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BRIEF NOTE

The steroid profile of Malaysian junior athletes during an out of competition season



Le profil stéroïdien des athlètes juniors malaisiens pendant une saison hors compétition

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KEYWORDS

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Summary

Objective. – Urinary steroids profiling was used to monitor the intake of endogenous anabolic androgenic steroids by athletes. However these profile can be influenced by different factors such as age, gender, ethnicity, bacterial and microorganism activities in urine. This paper discussed the difference of steroids profile between different populations and how reliable the cut-off values set by WADA to the steroids profile data obtained from present study.

Equipment and methods. – The urine samples were taken from 126 healthy and non-dope athletes which represent different genders, ethnicities and athletic status. The extraction procedures consisted of the solid phase extraction, enzymatic hydrolysis, liquid-liquid extraction and derivatization with trimethylsilyl. All the samples were analysed using gas chromatography coupled with mass spectrometry detector.

Results. – The comparison with other population (African, Caucasian and Hispanic) within the same sport genre showed a lower level of urinary steroids profile of Malaysian athlete population. Furthermore, the cut-off values set by WADA were too high compared to the data obtained from this study.

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MOTS CLÉS

Profil stéroïdien ;
Populations ;
Genre

Conclusion. – The lower level of steroids parameter of Malaysian athlete population could be hypothesised due to the polymorphism in the gene coding for UGT2B17, 5 α reductase, 5 β -reductase and/or hydroxysteroid dehydrogenases (HSD). The development of cut-off values by WADA needs to consider samples from various populations to ensure the validity of the values worldwide.

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Résumé

Objectif. – Le profil des stéroïdes urinaires a été utilisé pour surveiller la consommation de stéroïdes anabolisants androgènes endogènes par les athlètes. Cependant, ces profils peuvent être influencés par différents facteurs tels que l'âge, le sexe, l'appartenance ethnique, les activités bactériennes et des micro-organismes dans les urines. Ce document traite de la différence de profil des stéroïdes entre les différentes populations et de la fiabilité des valeurs limites fixées par l'AMA par rapport aux données de profil des stéroïdes obtenues dans le cadre de la présente étude.

Équipements et méthodes. – Les échantillons d'urine ont été prélevés sur 126 athlètes sains et non dopés qui représentent différents sexes, ethnies et statuts sportifs. Les procédés d'extraction comprenaient l'extraction en phase solide, l'hydrolyse enzymatique, l'extraction liquide-liquide et la dérivation au triméthylsilyle. Tous les échantillons ont été analysés par chromatographie en phase gazeuse couplée à un détecteur de spectrométrie de masse.

Résultats. – La comparaison avec d'autres populations (africaine, caucasienne et hispanique) dans le même genre sportif a montré un niveau inférieur de profil de stéroïdes urinaires de la population malaisienne athlète. En outre, les valeurs seuils fixées par l'AMA étaient trop élevées par rapport aux données obtenues dans le cadre de cette étude.

Conclusion. – Le niveau inférieur du paramètre des stéroïdes de la population des athlètes malaisiens pourrait être hypothétique en raison du polymorphisme du gène codant pour UGT2B17, 5 α réductase, 5 β -réductase et/ou hydroxysteroid déshydrogenases (HSD). L'élaboration des valeurs seuils par l'AMA doit tenir compte d'échantillons provenant de diverses populations pour garantir la validité des valeurs à l'échelle mondiale.

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1. Introduction

Endogenous anabolic androgenic steroids (EAAS) are among the substances that have been prohibited in sports. EAAS is a group of natural and synthetic compounds which chemically similar and mimic the action of endogenous steroid hormones such as testosterone. EAAS are used by the athlete especially athletes who involves in bodybuilding, weightlifting and track and field sports to build muscle, improve recovery and enhance performance.

Concentration of endogenous steroids produced in the body depends on various factors such as age, gender, ethnicity, bacterial and microorganism activities in urine [1]. Due to these reasons, this study was proposed to monitor the behaviour of endogenous steroid profile in Malaysian athlete populations. However, these paper will only focused on the difference of steroids profile between different population and how reliable the cut-off set by WADA to the steroids profile data obtained from these present study.

To achieve the aims, 126 athletes with different gender, ethnics, sports and age between 18 to 28 years old have been identified where 21 of them were a soccer player. All the subjects must involve in at least one sport activity at

university or national level. The urine sample for each subject was taken twice (morning urine and after meal urine) a week with at least 1 week apart from each sampling for three sessions. pH and SG of urine samples were checked and sample with pH and SG more than criteria stated in WADA Technical Document of EAAS [2] were excluded from further analysis. The sample were hydrolysed using *E. coli* β -glucuronidase enzyme, followed by liquid-liquid extraction and derivatisation using MSTFA. The extracted samples were then analysed using gas chromatography mass spectrometry in selected ion monitoring (SIM) mode to quantify all endogenous steroids that act as the important indicator for steroid abuse.

2. Summary of facts and results

All data obtained were analyses using SPSS 22.0 for Windows. Data of steroid parameters from 21 soccer athletes obtained from this study were compared with the data from various population obtained from previous study done by Strahm et al. [3] as shown in Table 1. This study has demonstrated that there was a deletion polymorphism in the *UGT2B17*

Table 1 Comparison of T, T/E, A/Et and 5 α -adiol/5 β -adiol from various population.

Steroids parameter	African (AF)		Asian (AS)		Caucasian (CA)		Hispanic (HI)		Malaysian	
	Median	Percentile 25–75	Median	Percentile 25–75	Median	Percentile 25–75	Median	Percentile 25–75	Median	Percentile 25–75
T	45.5	19.7–92.9	5	3.9–5.8	40.5	27.7–61.2	74.6	41.7–105.4	2.6	2.15–4.46
T/E	0.8	0.4–1.4	0.1	0.1–0.3	1.1	0.6–2.0	1.2	0.8–2.1	0.66	0.05–0.13
A/Et	1.3	1.1–1.6	1.5	1.3–2.1	1.5	1.2–1.8	1.7	1.4–2.0	1.48	1.25–1.87
5 α -adiol/5 β -adiol	0.3	0.2–0.6	0.9	0.8–1.1	0.4	0.2–0.7	0.4	0.3–0.6	0.91	0.79–1.03

Table 2 Comparison of cut-off value by WADA and 95% range from present study.

Steroid parameters	WADA (ng/mL)	Present study (ng/mL)
Androsterone	> 10,000	1398.68–2020.56
Etiocholanolone	> 10,000	952.80–1364.46
Testosterone	> 200	3.62–8.86
Epitestosterone	> 200	23.88–38.16
DHEA	> 100	25.20–38.37
T/E ratio	> 4	0.11–0.74

gene of Asian population [3]. The data shows that steroid profile of Malaysian athlete population from the present study were almost similar with Asian population from the previous study.

The comparison revealed a significantly lower T/E ratio in Malaysian population which suggested that the deletion polymorphism in the gene coding for UGT2B17 enzyme which involves in testosterone glucuronidation process might also occur in Malaysian population [1,4]. These support the findings from a previous study where lower values of testosterone, androsterone, etiocholanolone, 5 α -adiol and 5 β -adiol could be also related to 5 α -reductase/5 β -reductase [3] and/or hydroxysteroid dehydrogenases (HSD) enzymes activity. 5 α -reductase/5 β -reductase involves in conversion of testosterone to dihydrotestosterone (DHT) and HSD involves in two-way conversion of 5 α -adiol/5 β -adiol to DHT and androsterone/etiocholanolone. Besides UGT2B17, low testosterone level might be also related to high conversion of testosterone to DHT by 5 α -reductase/5 β -reductase. Low values of 5 α -adiol and 5 β -adiol could be due to lower conversion of DHT to 5 α -adiol and 5 β -adiol or, conversely, a higher reconversion of 5 α -adiol and 5 β -adiol to DHT [2]. Meanwhile, low values of androsterone and etiocholanolone could be related to lower conversion of 5 α -adiol and 5 β -adiol to androsterone and etiocholanolone or, conversely, a higher reconversion of androsterone and etiocholanolone to 5 α -adiol and 5 β -adiol. Additionally, lower concentration of androsterone, etiocholanolone, 5 α -adiol and 5 β -adiol in urine could also be due to the polymorphism of UGT2B17. There were no significant correlations between testosterone and androsterone, etiocholanolone, 5 α -adiol and 5 β -adiol. Therefore, additional studies are required to confirm the polymorphism of UGT2B17, 5 α -reductase, 5 β -reductase and HSD enzyme in Malaysian population. Additionally, environmental condition and dietary factors could also contribute to the differences in this steroids profile.

In this study, it was found that the range of steroid parameters of 126 athletes at 95% confidence interval obtained in this study were much lesser than the cut-off value of steroids set by WADA [2] (Table 2). These may open an opportunity for athlete to dope with certain dose of prohibited substance

that will not exceed the cut-off as stated by WADA. This could raise concern with the sports authorities where the results from doping testing would be wrongly interpreted as false negative. Therefore, it is best for each population to establish its own reference ranges.

3. Conclusion

This study demonstrated that geographical population may play an impact in the interpretation of a steroids profile. Moreover, the difference in gene expression in enzymes that are involved in steroids hormone metabolism in each individual could affect the steroids profiles which support the implementation of endocrinological passport by WADA. Thus, the used of any synthetic hormone or indirect androgen doping products such as aromatase inhibitors that could alter the endocrinological profile which may affect the production of steroids hormones can be detected.

In conclusion, steroid parameters range that have been developed in this study have demonstrated several significant differences between populations and more importantly with WADA cut-off values. The population reference range with 95% confidence interval of endogenous urinary steroids excreted in urine from a population representative of Malaysian athlete population obtained from this study could give a valuable information in doping control test which corresponding to Malaysian athletes as well as athletes from the South-East Asia region.

Disclosure of interest

The authors declare that they have no competing interest.

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