R E V I E W

Growth hormone doping: a review

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Abstract: The use of growth hormone (GH) as a performance enhancing substance was first promoted in lay publications, long before scientists fully acknowledged its benefits. It is thought athletes currently use GH to enhance their athletic performance and to accelerate the healing of sporting injuries. Over recent years, a number of high profile athletes have admitted to using GH. To date, there is only limited and weak evidence for its beneficial effects on performance. Nevertheless the "hype" around its effectiveness and the lack of a foolproof detection methodology that will detect its abuse longer than 24 hours after the last injection has encouraged its widespread use. This article reviews the current evidence of the ergogenic effects of GH along with the risks associated with its use. The review also examines methodologies, both currently available and in development for detecting its abuse.

Keywords: performance enhancing substance, GH, doping in sport, detection methods

Introduction

Growth hormone (GH) is a naturally occurring peptide hormone produced by the anterior pituitary gland. It plays a major role in maintaining body composition, well-being, physical performance, and cardiovascular health in adults as well as children. These properties have led to its abuse by professional sportsmen and women wishing to improve their athletic performance. Despite its apparent widespread use, until recently there was little scientific evidence to support its use as a performance enhancing agent. Recent studies, however, have suggested that GH increases both strength and sprint capacity.

This article reviews the current evidence of the ergogenic effects of GH along with the risks associated with its use. The review also examines methodologies, both currently available and in development for detecting its abuse.

PubMed and Google Scholar were searched for relevant use of the terms "growth hormone" and "abuse". Where further references were found within these articles, they were downloaded. This review also draws from the clinical and research experience of the three authors who have been working together within the GH-2004 project to develop a test for GH abuse for the last 9 years. Prior to that PHS was a member of the International Olympic Committee Medical Commission and led the GH-2000 project, the aim of which was to develop a test for GH abuse in time for the Sydney Olympic Games.

The evolution of growth hormone into a performance enhancing agent

The end of the 19th century saw the birth of organotherapy following a series of groundbreaking and controversial experiments undertaken by Charles Édouard

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Brown-Sequard, a renowned physiologist and neurologist. Brown-Sequard's unique experiments led the way for significant advancements in the field of endocrinology. His findings were presented for the first time to the Society of Biology in Paris in 1891, reporting significant restoration of his own strength following a 3-week program of self-injection of "first blood of the testicular veins; secondly semen; and thirdly juice from a testicle ... from a dog or a guinea pig".[1] Although many now believe that his findings may have been the result of a placebo effect, it is well accepted that the idea of hormone replacement and organotherapy was conceived as a result.

Brown-Sequard was the first to propose that "internal secretions ought to exist in animal tissues and that disease probably resulted from their lack". Brown-Sequard's research saw the beginning of a series of experiments throughout the Western world, investigating this method for rejuvenation as well as a treatment for various diseases.

It is unsurprising that this research was identified as an area that should be investigated as a means of improving athletic performance, and in 1894 Oskar Zoth, and Fritz Pregl were the first to consider this by assessing the effect of testicular extracts on muscular strength. With hindsight it is unlikely these testicular extracts contributed positively to the performance of athletes; however, Zoth and Pregl, while investigating the effect on athletic performance, provided the grounds where such research could be furthered.

Shortly after, in 1909, Harvey Cushing identified the etiology of acromegaly for the first time through a partial hypophysectomy, which also enabled him to suggest the potential treatment. This was the first time the role of pituitary hormones was considered and opened the way to our understanding of the role of GH on normal physiology.

GH was first extracted and purified from the human pituitary glands in 1945.[2] GH administration was shown to promote growth in hypopituitary animals and children with hypopituitarism who subsequently demonstrated a dramatic improvement in growth.[3] In adults, the beneficial effects of GH administration were first observed in 1962, when GH was noted to increase vigour, ambition and sense of well-being in a woman with adult hypopituitarism.[4]

Cadaveric growth hormone was the only source of the hormone until 1987 when the first recombinant version (methionyl human GH) became available. Pituitary-derived GH was subsequently found to be a source for the prion-induced Creutzfelt–Jacob disease and was withdrawn from the marketplace in 1985;[5] however, supplies of pituitary-derived GH continue to be available on the black market to this day.

It is not known exactly when GH was first used as a performance enhancing substance but the first published evidence we have is in *The Underground Steroid Handbook* by Daniel Duchaine, which appeared with a limited circulation in California in early 1982.[6] The cyclostyled newsletter encouraged athletes to use GH to enhance their performance. Specifically, Duchaine stated in his newsletter: "Wow, is this great stuff! It is the best for permanent muscle gains ... People who use it can expect to gain 30 to 40 pounds of muscle in 10 weeks".[6] Duchaine was known in the bodybuilding community as the "steroid guru" and was responsible for the development of many doping trends. Through experimentation on himself and other bodybuilders he was coaching, he advised athletes on the best substances and combinations for optimal performance.

Duchaine later retracted this statement, as he could not find any scientific studies that supported his claims. In his later book published in 1993 "Ultimate muscle mass" he stated that "I'd guess that almost 90% of all athletes taking STH [growth hormone] got no anabolic results from it (this includes at least two Mr Olympia competitors)".[6,7] However, the damage had already been done by that time as the use of GH was proliferating among the doping world. History shows that his earlier comments were the more accurate as we now know that GH is a potent anabolic agent particularly when combined with testosterone.[8]

Evidence of GH abuse in sport

There are no official incidence figures to show how common GH usage is amongst athletes or how popular it is in sporting circles owing to its illicit nature. One of the most well-known recorded examples of GH misuse involved Ben Johnson who admitted taking GH to boost his performance. Following Ben Johnson's admission, Justice Charles Dubin conducted one of the most searching investigations into the abuse of drugs in sports. During these hearings, the Canadian sprinter Angella Issanjenko also admitted using GH along with other drugs. According to the 900-page inquiry report,[9] it was concluded that the tight regulations limiting GH use had not prevented it from becoming widely available to athletes. Dubin also added in his conclusion that it appeared there was a growing trend of GH use as a performance enhancing agent.

In 1989 the International Olympic Committee (IOC) included GH in its prohibited substance list, as part of a new doping class of "peptide hormones and analogs" despite the lack of a legitimate test for human (h)GH.[10] However, this did not seem to deter GH misuse as a performance-enhancing substance. For many athletes it became the new drug of choice,[11] with some athletes calling the 1996 Olympics in Atlanta, USA, the "hGH games".[12]

Over the years, many accusations have been made against athletes claiming that they have been abusing GH. However, without a reliable detection method, the World Anti-Doping Agency (WADA) and the IOC have been unable to prove this. National Football League players, such as Bill Romanowski, and baseball players, Barry Bonds, Gary Sheffield, and Jason Giambi, are amongst some of the sportsmen accused of taking GH in the controversial book *Game of Shadows*, which was written following the undercover investigations of two San Francisco reporters.[13]

One of the most infamous pieces of evidence of GH abuse came after the raid on the Bay Area Laboratory Co-Operative's (BALCO) headquarters on September 3, 2003. Victor Conte, the owner of BALCO, claimed that he had supplied GH to many high-profile American athletes including Tim Montgomery and Marion Jones. Marion Jones, five-time Olympic medal winner, admitted in 2007 to using performance enhancing drugs including GH. She was later sentenced to a 6-month jail sentence for falsely denying administering performance-enhancing substances. Tim Montgomery allegedly admitted to taking GH before a US Federal grand jury and later faced a 2-year ban for doping offences. Conte was imprisoned for 4 months for his role in the scandal.[13]

GH is readily available to purchase over the internet, a factor that has led to its use becoming more widespread. As part of an investigation for the British Broadcasting Corporation, laboratory analysis of a batch of GH obtained over the internet by four-time Olympic gold medalist, Matthew Pinsent, confirmed that the purported GH was indeed of very high purity.[14]

Although Pfizer Pharmaceuticals was fined US$ 35 million for promoting hGH as an anti-aging and performance enhancing agent in 2007,[15] their advertisement is likely to have encouraged its abuse in the sporting arena. It is now illegal to prescribe, possess, or use GH for any unlicensed purpose in the US.

Physiological influences on the GH/insulin growth factor axis

To understand why athletes believe GH is a potent ergogenic aid, it is useful to review the physiology of GH. GH is secreted from the anterior pituitary gland under the regulation of the hypothalamic hormones, somatostatin, growth hormone releasing hormone (GHRH), and ghrelin. It is secreted in pulses and is rapidly cleared leading to highly variable circulating concentrations.

Gender

Over a 24-hour period, young women secrete 50% more GH than young men. In addition, in premenopausal women, GH production varies according to the menstrual cycle, approximately doubling in the late follicular phase compared with the early follicular and mid-luteal phases. This variation is thought to be related to changes in gonadal steroid production as GH pulse amplitude is positively correlated with serum estradiol and negatively correlated with serum progesterone.[16] After a glucose load, plasma GH levels are normally suppressed to greater extent in young men and young women in the early follicular phase of their menstrual cycle.

Nutrition

Body composition has a significant effect on GH production. Obese individuals have reduced mean GH concentrations, associated with decreased pulsatile GH secretion and a shorter GH half-life. The peak response of GH secretion to GHRH is reduced in obese subjects and this is reversible with fasting or weight loss. A possible explanation for this is related to obesity induced hyperinsulinemia, which may inhibit GH secretion, through enhanced negative-feedback inhibition by insulin growth factor-I (IGF-I).[17] The concentration of ghrelin, which acts to increase GH secretion, is reduced in obese people, but it is unclear whether this mediates the reduced GH secretion observed in visceral obesity.[18]

People with anorexia nervosa, by contrast, demonstrate increased basal and GHRH-stimulated GH release and elevated ghrelin levels. People with anorexia nervosa have low circulating IGF-I and IGFBP-3 concentration as a result of low hepatic insulin activity and can be viewed as developing a state of functional GH resistance. In healthy individuals, fasting increases GH production through an increase in GH pulse frequency and amplitude whereas re-feeding rapidly has the opposite effect suppressing fasting-enhanced GH secretion.

Age

GH is secreted at a low level in early childhood. Secretion increases gradually during childhood but increases three-fold during puberty, reaching a peak during late puberty. After early adulthood, GH production starts to decline at a rate of approximately 14% per decade of adult life.[19]

It is thought that the decline in GH secretion that occurs with increasing age is associated with a reduction in GH storage.[20] In parallel with the age related fall in GH secretion, serum IGF-I and insulin-like growth factor-binding protein-3 (IGFBP-3) concentration also decline,[21] though the concentration of the latter falls to a lesser degree than IGF-I and GH secretion.[22] In elderly individuals, the correlation between GH secretion and IGF-I and IGFBP-3 is less marked, suggesting that GH becomes less important in determining circulating IGF-I concentration with advancing age and instead other factors such as nutritional state, frailty, and chronic disease may play a greater role.

Exercise

Physical exercise plays an important role in the regulation of the GH-IGF-I axis[20] increasing GH secretion. The GH response to exercise is dependent on the duration and intensity of the exercise, the fitness of the exercising subject, and other environmental factors such as the ambient temperature during exercise[23] and body composition.[24]

The effect of exercise is greater in young women than postmenopausal women and men of any age. Furthermore, the same study showed that age diminishes the GH response to exercise and abolishes the young-adult gender difference.[25] The duration of exercise needed to induce GH secretion after physical exercise is inversely related to the exercise intensity.[26]

Anaerobic exercise is more stimulatory to GH release than continuous aerobic exercise, even if duration and workload are kept the same for the two types of exercise.[27] Although exercise is a powerful stimulus to GH release, the GH response to exercise is blunted in older and obese individuals.[24] This suggests that higher exercise intensities may be necessary to stimulate GH release in obese subjects to levels achieved in normal weight adults.

Benefits of GH in athletic performance

Many athletes believe that GH will improve their athletic performance; however, to date there is only a small amount of published evidence to support such claims in amateur young adult recreational athletes.[8,28] No published evidence exists in elite athletes. The majority of evidence showing the effect of GH administration comes from studies of GH replacement in GH-deficient adults.

The use of randomized clinical trials is currently accepted as the gold standard methodology for assessing the efficacy of medical products. It is difficult, however, to undertake such GH administration studies to demonstrate reliably whether GH increases muscle bulk and strength in the athletic population for ethical and legal reasons. Furthermore, it is probably the case that athletes use a 'trial of one' paradigm to learn the benefits of GH, as they know their performance precisely and by trying different diets, training regimens, and/or drugs they can deduce which ones improve performance by a level that they know exceeds their 'within-subject' variability.

Athletes abuse GH in combination with anabolic steroids and other performance enhancing substances, in varying concentrations during differing training and dietary regimens. Any change in performance could result from the synergistic action of these different interventions. These complex regimens are impossible to replicate in a single research trial, and therefore it seems likely that athletes are the ones who are best placed to address the question whether GH is performance enhancing. This approach was certainly used by the former East German coaches when athletes experimented with anabolic steroids.[29]

Effect of growth hormone administration
Body composition

Growth hormone has a key role in regulating body composition. An important property of GH is its potent lipolytic effect.[30,31] GH administration in people with GH deficiency reduces body fat mass and increases lean body mass significantly.[32] In a recent meta-analysis study it was concluded that GH administration in healthy athletes increase lean body mass by an average of 1.8 kg.[33]

GH increases lipolysis directly by reducing the action of a number of lipogenic enzymes[31] and indirectly by increasing the production of other lipolytic hormones, such as catecholamines and glucagon, and by increasing adipocyte adrenergic receptors expression. Overall these effects lead to increased body fat utilization.[34]

Muscle protein synthesis and strength

One of the best appreciated effects of GH is its effect on muscle anabolism. Reduced muscle mass and protein synthesis is seen when GH and IGF-I production are reduced.[35–39] GH induces glucose and amino acid uptake, and stimulates protein synthesis, possibly using the energy derived from its lipolytic activity.[31] By contrast GH has little effect on protein degradation. Instead insulin and to a lesser extent IGF-I inhibit protein degradation and this may explain why some athletes abuse insulin and GH together. The effects of GH on protein are mediated both indirectly via IGF-I and directly.[30]

The anabolic effect on protein and catabolic effect on fat leads to increased muscle mass at the expense of fat mass. Although these observations may lead athletes to believe that supraphysiological levels of GH will improve sporting performance, the contrary is suggested by acromegaly (a condition associated with GH hypersecretion) which is associated by muscle weakness, diabetes, and cardiomyopathy; however, acromegaly frequently remains undiagnosed for many years following its onset. During the early prediagnosis years, anecdotal evidence suggests that patients may experience increased strength and physical performance.[40]

Bone and connective tissue

GH plays an important role as an anabolic agent in connective tissue in human skeletal muscle and tendon, which provide a matrix to transmit force from individual muscle fibres to the bone.[41,42] Therefore, a strengthened connective tissue would give a stronger and more strain-resistant muscle and tendon unit. It is for this reason that GH has been used by athletes to treat muscle and tendon injuries. Similarly GH administration improves bone strength both through a direct effect and indirectly through increased intestinal calcium absorption and serum vitamin D concentrations.[43,44] It is important, however, to consider that in normal physiology, GH is secreted in pulses and this natural pulsatile and variable GH concentration cannot be replicated by a single GH dose, which will result in a single peak and will subsequently decline.

Previous work undertaken by Goldspink et al[45] showed in rabbit experiments that the IGF-I mRNA increased in the stretched or stimulated muscles but not in resting control muscles. Following cloning and sequencing of this RNA, it was concluded that this was derived from the insulin-like growth factor gene by alternative splicing. This is particularly relevant for understanding the etiology and the development of possible treatment in muscle wasting conditions such as sarcopenia.[46]

GH administration studies – what have we learnt?
GH administration in GH deficient patients

GH administration in both adults and children with GH deficiency increases muscle mass[47–49] but findings have been mixed regarding the effect of GH on muscle strength. A large number of GH administration studies have failed to show any significant improvements on muscle strength.[8,50–54] In these studies, GH was administered using a wide range of doses (10 µg/kg/day–40 µg/kg/day) and duration (4 hours–12 weeks). Consequently there is a significant debate about the relationship between muscle mass increase and increased muscle strength. It is possible that a longer treatment period of more than 6 months may be necessary to show performance benefits, similar to those used in trials of people with GH deficiency. GH administration significantly improves functional capacity and quality of life. The results of these studies have been discussed in recent reviews.[55,56]

GH administration in young athletes

Two recent studies have demonstrated a positive performance effect following GH administration to young male amateur athletes.[8,28]

In a study from Australia, the impact of GH administration was assessed on three performance metrics. These included a measure of anaerobic performance using the Wingate test, maximal strength using the isometric dead-lift test, and maximal explosive power using a single vertical jump height. The treatment period lasted 6 weeks and although no evidence was found to suggest an improved anaerobic capacity in women, a significant improvement was reported in men receiving GH either alone or in combination with testosterone. No significant effect was observed in levels of maximal strength or maximal explosive power in either gender or any treatment group.

A further study involving past abusers of anabolic steroids showed improved strength following GH administration.[28] The results of both studies should be treated with some caution as not all measures of performance were altered. Further studies are needed, particularly in women.

GH administration in the elderly population

The first study to demonstrate a performance effect in older men was a 6-month randomized GH administration trial.[57] The study found that while coadministration of GH with testosterone produced the most significant changes on the investigated variables, the administration of GH or testosterone alone produced significant results when compared to the placebo treatment. The second study involved a shorter GH administration period of 16 weeks and also examined the effects of its administration on performance.[58] This study concluded that GH administration resulted in significant gains in total and appendicular lean mass, muscle strength, and aerobic endurance which were accompanied by significant reductions in whole-body and trunk fat. The effects were enhanced with concomitant testosterone administration.

In addition, a further study has suggested that there is significant evidence to suggest that resistance training in combination with GH administration will lead to a significant increase in IGF-I gene expression and muscle strength.

Despite these findings, it may not be possible to extrapolate them to younger adults because of the marked differences in GH secretion between young and old.

Health risks related to GH abuse

The potential risks of GH use by athletes are not well appreciated because relevant epidemiological data are lacking. Both hGH and IGF-I are potent mitogenic and anti-apoptotic agents, and several reports have shown an association between IGF-I concentration and the incidence of breast, prostate, and colorectal cancers[59–61] but evidence that GH is carcinogenic is lacking.

In a critical evaluation of the safety of recombinant human GH, the GH and IGF Research Society concluded in a consensus statement that for the current approved indications, GH administration is safe. The society also concluded that there is currently insufficient evidence regarding the longer term safety of GH and it recommended that epidemiological studies were set up to monitor this closely.[62]

Acromegaly can cause insulin resistance, glucose intolerance, peripheral edema, and arthralgias and these may be experienced by athletes abusing GH in large quantities. Furthermore, GH abuse and concomitant use of anabolic steroids were identified as possible etiological factors in the development of bilateral internal laryngocoeles in an elite bodybuilder.[63]

A comprehensive review of adverse effects reported with supraphysiological GH levels is available in Buzzini 2007 and a summary of this has been provided in Table 1.[64] In addition to the health risks directly associated with the use of excess GH, there are additional risks associated with contamination if the GH is often sourced illegally. Although the availability of recombinant human growth hormone is expected to dominate the black market, some GH is extracted from cadavers and may be contaminated with the prion that causes Creutzfeldt–Jakob disease.

Challenges in developing a robust GH detection methodology

A further attraction to the use of GH by athletes is the lack of a reliable method to detect GH use more than 12 hours following its administration. Even though many efforts have been made to develop analytical methodology for distinguishing between endogenous GH and exogenous rhGH, it has proven

Table 1 Summary of adverse effects found to be associated with excess levels of growth hormone in patients suffering with acromegaly

Area of concern	Main symptoms
Cardiovascular	Reduced cardiac function
Pulmonary	Sleep apnea and respiratory failure
Musculoskeletal	Gigantism and increasing muscle weakness
Endocrine and metabolic	Increasing insulin resistance and the onset of diabetes mellitus
Neurological	Frequent headaches and instances of idiopathic intracranial hypertension
Malignancy	Possible increased risk of leukemia and solid tumors such as breast, colon, prostate, and endometrial cancer
Cosmetic	Coarsened facial appearance, abnormal enlargement of the extremities of the skeleton
Visceromegaly	Exhibited in organs such as tongue, thyroid gland, salivary glands, liver, spleen, kidney, and prostate

difficult to develop a reliable method. Detecting GH abuse by measurement of GH directly is not practical because of its pulsatile release and sensitivity to a number of external influences, including exercise and stress.

A single baseline or random measurement of circulating GH concentration has little diagnostic significance, either clinically or in the context of GH doping.[65] This was demonstrated using blood samples collected from 500 elite athletes in the period leading up to the Sydney Olympics, as part of the campaign *Io non rischio la salute* (I'm not risking my health) conducted by the Italian Olympic Committee (CONI) in 1999. There were a large number of blood samples, including one from a gold medalist, who had markedly high GH values. Following assessment of these findings using the serum concentrations of biochemical markers of the GH function, it was concluded that there was no evidence to suggest GH abuse.[66]

Developing a detection method for GH abuse using urinary GH measurements has also proven challenging because GH urinary excretion does not accurately reflect the blood concentration and can rise following exercise alongside the increased excretion of albumin and other proteins. Consequently, high urinary GH concentrations would not necessarily be indicative of GH doping. Nevertheless a significant amount of research has been invested in this area. This is because blood testing is relatively new in sports as this type of testing poses both potential logistical and legal issues.[67] To date, no official court has formally determined whether mandatory blood testing in sport for performance enhancing substances violates any internationally or nationally recognized individual rights to privacy

or bodily integrity. In terms of logistical challenges, blood collection is more difficult than urine specimen collection as blood samples call for additional handling to ensure they arrive at the anti-doping laboratory in a condition that allows for analysis to minimize risk. However, the use of blood over urine in anti-doping testing has the advantage that specimen manipulation is nearly impossible.[68]

Nevertheless, the development of a reliable urine detection test has proved an impossible task to date. Leger in 1995 concluded that GH excretion varies too widely, as assessed in normally growing and growth hormone-deficient children, to be used as a clinical GH quantification method.[69] Attempts to develop a GH detection test using GH measurements in urine by Saugy et al[70] have been unsuccessful. As well as the variability, the much lower GH concentration in urine compared with blood make measurement difficult. Circulating GH is cleared from the bloodstream through degradation, predominantly in the liver and kidney. In the kidney, GH is cleared principally by glomerular filtration, uptake and degradation in the proximal tubule, and minute quantities of intact GH, only 0.001%–0.010% of pituitary GH secretion, appear in the urine.

Two different but complementary approaches have been developed to detect GH abuse; both methodologies have merits and drawbacks depending on the testing scenario. The first is based on the detection of different pituitary GH isoforms and can detect athletes who have administered rhGH within 12–24 hours of the test; this methodology is therefore most suitable for unannounced out of competition testing. The second method relies on measurement of GH-dependent markers and has a longer window of opportunity of up to 2 weeks since last GH administration. This methodology would be suitable for both out and in competition testing scenarios.

The GH isoform method

This method is currently the only method approved by WADA and the IOC and was first implemented at the Athens Olympic Games in 2004.[71] This method involves the analysis of GH isoforms and was originally referred to as the "differential isoform assay method".

The pituitary gland secretes a number of GH isoforms; the most abundant is the 22 kDa isoform but there are other minor products of gene transcription and post-transcription modification. The most prominent of these is a 20 kDa isoform that normally circulates at concentrations averaging around 10% of the 22 kDa isoform. By contrast, exogenous rhGH contains only the 22 kDa isoform. When exogenous rhGH is administered, there is a marked decrease in the endogenous pituitary GH production because of negative feedback and a fall in non-22 kDa isoforms.[72]

The presence of different isoforms provides the basis for the detection method developed by Wu, Bidlingmaier, and Strasburger. This methodology distinguishes between circulating endogenous GH secretion and exogenous recombinant human GH (but not pituitary-derived GH) administration by measuring the ratio between the concentration of different isoforms.

The isoform test has been validated in a range of settings and findings have shown that the ratio of 22 k to total GH is stable across several populations regardless of their age, gender, body weight, or height.[73] It is not yet confirmed whether the ratio is stable across all ethnic groups although preliminary findings suggest little impact from ethnicity.[74] Acute bouts of exercise reduce the sensitivity of this test slightly but have no adverse impact on false positive rates.[75,76]

The major disadvantage of this method is that GH isoforms have short half-lives and spontaneous GH secretion returns to baseline 12–24 hours after the last dose of GH. This means that the window of opportunity for detection is short, between 12 and 24 hours.

The isoform method cannot detect pituitary-derived cadaveric GH doping (pituitary-derived GH from both animals and humans is still commercially available) or the abuse of GH secretagogues or IGF-I because the GH isoform ratio is unchanged in each of these situations. Other detection methodologies such as the GH biomarker approach would be required to identify doping in this scenario.

Catching the first GH cheat using the isoform test

Following its implementation in 2004, the first adverse analytical findings of the isoform test was in November 2009 when Terry Newton, a British rugby league player, tested positive.[77] Newton subsequently admitted to injecting GH in an attempt to recover from injuries and alleged that he knew a number of other rugby league players who were also using GH. Subsequently there have been further adverse analytical findings.

The low detection rate is likely to reflect its limited window of opportunity as most of the tests have been immediately postcompetition.

The GH biomarker approach

The second GH detection methodology currently being considered for implementation by WADA is the "GH marker" approach or "GH biomarker" approach. This method involves

the measurement of circulating concentrations of two GH-sensitive proteins, IGF-I, and Procollagen III N-Terminal Propeptide (PIIINP), which are known to increase in a dose-dependent manner following GH administration.[78–80]

The GH marker test utilizes gender specific mathematical functions which include the logged values of serum IGF-I and PIIINP to produce a detection GH-2000 score, named after the project that first established the method.[81,82] A positive test would be declared if the values exceeded those found in a normal healthy population of elite athletes.[83–85] The score was originally calibrated against a large sample of 813 elite athletes which was also used to assess any bias caused by the age of the individual or other characteristics of the athletes.[83]

The resulting age adjusted function for men is:

$$GH\text{-}2000 \text{ score} = -6.586 + 2.905 * \log(PIIINP) \\ + 2.100 * \log(IGF\text{-}1) - 101.737/age$$

where age is measured in years.

Similar calibration was undertaken for female elite athletes, leading to the age-adjusted derived score for women:

$$GH\text{-}2000 \text{ score} = -8.459 + 2.454 * \log(PIIINP) \\ + 2.195 * \log(IGF\text{-}I) - 73.666/age$$

Both formulae were standardized to give a mean 0 and standard deviation 1 over the population of elite athletes.

The marker approach has a major advantage over the isoform test in that the biomarkers are more stable in serum than GH but rise dramatically after GH administration and only slowly return to baseline therefore providing a longer "window of opportunity" to detect an athlete who has been abusing GH.

The test has been under development since 1996 and a large number of studies have been undertaken to ensure the GH marker methodology is robust and reliable and would withstand the scrutiny anticipated in a Court of Arbitration in Sport. The following areas have been assessed:

Responsiveness and disappearance kinetics following GH administration

The markers chosen for the GH test are required to respond to GH administration in both men and women and across a wide range of ethnic groups.

Double-blind placebo-controlled GH administration randomized controlled trials have been undertaken to assess the responsiveness of a range of markers to GH administration in male and female amateur athletes.[78,86,87] Results showed that the combination of IGF-I and PIIINP provides very good discrimination between GH administered individuals and those

who received placebo, without placing any particular group at an increased risk of a false positive.[78,81,88] A further double-blind GH administration study suggests that the response to GH in other ethnic groups does not differ significantly from white European amateur athletes.

The responsiveness varied between men and women but this was expected as it is known that there is sexual dimorphism in the GH-IGF axis. Women are more resistant to the actions of GH and therefore the increase in markers was smaller than that observed in men.[78,86] Nevertheless the responsiveness was sufficient to discriminate reliably between each of the treatment groups with reasonable sensitivity.

The disappearance kinetics for both IGF-I and PIIINP indicate that detection is possible for as long as 2 weeks after cessation of GH abuse.[88] In particular, PIIINP has a much longer half-life than GH and IGF-I thereby lengthening the 'window of opportunity' for detection.[89]

The impact of acute endurance-type exercise

As testing often occurs in the immediate postcompetition setting, it is important that exercise does not change IGF-I and PIIINP sufficiently to affect the performance of the test. During a GH administration study, IGF-I and PIIINP increased by 20% and 10.2%, respectively, following an acute bout of exercise but this increase was small in comparison with the much larger 300% increase in the markers that occurred following GH administration.[78,88]

The impact of intra-individual variability

The intra-individual variation (day-to-day stability) of the measured concentration of these markers should be small in placebo-treated individuals, thus allowing the determination of reliable physiological ranges.

Results from four longitudinal studies following athletes for a period of up to 12 months showed remarkably consistent results, with no apparent difference between amateur and elite athletes. The intra-individual variability for IGF-I varied between 13.9% and 16% while the variability for PIIINP varies from 12% to 19% which is no greater than the measurement error involved with immunoassay laboratory analysis.[90]

Impact of injury

Injuries are common in sport and these may cause an increase in PIIINP[91,92] and IGF-I.[93] In particular, skeletal injury alters PIIINP as this is a marker of soft tissue and bone turnover.[93,94] Following a tibial shaft fracture, bone turnover increases for at least 24 weeks and was accompanied by an increase in PIIINP.[91] Therefore, it was vital to ensure that no athlete

would be falsely accused of GH doping under the proposed methodology because of an injury.

A specifically designed study followed 143 men and 40 women after a sporting injury. There was no change in IGF-I over the 12-week follow-up, but PIIINP increased by approximately 20%, reaching a peak 2–3 weeks after injury. The magnitude and duration of this increase was dependent on the severity and nature of the injury but under no situation did the rise cause any false-positive readings in the proposed test.[95] Previous studies have shown with more serious illness or injury that IGF-I concentrations fall and so while the above study did not study the full range of athletic injury, it is also unlikely that more serious injuries would cause a false positive because any further increase in PIIINP would be offset by a reduction in IGF-I.

Impact of ethnicity

Published studies of 'non-elite athlete' healthy volunteers have found little difference in IGF-I concentrations between ethnic groups[96–98] but it was important to confirm this in elite athletes. Two independent research groups undertook studies to examine whether there was any evidence to suggest a difference in circulating IGF-I and PIIINP between ethnic groups. One of these studies was a cross-sectional study of elite athletes that showed that although there are small differences in the mean values between ethnic groups – for example, the IGF-I concentrations in Afro-Caribbean men are approximately 8.2% lower than white European men – nearly all values lie within the 99% prediction intervals for white European athletes, regardless of ethnic background.

Validation of methodology using independently collected data

A confirmatory study was undertaken as a way of assessing the performance of the GH marker approach using another independent population. When the male GH-2000 formula was applied to an independent dataset obtained from the Institut für Dopinganalytik und Sportbiochemie Kreischa, 90% of the individuals who had received GH were correctly identified and there were no false-positives; these findings were similar to the results found in the GH-2000 dataset from which the formula was developed.[79]

Impact of concomitant use of GH with other doping agents

It is believed that athletes abusing GH do so in combination with other performance enhancing substances, such as anabolic steroids. It is therefore important that any proposed

methodology will still be applicable in such a scenario. It is nearly impossible to replicate such circumstances in a research study, as the frequency, dosage, and combination of substances are not known but we can infer the outcome of such a scenario by examining the concomitant use of GH with other commonly used doping substances. A study performed in Australia showed that the post-GH rise in IGF-I was not suppressed by concomitant testosterone administration while the increase in PIIINP was larger.[80]

The need for assay standardization

The GH marker approach, just like the isoform test, currently relies on the use of immunoassays to quantify IGF-I and PIIINP. Following the completion of the original GH-2000 project, the IOC was advised in 1999 to establish assays within their own laboratory system[99] but they failed to heed this recommendation. Unlike the isoform test, the GH-2000 and GH-2004 research groups that developed the GH marker methodology have relied on commercial immunoassays resulting in limited control of any changes made to the reagents and the standards used in the assay. This coupled with the fact that there is currently no reliable international standard for PIIINP has meant that the numerical values obtained from different assays vary and are prone to change.

Assay manufacturers update their immunoassays over time and there is no standardization process for the development of immunoassays by different manufacturers. These are key areas that need to be clarified before introduction of the "marker" test. It is the nature of immunoassays that measurements are not absolute and inevitably are a function of the reagents and conditions used in the assay.

This has been a major stumbling block delaying the implementation of this methodology by WADA. WADA have now funded a significant research project aiming to address the impact of using commercial immunoassays by deriving assay specific cut-offs with modern assays for the GH markers. Following implementation of the tests, these cut-offs will be reviewed frequently to ensure such threshold levels remain relevant to the assays used at the time of testing.

Other GH detection methods – past and future

Over the years a number of other groups assessed whether other GH dependent biomarkers can detect GH abuse. The Institut für Dopinganalytik und Sportbiochemie in Kreischa, Germany, proposed a detection methodology through the

use of a discriminant function based on the concentrations of IGF-I, PIIINP, and IGFBP-3.[100] The Australian Japanese Consortium, which studied the effects of GH and testosterone, proposed using an alternative bone marker (carboxy terminal cross-linked telopeptide of type I collagen) to PIIINP because this had a better discrimination during the wash-out phase because of the longer half-life of the Type 1 collagen marker.[74] This approach has not been followed due to the lack of a second immunoassay which would be needed to comply with WADA laboratory guidance.

In the near future, mass spectrometry methods for IGF-I measurement will become available. Although immunoassays may continue to be used for screening because of the ease of use, it is likely that mass spectrometry methods will become the gold standard. It is unlikely, however, that such methods for the detection of PIIINP will be available for some time.

Serum proteomic profiling

Serum proteomic profiling using surface-enhanced laser desorption/ionization time-of-flight mass spectrometry can detect alterations in multiple proteins after GH administration. By studying protein profiles in blood samples from individuals who had received GH and controls, Chung et al identified the hemoglobin alpha-chain as a novel serum biomarker as it changed after hGH administration and provided good discrimination between the two groups.[101] This study has not been repeated and at present the sensitivity or specificity of this approach has not been assessed.

Surface plasmon resonance

Surface plasmon technology is a non-labeled optical methodology that measures the refractive index of small quantities of a material adsorbed onto a metal surface allowing measurement of mass.[87] The intention is that if successful this technology could be used to quantify the concentration of GH dependent markers, instead of using conventional immunoassays. However, early findings suggest that the use of this technology does not yield the same level of sensitivity as currently available commercial immunoassays.

The use of pharmaceutical markers in GH preparations

A recent publication[102] reviewed pharmaceutical proposals for enabling easier detection of GH abuse by modifying current GH pharmaceutical preparations. Three theoretical proposals have been put forward for this:

a. The development of a new epitope by altering the amino acid sequence of the protein whilst maintaining its biological activity. This could then be used in an immunoassay to quantify this protein.
b. Covalently attaching a marker to the protein that would be measurable.
c. The addition of a traceable marker to synthetic GH formulation that could be easily detected by a biological or chemical assay.

This approach suffers from the distinct risk that athletes will more than likely avoid the use of this formulation, opting for unregulated unmodified GH which would potentially pose even a greater health risk. There are also ethical concerns about exposing patients to a marker that has no medical benefit to them. Investment in this research area would require substantial efforts, and it would be more practical to concentrate efforts on refining detection methodologies currently under development.

The future of GH doping – gene doping

Athletes are continuing to seek new ways of improving their physical performance and patterns of performance enhancing drug abuse have changed as developments in anti-doping technology are made. Athletes have always been ahead of the "doping game" with athletes abusing GH 10 years before it was accepted as an effective medical treatment in adults.[31] It is thought that the next era of doping will be that of gene doping, where currently there are no methodologies for detecting such tools.

There are concerns that gene doping could be used (or might already be in use) in a variety of athletic settings[103] utilizing the advances in the treatment of serious diseases such as hemophilia[104] and X-linked severe combined immunodeficiency disease. WADA has defined gene or cell doping as the "nontherapeutic use of genes, genetic elements, and/or cells that have the capacity to enhance athletic performance".[105]

In sport, gene therapy might be used to heal tissue defects more efficiently following trauma thereby minimizing the effect of sport injuries. Furthermore, gene therapy has the potential to modify normal metabolism. Erythropoietin, hGH, insulin-like growth factor-1 (IGF-I), peroxisome proliferator activated receptor-delta, and myostatin inhibitor genes have all been identified as potential targets for gene doping.[106–108]

The use of gene therapy carries a number of potential risks partly as a result of the toxicity of the gene transfer vectors and partly because of uncontrolled transcription of the gene

itself. For example, overexpression of the erythropoietin gene may lead to increased blood viscosity of the blood and the risk of clotting.[103] The effects of gene therapy to increase IGF-I or GH production are unknown but may lead to problems seen in individuals with acromegaly or the development of cancer.

Detection of gene therapy abuse is difficult, mainly because any effects of the transferred gene are similar to endogenous function. It may be possible to detect the viral vector or the gene protein product if this is present in the circulation in supraphysiological quantities.

Conclusions

Despite a widespread belief that GH abuse will enhance sporting performance, there is limited evidence to support this. In addition, its abuse is associated with significant health risks and athletes should take this into consideration when contemplating its use for enhancing their performance. Despite a number of significant challenges, both physiological and logistical, there now are a number of promising GH detection methodologies under way. These will help to protect the health and safety of athletes, the integrity of sport, and the principles of fair play.

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