"Physiological and Analytical Factors affecting Carbon Isotope Ratios of Testosterone and Androstanediols"

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PROJECT REVIEW

The abuse of synthetic hormones, which also occur naturally in the human body is difficult to detect. These compounds are chemically identical. However, the carbon, of which these compounds are made, features two stable isotopes, atoms that exhibit slightly differing masses. The ratios of the stable carbon isotopes ^{13}C and ^{12}C are different in synthetic and natural hormones.

The most frequently abused hormone is testosterone (T), the principal male sex hormone. In fact, not the testosterone itself is analyzed for ^{13}C and ^{12}C, but metabolites that are found in the human urine. There are several of them and they have largely differing concentrations. The most abundant metabolites are androsterone (A) and etiocholanolone (E). Due to their large concentrations they can be analyzed with relative ease. But E and A also have sources other than T. Therefore A and E might exhibit only slightly altered isotope ratios following abuse of T.

Two other compounds, so-called androstanediols, AD and BD, are made virtually exclusively from T. It is also possible, if difficult, to analyze T itself. Interestingly, T as well as AD and BD show much larger variation of the isotope ratios in control subjects than A and E. Therefore the message of corresponding analyses is often not quite clear, although the three compounds certainly yield the most sensitive parameters for T doping.

The project aims to elucidate the biological and analytical factors that cause the scatter of the isotope ratios of T, AD, and BD. We thus want to be able to provide unequivocal results in case of T doping. We also expect a much better sensitivity of the method, so that T doping can also be detected when small amounts have been applied or when the administration has been performed a while ago.

Results and Conclusions

Analysis of the ratio of the two stable carbon isotopes 13C and 12C currently represents the methods choice to detect the illicit administration of synthetic steroids. Synthetic testosterone plays an outstanding role amongst these compounds. Typically, it betrays its origin in that it exhibits significantly reduced 13C/12C ratios as compared to endogenous steroid hormones.

The 13C/12C analytical procedure, however, needs not to be restricted to testosterone necessarily. By contrast, it will be even beneficial to extend the analysis to the main testosterone metabolites 5α-androstane-3α,17β-diol (5αAdiol) and 5β-androstane-3α,17β-diol (5βAdiol).

However, there is also natural variation of the relevant 13C/12C ratios. But few is known concerning the physiological factors that might take effect here. Moreover, the 13C/12C analysis of these steroids is challenging. Mostly because of their comparably

low abundances in human urine. Therefore sufficient separation and purification is challenging.

Consequently, an efficient analytical procedure for 13C/12C analysis of 5αAdiol and 5βAdiol had to be developed first. This method now allows for long-term precisions of 0.2‰ to 0.3‰ on the so-called VPDB scale. In turn, this was supposed to facilitate valid investigations of physiological effects.

It turned out that increased urinary concentrations of the androstanediols 5αAdiol and 5βAdiol and of testosterone generally result in increased 13C/12C ratios.

As an integrated parameter, we chose energy availability (EA) as a proxy for physical workload. EA is defined as the amount of energy available for the maintenance of physiological functions taking into account the energy expenditure for exercise. In general, higher EA results in lower 3C/12C ratios of the investigated steroids, but in particular of 5αAdiol. The effect appears less pronounced in males, however.

No immediate diurnal effects could be observed. Similarly, no systematic effects were observed in respect to the female menstrual cycle. However, there seems to be a tendency towards alternating 13C/12C ratios within few days. The latter effect seems to be cleared by oral contraception. Within given limits and analytical precision the 13C/12C ratios of the investigated steroids then seem to reflect a pure random process.

While still varying randomly in time, specifically the 13C/12C ratios of 5αAdiol appeared lowered in females using oral contraceptives