**The Use and Abuse of Growth Hormone in Sports**

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Abstract

GH is banned by the World Anti-Doping Agency (WADA) as a performance-enhancing anabolic agent. Doping with GH likely began in the early 1980s and become more prevalent with the advent of recombinant technology well before any scientific evidence of benefit. The expectation that GH improves physical function stems from its anabolic and lipolytic properties. Athletic performance depends on muscle strength and the energy required to power muscle function. In recreational athletes, GH selectively improves anaerobic sprint capacity but has not been proven to significantly enhance muscle strength, power nor VO2 max. GH is secreted as a family of isoform peptides in a pulsatile manner reflecting intermittent secretion and rapid clearance. Its anabolic actions are largely mediated by IGF-I, which stimulates whole body protein synthesis including skeletal muscle and collagen proteins. Two methods have been validated for detecting GH abuse in athletes. The first (Isoform Method) is based on distinguishing pure recombinant 22 kD GH from the heterogeneous isoforms secreted from the pituitary. The second (Marker Method) is based on measuring blood levels of GH responsive proteins, specifically IGF-I and N-terminal propeptide of type III collagen. Only a handful of athletes have been caught since the implementation of GH doping tests in 2004. The low rate likely reflects the limitation of in-competition testing using current methods. Improved detection rates may be achieved by more out-of-competition testing, introducing athletes’ biological passports and the development of novel methods. Governance, operational, technical and political factors influence the effectiveness of an antidoping program.

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# Introduction

Cheating in order to win a sporting event is as old as competition itself. The earliest records of doping in sport come from the Ancient Olympics games when athletes are reported to have taken figs to improve their performance (1). With the advent of modern pharmacology in the 20th century, many athletes began to experiment with cocktails of drugs to improve strength and overcome fatigue with little thought given to potential risk. Recombinant technology brought a new dimension of peptide hormone abuse, such as GH, to sports. Along-side these developments, has been the establishment of doping control in an attempt to stamp out cheating and provide protection against potential harm.

# History

## Growth Hormone

It is uncertain when athletes first started to use GH. It was initially promoted as a performance-enhancing drug in the first edition of ‘The Underground Steroid Handbook’ (2). It described GH as the newest and most potent anabolic substance of interest to athletes. This was seven years before the results of the first two randomised double-blind controlled trials of GH administration to people with GH deficiency were published in the peer-reviewed scientific literature. These trials showed marked effects on body composition (3,4) but by then, GH had become widely known in elite sport as a ‘doping agent’ often used in combination with testosterone or other androgenic anabolic steroids.

GH abuse in sport was brought to a worldwide audience following the enquiry into Ben Johnson’s disqualification in the Seoul Olympic Games of 1988 when he beat his arch rival Carl Lewis to win gold in the 100 metres only to lose the medal a few days later when his urine tested positive for the anabolic steroid stanozolol; he later admitted in court to have taken GH as well (5-7).

The popularity of GH as a drug of abuse in sport grew rapidly because it was undetectable by IOC Anti-Doping Laboratories even though there was no scientific evidence of performance benefit. In addition to the confession of Ben Johnson, other events pointed to its widespread abuse. Athletes had been caught smuggling vials of GH into countries where games are held (8); it disappeared from the manufacturer’s production line, became targeted in burglaries of pharmacies and even pharmaceutical distribution trucks and there was at least one instance where the mother of a child with GH-deficiency re-sold her son’s supply to an athlete (9).

## Anabolic Androgenic Steroids

The abuse of anabolic androgenic steroids (AAS) preceded that of GH by several decades and was well established by the time GH was first used. The first well-documented use of testosterone as a performance-enhancing drug was with the German rowing team in 1952 (10).

A program of state sponsored doping with AAS propelled East Germany (the former German Democratic Republic) from obscurity to prominence as a major sporting nation garnering a slew of gold medals and achieving 3rd place in the 1972 Munich Olympics medal table. The performance enhancing effects of AAS are exemplified by the chronology of shotput distances. Between 1956 and 1980, the winning distance in the Olympic women’s shot put increased from 15.28 m to 22.41 m. Since the introduction of effective testing in the early 1980s, the winning distance in the women’s shot put has fallen progressively and plateaued to the point where the winner in Rio de Janeiro in 2016 would not have made the final in Moscow in 1980 (Figure 1). Testosterone and other AAS are among the most commonly abused performance-enhancing drugs representing 44% of Adverse Analytical Findings in WADA-accredited laboratories in 2017 (https://www.wada-ama.org/sites/default/files/resources/files/2017\_anti-doping\_testing\_figures\_en\_0.pdf.)

# Doping Control

It was the deaths of the Danish cyclist Knud Jensen at the Rome Olympiad in 1960 and the British cyclist Tommy Simpson before the cameras in the 1967 Tour de France that finally signalled the need to control the abuse of substances in the quest of winning.

## The International Olympic Committee (IOC) Medical Commission

In response to the growing threat and adverse publicity of doping the IOC formed a Medical Commission in 1967 to establish an effective world-wide anti-doping system. The IOC established the first laboratory in Los Angeles, gradually developing a global network of accredited laboratories. The IOC Medical Commission established a list of banned substances that was regularly updated and known as The Prohibited List.

## The World Anti-Doping Agency (WADA)

Potential conflicts of interest between the organisers of the Olympic Games and the system run to prevent doping led to the creation of the World Anti-Doping Agency (WADA) in 1999. WADA was constituted and funded equally by the sport’s movement (largely the IOC) and national governments. WADA is responsible for funding and managing the network of IOC laboratories, running the whole of the world-wide anti-doping system.

### WADA Code

WADA has also taken over from the IOC in updating the List of Prohibited Substances annually (11). The decision to include a substance or method in the Prohibited List depends on the substance meeting two of three criteria:

* *The substance or method enhances sporting performance or has the potential to do so*
* *The substance or method may cause harm to the athlete or has the potential to do so*
* *The substance or method violates the spirit of sport*

From an endocrine perspective, the drugs appearing on the 2018 WADA List of Prohibited Substances are classified into four categories (Table 1). ‘GH, its fragments and releasing factors’ are listed in the second category under ‘Peptide Hormones, Growth Factors, Related Substances and Mimetics’.

**Therapeutic Use Exemption**

Recognizing that many of the listed hormones are used to treat medical conditions, WADA allows the use of banned substances for legitimate health indications through the process of Therapeutic Use Exemption (TUE). TUE is recognized for 19 conditions and this includes hormone-deficient states such as GH deficiency and hypogonadism as listed in Table 2.

# Physiology of Growth Hormone

## Secretion

Growth Hormone is produced and secreted in an episodic manner in multiple forms from the anterior pituitary gland, the major component being a fully translated 22 kD protein from the GH gene. It is this form that is produced by genetic engineering, also referred to as recombinant human GH.

## Biochemistry

### Growth Hormone Gene

Pituitary GH is the product of the hGH-N gene, which is one of a family of five highly conserved genes located on the long arm of human chromosome 17q22-24. The other four genes are somatomammotropin (hCS): hCS-L, hCS-A, hGH-V, and hCS-B.(12). The GH-N gene is expressed primarily in pituitary somatotroph cells but also to a very minor extent in the brain, immune cells, reproductive tract (breast, ovary, testis, prostate) gastrointestinal system and the lungs (12). Extra-pituitary GH subserves developmental and proliferative function acting in an autocrine and paracrine manner and insufficient to function in an endocrine manner. Extra-pituitary GH expression is strongly implicated in neoplastic transformation of breast, prostate and colonic neoplasms (13,14).

The hGH-N gene codes for a 22-kD (191–amino acid) protein, which is synthesized, stored, and secreted by somatotroph cells. In addition to 22 kD protein, the hGH-N gene produces different forms of GH through alternative splicing and post-translational modification and after secretion by proteolytic cleavage (15). A 20 kD GH isoform is produced from alternate splicing that excludes amino acid residues 32-46. Following translation, both isoforms may undergo further biochemical modifications by deamidation, acylation and glycosylation giving rise to ‘acidic’ forms of GH. After secretion, GH isoforms can form dimers and oligomers in plasma. The approximate proportions of 22 kD and 20 kD GH, their modified acidic, dimeric and oligomeric forms in the circulation are shown in Figure 2. Thus pituitary GH is not a single protein but a family of proteins derived from a single gene through transcriptional and translational modification (15).

The hGH-V, expressed exclusively in placental syncytiotrophoblasts, encodes a 22-kDa protein that emerges from mid-gestation. As hGH-V levels increase, pituitary GH secretion declines progressively from feedback regulation of the maternal hypothalamic-pituitary axis. Postpartum circulating hGH-V levels drop rapidly and are undetectable 1 hour after delivery (15).

## Clinical Chemistry

GH secretion is pulsatile and exhibits a diurnal rhythm with approximately two thirds of the total daily GH secretion produced at night triggered by the onset of slow-wave sleep. Secretory episodes are separated by troughs of minimal basal secretion during which GH is undetectable for more than 50% of a 24-hour period. Therefore, the amount of GH secreted cannot be gauged from a single blood measurement.

The amount of GH secreted varies throughout the lifespan. GH output rises at the onset of puberty increasing by two to threefold at late puberty and declines exponentially during the third decade of life, then progressively with advancing age to about 50% of that observed in the third decade (16). On average, the daily production of GH in the prepubertal state is 200 to 600 µg/day, rising to 1000 to 1800 µg/day at the peak of puberty. Women secrete about 50% more GH than men (16).

### Growth Hormone Isoforms

The major 22 kD and 20K kD GH isoforms are secreted simultaneously generating parallel peaks and troughs throughout the day (17). Representative 24 hour profiles for these two isoforms are shown in Figure 3A. Both forms are released in relatively constant proportion with that of 20 kD form circulating in concentrations of 10-15% of 22 KD GH (17,18). The proportion is preserved during stimulation of GH by hypoglycaemia (17) or by exercise (19). The secretion of GH from the pituitary is subject to feedback inhibition by GH itself. Figure 3B shows the suppression of 20 kD GH in normal volunteers after administration of 22 kD GH (17). This feedback inhibition forms the basis of the isoform test for GH abuse in sports (see below).

### Growth Hormone Binding Protein

GH is bound to a high affinity binding protein (GHBP) in blood. GHBP is derived from the cleaved extracellular domain of the GH receptor. Although the function of GHBP is not fully understood, there is strong evidence that it modulates the pharmacokinetics and distribution of GH (20,21). GH dissociates freely from the GHBP in accordance with the law of mass action. About 50% of GH is bound at low physiological levels, but most of the GH is unbound during a secretory pulse (21). GHBP in blood is not GH regulated because the concentration is not different between people with GH deficiency, those with normal GH secretion and people with acromegaly nor affected by GH administration (22,23). Thus GHBP is not a marker of GH status or action.

### Metabolic Clearance Rate

Growth hormone has a half-life in plasma of approximately 14-18 minutes and a metabolic clearance rate of approximately 135 ml/kg/hr (24-26). Most GH is cleared through internalisation, after binding to the growth hormone receptor. A proportion is also cleared by the kidneys where GH is filtered by the glomerulus, reabsorbed in the proximal tubule and degraded (27)

20 kD GH has a slightly different pharmacokinetic profile because of its propensity to dimerise resulting in a slightly longer T1/2 of 19-25 minutes (17,19). The clearance of oligomeric forms is reduced in proportion to the degree of oligomerisation with half-lives of 27 and 45 minutes for dimeric and oligomeric GH (28).

Following subcutaneous injection 22 kD GH reaches a peak of between 3-4 hours, disappearing with a T1/2 of about 4 hours and remains elevated in blood for approximately 10-12 hours (29). For 20 kD GH, a peak concentration is reached after 3-4 hours and disappears with a T1/2 of 1.9 – 2.9 hours (30).

### Urine

Circulating GH passes through the glomerulus into the proximal renal tubule where almost all is reabsorbed but extensively degraded. Less than 0.01% of filtered GH is excreted in urine (31). Urinary GH excretion is highly variable even within the same individuals from day to day with CVs of up to 60% (32). Less than 0.005% of administered GH is excreted in urine (25,33). This extremely low excretion and high variability works against urine being a sensitive body fluid for detecting changes in blood concentration. The concentration of urine GH is very low, in the pg/mL range; more than 100 fold lower than that in plasma and below the sensitivity of commercial GH assays (34). Strenuous exercise increases the renal excretion of various blood proteins including GH (35). Renal disease and injury, acute and chronic, reduces the tubular capacity for reabsorption, increasing urine loss of proteins including GH (34,36). For, these reasons, urine is unlikely to be a suitable body fluid for detecting GH abuse (27).

## Regulation

### Hypothalamic Control

The hypothalamus controls GH secretion through a coordinated interplay of simulation by GHRH and inhibition by somatostatin. This principle control system is modulated by a host of peripheral hormonal, nutritional, metabolic and physical cues. One of the most significant regulators of GH secretion is ghrelin. Ghrelin is a 28–amino acid peptide, which binds to a receptor, distinct from that of GHRH, termed the GH secretagogue (GHS) receptor (37). Ghrelin is synthesized in peripheral tissues, especially gastric mucosal in neuroendocrine cells, as well as in the hypothalamus. The ghrelin system has a much broader function beyond the stimulation of GH secretion, regulating appetite, energy balance, sleep-wake rhythm, gastric motility, glucose homeostasis, cell growth and cardiac function.

Considerable effort has gone into developing analogues of GHRH and of ghrelin for enhancing GH secretion for the therapy of short stature and as anabolic and anti-obesity therapy and to prevent the decline in physical and metabolic health that occurs with aging (38).

GHRH acutely stimulates GH secretion in a dose dependently manner, an effect lasting 2-3 hours. Several long acting analogues of GHRH have been synthesized but very few have been successfully developed for clinical use (39). Tesamorelin, a 44 amino acid analogue, is approved for treatment of HIV associated abdominal obesity (40). CJC-1295, a 29 amino acid analogue was under investigation for the treatment of lipodystrophy and GH deficiency (41) but withdrawn after a phase II clinical trial as a precaution. GHRH and ghrelin analogues are of considerable interest in the sporting black market.

Ghrelin also stimulates GH secretion acutely in a dose-dependent manner, an effect lasting 2-3 hours. In addition to GH, ghrelin evokes the stimulation of prolactin and ACTH but to a lesser extent. Small peptides known as GH releasing peptides (GHRPs), synthesised in the 1980s before the discovery of ghrelin and the GHS receptor, have formed the basis for the development of ghrelin analogues, now available as injectable and oral formulations (38) (42). Hexarelin, GHRP2 (pralmorelin) and GHRP6 are administered as injection. Several orally active, and selective ghrelin receptor agonists have been developed, including anamorelin (43), ibutamoren (MK-677) (44), ipamorelin (45), and macimorelin (46). In older people, 2 years of treatment with MK677 an oral analogue increased IGF-I and lean mass but not muscle strength (47). Macimorelin, an orally active analogue, is under investigation as a pharmacological test for GH deficiency (46). The GH releasing properties and biological effects of GHRH and ghrelin analogues are attractive to the sporting black market (48). These classes of GH releasing agents are banned by the WADA (Table 3) and during 2016 and 2017, GHRPs were detected 23 times in WADA accredited laboratories, more frequently than GH administration was detected.

Somatotrophs are also regulated by cholinergic and adrenergic neural pathways. Drugs with cholinergic agonist properties, such as pyridostigmine (49), and with agonist properties, such as clonidine, have been of interest as potential long- term enhancers of GH secretion. Although pyridostigmine amplifies GH release evoked by GHRH, its sustained effects on GH secretion have not been assessed. Clonidine acutely stimulates GH secretion in children, however, long term therapy fails to enhance growth in children (50). Clonidine is a weak acute stimulus of GH secretion in adults (51). Overall, there is scant evidence that neurotransmitter manipulation of GH secretion is therapeutically effective.

### Physiological Factors

#### Gonadal Steroids

Gonadal steroids are major regulators of the GH-IGF axis. GH secretion and IGF-I are reduced in men with hypogonadism (52). Androgen replacement enhances GH secretion resulting in a parallel increase in blood IGF-I levels. The stimulation of GH secretion requires aromatization to estrogens because the effect is abrogated by estrogen receptor antagonism and does not occur with non-aromatizable androgens.

In women, androgens also stimulate GH secretion, an effect also mediated by aromatization to estrogen (53). In women, GH status is unaffected by the menstrual cycle. In hypogonadal women, the impact of estrogen treatment on GH secretion is dependent on the route of delivery. When administered by the oral route, estrogens reduce IGF-I and increase GH blood levels whereas this does not occur with a non-oral route (54). This phenomenon arises from a first-pass effect of estrogen, which inhibits hepatic production of IGF-I, reducing central feedback inhibition resulting in an enhancement of GH secretion (55). In contrast, androgens drive GH secretion centrally increasing blood IGF-I levels, an effect mediated by a paracrine effect of estrogen. Thus androgens and oral estrogens induce similar enhancing effects on GH secretion but divergent effects on IGF-I. The observations that androgens enhance while oral estrogens attenuate the anabolic effects of GH (55-57) provide further evidence that IGF-I is a pivotal anabolic mediator of the GH system.

#### Nutrition

Micronutrients and nutrition are major effectors of GH secretion. Glucose and free fatty acids suppress GH secretion. The GH-releasing properties of some amino acids also form the basis of their marketing as supplements that boost muscle growth and performance. Amino acids, such as arginine, lysine and ornithine, can stimulate GH release when infused intravenously or administered orally. The GH response to amino acid administration has a high degree of inter-individual variability and is influenced by training status, sex, age, and diet (58). High oral doses are required to stimulate GH release frequently causing stomach discomfort and diarrhea. Oral arginine administered before exercise does not augment GH release induced by exercise (59). There is no evidence that oral supplementation of amino acids, before strength training increases muscle mass and strength to a greater extent than strength training alone (60), although protein supplements immediately following exercise may aid recovery.

GH secretion is also affected by the fasted and fed states as well as the general nutritional state. Nutrient deprivation enhances GH secretion within 12 hours, doubling by 48 hours. Under these nutritional conditions, insulin concentrations fall leading to diminished hepatic IGF-I production and a reduction in feedback inhibition of GH release (61). The enhancement in GH output during nutrient deprivation is a biological response that results in stimulation of lipolysis and subsequently increased lipid utilisation resulting in the sparing of protein (9). In the fed state, GH secretion is suppressed by the central feedback effects of glucose, fatty acid, insulin and IGF-I. Obesity is associated with a similar profile of reduced GH output, reversible with weight loss (62). The close relationship between GH secretion and insulin is a reflection of its important role as a metabolic hormone, regulating energy metabolism, substrate partitioning, usage and storage and the sparing of protein during nutrient deprivation.

#### Exercise

Exercise is a potent physiologic stimulus for GH release. There has been intensive study on the mechanisms and significance of exercise and GH secretion. Both resistance and endurance exercise enhance GH secretion with intensity and frequency being important factors (63). An exercise intensity above lactate threshold and for a minimum of 10 minutes appears to elicit the greatest stimulus to the secretion of GH. A program of endurance training at or above the lactate threshold enhanced pulsatile GH secretion (64). The mechanisms are not fully understood but include neural input, neuromuscular activity, a rise in lactate and in core body temperature (63,65). A less well-known effect of GH is the stimulation of sweat gland secretion important for thermoregulation. Skin exocrine function is reversibly impaired in adults with GH deficiency, who demonstrate a greater increase in core body temperature during physical exertion, limiting exercise tolerance (66). GH supplementation in healthy adults enhances lipolysis (67) and reduces oxidative loss of protein (68) during exercise providing evidence that exercise-evoked GH secretion brings metabolic and homeostatic benefits.

## Action

Daughaday made the seminal discovery that the growth promoting action of GH is mediated by the generation from the liver of IGF-I then called somatomedin (later shown to be the same as non-suppressible insulin-like activity), giving rise to the somatomedin hypothesis (69). The hypothesis that IGF-I was produced solely from the liver was challenged by in-vitro evidence that several tissues produced this growth factor (70). Not long after, Issakson and colleagues reported that local administration of human GH in-vivo to the cartilage growth plate of hypophysectomized rats accelerated longitudinal bone growth from local IGFI production (71,72). Elegant tissue specific knock out studies have since established that GH exerts regulatory control of body and tissue growth through a combination of endocrine and paracrine actions mediated by IGF-I (69).

GH also exerts actions that are independent of IGF-I. The lipolytic, gluconeogenic and anti-natriuretic actions do not involve IGF-I mediation in several tissues. Forearm studies in humans employing arteriovenous measurements have revealed that GH stimulates amino acid uptake directly into muscle (73). This effect occurs within 3-6 hours of a GH infusion without a change in IGF-I concentrations. Thus GH elaborates a range of effects on metabolism, body systems and body growth integrated through a mix of direct and indirect IGF-I-mediated action.

### Blood Markers of GH Action

Growth Hormone stimulates the secretion of a number of proteins from the liver and from peripheral tissues. Many GH responsive proteins have been investigated for their potential as sensitive and specific discriminants of GH use. These include IGF-I, IGF binding protein 3 (IGFBP3) and Acid Labile Subunit (ALS) produced from the liver and the collagen proteins N-terminal pro-peptide of type 1 procollagen (PINP), N-terminal pro-peptide of type III collagen (PIIINP) and Carboxyterminal telopeptide of type I collagen (ICTP) from bone and connective tissue. (74). Figure 4 shows a schematic representation of the stimulation of components of the IGF system and of bone proteins by endogenous or exogenous GH. In elite athletes, demographic factors account for significant variability in the blood levels of these markers, all falling significantly with age (75,76). Age was the major contribution to the variability, equivalent to >80% of the attributable variation in IGF-I and the collagen markers. Gender was the next major factor with the IGF axis markers all being significantly higher in women by 10-15%, and the collagen markers significantly higher in men by 3-10% (75,76). Ethnicity and sport type exert a minor influence of less than 5% (76). Therefore, a test based on IGF-I and the collagen markers must take age into account for men and women, while ethnicity and sport type are unlikely to be confounders for these markers.

### Protein Metabolism

Protein mass is not static but constantly turning over in a dynamic process of loss and synthesis. Approximately 0.6% of total body nitrogen is replenished each day, indicating total body protein is replaced every 160 days. Tracer studies employing steady state methodology have given insights into how GH regulates whole body protein anabolism. Whole body metabolism can be broken into three components: proteolysis, oxidation and synthesis in which the amino acid pools derived from proteolysis are oxidised or resynthesised into protein. Oxidation of amino acids provides a source of energy but represents irreversible loss of protein; it is therefore the hallmark of catabolism. GH directs amino acids from oxidative towards synthetic pathways (77). By contrast, insulin exerts an anti-catabolic action by reducing the rate of proteolysis (78,79). IGF-I elicits effects that are similar to GH, stimulating protein synthesis and reducing oxidation (80). This observation provides strong evidence that IGF-I plays an important role in mediating the effect of GH on whole body protein anabolism.

# Growth Hormone, Bioenergy and Skeletal Muscle Function

Skeletal muscles are specialised contractile tissues that control posture and physical activity while having an important role in energy metabolism. Their function is dependent on the composition and strength of fiber types that require energy to drive and sustain contractile work. The stimulation of muscle protein anabolism and growth by GH has led to widespread expectation that it increases muscle strength and power.

Muscle function is most commonly defined by strength and power, (81) endpoints reflecting overlapping but different aspects of muscle function. Strength is dependent on muscle size, type and properties of contractile proteins. Muscle power, a measure of work performed per unit time, is assessed in different ways that vary in duration. The energy required to support muscle work can be drawn from anaerobic or aerobic processes such as pre-formed stores or that generated from the oxidative metabolism of substrates (82). Muscle power is influenced by the availability of energy at the time of assessment. The recognition of mitochondrial myopathies as a class of functional muscle disorders arising from defects in mitochondrial respiratory chain enzymes highlights bioenergetics as an important mechanism influencing skeletal muscle function dependent on oxidative phosphorylation (83). The bioenergetics of muscle is an important player determining aspects of muscle function (84).

## Muscle Fiber

Skeletal muscle is composed of fibers that are made up of different proteins with distinct properties. Actin and myosin are functional proteins that subserve contractile function whereas tropomyosin and troponin are structural proteins that keep the contractile proteins in proper alignment and give muscle fibers elasticity and extensibility. Muscle fibers are classified by myosin heavy chain (MHC) isoforms mainly into two types. Type I fibers, also known as slow-twitch fibers, contain an abundance of mitochondria and rely on aerobic or oxidative pathways for energy. These fibers determine the endurance capacity of muscle. Type II fibers, also known as fast-twitch fibers, utilise energy from anaerobic or glycolytic pathways due to their low mitochondrial content. These fibers have high contractile force, but easy fatigue and thus support high intensity activities, such as high jump, weight lifting and sprinting.

There are few human studies investigating GH regulation of muscle fiber composition, and most of these entail small numbers. Most studies in adults with GH deficiency have reported no significant difference in fiber type distribution from matched healthy people (85,86). Fiber composition does not change significantly after six months of GH replacement therapy (86). There is insufficient evidence to support a role of GH in regulating type I or II fibers in human skeletal muscle.

## Bioenergetics of Muscle Function

The contractile function of skeletal muscle relies on an adequate supply of chemical energy. During muscle contraction, chemical energy is converted to mechanical energy that leads to movement. Figure 5 illustrates the metabolic processes involved in energy production in muscle and the concept of energy continuum during physical activity. In humans, chemical energy is available in the form of adenosine triphosphate (ATP), which is generated by two energy systems: anaerobic and aerobic (84,87). The anaerobic energy system relies on preformed ATP as phosphocreatine (PCr) stores or ATP production from anaerobic glycolysis, i.e. breakdown of glucose in the absence of oxygen. The aerobic energy system generates ATP from oxidation of metabolic fuels such as carbohydrates, lipids and proteins. In the cytoplasm, glycolysis leads to the production of pyruvate. In the absence of sufficient oxygen, pyruvate is reduced to lactate, which is released into the circulation and recycled to glucose in the liver. In tissues with adequate oxygen supply, pyruvate and fatty acid are converted to acetyl CoA in the mitochondria. Acetyl CoA undergoes oxidation via the tricarboxylic acid (TCA) cycle and the mitochondrial respiratory chain producing ATP. The amount of preformed ATP present in the muscle cells is only sufficient to sustain physical activity for the first 5-10 seconds; thereafter, anaerobic glycolysis provides energy for further 30-40seconds, when aerobic metabolism begins to take over and provides energy for prolonged sustained activity (84,87).

### Substrate Metabolism

Muscle function is dependent on the availability of metabolic fuels and its capacity to synthesize ATP. The energy synthesis from substrate utilization in exercising muscle is regulated by nutritional, genetic, and hormonal factors as well as physical training. GH stimulates lipolysis during resting condition (88) as well as exercise (89) leading to an increase in plasma fatty acid (FA) levels and consequently fat oxidation. GH also increases plasma glucose concentration by augmenting glycogenolysis (90) and gluconeogenesis (91). Thus, GH may enhance muscle function by increasing availability of FA and pyruvate as metabolic fuels for energy production. With exercise there is an increase in cardiac output and blood flow to exercising muscles. This local increase in perfusion can be substantial and it directs the supply of substrates to where they are most needed as well as helping clear lactate from the exercising muscle and transporting it to the liver for recycling into glucose (the Cori Cycle).

It is widely assumed that an increase in whole body lipid oxidation reflects the action of GH on skeletal muscle. This traditional thinking was challenged by studies in rodents as well as humans, suggesting GH action is tissue specific. Tollet-Egnell et al. reported that GH inhibits the expression of genes involved in lipid oxidation in skeletal muscle of rats (92). Evidence from a study of metabolic gene expression in skeletal muscle of adults with GH deficiency suggests that GH downregulates genes governing lipid metabolism (FA transport and β-oxidation) as well as, TCA cycle activity and mitochondrial respiration (93). For example, the expression of oxoglutarate dehydrogenase and succinate dehydrogenase complex B in the TCA cycle and ATP synthase and NADH (reduced nicotinamide adenine dinucleotide) dehydrogenase in the mitochondrial respiratory chain were reduced by up to 40%. Assuming these transcriptional changes reflect effects on protein expression, the findings suggest that GH inhibits oxidative metabolism of substrates favoring non-oxidative (anaerobic) pathways for ATP synthesis in skeletal muscle. This possibility is supported by a study in trained cyclists, in which GH use was associated with increased plasma lactate levels during moderate to intense exercise compared to placebo, implying an increased rate of anaerobic disposal of pyruvate (94).

In summary, GH effects on substrate metabolism are tissue specific. Recent evidence suggests that GH may promote non-oxidative or anaerobic substrate metabolism in skeletal muscle for ATP synthesis, findings contrary to its effects on whole body metabolism (95).

# Effects on Body Composition

In adults with GH-deficiency given replacement doses of GH, the stimulation of whole-body protein anabolism and of lipid utilisation translate into an increase of LBM and a diminution of fat mass (6,7). In a study that measured total body potassium to estimate changes in intracellular LBM in people with GH-deficiency treated with GH, this amounted to an approximately 5kg exchange of fat for LBM (7). The effects on body composition are highly reproducible and a consistent finding in children, adults and older people who are either GH deficient or sufficient (96). In young healthy adults, a meta-analysis of GH treatment drawn from 7 controlled trials of over 100 participants observed an average gain of 2.86 kg LBM and loss of 1.22 kg of fat mass (97). Another meta-analysis of 44 articles describing 27 study samples in 303 young fit adults who had received hGH reported that average LBM increased by 2.1 kg while fat mass decreased by -0.9 kg (98).

The LBM mass is a heterogeneous compartment comprising muscle, bone, viscera, connective tissue and fluid distributed in the extracellular and intracellular compartments. Most studies employ methods that do not allow the quantification of the sub-components of the lean compartment. A review of techniques for measurement of body composition is beyond the scope of this review. Conceptually, the fat-free compartment can be divided into bone and body cell mass and extracellular fluid. Thus, an apparent increase in lean mass can arise from an expanded extracellular fluid volume can be erroneously interpreted as a gain in muscle mass if the fluid component is not measured or known. This is a pertinent consideration in interpreting the effects of GH because of its potent sodium and thus fluid-retaining properties (99). In a study of recreational athletes, GH treatment for 8 weeks increased the lean mass by an average of 2.70 kg, of which approximately 70% was extracellular fluid (100). Thus, only 30% was attributable to a gain of body cell mass, of which muscle is albeit a major component. Changes in soft tissue composition and in particular the lean component require cautious appraisal and interpretation as the changes are unlikely always to reflect similar gains in muscle mass. Lean mass is not always muscle mass and interpretation of data based on imaging must bear the component of tissue fluid in mind.

# Effects on Physical Performance

This section reviews the outcomes of GH supplementation in athletes on four of the most common measures of physical performance: strength, power, endurance and sprint capacity. We provide information on the assessment methodologies and select double-blind placebo control trials for analysis, unless otherwise stated.

## Muscle Strength

Muscle strength is defined as maximal force (in newtons, N) or torque (in Newton-metres, N.m) that is generated by a muscle or a group of muscles during maximal voluntary contraction (MVC) (81). The force is determined by fast twitch type II muscle fibers relying on preformed ATP for energy (82). Muscle strength is commonly assessed by measuring the force or torque produced during an isometric or isokinetic contraction. Isometric strength is the MVC that can be developed against an immovable object without a change in joint angle, whilst isokinetic strength is a measure of torque/force through a range of motion in which limb is moving at a constant velocity (81).

There is clear evidence that long-term replacement of GH normalises muscle strength in adults with GH deficiency who have reduced isometric and isokinetic muscle strength (84). Whether the benefits are also seen in fit young adults, has been investigated in three double -blind placebo- controlled studies. involving 83 healthy young adults treated with approximately 2-3 mg/d of GH for between 6-12 weeks. These studies assessed biceps strength (101), quadriceps strength (101), the strength of seven muscle groups (102) and isometric dead lift (100). These studies reported no significant effect of GH on muscle strength (97) (Figure 6).

## Effect on Power

Muscle power is defined as work performed per unit of time and is expressed in joules per second or Watts. It is described in terms of aerobic and anaerobic power, depending on which energy source is predominantly used to do the work. Thus, muscle power can be assessed by measuring aerobic exercise capacity and anaerobic exercise capacity (84).

### Aerobic exercise capacity

Aerobic exercise capacity is a measure of endurance, i.e. the muscle’s ability to sustain work for prolonged period with energy provided principally from mitochondrial oxidation of substrates. In the athletic world, it supports activities such as marathon, football, tennis, while in day-to-day life, it relates to activities such as walking. It is determined by the measurement of maximal oxygen uptake (VO2max) in L/min or ml/kg/min or maximal aerobic power output in Watts or kilojoules during an incremental exercise test on a cycle ergometer or a treadmill (103).

Numerous double-blind placebo-controlled and long-term open label trials have reported the positive effects on aerobic exercise capacity in adults with GH deficiency (104). However, there is no convincing evidence that VO2max is affected by GH treatment in healthy young adults (84). Based on a review of three double-blind placebo-controlled studies assessing GH treatment in over 100 participants with doses of approximately 2-3 mg/d, there was no treatment effect over placebo (97) (Figure 6). The data indicate that GH supplementation in the doses used do not improve cardio-respiratory and muscle function in young healthy adults. Interestingly, in a double-blind placebo-controlled randomised trial in 56 recreational athletes, the administration of rhIGF-I combined with rhIGFBP-3 led to a 7% increase in VO2 max with no significant change in body composition (105). The reason(s) for the different outcomes between GH and IGF-I supplementation are unknown but merit further study.

### Anaerobic exercise capacity

Anaerobic exercise capacity is defined as the total amount of work performed during a maximal exhausting exercise of a short duration, that is powered by ATP supplied under anaerobic conditions (106). The Wingate test, which measures maximal power output over 30 seconds by cycle ergometry, is a widely used test of anaerobic capacity. Sporting activities that require short-term high intensity physical activity, such as sprinting, require considerable energy support from anaerobic ATP. All physical activities including activities of daily living also depend on anaerobic energy for initiation, for the first few seconds, before aerobic metabolism becomes the predominant energy source (107,108). Only one study has investigated the effects of GH on anaerobic exercise capacity (100). This double-blind placebo-controlled study in recreational athletes reported a significant improvement of 3.8% in anaerobic exercise capacity after GH therapy for 8 weeks, as assessed by the Wingate test. If translated to proportionate time reductions, the 3.8% could equate to an improvement of 0.4 second in a 10-second sprint over 100 meters or of 1.2 seconds in a 30-second swim over 50 meters (100). This improvement occurred without a significant change in body cell mass nor in muscle strength and power (jump height) suggesting that muscle anabolism is unlikely to explain the improvement in sprint capacity (Figure 7). Jump height represents instantaneous work whereas the Wingate test involves all-out intensive exercise on a cycle ergometer for 30 seconds. Although both tests measure anaerobic power, the energy required for jumping is drawn from phosphocreatine stores while that for the longer Wingate test, from phosphocreatine stores and ATP derived from glycolysis. Stimulation of ATP generation from anaerobic glycolysis enhances the production of lactate. The finding of higher lactate concentrations in people undergoing evaluation of physical capacity after GH treatment (109) provides evidence that the anaerobic energy system is stimulated by GH. In the study by Meinhardt and colleagues (100), GH treatment significantly improved sprint capacity without affecting muscle strength or aerobic capacity in the same athletes under the same conditions. Along with previous studies in athletes reporting that GH treatment did not improve muscle strength (101,102) nor endurance (109,110), the collective evidence indicate that GH exerts a selective ergogenic effect on sprint capacity.

These results in athletes stand in contrast to a report by Graham and colleagues who undertook the first study investigating whether GH improved physical function (111). They evaluated the impact of 6 days of GH treatment in men withdrawn from chronic anabolic-androgenic steroid use. They observed a beneficial effect on strength, endurance and sprint capacity compared to a similar group who did not receive GH. Conducted as an open label study, a placebo effect (see below) cannot be ruled out. The observation that GH may improve aspects of physical performance in a setting of abstinent anabolic steroid dependency requires confirmation in a blinded-placebo-controlled study.

## Dose and Duration

The collective published information on the performance outcomes of GH treatment are limited by the dose and duration of treatment and evaluation (84). These studies employ GH doses from 15-180 μg/day for up to 12 weeks. The study that detected an improvement in sprint capacity employed a dose of 2 mg/day, approximating 28 μg/kg/day for a 70 kg person for 8 weeks. The dose corresponds to about 2-3 times daily production rates in young adults. It is possible that higher doses for longer periods may have induced a greater effect on sprint capacity or a measurable improvement in strength and endurance. Conversely the ability to detect a small effect requires a much larger sample size. It is not known what doses are used covertly for doping and the cocktails with other substances, including anabolic steroids nor their combined effects.

### Polypharmacy

It is widely known that athletes commonly dope with a cocktail of prohibited substances and rarely with a single agent. When Dwain Chambers was caught doping he decided to aid UK Sport by ‘coming clean’ and describing his methods of doping with multiple drugs including GH supplied to him by Victor Conte of the infamous Bay Area Co-operative (BALCO) (112).

*What Chambers took...*

*THG - A previously undetectable designer steroid nicknamed 'the clear’. Used in off-season to accelerate healing and tissue repair.*

*Testosterone/epitestosterone - Again used during off-season. Rubbed into the skin, the substance was used to offset suppression of the naturally produced testosterone caused by the use of THG.
EPO (Erythropoietin) - A drug used predominantly by endurance athletes, it was injected once a week in the off-season to increase red blood cell count and oxygen carrying ability.*

 *hGH (Human Growth Hormone) - Injected three times a week by Chambers to aid muscle growth in off season.*

 *Insulin - Although primarily associated with the treatment of diabetes, Chambers used the substance after heavy weight sessions to speed up the transportation of sugar.*

 *Modafinil - Chambers would take a 200mg tablet to boost alertness and overcome fatigue.*

 *Liothyronine - produced naturally by the body’s thyroid gland, it was used to increase Chambers’ basic metabolic rate before races.*

This remarkable confession gives insight into the polypharmacy not uncommon in doping and known as “stacking”. The individual programmes are usually determined by the coaches and are pragmatic rather than scientific but based on experience, hearsay and ‘trials of one’ (113). From a medical perspective, although there may well be short-term gains in physical performance there must also be considerable short and long-term risks to health (114). There may be endocrine reasons behind some ‘stacking’ protocols – thus the combined administration of GH, testosterone and insulin is likely to have a greater effect than one component alone since physiologically they work synergistically in building and maintaining lean body mass (9,96).

## Sex Steroid Modulation

Sex steroids modulate the metabolic action of GH, with estrogens inhibiting and androgens enhancing. In women with GH deficiency, a greater dose of GH is required to normalise blood IGF-I levels. There is gender dimorphism on body composition in that the gains in lean mass and loss of fat mass with GH replacement are attenuated in women (53). In GH deficiency, the magnitude in gain of lean mass and loss of fat mass is less in women than in men for the same if not higher replacement dose of GH (115). In recreational athletes, the stimulation of IGF-I and collagen proteins by the same GH dose is greater in men than in women and amplified by co-administration of testosterone (116). In normal men, the anabolic effects of testosterone and GH on anabolism and lipolysis are additive (55) respectively increasing lean and reducing fat mass (100). Studies in older men have reported significant improvement in strength and endurance from combined treatment with GH and testosterone where GH alone was without significant effect (117,118). In athletes, six weeks of GH treatment improved sprint capacity in men, an effect that more than doubled by co-administration of testosterone (100). Thus there is strong evidence that testosterone enhances the action of GH. There are no published studies investigating the impact of estrogen on physical performance during GH therapy.

**Recovery from musculoskeletal injury**

GH may accelerate recovery from soft-tissue injury, based on the known effects of GH on connective tissue formation, as indicated by an increase in collagen synthesis markers (116,119). In animals, tendons heal faster after treatment with IGF-I, which increases following GH treatment (120). In healthy young men, 14 days of GH treatment increased matrix collagen synthesis by up to six-fold in skeletal muscle and tendon (121). The increased synthesis in muscle and tendon collagen suggests that GH could strengthen the supporting connective tissue of muscle.

## Psychological Benefits

There may be a psychological effect of substance administration through a placebo effect. It refers to a favorable outcome arising purely from the belief that one has received a beneficial treatment (122). Placebo treatment can modulate pain pathways, increase endogenous opioids, and influence the neuroendocrine and immune systems (123). Placebo treatment increases physical performance, pain endurance, and reduce muscle-fatigue perception (123-125). In their double-blind controlled study, Meinhardt et al evaluated the perceived and actual benefits in people allocated to placebo treatment. All participants completed a self-evaluation questionnaire along with physical performance testing before and after 8 weeks of treatment. The questionnaire inquired whether the participant thought they were on placebo or GH treatment and what impact they thought the treatment had on their performance, without knowledge of the performance data (126). Mean perceived performance scores were higher for incorrect guessers compared to correct guessers, however, there was a trend to significance only for sprint capacity (Figure 8). Mean changes in measured performance were higher in those who thought they were on GH with a significant increase for jump height (p=0.05). Nearly three times more men than women believed they were on active treatment (81% vs 31%). Compared to baseline, men who guessed incorrectly had significantly improved self-assessed scores (p<0.03) for all categories and also increased measured performance for VO2 max (p=0.03) and strength (p=0.06). For women, there were no significantly greater outcomes for those who guessed incorrectly compared to correctly. In short, athletes who believed they were on active treatment not only had a perceived improvement on performance, but also in measured physical performance. The effect was greater in men. In conclusion, a placebo effect may contribute to perceived and actual performance-enhancing effects of GH, particularly in men (126). However, GH treatment only imparted a beneficial effect on sprint capacity compared to placebo.

## Adverse Effects

The adverse effects from GH arise from its anti-natriuretic, metabolic and growth-promoting properties (127). Most of the acute adverse effects reported in healthy adults arise from fluid retention. These include edema, carpal tunnel syndrome, myalgia and arthralgias. The severity of these adverse effects may be worsened by concurrent abuse of anabolic steroids, with the magnitude of fluid retention (128) and the development of cardiac hypertrophy and myopathy (129,130).

Acromegaly is an appropriate model to gauge the potential harm of long-term GH excess. Among the long-term complications of acromegaly are cardiac hypertrophy, cardiomyopathy, muscle weakness (131,132), hypertension (133), arthropathy, (134,135) diabetes (134) and possible increased risk of malignancy (136). The abuse of GH carries an increased health risk, which could be even greater when taken as a cocktail with other substances. It is not possible to quantify the degree of harm because of the covert nature of the drug-abuse culture and the ethical difficulties of undertaking research in this area (127). Educational programs highlighting the dangers of drug abuse have been established by organizational and governance bodies and is an approach strongly endorsed by the WADA.

# Detecting Growth Hormone Abuse

Detecting GH abuse is not easy for several reasons (137):

* rhGH has identical structure to native GH
* GH has a short half-life
* GH is secreted in a pulsatile fashion with high peak and low trough values
* Exercise and stress, the situations in which anti-doping testing occur, are potent stimulators of GH secretion

There are two methods currently approved by WADA for detecting GH abuse.

## The Isoform Method

The Isoform method developed by Strasburger, Bidlingmaier and Wu in Germany (138) was the first WADA-validated method for detecting GH abuse. As discussed above, endogenous GH secretion involves in multiple isoforms while recombinant hGH pharmaceutical preparations contain only the 22-kDa isoform. The Isoform method is based on distinguishing pure recombinant 22kD GH from the heterogeneous isoforms secreted from the pituitary, which are suppressed after rhGH administration through negative feedback. The method depends on carefully selected antibodies to GH, one that is specific for the 22kDa isoform and another that does not distinguish between the isoforms and thus measures ‘total’ GH. The ratio between the two measures is used to detect the presence of rhGH (Figure 9).

WADA regulations stipulate that where immunoassays are used to detect analytes, two immunoassays for each analyte are required to ensure specificity of the assays. Thus, the test is marketed in kit forms A & B with two independent assays for 22 kD GH and total GH. The ratio between the 22 kD GH and total GH has to exceed given figures (that differ for kits A & B and between men and women) for both kits to result in an adverse analytical finding (positive result).

The isoform method was introduced during the Athens Olympic Games in 2004 but it was not until February 2010 after analysing more than 1000 samples that the first positive occurred (137). The very short window of opportunity of the method means that the only chance of catching a cheat is to perform the blood sampling up to 30 hours of an injection of rhGH (139). Another disadvantage of the method is that it cannot detect abuse of GH extracted from cadavers (which contains all the GH isoforms), which is believed to still be in circulation on the black market and has been confiscated by customs officers.

Despite the limitations, the Isoform Method is an endorsed and established method for detecting GH abuse. Technical advances are being developed to improve the availability, sustainability and cost of antibody production (140).

## The GH-2000 Bio-marker test

The GH-2000 bio-marker method was initially developed by the GH-2000 multi-national research team. The research was completed in March 1999 but political issues within IOC and concerns about the potential effects of ethnicity and injury prevented the test from being implemented at the Sydney Olympic Games (Sonksen 2009). After a short hiatus, further work was undertaken by the GH-2004 team based at the University of Southampton. These studies confirmed the applicability of the test to a worldwide sporting community, investigated the effects of injury, described the optimal pre-analytical conditions and developed decision limits for a variety of immunoassays to be used by WADA-accredited laboratories. The test was finally introduced at the 2012 Olympic and Paralympic Games in London where it caught two power lifters who were missed by the isoform method (141).

The method depends on the measurement of two GH-sensitive bio-markers, IGF-I and P-III-NP both of which increase in a dose-dependent manner after GH administration and can remain elevated for as long as a month after GH administration stops (142). The method depends on a discriminant function (one for men and one for women) that includes serum IGF-I and P-III-NP concentrations and athlete’s age. If the result of this equation exceeds a limit set at a probability of 1:10,000 risk of a false-positive, it becomes an Adverse Analytical Finding (AAF). The GH-2004 team has assessed the IGF-I and P-III-NP analyses and respective GH-2000 scores from samples from over 7000 men and 2000 women, obtained during routine anti-doping testing in 15 WADA approved laboratories around the world. Overall, these analyses have indicated that the original decision limits were robust but required minor refinement to accommodate some of the assay combinations for men.

Several approaches are currently being examined to improve the performance of the GH-2000 test. As the intra-individual variability of IGF-I, P-III-NP and consequently the GH-2000 score is significantly smaller than population variability, including the GH-2000 bio-markers as part of the athlete’s biological passport is likely to improve detection rates.

The use of commercial immunoassays is a major barrier to the implementation of the test, as predicted by the original GH-2000 team. Manufacturers change or withdraw immunoassays with little notice and indeed the original immunoassays used in the initial GH-2000 studies have been withdrawn. The validation of three IGF-I assays and two P-III-NP assays reduces the chance of the test being withdrawn if a manufacturer ceases production of the assay but it has led to six possible combinations of GH-2000 score. As each assay produces slightly different results, this leads to different GH-2000 scores depending on the combination used and so different decision limits are needed according to the pairing used.

The development of reliable mass spectrometry methods for both IGF-I and P-III-NP should eventually replace the use of commercial immunoassays. This will allow the anti-doping agencies to maintain control over assay performance and will remove the need for double testing. At present, an IGF-I mass spectrometry method is approved but this needs to be more widely adopted before it can replace IGF-I immunoassays completely. Development of a P-III-NP mass spectrometry method is under way but the complexity of the P‑III-NP molecule has made this a challenging venture.

## Alternative approaches to detecting GH abuse

Since the Isoform and Bio-marker tests have been in routine use very few positive results have been obtained. WADA reports that since 2010 there have been only 19 adverse analytical findings for GH by the isoform test and only two for the biomarker test since 2012. These figures are much lower than the estimated prevalence of GH-doping suggesting that most of the athletes taking GH illegally are able to avoid being tested during the tests’ ‘window of opportunity’. This emphasises the importance of unannounced out-of-competition testing; however, this is both costly and difficult to organise.

### Investigative biomarkers

Diacorin, a myokine, has recently been identified as a novel GH responsive protein. However, it is inferior to IGF-I as a marker of GH abuse (143). Several other biomarkers have been proposed to detect GH abuse including urinary c-peptide, FN1 gene, FN1 protein, RAB31 gene and RAB31 protein (144,145). Whether these markers prove more useful than IGF-I and P-III-NP remains to be validated. This is no small undertaking as it will be necessary to develop reliable methods to measure these markers as well as determining how the markers vary in normal physiology and pathology

### Gene Expression Profiling

Mitchell et al investigated the value of gene expression profiling in peripheral blood leukocytes *in-vivo* as a test for GH doping in humans (146). GH induced significant changes in leukocyte gene expression in both women and men. The maximal changes were merely a doubling for upregulated or halving for downregulated genes, similar in magnitude to the variation observed between individuals. The poor sensitivity and specificity indicate that gene expression profiling of peripheral blood leukocytes is unlikely to be a viable approach for the detection of GH doping.

### Proteomics

Proteomics offers a potentially more powerful method of detecting suitable GH-sensitive markers. Using proteomics Chung et al have identified haemoglobin  chain (147). By undertaking a proteomic analysis of plasma from people with GH-deficiency and acromegaly, and people administered GH, Kopchcik and colleagues found that α1 antitrypsin and transthyretin are stimulated, while apolipoprotein A1 and hemoglobin β subunit are inhibited by GH (148,149). Tan and colleagues used a proteomic approach to examine potential plasma biomarkers before, during and after an 8-week period of rhGH or placebo treatment of non-elite athletes (150). They discovered 8 rhGH-sensitive plasma proteins (2 of which had been examined by GH-2000; IGFBP3 & IGF-ALS) as candidate targets but their concentrations fell very quickly after rhGH was discontinued. One marker vitamin D binding protein was suppressed by GH and remained so during the washout phase and may prove to be of value. More work needs to be undertaken to determine if the changes in any of these proteins are sufficient to be robust markers of GH abuse.

### Urinary GH Isoforms

After concentrating GH from urine with hydrogel nanoparticles, it is possible to apply the GH-isoform method reagents to detect the presence of 22 kD rhGH (151). Although urine is not a good medium for mirroring blood GH (since renal clearance of GH is profoundly altered by exercise) it is possible that this method could prove useful as it relies on ratios and not absolute concentrations but much validation work is needed however. It is unlikely to add any useful information to the accepted blood-based method.

### MicroRNA as a Biomarker of GH administration

Circulating microRNAs in plasma are being studied for use as biomarkers of administration of GH (152). Four microRNAs were identified that were differentially expressed after administration of therapeutic doses of rhGH. Further validation studies will be needed to ascertain usefulness for detecting GH abuse in sport.

### Growth Hormone Secretagogues (GHS)

A host of secretagogues of GHRH and ghrelin have been developed, with analogues chemically modified for oral activity and prolonged action (see above and Table 3). Detection of GH secretagogues is easier than for GH as these are foreign substances and successful methods have already been developed. These secretagogues have been picked up by the WADA testing programme: In 2017, 19 adverse analytical findings (AAFs; ‘positives’) for GHS/GHRP were found in 49,521 (0.04%) urine analyses while in 8,348 urines tested for GHRH, none were positive.

### Growth Hormone Analogues

The last two decades have seen the development of long acting GH formulations to overcome the inconvenience of daily GH injections and reduce non-adherence of daily therapy. The first to market, Genentech’s Nutropin Depot, was discontinued in 2004 (approved by FDA in 1999) because of manufacturing difficulties, injection site reactions and initial burst release (153,154). There are currently several long-acting GH preparations in development. The various technologies employed are (i) depot formulations containing encapsulated GH polymer microspheres; (ii) pegylation of GH; (iii) non-covalent binding of GH to albumin; (iv) prodrug formulations and (v) GH fusion proteins (155). The frequency of administration for most is once a week. A number of these are undergoing advanced efficacy and safety testing in the clinic (156-160). These long acting analogues of GH lend themselves to detection by the isoform and GH marker methods.

### IGF-I

IGF-I is an attractive alternative to GH because IGF-I mediates many of the anabolic actions of GH (137). It promotes muscle protein synthesis and has beneficial effects on glycogen storage that could enhance performance. Recombinant human IGF-I is available for use in clinical practice and several other IGF-I compounds and IGF-I analogues have been advertised on the internet and may be available on the black market. The GH-2004 team has demonstrated an increase in VO2max following the use of IGF-I-IGFBP-3 for one month (105). The use of IGF-I can be detected by measuring the IGF-I concentration. The GH-2000 biomarker score also rises but IGF-I has a much lower effect on P-III-NP than GH (161).

### Gene therapy

There is considerable potential for gene therapy to enhance athletic performance and this has already been demonstrated in experimental animals with ‘Schwarzenegger’ and ‘Marathon’ mice (162). This is a rapidly developing field and experience has shown that amoral coaches and athletes are always looking for new methods of gaining advantage even if it includes significant risks to their health. This is a huge area beyond the scope of this paper but has been recently reviewed (163).

### Athlete’s Biological Passport

The Athlete’s Biological Passport (ABP) is a novel approach for the detection of doping (164). It is based on the inherent stability of many human physiological processes and measures of specific analytes of interest. The underlying concept is very similar to that of ‘personalised medicine’ that is exploiting personalised physiology as a forensic tool. The introduction of an ABP module for hematology in 2009 resulted in more than 100 athletes being sanctioned over the 2 year period 2008 to 2010 (165,166). An ABP ‘Steroidal Module’ was added in 2014. In the case of GH, this entails adding the blood levels of the GH responsive markers IGF-I and P-III-NP to the ABP (167). Two large studies have reported the intra-individual variability for serum IGF-I to be between 14-16% and for PIIINP 7-18% while the between-individuals ranges were 44-71% and 31-58% respectively (167,168). This ABP for GH doping is currently under development

# Legal Framework

The detection of, sanctions on and prosecution of GH doping sit within a complex governance framework that covers the full spectrum of doping in sports. An increasing number of international sports-related disputes with the absence of any independent authority overseeing sports dispute resolution, led to the formation of the he “Court of Arbitration for Sport” by the IOC in 1984. Subsequently there was a call for greater independence from the IOC. To satisfy this, CAS was reformed in 2003 by a Swiss Federal Tribunal (169). It is the ‘high court’ for all sporting disputes, including doping violations (Appendix 2).

# Political Imperatives

The cleaning up of sports doping operates under commercial and political forces.

## Business

Olympic Sports is big business run by private organisation Revenues from TV rights at Olympic Games account for 47% of income while ‘sponsorship’ accounts for another 45% (170). Their total revenue is kept very secret but income for the Winter Games in 2018 has been estimated at US$ 2billion (171). The Summer Olympic Games in Rio brought in US $4.1 billion and assuming this is around 47% of total income, places that at just over US $9 billion (172)(Figure 10). As publicity on doping is a commercial liability, conflicts of interests are at play in providing transparency on the cleanliness of big sports.

## State-Sponsored Doping

It was the exposure of the extent of the East German state-sponsored doping after the collapse of the Berlin Wall that shocked the world (173), More recently there has been involvement at the highest political level with systematic doping of Russian athletes in the Winter Olympic Games at Sochi in 2014 (174). Investigations by WADA (175) resulted in Russia being banned from the 2018 Winter Olympic Games in Pyeongchang, South Korea (176). However, in September 2018, WADA announced the lifting of a three-year suspension of Russia amidst much controversy. This decision has been viewed by UK Anti-Doping, chief executive Nicole Sapstead, as “WADA casting aside its responsibilities to clean athletes, sports fans and those who work tirelessly for clean sport". Professor Richard McLaren, whose report said Russia operated a state-sponsored doping programme, criticised WADA's decision by adding that ‘Politics is dictating this decision’ (https://www.bbc.com/sport/45565273).

## Lack of Cooperation

The real prevalence of doping in elite sport is unknown (177). Despite the increase in funding for anti-doping activities since the creation of WADA in 1999, the proportion of athletes caught doping through analysis of blood and urine samples has remained around 2% (Table 4). This figure appears too low figure given that the best estimates of the prevalence of doping in elite sport appears to be between 14 and 39% (178). The reasons for the discrepancy are difficult to understand and is the subject of an important report commissioned by WADA from a group of experts representing the key ‘stake-holders’ (179). An effective anti-doping strategy must include not only an effective laboratory but also close co-operation between a number of the other agencies capable of making a contribution. The situation is especially true for GH. Since implementation in 2004, only 19 have returned positive of over 15000 GH doping tests undertaken among athletes competing internationally and nationally. In the US, the National Football League (NFL) has not yet implemented random GH testing despite the NFL’s commitment to undertake this in 2011 in response to claims that half of the league players use GH. (https://sports.vice.com/en\_us/article/jpz3eg/living-in-the-dark-ages-why-scientists-say-the-nfls-hgh-test-is-bs). The ABP is likely to improve the efficiency in catching athletes who dopeas each athlete in the program will be tested regularly and the hope and expectation is that it will be easier to detect a shift in biomarker level that signals that a banned substance has been taken. Some countries have made doping a crime, for example Austria, France and Italy (180) while others have debated the issues and decided against doing so (181). In keeping with these recommendations, many anti-doping agencies have spread their net wider with some success.

# Conclusion

Performance enhancement is an enduring goal for athletes in search of fame and glory. Covert use of prohibited drugs is rampant, necessitating the establishment of an antidoping control agency, the WADA, for fair play. GH abuse in sports is widespread because of its perceived anabolic potency and the difficulty of detection. When evaluated in doses under controlled supervision, GH does not affect strength or endurance but selectively improves sprint capacity. However, the doses typically used in the underground and the way GH is combined with other doping agents are largely not known. The WADA has implemented two tests which have successfully detected GH abuse. GH secretagogues, agents that stimulate the secretion of GH are banned, however, there is no evidence as yet supporting performance benefit. The detection rate of GH abuse is low but may be improved by implementing out-of-competition testing, using biological passports and enhancing cooperation between jurisdictions. The success of GH and other anti-doping programs is influenced by commercial, marketing and political factors.

# Appendices

## Appendix 1: Athlete’s Biological Passport (182)

The fundamental principle of the Athlete Biological Passport (ABP) is to monitor selected biological variables over time that indirectly reveal the effects of doping rather than attempting to detect the doping substance or method itself.

Anti-doping organizations can integrate the Athlete Biological Passport into the larger framework of a robust anti-doping program in order to:

* Identify and target athletes for specific analytical testing by intelligent and timely interpretation of Passport data; and
* Pursue possible anti-doping rule violations based on an atypical passport, in accordance with Article 2.2: Use or attempted use by an athlete of a prohibited substance or a prohibited method of the [World Anti-Doping Code (Code)](https://www.wada-ama.org/en/what-we-do/the-code).

As the international independent organization responsible for coordinating and monitoring the global fight against doping in sport, WADA has taken the lead in the development of the Athlete Biological Passport concept. [WADA’s Athlete Biological Passport Operating Guidelines (ABP Guidelines)](https://www.wada-ama.org/en/resources/athlete-biological-passport/athlete-biological-passport-abp-operating-guidelines) were approved by [WADA’s Executive Committee](https://www.wada-ama.org/en/who-we-are/governance/executive-committee) and took effect on December 1st, 2009. This first version contained a standardized approach to the profiling of individual Athlete Haematological variables for the detection of blood doping (the ‘Haematological Module’ or ‘blood module’).

During the first three years of a bio-passport program of the Union Cycliste Internationale (UCI), 26 riders were found positive for erythropoietin (EPO). In twenty out of the 26 cases, it was the abnormal blood profile which raised suspicions leading to a targeted doping test (https://en.wikipedia.org/wiki/Biological\_passport#Cyclists\_sanctioned\_on\_basis\_of\_biological\_passports). [Manuel Beltran](https://en.wikipedia.org/wiki/Manuel_Beltran) (Liquigas) tested positive for [EPO](https://en.wikipedia.org/wiki/Erythropoietin) at the [2008 Tour de France](https://en.wikipedia.org/wiki/2008_Tour_de_France) in a targeted test after anomalies appeared in a blood sample taken at the start of the Tour. The pre-Tour blood samples were collected by the French Anti-doping Agency ([AFLD](https://en.wikipedia.org/wiki/Agence_Fran%C3%A7aise_de_Lutte_contre_le_Dopage)) and the results from the testings were submitted to the UCI to form part of their database of profiles for their biological passport programme.

WADA ABP Guidelines have been continuously refined and the ABP approach has been successfully integrated into the anti-doping strategies of numerous [International Federation (IF)](https://www.wada-ama.org/en/international-federations-if) and [National Anti-Doping Agency (NADO)](https://www.wada-ama.org/en/who-we-are/anti-doping-community/national-anti-doping-organizations-nado) programs, resulting in a significant increase in the number of adverse analytical findings (AAFs) that are a result of targeting by the ABP as well as a number of direct anti-doping rule violations (ADRVs).

The 4th version of the WADA ABP Guidelines, which were approved by WADA’s Executive Committee in November 2013, introduced a second module, the `Steroidal Module´, which became operational on January 1st, 2014. The Steroidal Module monitors selected urinary steroid concentrations over time in order to detect steroid doping.

WADA will continue to develop the ABP in consultation with stakeholders, by refining the present modules as well as adding new ones as they are finalized.

## Appendix 2: Legal Governance Framework for international Sports Federations.

The various Olympic Sports are governed by their international sports federations (IFs) which are independent but affiliated to the IOC. If they wish to be recognised by the IOC they must ensure that their statutes, practice and activities conform with the Olympic Charter. **The International Sports Federations are responsible for the integrity of their sport on the international level in accordance with the Paris Agreement**

The preamble of the so-called ‘Paris Agreement’ states that

*“with the aim of facilitating the resolution of disputes in the field of sport, an arbitration institution entitled the “Court of Arbitration for Sport" (hereinafter the CAS) has been created, and that, with the aim of ensuring the protection of the rights of the parties before the CAS and the absolute independence of this institution, the parties have decided by mutual agreement to create a Foundation for international sports-related arbitration, called the “International Council of Arbitration for Sport” (hereinafter the ICAS), under the aegis of which the CAS will henceforth be placed.”*

Organisation and structure of the ICAS and CAS:

1. The code of Sports-related Arbitration of 22nd November 1994 - governs the organisation and arbitration procedures of the CAS
2. The International Council of Arbitration for Sport (ICAS) – The ICAS is the supreme organ of the CAS. The main task of the ICAS is to safeguard the independence of the CAS and the rights of the parties. To this end, it looks after the administration and financing of the CAS.
3. The Court of Arbitration for Sport (CAS) - The CAS performs its functions through the intermediary of arbitrators, of whom there are at least 150, with the aid of its court office, which is headed by the Secretary General. One of the major new features following the reform of the CAS was the creation of two divisions: an “Ordinary Arbitration Division”, for sole-instance disputes submitted to the CAS, and an “Appeals Arbitration Division”, for disputes resulting from final-instance decisions taken by sports organisations. Each division is headed by a president.

CAS judges on two types of dispute that may be submitted to the court: those of a commercial nature, and those of a disciplinary nature, of which a large number are doping-related. Disciplinary cases are generally dealt with in the first instance by the competent sports authorities, and subsequently become the subject of an appeal to the CAS, which then acts as a court of last instance (169).

**Figure Legends**

Figure 1

Olympic shot-put distances for women since 1948 in relation to chronological history of doping with anabolic steroids

Figure 2

Proportion of GH isoforms secreted by the pituitary gland from post-transcriptional and post-translation modification. The isoforms are 22k GH, 20k GH as monomers, dimers and oligomers and acidic and acetylated forms of GH. Drawn from (15)

Figure 3

A. Concentration of 22k and 20k GH in a normal subject showing co-secretion of both isoforms over 24 hours. B. Concentrations of 22k and 20k GH in 6 normal subjects showing the suppression of 20k GH after a subcutaneous injection of rhGH (22k). From Leung et al (17)

Figure 4

Schematic representation of the stimulation by endogenous GH or exogenous GH (rh 22k GH) of proteins derived from the liver (IGF-I, IGFBP3 and ALS) or from bone (P-III-NP, PICP and ICTP).

IGFBP3 = IGF binding protein 3; ALS = acid labile subunit; P-III-P = N-terminal pro-peptide of type III collagen; PINP = N-terminal pro-peptide of type 1 procollagen; ICTP = Carboxyterminal telopeptide of type I collagen

Figure 5

Time course of the contributions by anaerobic and aerobic energy systems in the provision of energy as ATP during exercise. AA, amino acid; FA, fatty acid; TCA, tricarboxylic acid cycle; O2, oxygen.

Figure 6

Meta-analysis of the effect of GH treatment on muscle strength (top panel) and on maximum oxygen uptake (VO2 max) in placebo-controlled trials of recreational athletes.

Data are shown as forest plots with point estimates and 95% confidence limits. From Hermanssen et al (97).

Figure 7

GH effects on physical performance in recreational athletes. This figure illustrates the percent change after GH or placebo treatments in 96 subjects for four measures of physical performance: VO2 max, strength (dynamometry), jump height and Wingate test. Data from Meinhardt et al. (100).

Figure 8

Changes in measured performance in placebo-treated recreational athletes participating in a double-blind controlled study who thought they were on GH (black) and those who thought they were on placebo (white). The performance measures were VO2 max, dead lift dynamometry, jump height and sprint capacity (Wingate test) (126).

Figure 9

GH Isoform test: Changes in the mean ratio of 22 kD GH (Rec A) to pituitary GH (Pit A) after subcutaneous injection of 0,033 and 0.088 mg/kg of rhGH in 10 healthy subjects showing a rise in the ratios for up to 30 hours. Reproduced from Bidlingmaier et al. (139).

Figure 10

Monetary value of commercial rights of Olympic Games. From (172)

**Table 1:Drugs appearing on the 2018 WADA List of Prohibited substances**

(<https://www.wada-ama.org/sites/default/files/prohibited_list_2018_en.pdf>)

Substances and methods prohibited at all times

**S1 Anabolic Agents:**

Anabolic Androgenic Steroids (AAS)

Exogenous AAS

Endogenous AAS when administered exogenously

Other Anabolic Agents: Including but not limited to: Clenbuterol, SARMs

**S2 Peptide Hormones, Growth Factors, Related substances and mimetics:**

Erythropoietins (EPO)

EPO receptor agonists

Hypoxia-inducing factor (HIF) activating agents

GATA inhibitors

TGF-beta (TGF-β) inhibitors

Innate repair receptor agonists

Peptide hormones and hormone modulators

Chorionic gonadotrophin (CG) and Luteinizing Hormone (LH) and their releasing factors

Corticotrophins and their releasing factors

Growth hormone (GH), its fragments and releasing factors

Growth Factors and Growth Factor Modulators

**S3 Beta-2 Agonists:**

 Except:

Inhaled salbutamol – restricted amount

Inhaled formoterol – restricted amount

Inhaled salmeterol – restricted amount

**S4 Hormone and Metabolic Modulators:**

Aromatase inhibitors

Selective oestrogen receptor modulators (SERMS)

Other anti-oestrogen substances

Agents modifying myostatin function

Metabolic modulators

Activators of the AMP-activated protein kinase (AMPK)

Insulins and insulin-mimetics

Meldonium

Trimatazine

**Table 2: Conditions Included in Therapeutic Use Exemptions (TUEs)**

https://www.wada-ama.org/en/what-we-do/science-medical/therapeutic-use-exemptions

The following Medical information documents (also called TUE Physician Guidelines) are available from WADA:

ADHD (Attention Deficit Hyperactivity Disorder) in Children and Adults

Adrenal Insufficiency

Anaphylaxis

Asthma

Cardiovascular Conditions: The Therapeutic Use of Beta-blockers in Athletes

Diabetes Mellitus

Growth Hormone Deficiency in Adults

Growth Hormone Deficiency in Children and Adolescents

Infertility/Polycystic Ovarian Syndrome

Inflammatory Bowel Disease

Intravenous Infusion

Intrinsic Sleep Disorders

Male Hypogonadism

Musculoskeletal Conditions

Neuropathic Pain

Post Infectious Cough

Renal Transplantation

Sinusitis/Rhinosinusitis

Transgender Athletes

**Table 3: World Anti-Doping Agency List of Prohibited Substances. Growth Hormone is listed within the section on Peptide Hormones, Growth Factors, Related substances and Mimetics**

* Growth Hormone (GH), its fragments and releasing factors, including, but not limited to
* Growth Hormone fragments, e.g. AOD-9604 and hGH 176-191;
* Growth Hormone Releasing Hormone (GHRH) and its analogues, e.g. CJC-1293, CJC-1295, sermorelin and tesamorelin;
* Growth Hormone Secretagogues (GHS), e.g. lenomorelin (ghrelin) and its mimetics, e.g. anamorelin, ipamorelin, macimorelin and tabimorelin; GH-Releasing Peptides (GHRPs), e.g. alexamorelin, GHRP-1, GHRP-2 (pralmorelin), GHRP-3, GHRP-4, GHRP-5, GHRP-6, and examorelin (hexarelin).

**Table 4: Percentage of Adverse or Atypical Doping Tests Among Olympic and non-Olympic Sports from 2013-2017**

(https://www.wada-ama.org/en/resources/laboratories/anti-doping-testing-figures-report)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **2013** | **2014** | **2015** | **2016** | **2017** |
|  | **AAF1** | **Total2** | **AAF1** | **Total2** | **AAF1** | **Total2** | **AAF1** | **Total2** | **AAF1** | **Total2** |
| Olympic Sports\* | 0.97 | 1.94 | 0.77 | 0.99 | 0.83 | 1.64 | 1 | 1.26 | 0.77 | 0.82 |
| Non-Olympic Sports | 1.95 | 2.72 | 1.77 | 2.09 | 2.04 | 2.52 | 2.7 | 2.81 | 2.59 | 2.64 |
| Overall | 1.31 | 2.21 | 1.11 | 1.36 | 1.26 | 1.49 | 1.6 | 1.81 | 1.43 | 1.48 |

\* Olympic sport data include tests conducted in non-Olympic disciplines of the sport which are governed by an Olympic International Federation

1 AAF (Adverse Analytical Findings) do not include adjudicated or sanctioned Anti-Doping violations such as those who underwent the Therapeutic Use Exemption approval process.

2 Includes AAF and Atypical Findings.

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