British Journal of Pharmacology (2008) 154, 542–556
 0007–1188/08 $30.00
www.brjpharmacol.org

REVIEW

Growth hormone, IGF-I and insulin and their abuse in sport

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There is widespread anecdotal evidence that growth hormone (GH) is used by athletes for its anabolic and lipolytic properties. Although there is little evidence that GH improves performance in young healthy adults, randomized controlled studies carried out so far are inadequately designed to demonstrate this, not least because GH is often abused in combination with anabolic steroids and insulin. Some of the anabolic actions of GH are mediated through the generation of insulin-like growth factor-I (IGF-I), and it is believed that this is also being abused. Athletes are exposing themselves to potential harm by self-administering large doses of GH, IGF-I and insulin. The effects of excess GH are exemplified by acromegaly. IGF-I may mediate and cause some of these changes, but in addition, IGF-I may lead to profound hypoglycaemia, as indeed can insulin. Although GH is on the World Anti-doping Agency list of banned substances, the detection of abuse with GH is challenging. Two approaches have been developed to detect GH abuse. The first is based on an assessment of the effect of exogenous recombinant human GH on pituitary GH isoforms and the second is based on the measurement of markers of GH action. As a result, GH abuse can be detected with reasonable sensitivity and specificity. Testing for IGF-I and insulin is in its infancy, but the measurement of markers of GH action may also detect IGF-I usage, while urine mass spectroscopy has begun to identify the use of insulin analogues.
British Journal of Pharmacology (2008) **154,** 542–556; doi:10.1038/bjp.2008.99; published online 31 March 2008

Keywords: GH; IGF-I; insulin; sport; abuse

Abbreviations: FFA, free fatty acid; GH, growth hormone; IGF-I, insulin-like growth factor-I; IGFBP, IGF binding protein; P-III-P, type 3 pro-collagen; rhGH, recombinant human growth hormone

Introduction

It is widely believed that growth hormone (GH) has been used by sportsmen and women since the 1980s to improve their athletic performance (McHugh *et al.*, 2005) despite being banned for many years and appearing on the World Anti-Doping Agency list of banned substances.

The actions of GH that interest athletes are anabolic and lipolytic, leading to an increase in lean body mass and reduction in fat mass. Some of the anabolic GH actions are mediated through the generation of insulin-like growth factor-I (IGF-I), and there is anecdotal evidence that this too is being abused by athletes either alone or in combination with GH. The regulation of protein synthesis involves the synergistic actions of GH and IGF-I stimulating protein synthesis, while insulin simultaneously inhibits protein breakdown (Russell Jones *et al.*, 1993). GH stimulates protein synthesis through a mechanism that is separate and distinct from anabolic steroids and therefore it seems likely that their effects will be additive. This has led many athletes to combine GH with insulin and anabolic steroids (Sönksen, 2001).

The GH doses used by athletes are thought to be up to 10 times higher than those used by endocrinologists, and as such athletes are putting themselves at risk of harmful effects such as hypertension, diabetes and Creutzfeld–Jakob disease.

The detection of GH poses the greatest contemporary challenge to the anti-doping community. The detection of GH abuse has proved difficult for several reasons. Unlike many substances of abuse, such as synthetic anabolic steroids, GH is a naturally occurring substance. The demonstration of exogenous administration must therefore rely on finding concentrations exceeding normal physiological levels while excluding pathological causes such as acromegaly. This is made harder because GH is secreted in a pulsatile manner with exercise and stress being major stimulators of GH secretion (Prinz *et al.*, 1983; Savine and Sönksen, 2000). Consequently, GH concentrations are often at their highest in the immediate post-competition setting when most dope testing occurs. Recombinant human GH is almost identical

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Received 25 July 2007; revised 14 November 2007; accepted 3 March 2008; published online 31 March 2008

to pituitary GH, whereas cadaveric GH, which is in plentiful supply on the Internet, is indistinguishable from endogenously produced GH. Blood testing is needed for GH and IGF-I because less than 0.1% is excreted unchanged and even that is erratic, rendering urine testing unfeasible (Moreira-Andres *et al*., 1993).

The review will explore why athletes abuse GH, IGF-I and insulin and also the methodology that has been developed to catch the cheats.

GH abuse in sport

Growth hormone was first extracted from human pituitary glands in 1945 (Li *et al*., 1945). It was shown to promote growth in hypopituitary animals and was soon used to restore growth in children with hypopituitarism. How and where GH was first used as a doping agent is unknown, but the earliest publication to draw attention to it was Dan Duchaine's 'Underground Steroid handbook', which emerged from California in 1982 (Duchaine, 1982). Although it contains some fundamental errors, such as the recommendation and advertisement of animal GH for use in humans, the description of the actions of GH in this article was remarkably accurate and pre-dated adult endocrinology experience by about a decade. GH was described as the 'most expensive, most fashionable and least understood of the new athletic drugs. It has firmly established itself in power-lifting and within a few years will be a commonly used drug in all strength athletics.'

The most famous case of GH abuse in professional athletics came to light in 1988 after Ben Johnson won the 100 m gold medal at the Olympic Games in Seoul. He was subsequently disqualified after stanazolol was detected in his urine, but at a later hearing, both he and his coach Charley Francis admitted under oath that he had taken human GH in addition to anabolic steroids.

It is impossible to determine the precise prevalence of GH abuse among sportsmen and women, as much of our evidence comes from anecdotal reports (McHugh *et al*., 2005). Although initially advocated for strength disciplines, endurance athletes are also attracted to GH's lipolytic actions and reduced fat mass; in 1988, a large quantity of GH was found in a team car at the Tour de France.

There is evidence that adolescents are using GH. In a survey of two US high schools, 5% of male students admitted to having taken GH and nearly one-third knew someone who had taken GH (Rickert *et al*., 1992). Most GH users were unaware of its side effects and reported their first use between 14–15 years of age.

Growth hormone is an expensive drug and this has led some parents to sell GH prescribed to treat their child's GH deficiency on the black market. Officials have also misappropriated GH for their athletes. At the 1998 World Swimming Championships, Yuan Yuan, a Chinese swimmer, was stopped on entry into Perth with a suitcase full of GH that had been exported to China for therapeutic reasons.

A few athletes have admitted to taking GH. In a death-bed confession, Lyle Alzado, an American football player, admitted that 80% of American footballers have taken GH. In 2000, Australian discus champion Walter Reiterer claimed institutional and supervised usage of GH. With this in mind, it is interesting to note that 6 months before the Sydney Olympic Games, 1575 vials of GH were stolen from an importer's warehouse in Sydney.

More recently, Victor Conte, the owner of the Bay Area Laboratory Co-Operative, claimed that he had supplied GH to many high-profile American athletes including Tim Montgomery and Marion Jones. This admission came after the raid on the Bay Area Laboratory Co-Operative's headquarters on 3 September 2003, when evidence of systematic doping was found and many of the top names in athletics, baseball and American football were implicated in the scandal. Although many have denied taking GH, 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 (Fainaru-Wada and Williams, 2006).

The recent conviction of Sylvester Stallone, who was caught with GH in his possession on entering Australia, suggests that GH is readily available in athletic and body-building circles.

IGF-I abuse in sport

The prevalence of IGF-I abuse is probably much lower than for GH because, unlike GH, there is no readily available natural source, and therefore all IGF-I is obtained through recombinant DNA technology. Two companies currently market IGF-I, and these preparations have only recently received approval for use in humans to treat growth failure in children with severe primary IGF-I deficiency or with GH gene deletion who have developed neutralizing GH antibodies. The first product is Increlex or recombinant human IGF-I, manufactured by Tercica, and the second product manufactured by Insmed is Iplex, which differs from Increlex in that the recombinant human IGF-I is supplied bound to its major binding protein, IGFBP-3 (Kemp *et al*., 2006; Kemp, 2007).

Although in relatively short supply, other companies make IGF-I for cell culture and other uses, and this material could also become available to athletes. The wider availability of IGF-I, together with an appreciation of the efforts to detect GH abuse, is likely to increase its illicit use by athletes, despite being prohibited by the World Anti-Doping Agency.

Abuse of insulin in sport

We have only sketchy details about the use of insulin by professional athletes. It is alleged that short-acting insulin is being used in a haphazard way to increase muscle bulk in body builders, weight lifters and power lifters (Sönksen, 2001). After concerns raised by the Russian medical officer at the Nagano Olympic games, the International Olympic Committee immediately banned its use in those without diabetes. Athletes with insulin-requiring diabetes may use insulin with a medical exemption.

Physiology of the GH–IGF axis

Growth hormone is the most abundant pituitary hormone and is secreted in a pulsatile manner under the control of the hypothalamic hormones, GH-releasing hormone, somatostatin and ghrelin (Figure 1).

Regulation of GH secretion

Growth hormone-releasing hormone and ghrelin stimulate the synthesis and release of GH, whereas somatostatin is inhibitory in action (Veldhuis, 2003). Although ghrelin is secreted by the hypothalamus, the major source of ghrelin is the stomach and it is thought to be one mechanism that controls the GH response to eating (van der Lely *et al*., 2004). Circulating IGF-I reduces GH secretion through classical negative endocrine feedback (Carroll *et al*., 1997).

There are a number of physiological stimuli that increase or inhibit GH release, the most important of which are exercise and sleep (Savine and Sönksen, 2000). The highest GH peaks occur at night within the first hour of sleep during slow-wave sleep (Takahashi *et al*., 1968). Nutritional status regulates GH secretion both acutely and chronically; hypoglycaemia, reduced circulating free fatty acids (FFA) and higher amino-acid concentrations all increase GH secretion (Ho *et al*., 1988; Pombo *et al*., 1999), whereas in the longer term, GH secretion is increased in anorexia nervosa and decreased with obesity (Veldhuis *et al*., 1991; Argente *et al*., 1997).

Age and gender are important determinants of GH secretion (Savine and Sönksen, 2000). Secretion is highest during the pubertal growth spurt, and after the mid-20s, GH secretion decreases by 14% every decade (Toogood, 2003), and this decrease may be responsible for some of the age-related body composition changes (Holt *et al*., 2001). Women have higher baseline GH secretion but the pulses are not as high and are less erratic than in men (van den Berg *et al*., 1996).

The actions of GH and IGF-I

Growth hormone exerts its multiple metabolic and anabolic actions through binding to specific GH receptors that are found on every cell of the body (Holt, 2004). Following binding to the GH receptor, the tyrosine kinase Janus kinase 2 is activated and multitude of signalling cascades are initiated that result in the wide variety of biological responses, including cellular proliferation, differentiation and migration, prevention of apoptosis, cytoskeletal reorganization and regulation of metabolic pathways (Lanning and Carter-Su, 2006). Although a detailed description of these signalling cascades is beyond the scope of this review, it is worth remarking on the number of signalling proteins and pathways activated by GH, which include JAKs, signal transducers and activators of transcription, the mitogen-activated protein kinase pathway, and the phosphatidylinositol 3′-kinase pathway. Although these pathways are well described, the inter-relationship between the different pathways is not fully understood.

Growth hormone exerts most of its anabolic actions through the generation of circulating IGF-I (the somatomedin hypothesis) (Le Roith *et al*., 2001), the majority of which is produced in the liver. IGF-I may also act in a paracrine or autocrine fashion in response to GH action at other target tissues. Transgenic animals, in which the IGF-I gene has been selectively deleted in the liver and whose serum IGF-I is marked reduced, have led some to question the somatomedin hypothesis as these animals appear to grow normally (Sjogren *et al*., 1999; Yakar *et al*., 1999), even though with the

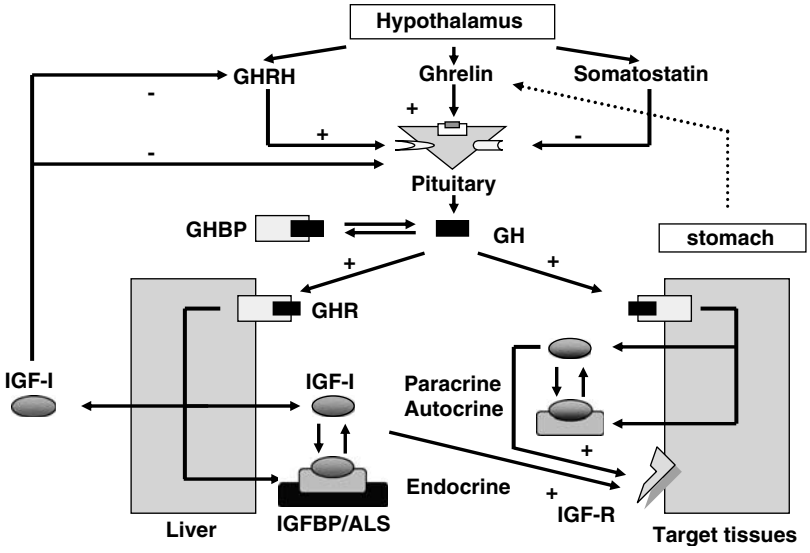

Figure 1 The growth hormone (GH)–insulin-like growth factor (IGF)-I axis. GH is secreted from the pituitary gland under the control of the hypothalamic hormones, somatostatin, Ghrelin and GH-releasing hormone (GHRH). GH circulates bound to its binding protein and acts through specific cell-surface receptors. The anabolic actions of GH are partially mediated by IGF-I. IGF-I acts through the IGF-I receptor in an autocrine, paracrine and classical endocrine mechanisms. Circulating IGF-I is almost entirely bound to a family of high-affinity binding proteins (IGFBPs) that coordinate and regulate the biological functions of the IGFs. IGF-I inhibits GHRH and GH secretion in a classical negative-feedback mechanism.

development of insulin resistance (Sjogren *et al*., 2001; Yakar *et al*., 2001). It is known, however, that the total amount of circulating IGF-I in an adult is not required for normal growth, as neonates have much lower circulating levels but grow very rapidly. The precise mechanisms regulating IGF-I bioavailability to the tissues are not yet understood but certainly involve IGF-binding proteins (IGFBPs). As IGFBP distribution in liver IGF-I knockout mice, as in newborn humans, is quite different from that in healthy adults, it has been argued that these data do not really challenge the view that endocrine IGF-I has a role in growth regulation.

Effects on whole body physiology

The physiological effects of GH are best examined by considering the condition of adult GH deficiency. Studies of hypopituitary individuals with appropriate replacement of thyroid, steroid and sex steroid replacement have shown that GH plays a pivotal role in body composition, well-being, physical performance and cardiovascular health (Cuneo *et al*., 1992; Carroll *et al*., 2000). In the absence of GH, lean tissue is lost and fat accumulates, and correspondingly waist-to-hip ratio increases as visceral fat increases (Table 1).

Effects on intermediate metabolism

Protein metabolism. Protein synthesis and degradation are each regulated by multiple hormonal and nutritional factors, and protein turnover in individual tissues and the whole body is in a state of constant flux. Insulin, GH and IGF-I have synergistic anabolic effects on protein metabolism (Figure 2) (Sönksen, 2001).

Growth hormone causes nitrogen retention, as shown by decreased urinary excretion rates of urea, creatinine and ammonium. In healthy humans, acute administration of GH modestly stimulates muscle and whole body protein synthesis (Fryburg *et al*., 1991). When GH is infused locally into the brachial artery, forearm muscle protein synthesis increases, whereas systemic IGF-I concentrations and whole body protein turnover are unchanged, indicating that GH stimulates protein synthesis directly as well as indirectly through IGF-I (Fryburg *et al*., 1991).

Insulin-like growth factor-I has an anabolic effect on protein metabolism, inhibiting whole body protein breakdown and stimulating protein synthesis (Fryburg, 1994). This effect is dependent on serum insulin and an adequate supply of amino acids. After IGF-I is administered systemically, serum insulin and amino acids decrease, the former through decreased production and the latter as a result of increased clearance from the blood. This attenuates the stimulation of whole body protein synthesis, but if the amino acids and insulin are replaced during the administration, the effect on protein synthesis by IGF-I is clearly seen (Russell Jones *et al*., 1994; Jacob *et al*., 1996).

Protein breakdown is inhibited by physiological concentrations of plasma insulin, the 'Chalonic' action of insulin (Umpleby and Sönksen, 1985; Tessari *et al*., 1986). In contrast, the rates of protein degradation are increased in cardiac and skeletal muscles in situations where insulin concentrations are low such as type 1 diabetes or starvation

Table 1 Clinical features of GH deficiency and effect of GH replacement

Effect of GH deficiency	*Effect of GH replacement*
Body composition	
Increased body fat	Decreased fat mass
Increased waist–hip ratio	Decreased waist–hip ratio
Increased visceral fat mass	Decreased visceral fat
Decreased lean body mass	Increased lean body mass
Decreased bone mineral density	Increased bone mineral density
Physical performance	
Decreased muscle mass	Increased muscle mass
Decreased muscle strength	Increased muscle strength
Decreased maximal exercise performance	Increased maximal exercise performance
Decreased maximum oxygen uptake	Increased maximal oxygen uptake, maximum power output, maximum heart rate and anaerobic threshold
Decreased maximum heart rate	Increased red cell mass
Psychological well-being	
Decreased ability to cope with daily life	Increased energy levels
Increased level of perceived health problems	Increased ability to participate in physical activities without tiring
Decreased physical and mental energy	Increased emotional reaction and social isolation scores
Decreased concentration skills	Increased perceived quality of life
Decreased initiative	Increased self-esteem
Increased social isolation	Decreased sleep requirement
Decreased self-esteem	
Decreased sex life	
Increased sleep requirement	
Cardiovascular system	
Increased prevalence of cardiovascular events	Increased left ventricular mass
Increased hypertension	Increased stroke volume
Decreased left ventricular mass	Increased cardiac output and resting heart rate
Decreased fibre shortening	Decreased diastolic blood pressure

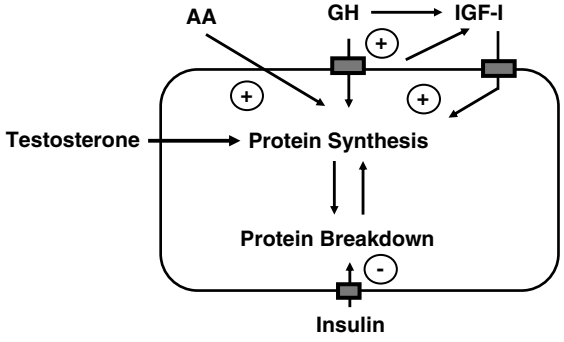

Figure 2 The synergistic action between insulin, IGF-I and GH in regulating protein synthesis. Without insulin, GH loses much (if not all) of its anabolic action. GH and IGF-I stimulate protein synthesis directly, whereas insulin is anabolic through inhibition of protein breakdown. The anabolic action of both GH and IGF-I appears to be mediated through induction of amino-acid transporters in the cell membrane. It is not yet clear how much of the action of IGF-I is through locally generated IGF-I ('autocrine' and 'paracrine') or through circulating IGF-I that is largely derived from the liver.

(Charlton and Nair, 1998). Although the rate of protein synthesis is reduced by 40–50% in young diabetic rats, a physiological anabolic effect of insulin on protein synthesis has not been confirmed in humans (Liu and Barrett, 2002).

The similarities between IGF-I and insulin suggest that these proteins act in a coordinated manner to regulate protein turnover. There are interesting differences, however, in their respective dose–response curves. Low physiological insulin concentrations inhibit protein breakdown and increase glucose disposal into skeletal muscle, whereas higher, non-physiological concentrations are required to stimulate protein synthesis (Louard *et al*., 1992). In contrast, increases in IGF-I that have no effect on glucose uptake stimulate protein synthesis, and higher concentrations are required to inhibit protein breakdown (Fryburg, 1994). During the last decade, there have been advances in the understanding of the intracellular signalling mechanisms of insulin and IGF-I, many of which are shared, such as insulin receptor substrate 1. The precise mechanism by which these similar but divergent pathways interact is not fully understood, but IGF-I, similar to GH, most likely acts through stimulating amino-acid uptake.

Lipolysis. The administration of GH to humans, either by continuous infusion or by a bolus injection, leads to stimulation of lipolysis and increased fasting FFA concentrations with the peak effect around 2–3 h after the injection (Hansen, 2002). These findings are in keeping with the changes in FFAs following physiological stimuli of GH secretion. In young healthy subjects, the nocturnal or exercise-induced peak of GH precedes the peak of FFAs by 2 h (Moller *et al*., 1995). Furthermore, during times of fasting or energy restriction, the lipolytic effect of GH is enhanced, although the effect is suppressed by co-administration of food or glucose (Moller *et al*., 2003). Acromegaly is associated with increased circulating FFAs, increased muscle uptake of FFAs and increased lipid oxidation.

Although GH receptors are abundantly expressed on adipocytes, it has been suggested that GH has a permissive effect on catecholamine-induced lipolysis (Hansen, 2002). *In vitro* studies have shown that GH has no direct lipolytic effect on human fat cells, but markedly increases the maximal lipolysis induced by catecholamines (Marcus *et al*., 1994).

Glucose homeostasis. The first observation that GH had an effect on glucose metabolism came in the 1930s when it was observed that hypophysectomy ameliorated the hyperglycaemia of experimental diabetes in dogs (Houssay and Biasotti, 1930). In both healthy subjects and those with type 1 diabetes, GH increases fasting hepatic glucose output, by increasing hepatic gluconeogenesis and glycogenolysis, and decreases peripheral glucose utilization through the inhibition of glycogen synthesis and glucose oxidation (Tamborlane *et al*., 1979; Bak *et al*., 1991; Fowelin *et al*., 1991, 1993, 1995). Patients with acromegaly develop insulin resistance and hyperinsulinaemia (Sönksen *et al*., 1967), and up to 40% become diabetic (Ezzat *et al*., 1994; Colao *et al*., 2000).

Although the effect on glucose homeostasis would appear to be disadvantageous for the athletes, it is worth bearing in mind that long-standing adult GH deficiency is associated with insulin resistance and that any acute effect on glucose homeostasis does not take into account changes in IGF-I, which also affects insulin sensitivity (Salomon *et al*., 1994).

Intravenous IGF-I causes hypoglycaemia in rats by stimulating peripheral glucose uptake, glycolysis and glycogen synthesis, although having only a minimal effect on hepatic glucose production (Jacob *et al*., 1989). In dogs, however, IGF-I has been shown to suppress hepatic glucose output, but to a lesser degree than insulin at the doses used, as well as increasing peripheral glucose utilization (Shojaee Moradie *et al*., 1995). As hepatic expression of the IGF-I receptor is reportedly low (Stefano *et al*., 2006), it is possible that this effect of IGF-I on hepatic glucose output is by an indirect mechanism. For example, IGF-I may bind the insulin receptor with low affinity (Holt *et al*., 2003). Alternatively, it may improve whole body insulin sensitivity by inhibiting the secretion of GH.

The effects of an intravenous IGF-I infusion in humans are similar to those described in animals and lead to hypoglycaemia (Zenobi *et al*., 1992). Insulin sensitivity increases with respect to glucose by IGF-I through increased peripheral glucose uptake and decreased hepatic glucose production (Boulware *et al*., 1994; Russell Jones *et al*., 1995). A subcutaneous infusion of IGF-I also causes hypoglycaemia, but the effect is slower in onset than insulin and decreases more slowly after the infusion was stopped, because of the presence of the IGF-binding proteins.

Bone metabolism. Growth hormone has profound effects on bone metabolism. GH deficiency is associated with osteopaenia, which is reversed by GH replacement (Gomez *et al*., 2000). Male subjects with osteoporosis have reduced GH peaks and low serum IGF-I concentrations, and furthermore, IGF-I concentrations correlate well with estimates of bone mineral density (Patel *et al*., 2005). In addition to a direct effect on bone stimulating the cycle of bone growth, there is evidence to suggest that GH and IGF-I may modify intestinal calcium absorption and serum 1,25 hydroxy vitamin D concentrations. Patients with acromegaly have increased intestinal calcium absorption (Lund *et al*., 1981), and GH therapy in pigs increases intestinal calcium absorption, probably through increased production of serum 1,25 hydroxy vitamin D (Chipman *et al*., 1980). GH replacement therapy in adult GHD leads to a short-term increase in serum 1,25 hydroxy vitamin D concentrations (Burstein *et al*., 1983). These data suggest that GH increases intestinal calcium absorption by its effects on renal 25 (OH)D 1-α-hydroxylase activity, but other mechanisms may also be involved (Halloran and Spencer, 1988).

Why do athletes abuse GH?

The importance of GH in adult physiology as well as in children was confirmed beyond doubt in 1989 by two independent studies undertaken in the UK and Denmark (Jorgensen *et al*., 1989; Salomon *et al*., 1989). Both groups

undertook double-blind, placebo-controlled trials in adults with hypopituitarism given appropriate replacement therapy with everything except GH. The studies showed remarkably congruous results after 6 months of GH treatment.

The most impressive finding was a change in and normalization of body composition with an average 6 kg increase in lean body mass, largely accounted by an increase in skeletal muscle, and a concomitant loss of fat. The body composition changes were accompanied by improvements in quality of life, particularly in the area of 'increased energy' and performance enhancements (McGauley *et al.*, 1990; Cuneo *et al.*, 1991a, b). Longer studies over the first 3 years of GH replacement showed that exercise performance continued to improve (Jorgensen *et al.*, 1994).

Does GH enhances performance in normal healthy young adults?
There has been considerable debate about the ability of GH to translate these body composition and other metabolic changes into improved performance (Rennie, 2003). Despite the theoretical benefits of GH, sceptics point to the condition of acromegaly and negative clinical studies as evidence of a lack of benefit.

Acromegaly—nature's experiment of GH excess
It has been argued that acromegaly, where there is oversecretion of GH usually from a pituitary adenoma, provides evidence that excess GH is not performance enhancing, as it is not associated with athletic prowess. Indeed, acromegaly is usually associated with muscle weakness rather than excessive strength (Table 2) (McNab and Khandwala, 2005). It needs to be appreciated, however, that acromegaly frequently remains undiagnosed for many years and the clinical presentation at diagnosis may not reflect earlier stages of the disease. Many patients if questioned carefully will give a history of increased strength in the first few years of their condition. Indeed, we know a rower who competed at an elite level during the early stages of his acromegaly. Not only was he one of the strongest crew, but he could also tolerate harder training sessions than his colleagues and recovered more quickly afterwards (PHS, unpublished data).

Although only a clinical case report, it illustrates that the timing and degree of GH excess are important for the physiological effect. Prolonged massive GH excess coupled frequently with deficiencies of other pituitary hormones, such as ACTH, may tell us little about the effects of lesser GH excess earlier in the natural history of the illness.

Table 2 Clinical features of acromegaly

Musculoskeletal (acromegaly unless indicated)
Increased stature (gigantism)
Protruding mandible (prognathia)
Teeth separation on lower jaw
Big tongue (macroglossia)
Enlarged forehead (frontal bossing)
Large hands and feet
May cause carpal tunnel syndrome
Osteoarthritis from abnormal joint loading
Cardiovascular
Dilated cardiomyopathy
Hypertension
Metabolic
Impaired glucose tolerance or diabetes
Skin
Thickened, greasy skin
Excessive sweating
General
Headaches
Tiredness

Can clinical trials provide us with the answer?
Although there have been many negative studies in this area, traditional randomized controlled trials that have the power to examine differences of 20–30% are ill suited to detect the much smaller differences that determine whether an individual wins a gold medal or not. Despite these difficulties, one trial showed that, in healthy, elderly men, the combination of GH and testosterone led to a 20% improvement in fitness as measured by maximal oxygen uptake (Giannoulis *et al.*, 2006), which was larger than with either compound alone. Very recently, a well designed and executed study in past abusers of anabolic steroids showed for the first time an ergogenic effect of GH in healthy young athletes (Graham *et al.*, 2008).

A further problem with the designs of our clinical trials is that they are designed to test one or at most two interventions. In reality, for the reasons described above, GH is frequently used by athletes in combination with insulin and anabolic steroids in varying concentrations during differing training and dietary regimens. It is impossible to control for all these variables within a single trial, and therefore it seems likely that the athletes using the '$n=1$' design are probably best placed to address the question whether GH is performance enhancing. This certainly appeared to be a potent weapon when abused by the former East German coaches (Franke and Berendonk, 1997).

A definitive answer to this question will probably never be found, as it would be difficult to obtain ethics approval for a suitable study. It should be remembered, however, that there were similar arguments about anabolic steroids 20 years ago that have subsequently been shown to have performance benefits.

Pharmacology of GH, IGF-I and insulin administration

The half-life of endogenously secreted GH is around 13 min (Sohmiya and Kato, 1992). It is rapidly cleared from the body by the liver, kidney and peripheral tissues through interaction with GH receptors. If recombinant human GH (rhGH) is administered intravenously, the half-life is similar to endogenously secreted GH (Refetoff and Sönksen, 1970; Haffner *et al.*, 1994). In practice, however, the pharmacokinetics of exogenously administered GH differs, as it is given by intermittent, usually daily, subcutaneous injection. Following injection, GH concentrations increase and reach a maximum concentration after about 2–6 h, depending on

the age and gender of the recipient (Kearns *et al.*, 1991; Janssen *et al.*, 1999). The estimated bioavailability of rhGH is 50–70%, because of degradation at the site of injection. This may explain why intramuscular injection results in a higher maximal and area-under-the-curve GH concentration than subcutaneous injection (Keller *et al.*, 2007). Thereafter, rhGH is rapidly cleared and GH is usually undetectable in women 12 hours after injection, even after high doses, while men have only low levels of GH. (Giannoulis *et al.*, 2005). There is quicker clearance of GH in women, probably reflecting their extra body fat that contains a high density of GH receptors (Vahl *et al.*, 1997). Longer-acting GH preparations are currently being developed.

The pharmacokinetics of IGF-I is complicated by the presence of a family of highly specific binding proteins (IGFBPs) that coordinate and regulate the biological functions of IGF-I. Less than 5% of serum IGF-I is free and most is bound in a ternary complex of IGF-I, IGFBP-3 and an acid-labile subunit (Figure 1). The half-life of free IGF-I is only a few minutes, whereas the half-lives of IGF-I bound in a binary and ternary complex are 20–30 min and 12–15 h, respectively (Guler *et al.*, 1989). When subcutaneous IGF-I is administered to healthy volunteers, the maximum concentration is achieved at about 7 h and half life is 20 h (Grahnen *et al.*, 1993). The half-life is prolonged when IGF-I is administered as a complex with IGFBP-3. In patients with severe GH insensitivity syndrome, IGF-I concentrations peaked between 15 and 19 h after the injection of the complex, and a single injection was effective in increasing IGF-I concentrations in these patients for a 24 h period (Camacho-Hubner *et al.*, 2006).

The half-life of intravenous insulin is only 4 min, but apart from the treatment of diabetic emergencies and possibly the replenishment of glycogen using an insulin clamp (Sönksen and Sönksen, 2000), pharmacologically administered insulin is through subcutaneous injection (Matthews *et al.*, 1985). Insulin manufacturers have developed many preparations of insulin including insulin analogues in the attempt to provide insulin replacement to people with diabetes in the most physiological way (Peterson, 2006). Consequently, the shortest-acting insulin analogues appear in the circulation within 5–10 min of injection and cleared within 4–6 h, whereas longer-acting insulins are present for over 24 h.

Potential adverse effects of GH administration

The side effects of GH administration to adults with GH deficiency are well documented, and any athlete receiving GH will potentially be at risk of these side effects (Powrie *et al.*, 1995). It is believed, however, that many athletes are using doses that are up to 10 times higher than those used therapeutically. The effects of chronically administering this dose of GH are unknown, but it would be reasonable to expect that athletes may develop some of the features of acromegaly with prolonged use (Table 2).

Sodium and fluid retention
Growth hormone causes fluid retention through its action on the kidney to promote sodium reabsorption (Powrie *et al.*, 1995; Moller *et al.*, 1999). This may be manifest as ankle swelling, hypertension and headache.

Diabetes
Patients with acromegaly develop insulin resistance and hyperinsulinaemia, and up to 40% become diabetic (Sönksen *et al.*, 1967; Ezzat *et al.*, 1994; Colao *et al.*, 2000).

Cardiomyopathy
Cardiovascular complications are a major cause of morbidity and mortality in patients with acromegaly (Colao *et al.*, 2001). The excess of GH and IGF-I causes a specific derangement of cardiomyocytes, leading to abnormalities in cardiac muscle structure and function, inducing a specific cardiomyopathy. In the early phase, there is a hyperkinetic syndrome, characterized by increased heart rate and systolic output. Two-thirds of patients have concentric cardiac hypertrophy and this is commonly associated with diastolic dysfunction and eventually with impaired systolic function leading to heart failure, if the acromegaly is left untreated. In addition, abnormalities of cardiac rhythm and those of heart valves have also been described. The coexistence of arterial hypertension and diabetes may further aggravate acromegalic cardiomyopathy. It is alleged that the American sprinter, Florence Griffith-Joyner (Flo Jo), purchased GH from fellow sprinter Darrell Robinson. When Flo Jo died at the age of 38 years, her heart was enlarged consistent with cardiomyopathy (Sullivan, 1998).

Cancer
Although controversial, the consensus statement of the Growth Hormone Research Society (2001) was that there is no increased risk of cancer when GH is given at physiological replacement doses (2001). There is evidence to suggest that acromegaly, where GH levels have been much higher than physiological doses for many years, may be associated with increased rates of colorectal, thyroid, breast and prostate cancers (Jenkins *et al.*, 2006).

Creutzfeld–Jakob disease
Initially, the only source of GH came from extracts of human pituitary glands, and tragically, this was discovered to be a source for the prion-induced Creutzfelt–Jacob disease (Brown *et al.*, 1985). As a result, pituitary-derived GH was withdrawal from the market place in 1985 and was replaced with recombinant human GH in 1987. Despite the dangers, supplies of pituitary-derived GH continue to be available on the black market to this day, and athletes continue to use this and thus a case of Creutzfelt–Jacob disease may emerge in an elite athlete at some time in the future.

Potential adverse effects of IGF-I and insulin administration

We have only limited experience with the use of exogenous IGF-I, and so most of the known side effects relate to

short-term usage only. It seems reasonable to hypothesize, however, that many of the longer-term effects of GH administration would also occur with IGF-I, as the anabolic effects of GH are closely related to the production of IGF-I in different tissues.

In the clinical trials, the commonest short-term side effects are oedema, headache, arthralgia, jaw pain and hypoglycaemia (Kemp *et al.*, 2006; Kemp, 2007). These appear to be more marked when IGF-I is used alone as the recombinant human IGFBP-3 seems to buffer the acute effects of IGF-I.

The side effects of insulin are well documented from our experience in treating people with diabetes. The most commonly experienced side effect is hypoglycaemia. Weight gain is also a problem in people with diabetes, but this is probably less of an issue for athletes whose diet and training regimens are closely monitored.

Detection of GH abuse

Two complementary approaches have been investigated to detect GH abuse: the first is based on the detection of different pituitary GH isoforms, whereas the second relies on measurement of GH-dependent markers. The very different approaches are viewed as a major strength, as their different properties mean that they are useful in different situations.

The isoform or differential immunoassay method

Growth hormone exists as multiple isoforms; 70% of circulating GH is in the form of a 22-kilo Dalton (kDa) polypeptide, whereas 5–10% occurs as a 20 kDa isoform, from mRNA splicing. Dimers and oligomers of GH exist as do acidic, desaminated, acylated and fragmented forms (Baumann, 1999). The differential immunoassay approach is based on the principle that endogenous GH occurs as a number of isoforms, whereas rhGH contains only the 22 kDa isoform (Figure 3). When rhGH is administered in sufficiently high doses, there is a suppression of endogenous GH secretion through negative feedback to the pituitary, and therefore the ratio between 22 kDa GH and non-22 kDa GH is increased (Bidlingmaier *et al.*, 2003). This change in ratio can be detected with specific immunoassays that distinguish the different isoforms.

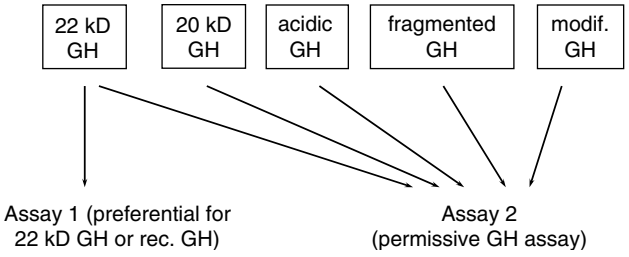

Figure 3 Principle of the isoform method. rhGH contains 22 kDa and this is specifically recognized by assay 1. Pituitary GH contains multiple isoforms and these are recognized by assay 2. When rhGH is administered, endogenous production of pituitary GH decreases, and therefore the ratio between assay 1 and assay 2 increases after rhGH administration.

The isoform method was first established by Christian Strasburger and Martin Bidlingmaier in Germany by employing one assay that specifically measured 22 kDa GH and another permissive assay that measured all GH isoforms (Figure 3). A slightly different approach has been adopted by an Australian Japanese Consortium that developed assays that specifically measure either 22 or 20 kDa GH (Momomura *et al.*, 2000).

When the German method is applied to a normal population, the ratio between 22 kDa and total GH is less than 1 with a normal distribution of values, whereas individuals receiving GH have values that are greater than one (Figure 4) (Wu *et al.*, 1999). Age, sex, sporting discipline, ethnicity and pathological states do not seem to affect the relative proportions of GH isoforms (Holt, 2007), but exercise causes a transient relative increase in the 22 kDa isoform, thereby lowering the sensitivity of the test if samples are taken immediately after competition (Wallace *et al.*, 2001a, b).

The short half-life and rapid clearance of rhGH, even when injected subcutaneously, means that the 'window of opportunity' for detection of GH doping with this test is less than 36 h (Keller *et al.*, 2007). As GH is usually administered in the evening, GH is frequently undetectable in a blood sample taken the following day (Giannoulis *et al.*, 2005). The 20 kDa GH remains suppressed for 14–30 h in women depending on the dose used, whereas in men, 20 kDa GH remains undetectable for up to 36 h (Keller *et al.*, 2007). Spontaneous GH secretion returns 48 h after the last dose of rhGH treatment (Wu *et al.*, 1990). Consequently, any athlete who stops administering GH several days before the competition will not be detected. Thus the isoform method is unlikely to catch a GH abuser in the classical 'post competition' dope testing scenario, and the optimal use of this method must be in unannounced 'out of competition' testing. This method does not detect individuals receiving cadaveric GH, IGF-I or GH secretagogues.

This test was introduced at the Athens and Turin Olympic Games and no positives were detected in samples taken 'post

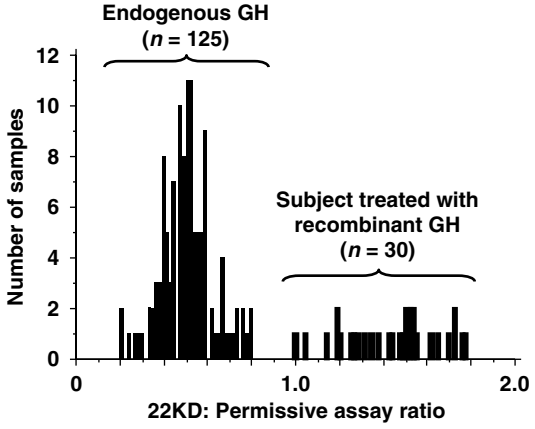

Figure 4 Ratio between 22 kDa-hGH assay and total hGH assay in serum samples obtained from 125 controls and 30 individuals treated with rhGH. Mean values are 1.43 ± 0.21 for treated individuals and 0.50 ± 0.12 for controls ($P < 0.0001$). Adapted from Wu *et al.* (1999). Figure reproduced with the permission of Christian Strasburger.

competition'. One of the World Anti-Doping Agency rules for the use of immunoassays is that two antibodies recognizing different epitopes are needed for each analyte. The German group had carefully characterized their assays and antibodies, and this may have been an overriding factor in the decision to implement the test.

The GH-dependent marker method

Growth hormone administration leads to the alteration of the concentrations or ratios of several serum proteins, and this change may be used as a means of detecting exogenous GH. An ideal marker or combination of markers would have well-defined reference ranges, would change in response to GH administration and would remain altered after GH has been discontinued (Holt, 2007). The marker should be largely unaffected by other regulators of GH secretion such as exercise or injury and should be validated across populations.

This approach was pioneered by the large multi-centre GH-2000 project coordinated by Peter Sönksen with funding from the European Union under their BIOMED 2 initiative, with additional funding from the International Olympic Committee and the rhGH manufacturers Novo Nordisk and Pharmacia. The aim was to develop a test in time for the Sydney Olympic Games. It had three main components, the first of which was a cross-sectional study of elite athletes at National or International events to establish a reference range of selected markers of GH action (Healy *et al.*, 2005). Blood samples taken within 2 h of competitions showed that the markers were dependent on age as is the case in the general population, but in contrast, sporting discipline, gender and body shape had little effect. The findings of this study were subsequently confirmed by the Australian Japanese consortium led by Ken Ho. In a study of 1103 elite athletes sampled out of competition, less than 10% of the total variance of the markers was explained by gender, sporting discipline, ethnicity and body mass index, whereas age contributed to between 20 and 35% of the variance (Nelson *et al.*, 2006).

The second component of the GH-2000 project was the 'wash-out' study (Wallace *et al.*, 1999, 2000). When the project was conceived, 25 potential markers of GH action were considered. The aim of the 'wash-out' study was to narrow this number down to the most suitable markers for a more in-depth analysis. rhGH was administered to recreational male athletes for 1 week, with blood samples collected during and after the GH administration. Subjects also undertook exercise tests to assess the potential effect of 'competition' on the markers. Nine markers, either members of the IGF–IGF-binding protein axis or markers of bone and soft tissue turnover, were then selected for analysis in the third component of the GH-2000 project. This was a 28-day GH administration study involving self-administered rhGH at two doses to 102 recreational athletes under double-blind, placebo-controlled conditions to evaluate the potential markers for their ability to discriminate active drug from placebo and to assess the 'window of opportunity' when the test remained positive after rhGH was stopped (Dall *et al.*, 2000; Longobardi *et al.*, 2000).

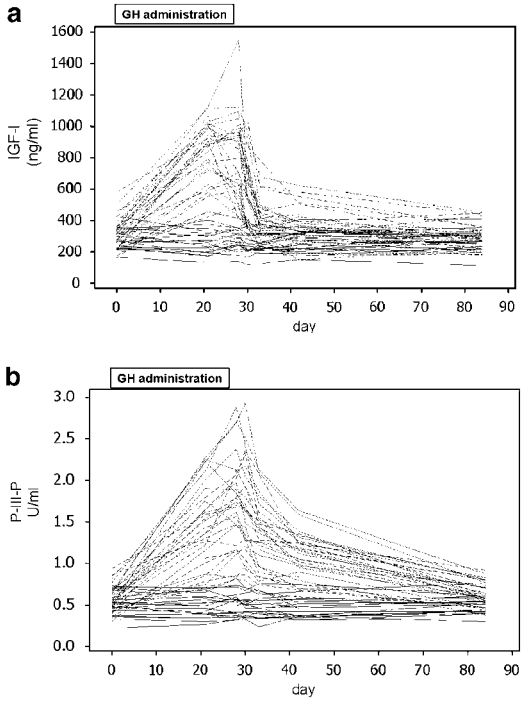

Figure 5 Change in IGF-I (**a**) and P-III-P (**b**) following the administration of GH or placebo for 28 days to 50 healthy male volunteers.

From these studies, the GH-2000 project proposed a test based on IGF-I and type 3 pro-collagen (P-III-P) (Powrie *et al.*, 2007) (Figure 5). These markers were chosen because they provided the best discrimination between individuals receiving GH or placebo during the randomized controlled trial. They exhibit little diurnal or day-to-day variation and are largely unaffected by exercise or gender (McHugh *et al.*, 2005). In the wash-out study, IGF-I and P-III-P increased 20 and 10.2%, respectively, following exercise, but this increase was small in comparison with the larger 300% increase in the markers with GH (Wallace *et al.*, 1999, 2000). Although discrimination was the prime reason for the selection, it is important to note that these proteins are produced by different tissues, thereby reducing the number of pathological conditions that could lead to an elevation in both markers and potential false-positives.

It is known that there is sexual dimorphism in the GH–IGF axis. There are small differences in IGF-I and P-III-P concentrations in elite male and female athletes (Healy *et al.*, 2005), although gender explained around 1% of the overall variance in these markers (Nelson *et al.*, 2006). Women are known to be inherently more resistant to the actions of GH and so the increase in markers is less pronounced in women than in men (Dall *et al.*, 2000; Longobardi *et al.*, 2000). This potential disadvantage may be offset because women may need to receive higher doses to obtain a performance-enhancing benefit.

Although a single marker could be used, by combining markers in conjunction with gender-specific equations, 'discriminant functions', the sensitivity and specificity of

the ability to detect GH abuse can be improved compared with single-marker analysis (Powrie *et al.*, 2007).

The procedure used to generate the discriminant functions involves splitting the available data into two: a 'training' set of data is used to calculate the discriminant function and a 'confirmatory' set is then used to validate the sensitivity and specificity of the discriminant function. The confirmatory set required to ensure the model is applicable to the general population and not just the 'training' set.

The sensitivity of any test is dependent on the specificity. Standard medical practice accepts as 'normal' values those being within two standard deviations of the mean, but by definition, 5% of the population lie outside the 'normal range'. This creates an unacceptably high false-positive rate if applied to athletes. The specificity to be used has not been determined by the anti-doping authorities, but nevertheless the GH-2000 formulae show reasonable sensitivity even up to false-positive rates of 1 in 10 000 and beyond (Figure 6). The formula has been modified more recently to take into account the effect of age to prevent younger athletes from being placed at a disadvantage (Powrie *et al.*, 2007).

The results of the GH-2000 project were presented at an International Olympic Committee workshop in Rome in March 1999 to review critically and assure the quality of the results. The conclusion of the workshop was strong support for the methodology, but it was felt that several issues needed to be addressed before the test could be fully implemented at an Olympic games. The biggest issue was related to potential ethnic effects of GH, as the vast majority of volunteers in the GH-2000 study were white Europeans. It was felt that injury could confound the test and further work was needed to develop immunoassays owned by International Olympic Committee and

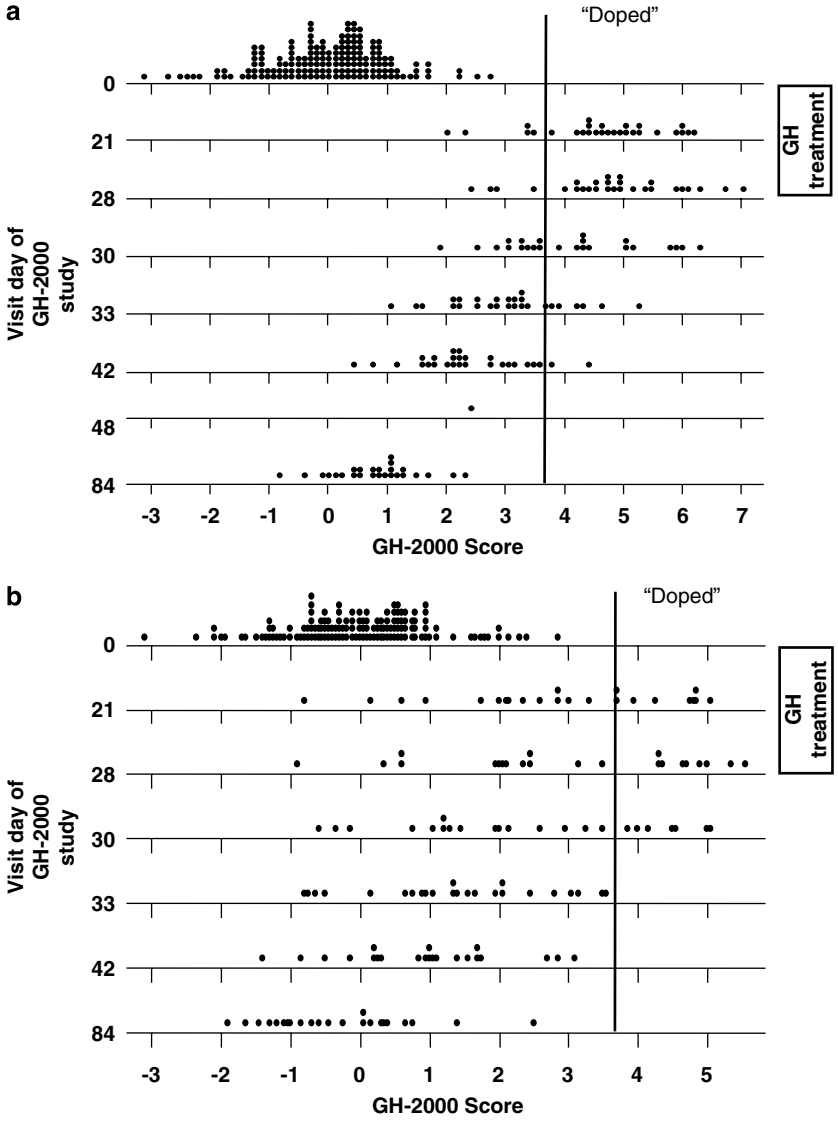

Figure 6 Change in GH-2000 score in men (**a**) and women (**b**) following 28 days of GH administration. Dotplot of the standardized scores for each visit day of the studies by group and data set. The mean of a normal population is 0 and the standard deviation is 1. Using a pre-defined sensitivity of 1 in 10 000, samples with a score of >3.7 are identified correctly as receiving GH (labelled as 'doped'). Note none of the baseline or placebo values are above 3.7.

subsequently World Anti-Doping Agency to prevent arbitrary changes being made to commercially owned immunoassays.

Apart from the assay development, these issues have largely been addressed by the GH-2004 study. A further cross-sectional study of elite athletes has shown 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 the values lie within the 99% prediction intervals for white European athletes, regardless of ethnic background. A further double-blind GH administration study suggests that the response to GH in other ethnic groups is similar to white European amateur athletes. The effect of injury was systemically examined by the GH-2004 team who followed 143 men and 40 women following a sporting injury. There was no change in IGF-I over the 12-week follow-up, but P-III-P increased by approximately 20%, reaching a peak 2–3 weeks after injury. This, however, did not cause any false-positive readings in the proposed test combining IGF-I with P-III-P.

Several other groups have also examined the use of GH-dependent markers, the first of which pre-dated the GH-2000 study. In this first study, the ratio of IGFBP-2 to IGFBP-3 was found to discriminate between those taking GH or placebo (Kicman *et al.*, 1997). These findings were not supported by the GH-2000 study, although several other groups have confirmed the utility of IGF-I and P-III-P. The Institut für Dopinganalytik und Sportbiochemie in Kreischa, Germany, undertook a 14-day GH administration study in amateur male athletes and derived a discriminant function based on IGF-I, P-III-P and IGFBP-3 (Kniess *et al.*, 2003). Most recently, the Australian Japanese Consortium presented the results of an 8-week GH administration study at the American Endocrine Society meeting in Toronto in June 2007. This also confirmed the value of IGF-I and P-III-P, although this suggested that an alternative bone marker (carboxyterminal cross-linked telopeptide of type I collagen) might provide better discrimination during the wash-out phase. This study also examined the effect of co-administration of anabolic steroids in males and showed that there were additive effects on P-III-P.

These confirmatory studies are important because it is unknown how well these GH-2000 formulae will perform in 'real life', where the patterns and doses of GH abused by athletes are unclear. When the male GH-2000 formula was applied to an independent data set 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, findings that were identical to those of the GH-2000 data when the formula was used (Erotokritou-Mulligan *et al.*, 2007).

Although this methodology has been rigorously tested, the development of World Anti-Doping Agency-owned immunoassays has lagged behind the science underpinning the method, despite the International Olympic Committee having been made aware of the need for these assays before the worldwide introduction of this test.

Future technologies to detect GH

Surface plasmon resonance

Surface plasmon technology is a non-labelled optical methodology that measures the refractive index of small quantities of a material absorbed onto a metal surface allowing measurement of mass. This technology is being applied for the detection of GH and dependent markers by the Barcelona anti-doping laboratory, but at present does not yield the same sensitivity as conventional immunoassays.

Mass spectrometry

Surface-enhanced laser desorption/ionization–time-of-flight mass spectrometry is a proteomic technique in which proteins are bound to proprietary protein chips with different types of adsorptive surfaces. It can be used to analyse peptide and protein expression patterns in a variety of clinical and biological samples, and biomarker discovery can be achieved by comparing the protein profiles obtained from control and patient groups to elucidate differences in protein expression. This technique has been applied to the detection of GH abuse to find potential new markers of GH abuse, such as the haemoglobin α-chain (Chung *et al.*, 2006), but the sensitivity is insufficient to deal with analyses of IGF-I and P-III-P.

The detection of IGF-I

At present, there are no technologies to detect IGF-I abuse, but it is reasonable to adopt a similar approach as the GH-dependent marker test, and this is currently being evaluated by the GH-2004 team. Similarly, this approach should detect athletes abusing GH secretagogues.

The detection of insulin

The challenges of detecting insulin are in many ways similar to GH in that insulin is a naturally occurring pulsatile peptide hormone. At present, there are no methods to detect endogenous insulin abuse but urinary mass spectroscopy may be useful to detect the presence of analogue insulin. This technique involves concentration of the urine and followed by isolation by immunoaffinity chromatography. The eluate may then be analysed using microbore liquid chromatography/tandem mass spectrometry that produces characteristic product spectra obtained from the analogues that are distinguishable from human insulin (Thevis *et al.*, 2006; Thomas *et al.*, 2007). Some insulin analogues are handled differently from insulin and excreted in much higher quantities, which may facilitate this approach (Tompkins *et al.*, 1981).

Future challenges

A major challenge for the future is the use of gene doping, where DNA is incorporated into target tissues, such as

skeletal muscle, with or without the aid of a vector, such as an adenovirus. Expression of the gene may then lead to enhanced local production of an anabolic substance such as IGF-I.

Proof-of-concept experiments have been undertaken in animals in which injection of a recombinant adeno-associated virus genetically manipulated to induce myocyte overexpression of IGF-I in young mice induced a 15% increase in muscle mass and a 14% increase in muscle strength without inducing a systemic increase in IGF-I (Barton-Davis *et al.*, 1998).

It is unclear whether this technology is being used by athletes; certainly, it has not been used in clinical practice, where it is highly needed despite significant investment. Anecdotal evidence, however, suggests that athletes are considering its use and this would provide new challenges to the anti-doping community. Traditional blood and urine testing may be of no benefit if gene doping causes no change in serum concentrations of the relevant proteins. Although the detection of vectors may be possible and changes in blood markers may occur, new technologies will be needed to catch this new form of doping.

Conclusion

Doping with GH and its related protein IGF-I remains a major challenge for those working in anti-doping. Anecdotal evidence suggests that abuse with GH and insulin is common, whereas the abuse of IGF-I is set to increase. There are compelling physiological reasons to explain why GH may have performance benefits. The athletes are risking long-term harm by using these drugs. Over the last decade, there have been major advances in methodologies to detect GH, and this should mean that once World Anti-Doping Agency has established suitable assays for IGF-I and P-III-P in its worldwide network of laboratories, athletes will no longer be able to cheat by taking GH without being caught.

Acknowledgements

The GH-2004 project is funded by the United States Anti-Doping Agency and the World Anti-Doping Agency. Our thanks go to the rest of the GH-2004 team: Eryl Bassett, Ioulietta Erotokritou-Mulligan, David Cowan, Christiaan Bartlett and Cathy McHugh. The GH-2004 study was undertaken in the Wellcome Trust Clinical Research Facility (WT-CRF) at Southampton General Hospital and we acknowledge the support of the WT-CRF nurses and Southampton medical students who have supported the study. We also pay tribute to our scientific collaborators, Astrid Kniess, Ken Ho, Anne Nelson, Christian Strasburger and Martin Bidlingmaier.

Conflict of interest

RIGH and PHS have received research funding from the World Anti-Doping Agency and US Anti-Doping Agency.

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