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Editorials

Detection of Illicit Use of Growth Hormone

Ulf-Håkan Stenman[1]

Illicit use of performance-enhancing drugs is a major problem in sports. Since 1967, the International Olympic Committee and the World Anti-Doping Agency (WADA)[2] have issued continuously updated lists of prohibited substances. In 1989, the first polypeptide hormones, human growth hormone (hGH), human chorionic gonadotropin (hCG), and adrenocorticotropin, were included in the list *(1, 2)*. Presently, substances increasing the release of hGH and insulin-like growth factor 1 (IGF-1), which mediates the action of hGH, are also banned *(3)*. WADA has approved test methods for detection of most prohibited substances, but although hGH is widely used for doping, methods for detecting it are not available *(3, 4)*. In this issue of *Clinical Chemistry*, Bidlingmaier et al. *(5)* describe an approach that is potentially useful for this purpose. It is based on the use of immunoassays that preferentially recognize pituitary (phGH) and recombinant (rhGH) hGH, the latter being the form most probably used for doping *(5)*. hGH exerts its effects both directly on end organs and by inducing expression of IGF-1 *(3)*. The combined effect of these is reflected by changes in markers of bone and soft tissue metabolism. Methods for detection of GH administration based on increased serum concentrations of procollagen III peptide and IGF-1 have also been developed *(6)*, but standardizing these methods is demanding and they have not yet been applied to doping control *(3)*.

Use of hGH for doping started to increase when rhGH became available around 1990, but cadaverous hGH was used before this. Ben Johnson, who was disqualified for doping in the 1988 summer Olympics, later admitted that he had used hGH in addition to anabolic steroids. The use of hGH for doping is based on its favorable anabolic and lipolytic effects on subjects with hGH deficiency *(3)*, but scientific evidence for a positive effect of pharmacological doses of hGH on athletic performance in healthy subjects is scarce *(4)*. In spite of this, it is obvious that illicit use of GH by athletes is widespread *(3)*, and anecdotal evidence suggests that doses 10-fold higher than those used therapeutically are being used *(3)*. The harmful effects of high GH concentrations in patients with acromegaly are well known, but little is known about the long-term effects caused by self-administration of GH. It has been suggested that abuse of hGH could be responsible for the premature cardiac death of a female top athlete *(3)*. Methods for controlling illicit use of this hormone are therefore important.

Administration of rhGH suppresses secretion of phGH while the plasma concentration of rhGH increases. Therefore the use of rhGH can be detected with assays differentiating between these forms. However, development of such assays is demanding: the primary structure of rhGH is identical to the major form of phGH in circulation, i.e., the 191–amino acid, 22-kDa form. In addition to this form, the pituitary secretes several structurally different variants, e.g., a 20-kDa splice variant (about 15% to 20% of total GH), and acidic forms (about 10%). About 40% of these occur in circulation as dimers and oligomers and part as complexes with binding proteins, the major one consisting of the extracellular part of the GH receptor *(7)*. The method developed by Bidlingmaier et al. *(5)* is based on the use of immunoassays that preferentially recognize either mono- and oligomeric phGH variants or 22-kDa rhGH, respectively. The assays use antibodies raised by immunizing mice with either rhGH or phGH and careful selection of monoclonal antibodies that preferentially recognizes either one of these. The unbound monomeric 22-kDa form represents only about 20% of phGH in circulation *(7)* and thus the 3-fold difference in the apparent concentrations of phGH and rhGH observed is plausible if secretion of hGH by the pituitary is nearly completely suppressed after rhGH administration. Other forms of phGH, i.e., 20-kDa phGH and complexes with GH-binding proteins, are not recognized by the assays used and do not affect the results *(5)*.

The authors have mapped the epitopes of the antibodies used, but this explains only part of the differences in antibody specificity. It is conceivable that antibodies to rhGH have reduced affinity for dimeric and oligomeric phGH, in which some epitopes may be partially hidden. However, the mechanism causing reduced recognition of rhGH by antibodies raised against

[1] Department of Clinical Chemistry, Biomedicum, Helsinki University Central Hospital, PB 700, FIN-00029 Helsinki University.
Address correspondence to the author at: Helsinki University Central Hospital, Department of Clinical Chemistry, FIN-00290 Helsinki, Finland. Fax +358-9-5054653; e-mail ulf-hakan.stenman@hus.fi.
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[2] Nonstandard abbreviations: WADA, World Anti-Doping Agency; hGH, human growth hormone; hCG, human chorionic gonadotropin; IGF-1, insulin-like growth factor 1; phGH, pituitary hGH; rhGH, recombinant hGH.

phGH is not clear, because the 22-kDa forms of these are supposed to have identical primary structures *(8)*. This may suggest that minor differences in protein folding affect tertiary structure and immunoreactivity. If this is the case, preparations from different manufacturers may behave differently. On the Internet, a large number of companies offer growth hormone preparations, but the origin of these is obscure and their characteristics may differ from those used in the present study.

The findings of Bidlingmaier et al. are also of relevance for standardization of hGH assays, for which poor between-method comparability is a problem. To solve this problem, a consensus panel has endorsed replacement of the earlier WHO standard (first International Reference Preparation of hGH 80/505) with the second International Standard for GH (WHO International Standard 98/574), which consists of rhGH *(9)*. Whereas this may solve one problem, it introduces another, i.e., differences in immunological properties between natural and recombinant hCG. Before introduction of a new hGH standard, this problem needs to be solved by ascertaining that the immunoassays used clinically detect recombinant and pituitary hGH equally. The use of recombinant proteins as standards is also an issue for other hormone assays. rhCG is being evaluated as a possible replacement of the present fourth International Standard for hCG, which consists of placental hCG isolated from urine of pregnant women. hCG is heavily glycosylated, and the carbohydrate structure of placental hCG differs from that of rhCG produced in Chinese hamster ovary cells *(10)*. If rhCG is adopted as a new standard, it will be important to ascertain that it is recognized in the same way as pregnancy hCG by the immunoassays used.

Administration of rhGH by subcutaneous injection can be detected for 24–36 h by the dual assay of rhGH and phGH *(5)*. Because the effect of GH on athletic performance can be expected to be slow and prolonged, administration of GH within the time window of detection does probably not have any positive effect during a competition. Thus detection of illicit use of rhGH can be expected to be most valuable when used out of competition. This probably also holds true for abuse of erythropoietin, but nevertheless several athletes have tested positive in samples obtained in connection with competitions. Thus the new approach to detect illicit hGH use deserves to be tested in practice.

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