

## PROJECT REVIEW

### **“Defining Interaction Between Anabolic and Peptide hormones Requirements for a Robust Test for Growth Hormone Doping”**

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Tests for detecting abuse of peptide hormones such as growth hormone (GH) in sport have proved elusive. However, there has recently been considerable progress in this field. Following the work of the GH 2000 project, two approaches were proposed for detecting GH doping; one using indirect markers of GH action and one based on the quantitation of GH isoforms secreted from the pituitary gland [1-3].

The difficulties associated with development of tests for doping are compounded by the fact that athletes frequently take performance-enhancing agents (PHAs) from more than one class. A PHA acting on one system can influence the activity of a different system. How physiological interactions between biological systems may alter the result of a doping test has not received detailed attention. For example, anabolic steroids stimulate the GH system [4, 5], and both anabolic steroids and GH stimulate erythropoiesis [6, 7]. Physiologic and pharmacologic interactions of this nature will have an impact on the predictive value of a test. Therefore, the validity of a test is dependent on basic information on how one P1-IA interferes with tests for another group.

An alternate approach for detecting exogenous GH use is based on the difference between recombinant human GH (r-hGH) and pituitary GH, which consists of different molecular isoforms including 17kD (17K) and 20 kD GH (20K). Initial work using subtraction assays indicates that GH doping can be reliably detected using this method [3]; however, isoform specific assays are preferable. We have developed a highly sensitive and specific immunoassay for 20K-hGH [8] and have also developed an immunoassay for 17K-hGH [9]. Use of 20K- and 17K-hGH together is likely to provide a more robust measure of GH abuse than use of one isoform alone.

Our Consortium is uniquely placed to conduct these investigations. The Australian Sports Drug Testing Laboratory (ASDTh) is IOC accredited, and has extensive experience of drug testing in the Olympic setting. Members of the Consortium are internationally recognised leaders in their field and each provides complementary expertise within this project. These areas include GH physiology, sex steroid interactions and clinical research facilities to undertake interventional studies (Prof Ho and co-workers), 20K GH assay development (Prof Irie and co-workers), 17K GH assay development (Prof Ho and co-workers), insulin-like growth factor-I (IGF-I) and IGF-binding proteins measurement (Prof Baxter and co-workers), anabolic steroid and androgen pharmacology (Prof Handelsman) and EPO detection (Dr.

Kazlauskas and co-workers). Several members have already established a network for large scale collection of samples and links with National sporting bodies (Prof Irie and Dr.Kazlauskas).

We have already collected more than 4000 serum samples from elite athletes as part of the EPO 2000 project involving the ASDTh. Multiple samples were obtained from more than 1100 athletes over a two week period. These athletes represented a wide variety of nationalities and ethnic groups. This archival collection is a powerful resource for the ultimate validation of tests which will be selected for their potential from studies of pharmacologic interactions, and will define the reference data for the application of these tests.

## **Defining interactions between anabolic and peptide hormones: requirements for a robust test for growth hormone doping**

### **Results and Conclusions**

This summarizes the results obtained in our project **to develop a robust test for doping in sport with growth hormone (GH)**, based on the detection of (i) GH-responsive proteins and (ii) pituitary GH isoforms in blood. This involved the conduct of two major studies:

- (a) a cross-sectional study of over 1000 elite athletes to determine the potential influence of demographic factors (**Demographic Study**)
- (b) a prospective intervention study in recreational athletes to identify the most sensitive GH-responsive markers and the potential influence of co-administration of androgens (**Intervention Study**)

The **main achievements** are:

#### ***Demographic Study***

- (i) Publication of the study characterising the influence of demographic factors and sport type on GH-responsive markers in the leading scientific journal in the field.
- (ii) Analysis of the influence of demographic factors on pituitary GH isoforms (20K and 22K GH).
- (iii) Addition to (i) and (ii) above, by recruitment of young elite athletes and Japanese elite athletes

#### ***GH/testosterone Intervention Study.***

- (i) Completion of a major placebo-controlled GH and testosterone administration study in over 90 recreational athletes.
- (ii) Measurement of IGF markers, collagen markers and GH isoforms, and a preliminary analysis of completed markers.

The **key findings** are:

#### ***Demographic) Study***

- (i) For GH-responsive markers, age and gender were the major determinants of variability, except for IGFBP-3 and ALS. Tests for GH doping based on IGF-I and on collagen markers must take age into account and ethnicity need not be considered for these markers.
- (ii) For pituitary GH isoforms, the relative concentrations of 20K and 22K GH, are minimally influenced by demographic factors. The stability of the isoform ratio to the effects of these factors renders it a promising measure of exogenous 22K GH abuse.
- (iii) Additional data from young athletes and Japanese athletes have further consolidated the age-related changes in the GH-responsive markers, particularly in early adolescence.

### ***Intervention Study.***

From a preliminary analysis of the IGF axis markers (IGF-I, IGFBP-3 and ALS) and PIIINP, the responses were greater in men than in women for all markers, and testosterone enhanced the response of PIIINP to GH, but not the responses of the IGF axis markers to GH.

### **Publications**

- 1- Influence of demographic factors and sport type on growth hormone-responsive markers in elite athletes. Nelson AE, Howe CJ, Nguyen TV, Leung K-C, Trout GJ, Seibel MJ, Baxter RC, Handelsman DJ, Kazlauskas R, Ho KK. *Journal of Clinical Endocrinology and Metabolism*, in press.
- 2- The Relationship between Body Composition and Physical Performance: a Study in Young Recreational Athletes. Meinhardt U, Hansen JL, Nelson AE, Walker IH, Graham K, Ho KKY. US Endocrine Society Meeting, Boston, June 2006.
- 3- The influence of demographic factors on the ratio of 20-kDa and 22-kDa GH isoforms and the utility of the ratio for detection of GH doping in sport. Anne E Nelson, Makoto Ueki, Tuan V Nguyen, Kin-Chuen Leung, Christopher J Howe, Graham J Trout, Minoru Irie, Robert C Baxter, David J Handelsman, Rymantas Kazlauskas and Ken K Ho. Third international Congress of the GRS and IGF Society, Kobe 2006; Endocrine Society of Australia Meeting, Gold Coast, August 2006
- 4- The Relationship between Body Composition and Physical Performance: a Study in Young Recreational Athletes. Meinhardt U, Hansen JL, Nelson AE, Walker IH, Graham K, Ho KKY. Endocrine Society of Australia Meeting, Gold Coast, August 2006