

"GH isoforms measured by the differential immunoassays in early and late pregnancy, in patients with pituitary diseases and diabetes"

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Project Review

Research in recent years demonstrated that the analysis of changes in the hGH isoform pattern occurring after administration of recombinant hGH can be used to detect hGH doping. Two pairs of immunoassays based on monoclonal antibodies have been developed, which can be used as two independent tests to measure the relative abundance of the monomeric 22 kD isoform over all other isoforms in a serum sample. Practically, a ratio is calculated between the results from the "rec" assay (employing antibodies preferentially recognizing the 22kDa isoform) and the "pit" assay (employing antibodies recognizing a wide spectrum of pituitary isoforms of GH). Details of the method have been published (Wu et al., 1999; Bidlingmaier et al. 2000, 2001, 2003, 2007, 2009). The method has been implemented in most WADA accredited laboratories and could theoretically be used for detecting cheating athletes.

An important aspect to make test results court proof is the reliability and quality of the normative data underlying the cut-off criteria developed to define an adverse analytical finding. Several studies have been performed to investigate the isoform composition and the respective rec/pit ratios in samples taken at rest and after exercise in healthy subjects, recreational and elite athletes from several sports disciplines and from different ethnic background. The differences found in the rec/pit ratios between the groups were comparably small, and it could be clearly shown that injection of rhGH leads to a strong increase in the ratio exceeding the normal variability.

To further strengthen the scientific evidence for the cut-off, the project proposed here aims to investigate the rec/pit ratio under clinical conditions which might be used as an argument by an athlete to explain an adverse analytical finding (=increased ratio) in front of a court. Investigations of molecular isoforms of hGH under different pathological conditions are scarce. Some papers exist about the 20 kD isoform in acromegaly, when endogenous GH secretion is increased (Murakami 2000, 2004; Tsushima 1999; Boguszewski 1997). The findings were not consistent, pointing to a similar variability in the isoforms in acromegalic patients as in normal subjects. However, the assays used in these papers are completely independent from the isoform assays used in WADA accredited laboratories. So, it remains to be clarified if specific pituitary diseases, but also other conditions where hGH secretion is affected, have an influence on the rec/pit ratios.

In the proposed project, samples from different clinical studies will be investigated. The conditions of interest are:

- early pregnancy (40 samples).
- acromegaly (30 samples)

- other pituitary diseases (non-functioning adenomas, Cushing adenomas etc., 20 samples total)
- diabetes (both, type I and type II, 20 samples total)

The samples will be analysed by both rec/pit assay combinations (termed "kit1" and "kit2"). The two kits involve different antibodies and are used for screening and confirmation test procedures in WADA accredited laboratories.

Results and Conclusions

From the data obtained during the project it is reasonable to conclude that diabetes and cortisol producing pituitary adenomas (Cushings disease) have no detectable impact upon the GH isoform pattern in serum samples as measured by the rec- and pit-assays used for the differential immunoassay approach.

During pregnancy, where pituitary GH secretion is gradually reduced and placental GH secretion increased with increasing gestational age, the situation is different for kit 1 and 2: Whereas the antibodies used in the rec2- and pit2-assay as well as the pit1-assay apparently are not influenced by the changes in the molecular forms of circulating GH, the presence of GHV seems to reduce the signal obtained from the rec1-assay. Overall, this leads to a remarkable reduction in the rec1/pit1-ratio especially after gestational week 10/11, probably because GHV concentrations reach higher levels. However, it is important that there is no risk of a false positive doping test result arising from this situation: Although for ethical reasons it is impossible to formally test recombinant GH applications in pregnant females, the only possible impact of the phenomenon on a doping test would be false negative test results because of falsely low ratios.

In active acromegaly, there was an intriguing difference between the findings in females and males: Whereas the ratios in female patients were completely normal, the mean ratios in males were somewhat higher than expected. However the samples were collected over several years, and not in a controlled doping test setting. Therefore, results have to be interpreted with caution. Nevertheless - although the vast majority of the ratio results were in the expected range, and even the highest ratios were far below the cut offs used to define an AAF - the observation in males deserves further investigation. It is noteworthy that the highest ratio was observed in a patient with a particular aggressive and big tumour, which was continuously growing and required repeated surgery plus cranial irradiation therapy in addition to medical treatment. Because of the paucity of scientific studies investigating the GH isoform composition in acromegaly it cannot be ruled out *a priori* that some subtypes of pituitary tumors preferentially synthesize the 22 kD GH isoform. Alternatively, the continuous presence of high circulating GH levels might affect the formation of dimers and multimers of GH in selected patients, thereby potentially leading to altered rec/pit ratios.

However, it is clear that the results presented here come from severely ill patients suffering from an extremely rare, but easily detectable disease because of the dramatic consequences of the pituitary tumors. Even if this is a population of extreme cases, no rec/pit ratio came into the range seen after recombinant GH administration. Therefore, the observation seems to be of some scientific interest but very likely does not represent a real practical problem in doping controls.