sec break after 15 min in order to take a blood sample at the earlobe. The intensity of exercise was pre-established at a heart rate corresponding to 65 % (± 5 %) of the target heart rate as calculated by the Karvonen formula [17]. Heart rate was recorded continuously by a Polar® heart rate monitor. The total group of 15 individuals was randomly divided into three subgroups: a running group, a cycling group, and a bench-stepping group. Subjects performed one of the three tests.

The cycling test was performed on a computerized cycle ergometer (Lode Excalibur Sport V 2.0, Groningen, Netherlands). The

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Table **1** Mean age, weight, heigth and heart rate (lower and upper threshold) for each of the three groups and the total mean

	Running group (n = 5)	*Cycling group (n = 5)*	*Stepping group (n = 5)*	*Total mean (n = 15)*
Age (yr)	25.4 ± 2.6	25.8 ± 5.3	25.6 ± 3.3	25.6 ± 3.6
Weight (kg)	72.3 ± 6.9	77.7 ± 6.5	70.3 ± 8.6	73.4 ± 8.6
Height (cm)	179.3 ± 4.2	178.8 ± 5.4	182.5 ± 8.0	180.2 ± 5.9
Target heart rate (beats/min)	149.1 ± 5.2	149.1 ± 2.9	147.0 ± 2.9	148.4 ± 3.7

Table **2** Likert scale score and lactate concentration of pp B compared to mean group

	pp B	*TG*
Likert scales	4	0.27 (± 1.03)
Lactate (mmol/l)	1.16	0.66 (± 0.37)

pp B, subject B; TG, mean baseline values (± SD) of the total group (n = 15)

starting work rate was 200 Watt. During the test, the resistance was adjusted in order to keep the heart rate at 65% (± 5%) of the target heart rate.

The 5 subjects of the running group ran on a treadmill (Woodway GmbH, Ergo ELG 55, Weil am Rhein, Germany) at a beginning speed of 11 km/h. The running speed was adapted to stay at a heart rate equal to 65% (± 5%) of the target heart rate.

Subjects performed eccentric muscle actions during 30 min of bench-stepping. The step height was 46 cm (average knee height) at a beginning rate of 100 steps/min. Subjects began using the right leg as the leading leg and changed their leading leg every 7.5 min. The stepping speed was adjusted in order to keep the heart rate at 65% (± 5%) of the target heart rate.

A total of 45 urine samples were collected from the 15 subjects. Each subject gave an urine sample before and 1 and 2 hours after the end of exercise. The collected urine samples were stored at – 20 °C awaiting analysis. Samples were analyzed and quantified for the presence of 19-NA, and 19-NE, using gas-chromatography-tandem mass spectrometry (GC-MS-MS) as described elsewhere [30]. For this method the daughter product spectra at a level of 0.1 ng/ml complied with the mass spectral criteria as defined by the International Olympic Committee for positive identification of a doping substance. The limit of detection of the method was 0.05 ng/ml.

Five blood samples were drawn from the earlobe of every subject before the exercise, 15 min after the start of the exercise, immediately after the exercise and 1 and 2 hours after the end of the exercise. The blood samples were stored at – 15 °C before analysis and lactate concentrations were determined enzymatically (Medingen, model ESAT 6660 Lactat).

Each of the 15 subjects were asked to rate their muscle soreness before and 12 h after the test, on a ratio scale (Likert scale of muscle soreness) of zero to six, where zero is the complete absence of soreness and six is the most severe pain possible because of soreness [14, 31].

All data were statistically analyzed with Statistical Package for the Social Sciences (SPSS 10.0) (Inc., Chicago, IL, USA). A one-way ANOVA was used to determine if the 19-NA and 19-NE concentrations were different between baseline and post exercise. If the concentration was different a Scheffé-test was used for post hoc comparison. For the statistical analyses of the soreness rating pre- and post exercise muscle soreness scores were analyed by a Students *t*-test. Significance was set at $p < 0.05$.

Results

Thirty minutes of exercise at 65% (± 5%) of the target heart rate did not influence the 19-NA or 19-NE excretion, neither type of exercise significantly modified urinary 19-NA or 19-NE levels. The mean ± SD of the specific gravity of all urine samples (U_{sg}) was 1022 kg/l ± 0.006 kg/l, indicating a "normal" hydration status of the athletes [23].

19-NA and 19-NE concentrations were under the detection limit in all but one sample. This sample was taken pre-exercise and had a 19-NA concentration of 0.13 ng/ml. The subject complained of muscle soreness. On the Likert scale of muscle soreness, before exercise he had a score of 4 "a light pain when walking on a flat surface/painful" and a basal lacate concentration of 1.16 mmol/l (Table **2**). After his cycling test neither 19-NA nor 19-NE could be detected.

In all subjects, basal lactate concentrations were normal and showed the expected changes during exercise (Fig. **1**).

Fig. **2** shows that all subjects from the bench-stepping group had no muscle soreness prior to exercise (pre-exercise) but suffered from muscle soreness 12 hours after exercise (post-exercise). The increase in muscle soreness, between pre- and post exercise, for the bench-stepping group was statistically significant ($p = 0.018$). Cycling and running at 65% (± 5%) of the target heart rate did not cause muscle soreness as objectified on the Likert scale of muscle soreness.

Discussion

Our study is the first controlled experiment providing data on the effect of concentric and eccentric exercise on 19-NA and 19-NE secretion. The results of this study confirm that a 30 min submaximal standardized exercise does not increase 19-NA and 19-NE concentration.

Surprisingly one subject had a baseline endogenous 19-NA concentration of 0.13 ng/ml, which is in accordance with baseline

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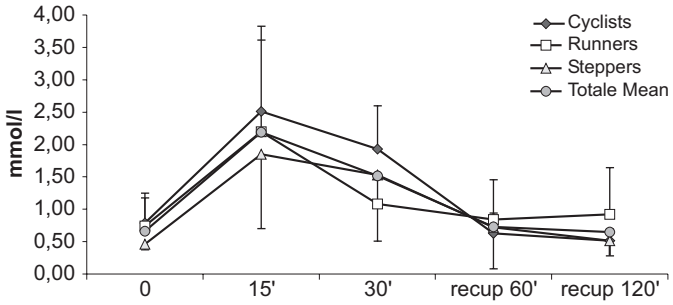

Fig. **1** Lactate concentrations (mmol/l) before, 15 min after the start, and immediately after exercise and 1 and 2 hours after the end of the exercise. Values are expressed as mean ± SD. No significant differences between groups were found.

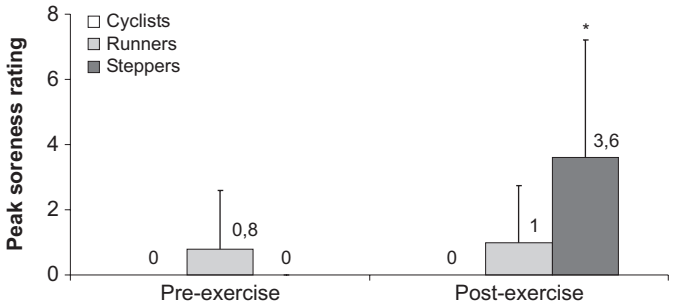

Fig. **2** Perceived muscle soreness on the Likert scale of muscle soreness, before and 12 hours post exercise. Values are expressed as mean ± SD. The increase in muscle soreness, between pre- and post exercise, for the bench-stepping group is statistically significant. * Indicates difference between pre- & post exercise ($p < 0.05$).

concentrations in other independent studies with more subjects [4,6,10,19,20], but still under the limits of the international anti-doping organizations.

He complained of muscle soreness, objectified with the Likert scale of muscle soreness and with the high baseline lactate concentration. No other subjects complained of muscle soreness and none of them had a score higher than 0 on the Likert scale of muscle soreness or had a lactate concentration higher than 0.51 mmol/l.

In 44 of the 45 urine samples, no 19-NA or 19-NE could be detected. Two conclusions can be drawn. 19-NA and 19-NE concentrations were below the limit of detection (0.05 ng/ml) or those two metabolites are not endogenously produced in non-treated athletes.

A 30 min standardized concentric or eccentric exercise, at 65% (± 5%) of the target heart rate, as in our experiment, does not stimulate the endogenous 19-NA or 19-NE production. Our results are in accordance with Schmitt et al. [28] who used an exercise to exhaustion, as indicated by clinical and biological parameters, during aerobic endurance test and explosive anaerobic test and in contrast with the results from Robinson et al. [26] and Le Bizec et al. [21] that showed an increased 19-NA and 19-NE concentration after a soccer game, a non-standardized 90 min effort with a 10 min break.

The discrepancy with these two latter studies could also be caused by the different exercise mode. Our exercise protocol used a 30 min moderate exercise, while a soccer match typically consists of 90 min intermittent high intensity exercise.

Although Le Bizec et al. [20] suggested that a certain degree of "competition stress" is needed to raise the 19-NA and 19-NE concentration, Robinson et al. [26] did not exclude that the athletes might have "*taken something*" during or just before the game, because the 19-NA and 19-NE metabolites were only detectable at the end of the exercise. Moreover, the high sensitivity of the instrumentation in this and recent other studies [4,6,20,22] allows for the detection of low concentrations of 19-NA and 19-NE, that can be released from fat tissue by strenuous exercise even months after injection of nandrolone esters. Because Le Bizec et al. [20] and Robinson et al. [26] used randomly selected competing players instead of well-controlled subjects this factor can not be excluded as a cause for the detection of 19-NA and 19-NE.

Conclusion

Our study provides additional evidence that in male athletes, during submaximal exercise, the urinary excretion of nandrolone metabolites remains far below the IOC treshold of 2 ng/ml. Exercise does not induce endogenous nandrolone secretion. Consequently, the detection in male athletes of urinary nandrolone metabolites above the 2 ng/ml treshold is strongly in favour of exogenous nandrolone use.

References

[1] Carreau S, Bourguiba S, Lambard S, Galeraud-Denis I. Testicular aromatase. J Soc Biol 2002; 196: 241 – 244
[2] Catlin DH, Leder BZ, Ahrens B, Starcevic B, Hatton CK, Green GA, Finkelstein JS. Trace contamination of over-the-counter androstenedione and positive urine test results for a nandrolone metabolite. JAMA 2000; 284: 2618 – 2621
[3] Catlin DH. Use and abuse of anabolic steroids. In: De Groot LJ, Jameson JL (eds). Endocrinology. 4th ed. Vol. 3. Philadelphia: Saunders Company, 2001: 2243 – 2252
[4] Ciardi M, Ciccoli R, Barbarulo MV, Nicoletti R. Presence of norandrosterone in "normal" urine samples. In: Schänzer W, Geyer H, Gotzmann A, Mareck-Engelke U (eds). Recent Advances in Doping Analysis (6), Proceeding of the Manfred Donike Workshop, 16th Cologne Workshop on Dope Analysis. 15th to 20th March, 1998: 97 – 105
[5] Dehennin L, Jondet M, Scholler R. Androgen and 19-norsteroid profiles in human preovulatory follicles from stimulated cycles: an isotope dilution-mass spectrometry study. J Steroid Biochem 1987; 26: 399 – 405
[6] Dehennin L, Bonnaire Y, Plou P. Urinary excretion of 19-norandrosterone of endogenous origin in man: quantitative analysis by gas chromatography-mass spectrometry. J Chromatogr B 1999; 721: 301 – 307
[7] Dehennin L, Bonnaire Y, Plou P. Human nutritional supplements in the horse: comparative effects of 19-norandrostenedione and 19-norandrostenediol on the 19-norsteroid profile and consequences for doping control. J Chromatogr B Analyt Technol Biomed Life Sci 2002; 766: 257 – 263
[8] Delbeke FT, Destmet N, Debackere M. The abuse of doping agents in competing body builders in Flanders (1988 – 1993). Int J Sports Med 1995; 16: 66 – 70
[9] Delbeke FT, Van Eenoo P, Van Thuyne W, Desmet N. Prohormones and sport. J Steroid Biochem Mol Biol 2003; 83: 245 – 251

Zeitschrift IJSM sm142
DOI ■
Satzbetrieb Ziegler + Müller
Verlag Thieme/Hentze
Datum 26.01.2004

[10] Galán Martin AM, Maynar Marino JI, Garcia de Tiedra MP, Rivero Marabé JJ, Caballero Loscos MJ, Maynar Marino M. Determination of nandrolone and metabolites in urine samples from sedentary persons and sportsmen. J Chromatogr B 2001; 761: 229 – 236
[11] Geusens P. Nandrolone decanoate: Pharmacological properties and therapeutic use in osteoporosis. Clin Rheumatol 1995; 14: 32 – 39
[12] Green GA, Catlin DH, Starcevic B. Analysis of over-the-counter dietary supplements. Clin J Sport Med 2001; 11: 254 – 259
[13] Hargens F. Androgenic-anabolic steroid use in strength athletes; effects on body composition and cardiovascular system. Doctoral Dissertation. Maastricht: University Press, 2001
[14] High DM, Howley ET. The effects of static stretching and warm-up on prevention of delayed-onset muscle soreness. Res Q Exerc Sport 1989; 60: 357 – 361
[15] Honour JW. Steroid abuse in female athletes. Curr Opin Obstet Gynecol 1997; 9: 181 – 186
[16] Kamber M, Baume N, Saugy M, Rivier L. Nutritional supplements as a source for positive doping cases? Int J Sport Nutr Exerc Metab 2001; 11: 258 – 263
[17] Karvonen J. The effects of training on the heart rate. Ann Med Exp Biol Fenn 1957; 35: 307 – 315
[18] Kendall B, Eston R. Exercise-induced muscle damage and the potential protective role of estrogen. Sports Med 2002; 32: 103 – 123
[19] Kintz P, Cirimele V, Ludes B. Norandrosterone et noretiocholanolone: les métabolites révélateurs. Acta Clin Belg 1999; 1: 68 – 73
[20] Le Bizec B, Monteau F, Gaudin I, André F. Evidence for the presence of endogenous 19-norandrosterone in human urine. J Chromatogr B 1999; 723: 157 – 172
[21] Le Bizec B, Bryand F, Gaudin I, Monteau F, Poulain F, André F. Endogenous nandrolone metabolites in human urine: preliminary results to discriminate between endogenous and exogenous origin. Steroids 2002; 67: 105 – 110
[22] Özer D, Temizer A. The determination of nandrolone and its metabolites in urine by gas chromatography-mass spectrometry. Eur J Drug Metab Pharmacokin 1997; 22: 421 – 425
[23] Phipps WJ. Medical-Surgical Nursing: Concepts and Clinical Practice. St. Louis: Mosby, 1995: 545
[24] Pipe A, Ayotte C. Nutritional supplements and doping. Clin J Sport Med 2002; 12: 245 – 249
[25] Reznik Y, Dehennin L, Coffin C, Mahoudeau J, Leymarie P. Urinary nandrolone metabolites of endogenous origin in man: a confirmation by output regulation under human chorionic gonadotropin stimulation. J Clin Endocrinol Metab 2001; 86: 146 – 150
[26] Robinson N, Taroni F, Saugy M, Ayotte C, Mangin P, Dvorak J. Detection of nandrolone metabolites in urine after a football game in professional and amateur players: a Bayesian comparison. Forens Sci Int 2001; 122: 130 – 135
[27] Schänzer W. Metabolism of anabolic androgenic steroids. Clin Chem 1996; 42: 1001 – 1020
[28] Schmitt N, Flament M-M, Goubault C, Legros P, Grenier-Loustalot MF, Denjean A. Nandrolone excretion is not increased by exhaustive exercise in trained athletes. Med Sci Sports Exerc 2002; 34: 1436 – 1439
[29] Uralets VP, Gillette PA. Over-the-counter anabolic steroids 4-androsten-3,17-dione; 4-androsten-3 beta, 17 beta-diol; and 19-nor-4-androsten-3,17-dione: excretion studies in men. J Anal Toxicol 1999; 23: 357 – 366
[30] Van Eenoo P, Delbeke FT, de Jong FH, De Backer P. Endogenous origin of norandrosterone in female urine: indirect evidence for the production of 19-norsteroids as by-products in the conversion from androgen to estrogen. J Steroid Biochem Mol Biol 2001; 78: 351 – 357
[31] Vickers AJ. Time course of muscle soreness following different types of exercise. BMC Musculoskeletal Disorders 2001; 2: 5

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