PROJECT REVIEW

"Confirmation of Doping with Natural Androgens by Isotope Ratio Mass Spectrometry; Simplifying the Analytical Procedure and Increasing the Evidential Power"

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In nature, different forms of carbon atoms exist called isotopes. The most common carbon atom has a weight of 12 but a smaller amount naturally exists as a heavier weight of 13. In testosterone, the proportion of carbon-13 to carbon-12 present, referred to as the carbon-13/carbon-12 ratio (13C/12C), can be determined by an instrument called a gas chromatograph-combustion-isotope ratio mass spectrometer (GC-C-IRMS) The 13C/12C content of testosterone produced in our bodies is ultimately made from carbon broken down from our dietary intake. However, pharmaceutical testosterone is synthesized from soya-plant material that has low 13C/12C ratio. A similarly low 13C/12C ratio of testosterone in urine therefore indicates that the testosterone is of pharmaceutical origin, i.e. a doping offence has occurred. The amount of testosterone in urine is very small, even following its administration, and the separation required to purify and concentrate it for IRMS analysis is time consuming and laborious. A simple and rapid preparatory technique is highly desirable. We will manufacture polymers, which are imprinted with the molecular shape of testosterone, so that testosterone from urine can quickly fit into these imprints, like pieces from a jigsaw. The testosterone can then be easily removed for analysis by GC-C-IRMS. Further development will allow the polymer to be coated onto a small glass bar for stirring in the sample itself prior to the bar being directly transferred into a heated inlet (called a thermal desorption unit) on the GC-C-IRMS, where all the testosterone is vaporized for analysis. This 'on-line process' is very rapid and all the isolated testosterone is analyzed thus increasing sensitivity. Finally, to prove that an abnormal 13C/12C ratio of the testosterone targeted is not because of impurities present, we intend to simultaneously analyze a portion of the sample with a standard type of spectrometer (quadrupole) attached to the GC-C-IRMS.

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Results and Conclusion

Comparison of WADA statistics for adverse findings with those of ten years earlier shows that there has been little change in the ranking of anabolic steroids, with testosterone continuing to account for the most common finding. It is analytically more challenging to prove the administration of testosterone, as it is also naturally produced within the body. An analytical approach that can distinguish administered testosterone from that naturally-produced is by determining the carbon fingerprint of testosterone present in urine. Testosterone has 19 carbon atoms, but collectively the carbon atoms in pharmaceutical testosterone are less heavy than testosterone produced in our body. The difference in heaviness is due to whether or not a carbon atom is present with an extra neutron in its nucleus, that is whether it is the heavier ‘carbon-13’ rather than the ‘carbon-12’. The relative amount of carbon-13 to carbon-12 present, referred to as the carbon-13/carbon-12 ratio ($^{13}C/^{12}C$), can be determined by an instrument called a gas chromatograph-combustion-isotope ratio mass spectrometer (GC-C-IRMS). For doping control purposes, this approach is used to prove whether an athlete has broken the rules by administering testosterone.

The concentration of testosterone in urine is very small, even following its administration (doping), and extensive sample work-up is required to purify and concentrate the testosterone from urine prior to carbon isotope analysis. To reduce the labor and simplify the process, the WADA-accredited at King’s College London (UK) in collaboration with the University of Leeds, manufactured a polymer (a macromolecule) into which the molecular shape of testosterone was imprinted, so that testosterone molecules from urine can quickly fit into these imprints, like jigsaw pieces. The testosterone can then be easily removed for analysis by GC-C-IRMS. The work was challenging, but ultimately successful. Even so, to make the process effective, more R & D work is required, so that an optimized polymer can then be applied by loading into small cartridges through which urine can flow with the testosterone being easily extracted for analysis. In addition to the polymer investigation, the researchers also reconfigured the design of the instrument so that

enhanced steroid purification can also be performed within the GC-C-IRMS, a process called multi-dimensional gas chromatography, and they added a different type of mass spectrometer (quadrupole analyzer) to assist with detection. These modifications to the instrument, which are cost-effective and simple to adopt by other laboratories, adds to the certainty that the testosterone measured by isotope analysis is pure, in keeping with the gold standard approach by WADA-accredited laboratories.

Financial Report

All of the WADA funding was spent, as specified in the grant application. Research Contracts from King's College London will communicate the financial report directly to WADA (this is common practice, apparently).