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Detecting the abuse of testosterone and its precursors in sports urine tests

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Since the early 1980s, the detection of testosterone (T) administration has been based on T/E ("the TE ratio"), the urinary ratio of T to an epimer with no known function, epitestosterone (E). This is because the average T/E is about 1 in normal men and it increases temporarily after T administration. The reporting cut-off set by the International Olympic Committee (IOC) was six.

Yet T detection remained a challenge because the screening and confirmation technique used for anabolic androgenic steroids, gas chromatography-mass spectrometry (GC-MS), cannot distinguish pharmaceutical T from endogenous T normally produced in the human body. Urine tests based on a T/E cut-off may miss cases with T/E<cut-off in which T (or both T and E) might have been used, or lead to reporting cases in which the elevated T/E might be natural.

Therefore, T/E>6 constituted an offence *unless* there was evidence that this ratio was due to a physiological or pathological condition. For some years the IOC gave special consideration to cases with 6<T/E<10. The approach to distinguishing "naturally elevated T/Es" from doping cases was longitudinal testing, because T/E in drug-free men is stable, whereas T intake causes a spike. To plot an athlete's T/E over time, anti-doping organizations might ask the lab to retrieve past data or subject the athlete to one or two short-notice follow-up tests within two months. In 2004, the IOC passed the responsibility for the prohibited list to WADA and one year later, WADA lowered the T/E cut-off from six to four.

The additional approaches to distinguishing T users from non-users are more or less practical or available in anti-doping labs. They include measuring related urinary hormones such as luteinizing hormone (LH), subjecting the individual to a diagnostic "ketoconazole test," reviewing medical history or endocrine evaluations, and most importantly, Isotope Ratio Mass Spectrometry (IRMS) testing, also known in sports as Carbon Isotope Ratio (CIR) testing.

The CIR reflects $^{13}C/^{12}C$. Most carbon atoms in nature are ^{12}C, but approximately 1.1 per cent of them are ^{13}C. Fortunately for anti-doping scientists, there is a measurable difference in ^{13}C content between endogenous and pharmaceutical testosterone (T) because they arise from different pathways. Endogenous T is made by the body from cholesterol. Pharmaceutical T is made by semisynthesis from plant steroids.

CIR testing begins with several hours of urine sample preparation. The urine extracts are analyzed by gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS).

In the GC step, only the retention time identifies each target analyte because the GC eluate is completely pyrolyzed in the subsequent combustion step. The combustion step oxidizes all the carbon atoms in the compound of interest to carbon dioxide, CO_2. The isotope ratio mass spectrometry step measures only m/z 44, 45, and 46 for CO_2, and calculates how much of the carbon was ^{13}C or ^{12}C.

The end result is not the absolute $^{13}C/^{12}C$ ratio, but the difference, or $\delta^{13}C$ (delta) value, between the $^{13}C/^{12}C$ ratio of the sample and that of an international standard. For example, a $\delta^{13}C$ value of -23‰ (per mil) for natural T means that it contains that much less ^{13}C than the standard: 23 fewer parts per

thousand. Pharmaceutical T contains less ^{13}C than endogenous T and has a more negative $\delta^{13}C$ value, for example –30‰.

CIR testing can help resolve far more than cases of suspected T abuse. Following pharmaceutical T administration, the $\delta^{13}C$ values of urinary T metabolites will become more negative. In contrast, the $\delta^{13}C$ values of T precursors, or of endogenous steroids not involved in T metabolism, will not change. Such compounds can be used as endogenous references. A gap in $\delta^{13}C$ value between T or its metabolites and an endogenous reference compound indicates abuse of T or of any steroid in its metabolism. Looking for such gaps is a superior approach because the $\delta^{13}C$ value of T itself in a non-user might be affected by factors such as diet and be difficult to interpret alone, and because such gaps reveal the abuse of any one of many T precursors and metabolites. CIR testing has indeed been applied to various T precursors, T metabolites, and endogenous reference compounds.

When the test was first introduced in the 1990s, it was used to obtain additional information to help decide whether an elevated T/E, perhaps together with T/E values over time, other urine tests, or a medical history, were consistent with a naturally elevated T/E. By 2004, WADA rules made it possible for anti-doping laboratories to report an adverse finding indicative of misuse of male hormones based solely on CIR results.

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Abstract (and full text PDF for a fee to the publisher)

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