PROJECT REVIEW

"Endogenous Testosterone, Testosterone Precursors And Metabolites; 19-Nor-Steroids and Establishment of Normal Urinary Levels Of These And Related Compounds"

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Recently, a sizeable number of urine samples analysed by official laboratories tested positive (>2ngIml) for the nandrolone metabolites 1 9-nortestosterone (19-NT), 1 9-norandrosterone (19-NA) and 19-noretiocholanolone (19-NE) (1-6). The large number of positive tests in such a short period of time, combined with the fact that some sports involved had not previously been associated with the abuse of anabolic-androgenic steroids (e.g. judo, handball, football and figure skating) have triggered investigations into the possible endogenous production of these metabolites in the adult male *(1,3).* The JOC and other bodies have concluded that more studies are necessary to fully elucidate the metabolic fate of anabolic-androgenic steroids (AAS) for the parent hormone, its precursors and metabolites.

Nandrolone is an AAS that acts in a manner similar to testosterone on many reproductive and non-reproductive target tissues (7). It contributes to the development of male secondary sex characteristics (androgenic) and muscle mass and strength (anabolic). Because most AAS are metabolised extensively, the parent steroids are only detected in the blood for a short period of time following administration. Therefore, detection of the metabolites is of crucial importance in determining over what time period the abuse has occurred (6). The issue of detection is further confused by studies which have indicated nandrolone and its metabolites are produced naturally in men (8). in particular, following intense exercise, there is a relative increase in concentration of nandrolone metabolites in urine (1). Moreover, the consumption of nutritional supplements containing 19-NA, a precursor of nandrolone which is available over the counter in the USA as a nutritional "supplement" for the enhancement of physical performance (9), produced levels of 19-NA and 19-NE in urine similar to those shown after illicit nandrolone administration and could be detected 7-10 days after a 50 mg single oral dose (2).

It has also been argued that too little is known about possible endogenous sources of nandrolone for the testing laboratories to be confident about setting their threshold and that the studies already undertaken have recruited too few subjects. The methods of measurement for most of these steroids are currently cumbersome, time-consuming and require highly trained personnel. Therefore, our proposal has two strands. In the first instance we propose to develop ELISA-based methods (Enzyme Linked Immuno-Sorbant Assay) for testosterone, nandrolone and some of their rnetabolites to allow routine and rapid screening of urine samples taken from

athletes. In conjunction, an HPLC anabolic-androgenic steroids profile will be established for use as a reference for the above immunoassay methods. Secondly, we intend to establish normal ranges for endogenous testosterone, testosterone precursors and metabolites in urine in a number of disparate populations.

Development of an HPLCprofilefor anabolic-androgenic steroids

We will optimise the existing HIPLC system (Waters, Cheshire, 13K) in the Department of Dietetics, Nutrition and Biological Sciences, Queen Margaret University College, so that it can detect and identify various AAS. In order to develop reference criteria the system will first be calibrated against authentic androgenic and tritiated-labelled steroids (9). Following that, urine samples can be diluted appropriately and extracted with dichloromethane. After concentration, the residues will be dissolved in the HPLC mobile phase and aliquots injected on the column (Allure column or C 18 column). The HPLC system will then be utilised to identify various AAS: testosterone, testosterone precursors, testosterone metabolites and other related substances. This procedure will allow us to develop a profile in which any unknown peaks can be identified and investigated. The results of steroid levels (e.g. testosterone and nandrolone) obtained by the HPLC system can then be correlated with established methods such as the GC-MS (10)

Development of ELISA methods for the measurement of testosterone and nandrolone in urine samples

The measurement of testosterone (a clinically important steroid) and nandrolone, a potent and widely abused steroid which is detectable in urine samples, has to be quick, simple and routine. Therefore, we are going to develop ELISA (Enzyme Linked Immuno-Sorbant Assay) methods to ascertain the levels of these steroids, in urine, on a routine basis. This will enable us to screen large number of samples within a short time at relatively low cost and without the need for highly skilled personnel. The principle of the ELISA could be either a direct method

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Results and conclusions

Highly sensitive and specific Enzyme-Linked Immuno Sorbent Assays (ELISA) have been developed and applied to measure endogenous nandrolone, free testosterone and total testosterone in urine samples. Two clinical studies were performed on normal healthy volunteers who were known not to have taken anabolic steroids. The first one aimed to establish normal ranges for urinary nandrolone and testosterone levels in non-exercising subjects and those who routinely exercise for leisure purposes. The second study investigated the effect of a controlled single bout of exercise on urinary levels of endogenous nandrolone, free and total testosterone in female and male volunteers.

An HPLC system that can separate some important anabolic androgenic steroids from glucocorticoids has been developed and established in our laboratories, and work is underway to analyse the urine samples collected from our volunteers under different conditions by this system. Eventually we could produce a correlation between the ELISA results obtained with or without the HPLC separation. Work is still in progress to estimate the low levels of endogenous nandrolone metabolites; 19-norandrosterone and 19-norethiocholanolone, testosterone and testosterone precursors in the urine samples taken from our volunteers in the 2 studies, by a GC-MS system. The results obtained will be compared to those of the immunoassay technique for endogenous nandrolone.

Publications and poster presentations

1) Al-Dujaili EAS and Skinner A, The effect of dietary fat intake on salivary and urine testosterone levels in resistance trained men.
   5th International Conference on Nutrition and Fitness, Athens, Greece, June 2004.