IN THE COURT OF ARBITRATION FOR SPORT

FLOYD LANDIS	)	
	)	
Appellant,	)	
	)	
V.	)	**CAS 2007/A/1394**
	)	
UNITED STATES ANTI-DOPING AGENCY	)	
	)	
Respondent.	)	
	)	

WITNESS STATEMENT OF CEDRIC SHACKLETON

My name is Cedric Shackleton. My address is 2723 8th Street, Berkeley, CA.

Resume:

Education: PhD in Clinical Biochemistry, University of Edinburgh 1969 Doctor of Science (Endocrinology), University of St Andrews 1988.

Work History: 1964-1969 Clinical Chemist, The Edinburgh Royal Infirmary; 1969-1978, Head of the Steroid Research Unit, the Clinical Research Centre, London; 1972-1978, Senior Lecturer the Institute of Child Health, London; 1978-1983, Researcher the University of California, Berkeley and San Francisco.

Present: Professor of Medical Sciences, the University of Birmingham, UK; and Senior Research Biochemist, Children's Hospital Oakland Research Institute.

Publication record: I have 209 peer-reviewed publications listed (NIH, PubMed recorded), primarily on steroid metabolism.

Research Interests: I have been working on steroid metabolism for 44 years. In the context of this case I have been utilizing mass spectrometry in steroid doping control intermittently since 1974 [see Ward, (Shackleton) et al., British J. Sports Med. 1975; 9:93-7]. I contributed to the development of the Carbon Isotope technique in the 1990s, particularly the focus on epimeric androstanediols as primary analytes [eg., Shackleton et al, 1997 "Steroids" 1997; 62:379-87]. With regard to a central topic of this appeal, the formation of 5ά-and 5β-reduced metabolites, I have extensively studied the degradation of steroids by the enzymes 5ά-and 5β-reductase, particularly in patients with the congenital defects of these enzymes (eg. for 5ά-reductase, see Peterson (Shackleton) et al. Clin Endocrinol 1985; 23: 43-53; and for 5β-reductase (see Palermo (Shackleton) et al, 2007; "Steroids" in press).

Professional Societies: Membership in the "Endocrine Society", the "Society for Endrocrinology" (UK), the European Society for Pediatric Endocrinology, the American Society for Mass Spectrometry.

Editorial Responsibilities: For 10 years I was one of four editors of the Journal "Steroids" so critically reviewed multiple articles submitted for publication. I frequently review articles for "The European Journal for Endocrinology", "Steroids" and the "Journal of Clinical Endocrinology and Metabolism.

Abbreviations: In this document, 5ά-androstanediol is sometimes referred to as 5ά; 5β-androstanediol as 5β; and pregnanediol as Pdiol.

Opinion:

There is no explanation for a ^{13}C difference between 5ά-diol and Pdiol in the range of -6 other than the administration of testosterone or one of its precursors.

The steroid metabolites 5ά, 5β and Pdiol in a normal individual are all the product of food and drink consumed by that individual. Different food sources have different ^{13}C values. The metabolites 5ά, 5β and Pdiol are each produced by a variety of metabolic pathways which are not identical. The metabolic production of 5ά and 5β are influenced by the application of exogenous testosterone or its precursors. Pdiol is not. IRMS analysis identifies differences in the ^{13}C value of an endogenous reference compound, like Pdiol, which is not influenced by testosterone administration, and ^{13}C values of those metabolites such as 5ά and 5β which can be affected. In an individual who has not doped, there will not be a significant ^{13}C difference between Pdiol and either 5ά or 5β. There is nothing that an athlete can eat or drink, other than testosterone or its precursors, that cause significant differences in these values. In my opinion, there is no question that an athlete who has a difference in ^{13}C values between 5ά and Pdiol in the range of -6, as in this case, has used exogenous testosterone, DHT, or another testosterone precursor.

The IRMS data were perfectly in agreement with known science thus allowing ^{13}C values to be different for both epimers 5ά and 5β.

1. Enzymes utilized in the formation of both 5ά and 5βare not related. The androstanediols 5ά and 5β are formed by entirely different pathways under no common control (Figure 1). 5ά-Reduced steroids are made from precursor molecules such as testosterone (but not exclusively) by two 5ά-reductase enzymes, called SRD5A1 (chromosome 5 p 15) and SRD5A2 (chromosome 2 p 23) which are coded by different genes on different chromosomes. Of the two 5ά-reductases, SRD5A2 is expressed (means is present) in sex organs such as the prostate, or in skin, as well as liver. The other, SRD5A1, is primarily located in liver. 5β Reduced steroids are formed by a third entirely different, and primarily hepatic (liver) enzyme AKR1D1, also coded by a separate gene on a different chromosome (chromosome 6). In addition to being coded by different genes these enzymes have different chemical structures. There are human genetic disorders I have studied where patients can be completely deficient in one or the other, 5ά- or 5β-reductase. All normal individuals will have their own enzyme activity values for each of these enzymes dependent on their genetic make-up. Thus, there is little or no connection between the mechanism for formation of the two androstanediols apart from their acutely increased

production because of an excess of a common precursor (eg, testosterone) which must be disposed of.

In the reduction (adding hydrogen) of testosterone differences in activity of these three reductases between individuals is sufficient to result in differences in the relative amount of 5ά and 5β steroids produced.

2. Differences in 5ά and 5β are supported by peer-reviewed studies. The Landis Sample A difference between 5ά-diol-Pdiol and 5β-diol-Pdiol was -3.99 and for sample B the value was -3.74. At page 56 of the Landis brief, it is stated "these differences are **far greater** than the testosterone metabolites in **any** peer reviewed study." That is clearly an erroneous statement because Subject "A" in the "peer-reviewed" paper of Aguilera et al (J Chromat B 727 (1999) 95-105) had a difference of 4.3 between the 5ά and 5β androstanediols. Other studies also show significant differences in 5ά and 5β metabolites in some individuals.

3. There are multiple origins of the androstanediols (including testosterone). Testosterone is only one of the precursors (parent compunds) of androstanediols, under normal conditions a minor one (origin 1 on the attached Figure). In non-doping individuals, the bulk of these steroids (and even more so for the secondary analytes androsterone and etiocholanolone) is made from adrenal steroids such as DHEA and 17-hydroxyprogesterone (origins 2 and 3 on the attached Figure). How much of each of the 5ά- and 5β-androstanediol comes from testosterone or adrenal steroids will vary between individuals. Also, gradually in the hours and days following a single testosterone administration the adrenal steroid contribution to the urinary androstanediols will get greater; as the suppression effect of testosterone administration abates. How this affects **each** androstanediol need not be synchronized.

4. When will 5ά and 5β "delta" values be very similar and when will they be different?

(a) Delta values will be very similar or parallel soon after receiving a **large** dose of testosterone or a precursor. At that point, the metabolic enzymes are swamped and are working at full capacity to metabolize (get rid of) the excess testosterone in the system. Also at that point, sources of androstanediols other than exogenous testosterone effectively get supressed (shut-out), ie, endogenous testosterone, adrenal gland steroids. The delta values will stay this way until the precursor (testosterone) becomes depleted and the enzymes are back to competing for it. It is important to recognize that frequently our laboratory experiments have been carried out with much bigger doses than even an athlete may take. Our [Shackleton et al, Steroids 62:1997:379-387] study used 180mg of testosterone, a figure close to 50x the normal testosterone production rate for males! It is no wonder that the 5-reductases are working at maximum capacity for days.

(b) The delta values need not be identical in the period following a large drug dose. Note the situation in Shackleton study Fig 4, subject 3, day 4; the 5ά has reached maximum negativity while the 5β keeps increasing its negativity. Fig 4, subject 2, the 5β is returning to normal before the 5ά. In the other subjects, the negativity of the 5ά decreases first, the 5β negativity stays longer.

(c) The androstanediols need not behave the same in terms of delta/delta negativity in the period **after the extended peak of negativity** (recovery period). It is important to note that

while both androstanediols may be returning to normal "delta" value they need **not** be doing it in parallel; they may in fact be **diverging,** see subjects 4 and 5 (days 8 onwards) in Shackleton paper. If urine collections in that study had been continued past 16 days in subject 4 there may well have been delta/delta differences greater than 4. The negativity decreases because gradually the non-exogenous precursors of androstanediols are returning to prominence.

The delta values for each androstanediol **can** be going in opposite directions, one increasing negativity, one decreasing. See Shackleton study Fig 4, subject 3, days 4-8.

(d) The delta/delta values of 5ά and 5β can be markedly different from each other if a **smaller** dose of testosterone is used. In this situation neither 5ά-or 5β-reductase may approach their capacity and there can be differences in delta/delta values of the androstanediols at all times following administration. The differences will vary markedly dependent on the hours lapsing since administration.

(e) If the 5ά has much **greater** negativity than the 5β, and particularly if the pregnanediol endogenous reference compound has **no** increased negativity, then one must consider that the subject may be doping with dihydrotestosterone (DHT), a 5ά-reduced metabolite of testosterone available as a transdermal cream (Andractim). Alternatively, it's possible an athlete would use both T and DHT. That scenario would give negativity to both diols, but the 5ά would probably have greater negativity. In this context the importance of the route of administration becomes important. Skin has 5ά-reductase which would likely increase the amount of 5άdiol produced. By contrast, this tissue has no 5β-reductase. In fact Schaenzer and co-workers (2007) showed that application of a testosterone cream did favor the production of the 5ά metabolite which resulted in greater negativity of the delta value for the 5άά Oral administration appears to favor the production of the 5βdiol because this dominates in hepatic metabolism. (Maitre study).

(f) As previously mentioned, everybody has their own pattern of metabolism in terms of 5ά- and 5β reduction, and the influence of adrenal steroids as androstanediol precursors. These differences would be masked at the height of metabolism after a **large** dose of exogenous steroid. Everybody would have near maximally negative values for both androstanediols. However, after a dose that is closer to the body's normal testosterone production like the recommended dosage of testosterone gel or oral testosterone or the application of DHT cream, individual metabolic patterns favoring 5ά or 5β could be seen shortly after administration. Even with larger doses, differences in 5ά and 5β could be expected over time. The specific time would depend on the metabolic preferences of the individual, the excretion rate of the particular type of testosterone, and individual excretion rates.

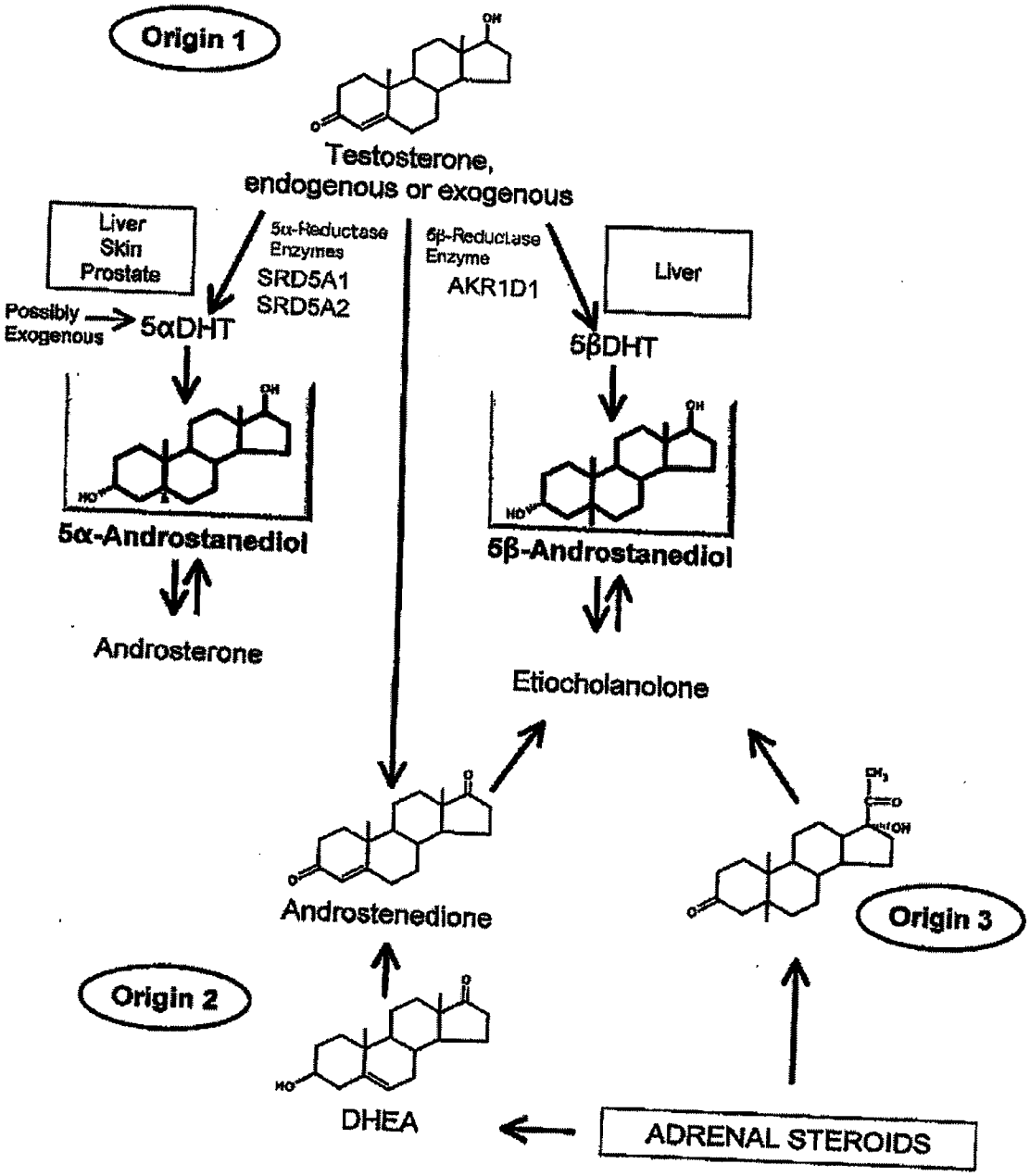
(g) Note that all of the studies involve a single type of testosterone application (gel or oral or injection) in significant amounts. An athlete who is trying to avoid detection may very well dope with a combination of substances or smaller doses.

In my testimony at the previous hearing, I offered my opinion that the chromatograms of Mr. Landis's Stage 17 Sample were of good quality and were reliable. That continues to be my opinion. However, Drs. Brenna, Matthews and Jumeau all have much more experience in IRMS chromatography than I do.

Summary:

There are multiple scenarios when either 5ά or 5β negativity are very different following steroid administration. There is no reason why a negativity difference greater than 4 is not completely appropriate (and within "known-science") following a testosterone and/or DHT administration.

The Multiple Origins of Andostanediol, Androsterone and Etiocholanolone



This is a simplified chart and does not contain all known metabolic and synthetic reactions.

I declare under penalty of perjury of the laws of California and New York that the foregoing is true and accurate. This statement was signed on March 7, 2008, in Berkeley, California.

Cedric Shackleton