

## PROJECT REVIEW

### **“Pharmacokinetics of pharmaceutical testosterone and TIE in subjects with low and high baseline T/Es: assessment of the ethnic differences and of the sensitivity of various carbon isotope ratio methods relative to TIE”**

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Over 98 percent of the carbon atoms in nature are  $^{12}\text{C}$  but about 1.1% are  $^{13}\text{C}$ , an isotope of carbon. If molecules of testosterone (T) or any other steroid are completely burned in a furnace at  $800\text{ }^{\circ}\text{C}$ , the molecules are combusted to carbon dioxide and water. The gas chromatography-combustion-isotope ratio mass spectrometer (GC-C-IRMS) instrument measures the  $^{13}\text{C}/^{12}\text{C}$  ratio of the combusted steroid, relative to a standard. The steroids are first extracted from urine, then separated in the GC, then combusted in the furnace, and finally the  $\delta^{13}\text{C}$  value is determined.

There is a measurable difference in  $\delta^{13}\text{C}$  values between pharmaceutical T (- 30 ‰) manufactured from soy compounds and T biosynthesized by the human body (- 25 ‰). This arises from differential ‘fixing’ of ‘in soy and related plants.’ Thus by determining the  $\delta^{13}\text{C}$  value of urinary T one can determine its origin. This brief overview greatly oversimplifies an exceedingly complex method which is highly dependent on the underlying analytical methodology. If the extraction and chromatography steps are not perfectly executed to provide complete baseline separation of the steroids, the results will be unintelligible.<sup>2</sup>

Correct interpretation of the  $\delta^{13}\text{C}$  values requires an understanding of factors that influence them. This proposal focuses on clinical studies that probe the underlying physiology and pharmacology of the method. This study is concerned with endocrinology, steroid metabolism and pharmacokinetics. In the past seven years we have developed and optimized the IRMS method,<sup>25</sup> determined  $\delta^{13}\text{C}$  values on various urinary steroids, validated our screening method which determines  $\delta^{13}\text{C}$  values on androsterone and etiocholanolone,<sup>4</sup> and validated a confirmation method which determines  $\delta^{13}\text{C}$  values of three urinary diols.<sup>5</sup> We have also presented evidence that the IRMS method is useful for distinguishing cases of naturally elevated TIE<sup>5</sup> from T users. Others have confirmed<sup>6</sup> and extended our studies<sup>8</sup> (Page limitations prevent citing all the relevant publications.) Our results show, as expected, that T administration lowers  $\delta^{13}\text{C}$  values of urine T metabolites. All the previous studies delve into the method, its efficacy, and the  $\delta^{13}\text{C}$  values of healthy subjects, whereas the study proposed herein probes the underlying physiology, endocrinology, and pharmacology of

the application of the IRMS method.

It is reported **9,11** that some individuals do not experience an increase in *T/E* when T or related steroids are administered. This lack of response is often attributed to the Asian ethnic group, although we have found that some non-Asians also do not respond to T. We believe, but have yet to unequivocally prove,<sup>9</sup> that the population we study (U.S. students) is bimodal with respect to baseline T/E and that this is one determinant of the response to T. The high mode (HM-T/E) group is characterized by relatively high baseline T and E excretion rates, whereas the opposite is the case for the low mode (*LM-T/E*) group.

## **Pharmacokinetics of Pharmaceutical Testosterone and T/Es in subjects with Low and High Baseline T/Es: assessment of the ethnic differences and of the sensitivity of various carbon isotope ratio methods relative to T/E.**

### **Results and Conclusions**

We investigated the hypothesis that a population of males might exist that could dope with testosterone and yet not be detected by the conventional TE that is used in doping control to detect testosterone users.

Twelve healthy male subjects were stratified as high mode TE (HM) or low mode (LM) based on their resting baseline urine TE. All received a three-day continuous intravenous infusion of testosterone. Serum and urine were collected for one day prior to the testosterone infusion, during the infusion, and for two days following the infusion.

The main hypotheses were that subjects with low TEs would be difficult to detect by the conventional TE screen used in doping control and that the carbon isotope ratio method would be a superior method for the conventional screening method. The hypothesis proved to be correct.

The excretion rates of all steroids increased during the first day of the infusion when the dose was equivalent to the normal production rate of testosterone. When the dose was increased to double and three times the normal production rate, the excretion rates increased still more. While the rates increased for both the HM and LM groups, in the case of the HM subjects the increases occurred earlier and were higher. In fact 5 of the 6 LM subjects never achieved a TE greater than 4 while the TEs exceeded 4 in all the HM subjects.

Based on other studies we expected the testosterone infusions would lower the concentration of urinary epitestosterone, however the opposite was found: the epitestosterone levels increased. This unexpected finding raises a number of intriguing questions about the control mechanisms for epitestosterone.

This study provides convincing evidence that all males do not metabolize testosterone in the same fashion and that at least two subgroups exist. Those with low TEs have low testosterone excretion rates and in general are not able to increase the rate above 5 $\mu$ g/h. These subjects are, in effect, protected from detection by the conventional doping control TE method. Further studies are needed to define these groups.

The carbon isotope ratio method detects all testosterone users in both the HM and LM groups and is therefore superior to the conventional TE method. In addition the carbon isotope ratio method is known to detect the use of other doping agents that are difficult to detect by the conventional TE method.