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Growth hormone doping tests
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·*Review*·

A robust test for growth hormone doping – present status and future prospects

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Abstract

Although doping with growth hormone (GH) is banned, there is anecdotal evidence that it is widely abused. GH is reportedly used often in combination with anabolic steroids at high doses for several months. Development of a robust test for GH has been challenging because recombinant human 22 kDa (22K) GH used in doping is indistinguishable analytically from endogenous GH and there are wide physiological fluctuations in circulating GH concentrations. One approach to GH testing is based on measurement of different circulating GH isoforms using immunoassays that differentiate between 22K and other GH isoforms. Administration of 22K GH results in a change in its abundance relative to other endogenous pituitary GH isoforms. The differential isoform method has been implemented; however, its utility is limited because of the short window of opportunity of detection. The second approach, which will extend the window of opportunity of detection, is based on the detection of increased levels of circulating GH-responsive proteins, such as insulin-like growth factor (IGF) axis and collagen peptides. Age and gender are the major determinants of variability for IGF-I and the collagen markers; therefore, a test based on these markers must take age into account for men and women. Extensive data is now available that validates the GH-responsive marker approach and implementation is now largely dependent on establishing an assured supply of standardized assays. Future directions will include more widespread implementation of both approaches by the World Anti-Doping Agency, possible use of other platforms for measurement and an athlete's passport to establish individual reference levels for biological parameters such as GH-responsive markers. Novel approaches include gene expression and proteomic profiling. *(Asian J Androl 2008 May; 10: 416–425)*

Keywords: growth hormone; doping; athletes; isoforms; growth hormone-responsive markers

1 Introduction

Despite being banned by the World Anti-Doping Agency (WADA), there is widespread abuse of growth hormone (GH), which is often used together with other banned substances such as anabolic steroids. A robust test is required to enforce the ban on GH; however, developing a test for a naturally-occurring polypeptide such as GH has been a challenge. This review examines the two main current approaches for GH detection based on isoforms of GH and on serum GH-responsive markers, describing the basis of each approach and its current status as a doping test. Future directions in the application of these tests, together with novel approaches being undertaken are also considered.

2 Abuse of GH in sport

There is anecdotal evidence that GH is widely abused, as indicated by the number of website hits for GH supply and by customs and police drugs seizures. The abuse of GH by athletes is probably a result of the immense pressure to perform in sport, which is reflected in a frequently-cited survey in which 98% of athletes said they would take a performance-enhancing substance that would guarantee an Olympic medal if they could not be caught [1]. Amazingly, when asked if they would take the drug if there were a guarantee that they would not get caught and would win every competition for the next

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5 years, even if they then died from its adverse effects, 50% also replied yes [1].

The abuse of GH may start at young ages. An early survey of 10th grade boys in the USA indicated that 5% had taken GH, with more than half using GH in conjunction with steroids [2]. Large surveys of US secondary school students have since reported an increase in the use of anabolic steroids in the late 1990s, followed by a subsequent decline in prevalence of their use [3]. Although the most recent National Collegiate Athletic Association survey of college athletes in the USA also indicates decreased use of anabolic steroids, 1.2% of the athletes reported using GH in the past 12 months [4]. Doses used by athletes are estimated to range from 3 mg to 8 mg daily for 3–4 days per week, often used in combination with other doping agents [5], resulting in average daily doses of 1–2 mg GH, approximately 2–3 times the daily endogenous pituitary secretion. "Polypharmacy" is widely practiced and GH is reportedly used in particular with anabolic steroids. A web-based survey reports the use of GH (1–10 mg/day) and insulin together with anabolic androgenic steroids (AAS) by 25% of AAS users [6]. The AAS abusers typically "stack" with several AAS, with 60% of those surveyed using > 1 000 mg AAS/week. A typical "complex cycle" reported consisted of high doses for a long period: 3 500 mg AAS/week together with 2 mg/day GH for 20 weeks [6]. Another web-based survey of weightlifters and body builders reports use of GH together with anabolic steroids by 5% of steroid users [7].

To date, there has been a lack of evidence that GH actually improves performance in athletes. Although beneficial effects have been demonstrated in adults with GH deficiency, in healthy young adults the balance of evidence has been against any beneficial effect of GH on strength and fitness [8–10]. Although GH has been demonstrated to induce a measurable protein anabolic effect in athletes [11], there has been no evidence from double-blind placebo-controlled studies to indicate that GH enhances muscle strength or performance in trained adult athletes [12, 13].

There are, however, adverse effects of long-term abuse of GH, broadly related to its known physiological effects on metabolism and growth. These include insulin resistance and increased risk of diabetes in susceptible individuals, fluid retention resulting in oedema, carpal tunnel syndrome or athralgia, and possibly cardiomyopathy and increased risk of malignancy [14]. The severity of these adverse effects might be worsened by concurrent abuse of anabolic steroids, which could have synergistic effects with GH, such as effects on fluid retention [15] and effects in power athletes on the myocardium [16, 17]. Because of the health risks that it poses to athletes and its potential to enhance sports performance, in addition to violating the spirit of sports, GH is listed in the 2008 Prohibited List (http://www.wada-ama.org/rtecontent/document/2008_List_En.pdf) as prohibited by the World Anti-Doping Code at all times, both in-competition and out-of-competition.

3 Challenges in developing a robust test

Developing a robust test for detecting GH abuse has been a challenge. Most current doping tests use urine, which until recently was the only body fluid available for sports doping testing. Urine is easily obtained, and in relatively large volumes compared to blood samples. However, the concentration of GH in urine is very low, at levels approximately 0.1%–1% of that found in blood. In addition, it is variable, with much of the variability not accounted for by the variations in serum GH [18, 19]. Although urinary GH concentration increases after administration of exogenous GH, increases can also occur following exercise [20]. For these reasons, urinary testing for GH is unlikely to be successful.

Detection of a naturally-occurring polypeptide, such as GH, is challenging because recombinant human 22 kDa (22K) GH available commercially and used in doping has the identical amino acid sequence to the 22K GH isoform secreted endogenously by the pituitary gland and is indistinguishable from it using current analytical methods. Differences in glycosylation patterns have been used to distinguish between exogenous recombinant hormone and endogenously secreted hormone as the basis for a test for erythropoietin [21]; however, this is not currently feasible for GH, which does not have N-linked glycosylation sites in the 1–191 sequence.

GH has a short half-life of 15–20 min in the circulation and exogenous GH administered by injection disappears rapidly from the circulation [22]. The circulating concentrations of GH also vary widely, because GH is secreted from the pituitary in a pulsatile manner and is regulated by several factors, including sleep, exercise and stress [23]. Exercise is a major stimulus to GH secretion: plasma concentrations can increase up to 10-fold, with the increases in GH dependent on the duration, intensity and nature of the exercise [10]. Therefore, because of the widely fluctuating physiological GH concentrations, in particular in response to exercise, increases in circulating GH are not specific for exogenous GH administration and direct measurement of total circulating GH cannot be used for a robust GH test.

4 Physiological basis for GH tests

The first approach to testing for GH doping is based on the physiology of GH secretion. GH is secreted by

the pituitary and circulates as a number of different isoforms [24]. GH expressed in the pituitary from the GH-N gene is subject to alternate splicing into different isoforms, post-translational modifications, proteolysis, formation of oligomers and binding to GH-binding proteins [24, 25]. The 191 amino acid 22K GH isoform is the most abundant form of GH, comprising approximately 50% of circulating GH in the monomeric form. The 20 kDa (20K) GH isoform lacks 15 amino acids (corresponding to residues 32–46 of the 1–191 sequence) and results from alternative splicing of the GH-N gene. 20K GH is the second most abundant monomer in the circulation (approximately 10%–15%) and has a longer half-life in the circulation compared to 22K GH [26]. Other 17.08 kDa and 17.84 kDa splice variants have been identified by proteomic analysis in human pituitary at low abundance (< 4% total pituitary GH) [27]. In addition to splice variants, other isoforms of GH differ in post-translational modifications, including acetylation, deamidation and phosphorylation [27]. Proteolytic fragments of GH have also been described, including 5 kDa and 17 kDa human GH [28]. The different isoforms of GH also form oligomers (dimers, trimers, tetramers, pentamers and possibly higher oligomers) in homopolymeric and heteropolymeric combinations, which comprise approximately 30% of circulating GH. Links between the components of the oligomers can be disulphide or other covalent links, or non-covalent. Furthermore, in the circulation, complexes form between 22K and 20K GH and GH binding proteins [24].

Negative feedback regulation by circulating GH and IGF-I inhibits the secretion of pituitary GH [29]; therefore, administration of GH results in reduced concentrations of other endogenous GH isoforms secreted by the pituitary [26]. Injection of exogenous recombinant 22K GH results in increased circulating concentrations of 22K GH and an increase, therefore, in its relative abundance because of the decrease in other endogenous pituitary isoforms. The change in the ratio between serum concentrations of 22K GH and other pituitary-derived isoforms of GH (Figure 1) forms the basis of one approach to testing for GH [30, 31].

The second current approach to a GH doping test is based on the physiological effects of GH that result in increased circulating concentrations of proteins that have a longer half-life and a more stable serum concentration than GH (Figure 1). GH stimulates production of insulin-like growth factor-I (IGF-I), which mediates many of the anabolic actions of GH, both by the liver, which is the main source of circulating IGF-I, and in other tissues where it has autocrine and paracrine effects. GH also stimulates the hepatic production of IGF binding protein-3 (IGFBP-3) and the acid labile subunit (ALS),

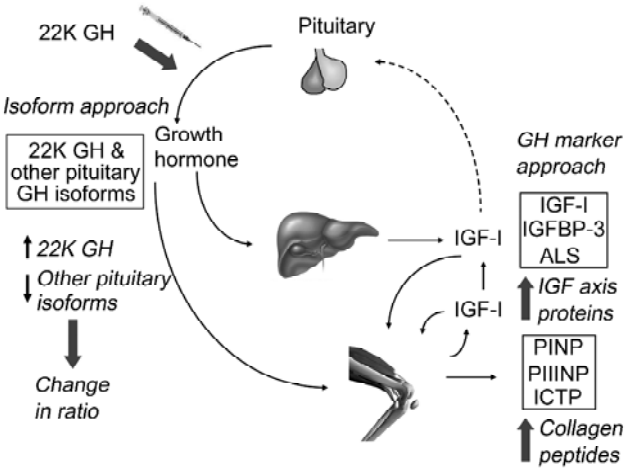

Figure 1. Physiological basis for the isoform and growth hormone (GH)-marker approaches to GH tests. GH is secreted from the pituitary and circulates as a number of different isoforms. The isoform approach to GH testing is based on the negative feedback that inhibits pituitary secretion of endogenous GH following GH administration. This results in a change in the ratio between serum concentrations of 22 kDa (22K) GH and other pituitary-derived isoforms of GH. GH stimulates the production of IGF-I together with its circulating binding partners insulin-like growth factor binding protein-3 (IGFBP-3) and the acid labile subunit (ALS), and also stimulates bone and connective tissue turnover, resulting in increased levels of specific collagen peptides (PINP, PIIINP, ICTP) related to collagen synthesis and degradation. The GH marker approach is based on increases in response to GH in the circulating concentrations of IGF axis and collagen peptides that have relatively long half-lives and stable concentrations.

which together with IGF-I form the circulating ternary complex [32, 33]. Therefore, in response to GH, the serum concentrations of these IGF axis proteins increase. GH also stimulates bone and connective tissue turnover both directly and via IGF-I, resulting in increased concentrations of specific collagen peptides related to collagen synthesis and degradation [34]. These include the marker of bone formation (N-terminal propeptide of type I procollagen [PINP]), the marker of bone resorption (C-terminal telopeptide of type I collagen [ICTP]) and the marker of connective tissue synthesis (N-terminal propeptide of type III procollagen [abbreviated as PIIINP or PIIIP, referring to measurements by different assays]) [35]. The half-lives of the IGF axis proteins and collagen markers, which range from 90 h to > 500 h [36], are considerably longer than that of GH. The increases in the serum concentrations of these GH-responsive markers form the basis of the second approach to GH testing [37].

5 GH isoform approach

Application of the GH isoform approach to doping testing has been made possible by the development of immunoassays that differentiate between the isoforms of GH, in particular between 22K GH and other GH isoforms. The Strasburger group has developed a method based on two immunoassays that distinguish between recombinant 22K GH and all endogenous GH isoforms using specific monoclonal antibodies (MAbs) [30, 38]. One of the assays uses an MAb that preferentially recognizes recombinant 22K GH (Rec-GH), and the second assay uses an MAb that is permissive and recognizes all pituitary isoforms (Pit-GH). Sandwich-type immunoassays using a microtitre-plate format have been established using these specific MAb as capture antibodies. The ratio of the measurements from the Rec-GH and Pit-GH assays (Rec : Pit ratio) indicates the relative abundance of 22K GH. Because of the different affinities of the antibodies for 22K GH and pituitary GH, the absolute concentrations are measured differently by each assay. This can result in ratios greater than 1.0, which indicate a relatively higher proportion of 22K GH, rather than a 22K GH content greater than 100%, which is theoretically impossible [30].

Following administration of recombinant 22K GH, there is an increase in the relative abundance of 22K GH compared to the other forms of GH, and the Rec : Pit ratio is increased. Good separation of the Rec : Pit ratio for GH-treated versus control samples (Rec : Pit ratio GH: 1.43 ± 0.21 *vs.* control 0.50 ± 0.12, mean ± SD) has been reported [38]. The window of opportunity for detection is relatively short, possibly up to 24–36 h after the last GH injection [30]. To meet the requirement of WADA for a confirmatory test for any immunological assay using a different antibody that recognizes a different epitope of the peptide or protein being assayed, the Strasburger group has further established another pair of Rec and Pit assays using different specific MAbs [39].

An alternate isoform-based method for detection of exogenous GH has been developed using measurement of the specific 20K GH isoform, together with measurement of 22K GH. Specific MAbs to 20K GH have been raised that do not cross-react with 22K GH and a specific sandwich ELISA established for the measurement of 20K GH in human serum [40, 41]. Co-secretion of 20K GH with 22K GH, with peaks of secretion coinciding during the day, has been demonstrated, indicating that under normal physiological conditions, the circulating concentration of 20K is in a constant proportion to 22K GH [26, 41] (Figure 2). Administration of exogenous GH, however, results in rapid reduction of 20K GH concentration, due to negative feedback regulation on pituitary secretion of 20K GH (Figure 2). Following injection of exogenous 22K GH, the increase in circulating 22K and the reduction in 20K GH result in a rapid change in the ratio of 22K to 20 K GH for up to 24 h [26]. Current studies from our group on the changes in the ratio following daily injections of 2 mg GH for 8 weeks also suggest that the window of opportunity for detection might be within 24 h of injection (unpublished results).

The ratio between 22K and 20K GH is relatively stable, with little effect of age, gender, body weight or height in the general population [41]. Using a study group of nearly 1 000 elite athletes from four major ethnic groups, our group has shown that the effect of age, gender, body mass index (BMI), ethnicity and sport type on the 22K/20K ratio is minimal [42]. The stability of the ratio to the effect of demographic factors and sport type renders it a promising measure of exogenous GH abuse. The effect of exercise on the isoform approach to detection of GH has been investigated in a study of male athletes, in which all the molecular isoforms of GH measured increased with acute exercise. The proportion of non-22K GH isoforms increased after exercise due in part to the slower disappearance rates of 20K and possibly other isoforms [43], indicating that the effect of exercise would likely be false negatives, rather than erroneous false positives. Supraphysiological doses of GH administered to male athletes suppressed exercise-stimulated endogenous GH isoforms, which also supports the use of the isoform approach [31].

A major limitation of the isoform approach is the short window of opportunity of detection of possibly 24–36 h after injection, which limits its use primarily to no-advance-notice out-of-competition testing. In addition, the isoform approach can only detect 22K GH, and does not detect administration of pituitary-derived GH, IGF-I or GH secretagogues. The differential isoform method was implemented by WADA for the 2004 (Athens) and 2006 (Turin) Olympic Games. To date, however, there have been no irregular findings from sports samples tested using this method, because of the short window of opportunity for detection, which makes detection unlikely during competition periods. More widespread implementation of the method has been limited by the availability of the assay materials. Commercial kits have now been developed and it is anticipated that the differential isoform approach will soon be implemented by many anti-doping agencies and testing laboratories.

6 GH-responsive marker approach

The GH-responsive marker approach, based on detecting increased levels of GH-responsive proteins in blood, has the advantage of a longer window of opportunity for detection than the isoform-based approach. The collaborative GH-2000 group pioneered the evaluation of

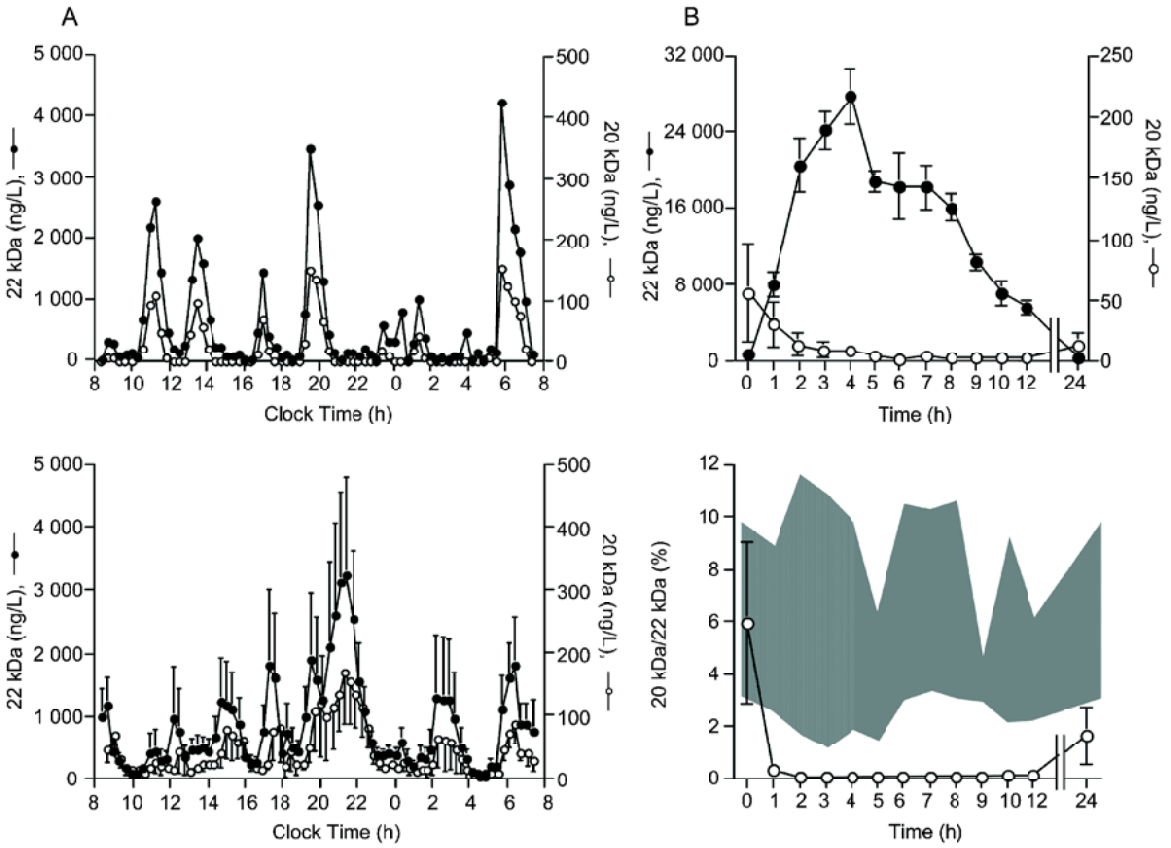

Figure 2. Secretion profiles of 20 kDa (20K) and 22 kDa (22K) growth hormone (GH) in normal subjects and following GH administration. (A): The 24-h secretion profiles of 20K and 22K GH in normal subjects are shown as representative profiles for an individual and as the plot of mean ± SE values of eight subjects. (B): Effects of exogenous 22K GH administration in normal subjects are shown as plots of the 20K and 22K GH concentrations, and as the ratio of 20K/22K plotted against the normal range (shaded area), which comprises mean ± SD values derived from the 24-h secretion profile study. The fall in the 20K/22K ratio arises from suppression of 20K GH and from the increase in circulating 22K GH in blood after exogenous 22K GH administration. Used with permission from Leung *et al.* [26].

serum IGF axis makers: IGF-I, IGFBP-1, IGFBP-2, IGFBP-3 and ALS, and serum markers of bone and connective tissue turnover: osteocalcin, bone-specific alkaline phosphatase, C-terminal propeptide of type I collagen (PICP), ICTP and PIIIP [44]. Parallel studies have been performed to determine the effect of exercise on these markers. Although acute exercise transiently increased IGF-I, IGFBP-3 and ALS, the increases were much smaller than those in response to GH administration alone [45]. The same was true for osteocalcin, PICP, ICTP and PIIIP, the responses of which were greater and more prolonged following GH than after acute exercise [46].

The effect of administration of GH for 4 weeks on these GH-responsive markers was examined in a randomized double-blind placebo-controlled study in 99 young athletically trained men and women, using two doses of GH: 0.033 mg/kg/day and 0.067 mg/kg/day. The IGF axis proteins IGF-I, IGFBP-3 and ALS all increased in response to GH, with the greatest response in IGF-I. Men were significantly more responsive than women. All IGF proteins had returned to baseline within a few days of cessation of treatment, except for IGF-I, which was elevated for longer in men [47]. All the markers of bone and connective tissue turnover increased in response to GH, with ICTP and PIIIP exhibiting the greatest responses, and peak increments being greater in men than in women. Osteocalcin, ICTP and PIIIP remained significantly elevated for up to 8 weeks after cessation of treatment, which clearly indicates the potential for a longer window of opportunity for detection using these markers [48]. Other placebo-controlled administration studies have also shown the potential for IGF axis and collagen peptides as markers of GH administration [49,

50]. A recent evaluation of IGFBP-4 and IGFBP-5 indicates that they will not be useful as IGF-I independent markers [51]. In a double-blind placebo-controlled study of GH administration to recreational athletes, our group recently showed that the response to GH is greater in men than in women for IGF-I, IGFBP-3, ALS and collagen markers, with the peak response being greater for IGF-I and for PIIINP. The collagen markers remained elevated for longer than the IGF axis markers, indicating the potential for an extended window of detection using the collagen peptides ICTP and PIIINP [52].

The application of this method based on GH-responsive markers requires extensive normative data in elite athletes to identify the factors influencing their levels in blood and to establish normal reference ranges. In a large cross-sectional study of IGF-I, IGFBP-3, ALS, PINP, ICTP and PIIINP in over 1 000 elite athletes from 12 countries representing four major ethnic groups, we reported that age and gender are the major determinants of variability for IGF-I and the collagen peptides, whereas ethnicity accounts for less than 6% of the attributable variation, except for IGFBP-3 and ALS [53]. There is a significant negative correlation between age and all these GH-responsive markers, similar to the correlation seen in the general population [54, 55] and age is the major contributor to variability, especially for the collagen peptides (Figure 3). There are significant differences between men and women; however, the contribution of

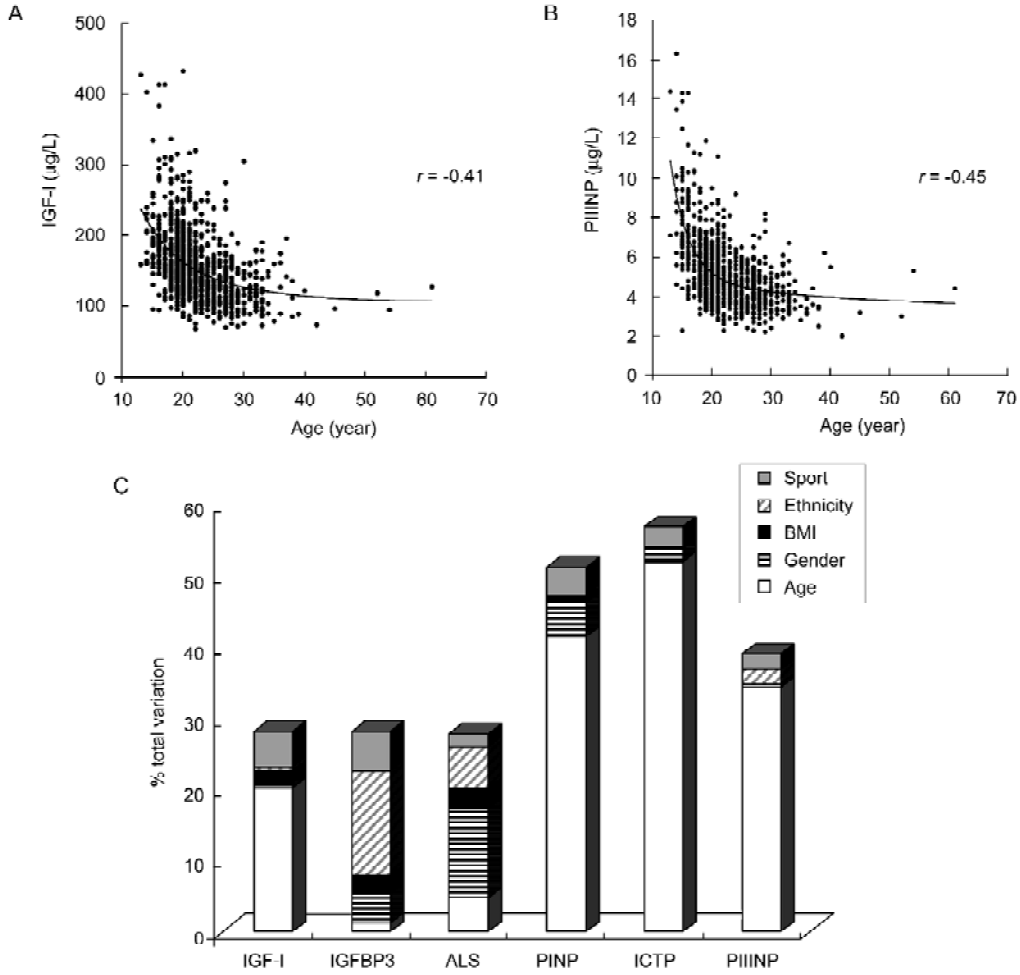

Figure 3. Factors influencing growth hormone (GH)-responsive markers in elite athletes. The relationship between age and IGF-I and PIIINP. The measurement for each individual is plotted against age for IGF-I (A) and N-terminal propeptide of type III procollagenPIIINP (B). The lines indicate the function that best fits the relationship between the reciprocal of age and the marker. (C): Multiple regression analysis. The contribution of age, gender, body mass index (BMI), ethnicity and sporting type to the total variation is shown for each marker, for the analysis of 995 athletes from seven sporting groups. Adapted with permission from Nelson *et al.* [53], copyright 2006, The Endocrine Society.

gender is smaller than that of age, except for IGFBP-3 and ALS. The contributions of BMI and sport type are both modest compared with those of age and gender (Figure 3). Therefore, our study of elite athletes in the out-of-competition setting indicates that a test based on IGF-I and the collagen markers must take age into account for men and women, and that ethnicity is unlikely to be a confounder for IGF-I and the collagen markers [53]. Our findings on the influence of age, gender, BMI and sport type have also been confirmed in a study of mostly Caucasian elite athletes in the post-competition setting [56], which also concludes that sport category is not a significant predictor compared to age and gender.

The successful application of the markers approach also requires data on the within-subject variability of the IGF axis and collagen peptides over time. Examination of short-term variability in our cohort of over 1 000 elite athletes shows that the within-subject variability is less for the collagen markers and for IGFBP-3 and ALS, than for IGF-I (Nguyen TV *et al.*, unpublished data). Statistical modelling, such as the Bayesian approach, and use of multiple measurements might, therefore, assist in the application of the marker approach to doping tests. Further data on longer-term within-subject variability, which has been addressed by the GH-2000 group [44], is also required. The effect of injury on the collagen peptides in athletes also warrants investigation. Distinct changes in serum biochemical bone markers, both in the early stages after fracture and up to several weeks later, have been described following lower limb fractures, as a result of bone remodeling and collagen III synthesis in fracture healing [57, 58]. Preliminary studies in subjects from sport injury clinics have also been described by the GH-2000 group [44], and larger studies are underway.

A robust test for GH must also take into account the possible confounding effects of multiple performance-enhancing substances that are used by athletes that practice polypharmacy. We investigated the effect of administration of recombinant human erythropoietin (r-HuEPO) on GH-responsive markers in young male recreational athletes and found no significant treatment effect compared to baseline on IGF-I, IGFBP-3, ALS, PINP, ICTP or PIIINP [59]. Therefore, use of r-HuEPO by athletes should not affect the validity of a test using these IGF axis and collagen markers. We have also recently investigated the effect of testosterone on GH-responsive markers in a double blind placebo-controlled study of recreational athletes. Testosterone alone did not affect IGF-I, IGFBP-3 or ALS and only modestly increased PINP, ICTP and PIIINP. Combined administration of testosterone with GH did not affect the sensitivity of the markers to GH alone, except for PIIINP, where combined treatment significantly increased the peak response to GH [52].

A GH doping test based on the GH-responsive markers should not rely on a single marker, but use a combination of markers. The different pharmacodynamic profiles of the IGF axis and collagen markers have been indicated particularly by studies with extended washout periods, showing the prolonged elevation of collagen markers after cessation of GH administration [47–49, 52]. This indicates the benefits of using a combination of several markers to detect GH doping both during active administration and during washout. Combinations of IGF-I, IGFBP-3, PIIINP and ICTP have been proposed [37, 49, 50]. In our cross-sectional study, no individuals had extreme values (outside the 99% reference interval) both for IGF-I and for the collagen markers in the same sample, which confirms that the use of IGF-I and a collagen marker will increase the specificity of the test [53]. Algorithms based on IGF-I and PIIINP show promise in discriminating GH-treated from placebo-treated subjects, with low false positive rates in particular when sex-specific algorithms including age are used to account for the effects of age and gender on these markers [37, 49, 60]. Our recent GH administration study highlights the potential of IGF-I, PIIINP and ICTP in combination as promising discriminators of GH administration against our reference population of elite athletes, both during treatment and for up to several weeks following treatment, because of the longer time course of the collagen marker responses (Nelson AE *et al.*, unpublished results).

In summary, implementation of the GH-responsive marker approach will clearly extend the window of opportunity for the detection of GH. This method also has the potential to detect abuse of other agents, such as cadaveric pituitary GH, which is reportedly still a source of illicit GH, although no longer used clinically because of the risk of transmission of Creutzfeldt-Jakob disease, recombinant placental GH, GH secretagogues and IGF-I. Extensive data is now available that validates the use of the GH-marker approach, although some additional data is still required. The main hurdles to be overcome in the implementation of the markers approach as a doping test are technical and logistic issues, in particular those relating to ensuring availability to testing laboratories of standardized assays with assured supplies of antibodies.

7 Novel approaches to the detection of GH

Novel approaches are being investigated to identify new markers or profiles of gene expression or proteins that are diagnostic of GH use. One line of investigation is the study of gene expression in peripheral blood leucocytes, because leucocytes are the only source of genetic material readily available from the current testing substrates. There is strong evidence that GH regulates various aspects of the immune system and that leucocytes

also respond directly both to GH and to IGF-I [61]; therefore, GH administration can be expected to result in gene expression changes in leucocytes, potentially with a long time course because of secondary effects such as those via IGF-I. A pilot study has examined changes in expression of selected genes in human haematopoietic cells treated with GH using real time polymerase chain reaction [62]. We are currently investigating gene expression in peripheral blood leucocytes from subjects following treatment with GH *in vivo*, to determine a diagnostic gene expression "fingerprint". We are using Affymetrix microarray gene profiling to examine genome-wide gene expression of RNA extracted from leucocytes [63]. The methodology established might also be useful for detecting abnormal gene expression in response to gene doping.

Proteomic methods are also being applied to serum to investigate novel protein markers or diagnostic profiles. Protein expression profiles have been studied in serum using surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS), in which proteins are bound to proprietary protein chips with different adsorptive surfaces, then mass to charge ratios determined following ionization of the bound proteins. SELDI-TOF analysis has indicated differences in the serum protein profiles from subjects treated with GH compared to placebo and has identified haemoglobin α-chain as a single biomarker classifier [64], thus demonstrating the potential for this method for GH doping detection.

8 Conclusions and future directions

Robust tests for GH detection are required to enforce bans on GH and to deter its use in sport. Two current approaches to testing for GH based on measurement of GH isoforms and on GH-responsive markers have been extensively developed. These tests will likely be used in a complementary manner, due to their different windows of opportunity for detection characteristics. WADA has implemented one method based on differential GH isoforms to a limited extent and with the development of commercial assays, the test should soon be more widely implemented. The GH-marker approach will extend the window of opportunity for detection and can detect other forms of GH and GH secretagogues. Its implementation is dependent largely on establishing the availability of standardized assays with assured supplies of antibodies to testing laboratories.

Future directions for research might include the use of other platforms for the measurement of the GH isoforms and GH-markers used in the current approaches. New immunoassay platforms, including multiplexed particle-based flow cytometric assays, such as the Luminex system [65] represent technical advances that will enhance efficiency and sensitivity. Mass spectrometry methods may also be applied to quantification of GH isoforms and of GH-responsive markers. Recently, quantification of IGF-I and IGFBP-3 in human serum by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using synthetic stable-isotope labeled peptides as internal standards [66] and a mass-spectrometry-based assay for rat PINP [67] have been described.

The use of an "athlete's passport" that documents measurements of biological parameters over time has been proposed both in the wider context of monitoring the health of the athlete and to assist in the detection of banned substances, as recently described for erythropoietin [68]. It is proposed that to create the "passport", the athlete would take a baseline test that would provide reference levels for the individual. The "passport" would enable detection of abnormal levels that differ to the baseline for that athlete, rather than comparing the levels to normative ranges alone. This has the potential to increase the sensitivity of methods, particularly those based on biomakers such as the GH-responsive marker approach.

In conclusion, robust tests should soon be in place to detect GH and to enforce the ban on its abuse. Commercial assays are now available that will enable wide implementation of the isoform-based approach. The GH-responsive marker approach will extend the window of opportunity for detection of GH, and the technical hurdles to its implementation are currently being addressed by the anti-doping authorities. In addition to further development of these two approaches, novel approaches must continue to be pursued in order to expand the repertoire of testing approaches available and to maintain deterrence of GH doping.

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