Growth Hormone Doping in Sports: A Critical Review of Use and Detection Strategies

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GH is believed to be widely employed in sports as a performance-enhancing substance. Its use in athletic competition is banned by the World Anti-Doping Agency, and athletes are required to submit to testing for GH exposure. Detection of GH doping is challenging for several reasons including identity/similarity of exogenous to endogenous GH, short half-life, complex and fluctuating secretory dynamics of GH, and a very low urinary excretion rate. The detection test currently in use (GH isoform test) exploits the difference between recombinant GH (pure 22K-GH) and the heterogeneous nature of endogenous GH (several isoforms). Its main limitation is the short window of opportunity for detection (~12–24 h after the last GH dose). A second test to be implemented soon (the biomarker test) is based on stimulation of IGF-I and collagen III synthesis by GH. It has a longer window of opportunity (1–2 wk) but is less specific and presents a variety of technical challenges. GH doping in a larger sense also includes doping with GH secretagogues and IGF-I and its analogs. The scientific evidence for the ergogenicity of GH is weak, a fact that is not widely appreciated in athletic circles or by the general public. Also insufficiently appreciated is the risk of serious health consequences associated with high-dose, prolonged GH use. This review discusses the GH biology relevant to GH doping; the virtues and limitations of detection tests in blood, urine, and saliva; secretagogue efficacy; IGF-I doping; and information about the effectiveness of GH as a performance-enhancing agent. **(*Endocrine Reviews* 33: 155–186, 2012)**

I. Introduction

The use of GH as a performance-enhancing agent is believed to be widespread among both professional athletes and adolescents participating in sports (1–4). GH is classified as a prohibited substance on the World Anti-Doping Agency (WADA) Prohibited List [http://www.wada-ama.org/en/World-Anti-Doping-Program/Sports-and-Anti-Doping-Organizations/International-Standards/Prohibited-List/]. Aspects of GH that are attractive to athletes are its purported ergogenic activity, aid in recovery from injury, and "undetectablity" (Table 1). A detailed time line of the use of GH in sports is presented in Holt *et al.* (5). This review critically evaluates the scientific underpinnings of GH use in

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Abbreviations: ALS, Acid-labile subunit; CS, chorionic somatomammotropin; GHBP, GH binding protein; GHR, GH receptor; GHRP, GH-releasing peptide; GHS, GH secretagogue; hGH, human GH; IGFBP, IGF-binding protein; IRMA, immunoradiometric assay; mol wt, molecular weight; MS, mass spectrometry or mass spectrometric; P-III-NP, procollagen type III amino-terminal propeptide.

TABLE 1. Rationales given for using GH as a doping agent in sports

GH is ergogenic (performance-enhancing)
GH is the master anabolic hormone
GH increases skeletal muscle mass — and hence strength and endurance
GH enhances assimilation of nutrients to build tissues
GH is lipolytic, with calories liberated from adipose tissue redirected to build muscle and to be utilized as metabolic fuel
GH accelerates recovery from sports injuries
GH causes beneficial weight loss
The use of GH, a natural substance, cannot be detected in antidoping tests

sports, with particular emphasis on strategies and methods of detection of exogenous GH administration.

II. Background Information on GH Structure, Function, and Regulation

GH is a pituitary polypeptide hormone with anabolic and growth-promoting activity. Both its structure and function are species-specific. The only GH with bioactivity in humans is human GH (hGH) or the closely related primate GH (6, 7). In contrast, hGH is biologically active in a number of lower species, a feature that has been termed "one-way species specificity." hGH also has lactogenic activity, a feature that is lacking in nonprimate GH. This review will only discuss hGH because animal GH are not pertinent in the context of doping in humans.

A. GH genes

The human genome contains five GH-related genes, located in the GH gene cluster on chromosome 17q24.2. This locus occupies approximately 47 kilobases and contains two GH genes—*GH-N* (or *GH1*) and *GH-V* (or *GH2*)—as well as the related chorionic somatomammotropin (CS) (also known as placental lactogen) genes (8). These multiple genes are believed to have arisen by gene duplication. Each of the five genes in the cluster is composed of five exons and four introns. The *GH-N* gene is expressed in pituitary somatotrope cells and, to a minor extent, in lymphocytes, whereas *GH-V* and *CS* genes are expressed in the placenta. The level of GH gene expression in lymphocytes may be sufficient to play a local paracrine/autocrine immunoregulatory role, but it is insufficient to fulfill a hormonal role at distant sites. In the absence of pituitary (or placental) GH gene expression, there is no detectable GH in blood, and the clinical features of severe GH deficiency ensue.

B. Primary GH gene products

The main product of the *GH-N* gene is a 191-amino acid, 22,129 molecular weight (mol wt), single chain, simple (unmodified) protein with two disulfide bridges (Fig. 1). It is the prototype pituitary GH and is known as 22K-GH. It is also the recombinant GH available for therapeutic use (and for doping purposes). Another GH isoform, the 20K-GH variant, is also derived from the *GH-N* gene by alternative mRNA splicing (9); it has a structure analogous to 22K-GH, except for the deletion of internal residues 32–46. It has 176 amino acids and a mol wt of 20,274. It arises from the use of an alternative splice site in exon 3 and is expressed at 5–10% of the expression level of 22K-GH. A third isoform (17.5K-GH), arising from skipping of exon 3 and lacking residues 32–71, has been proposed as an additional GH variant based on the finding of a transcript (10). This form has not been shown to be expressed in significant amounts under normal physiological conditions.

The *GH-V* gene product, GH-V, GH2 or placental GH, is a 191-amino acid, 22,321 mol wt, single chain protein with two disulfide bridges, similar in structure to 22K-GH (Fig. 1). Its sequence differs from that of 22K-GH at 13 amino acid positions. It contains a consensus sequence for N-glycosylation at position 140 and exists as both a glycosylated and a nonglycosylated form. The *GH-V* gene does not produce significant amounts of a 20K variant (11, 12). GH-V is exclusively produced by the placenta and during pregnancy progressively supplants GH-N in the maternal circulation (13, 14). It has similar somatogenic activity as GH-N but has reduced lactogenic activity (15–17).

CS is also produced by the placenta in considerable amounts. It has about 85% structural homology with GH, but has no significant somatogenic bioactivity. GH-V and CS will not be further discussed in this review because they have limited relevance for GH doping. Thus, the term "GH" will refer to hGH-N and its isoforms.

C. GH isoforms

GH is not a single protein, but consists of several molecular variants (isoforms). A detailed treatise on GH isoforms has recently been published (18); a synopsis tailored to the purposes of the current review follows (Table 2). The principal and most abundant GH form in pituitary and blood is monomeric 22K-GH. This is also the isoform produced commercially for therapeutic purposes, known as "recombinant GH." Because of its availability, it is also the form typically used for GH doping. The 20,000 mol wt variant, known as 20K-GH, is the second most abundant isoform in pituitary and plasma (5–10% of total GH) (19, 20). It has a propensity to dimerize, and its dimer is enriched compared to the 22K-GH-dimer (19, 21, 22). Recombinant 20K-GH has been produced pharmaceutically (23) but was never developed for therapeutic use. Whether

Figure 1.

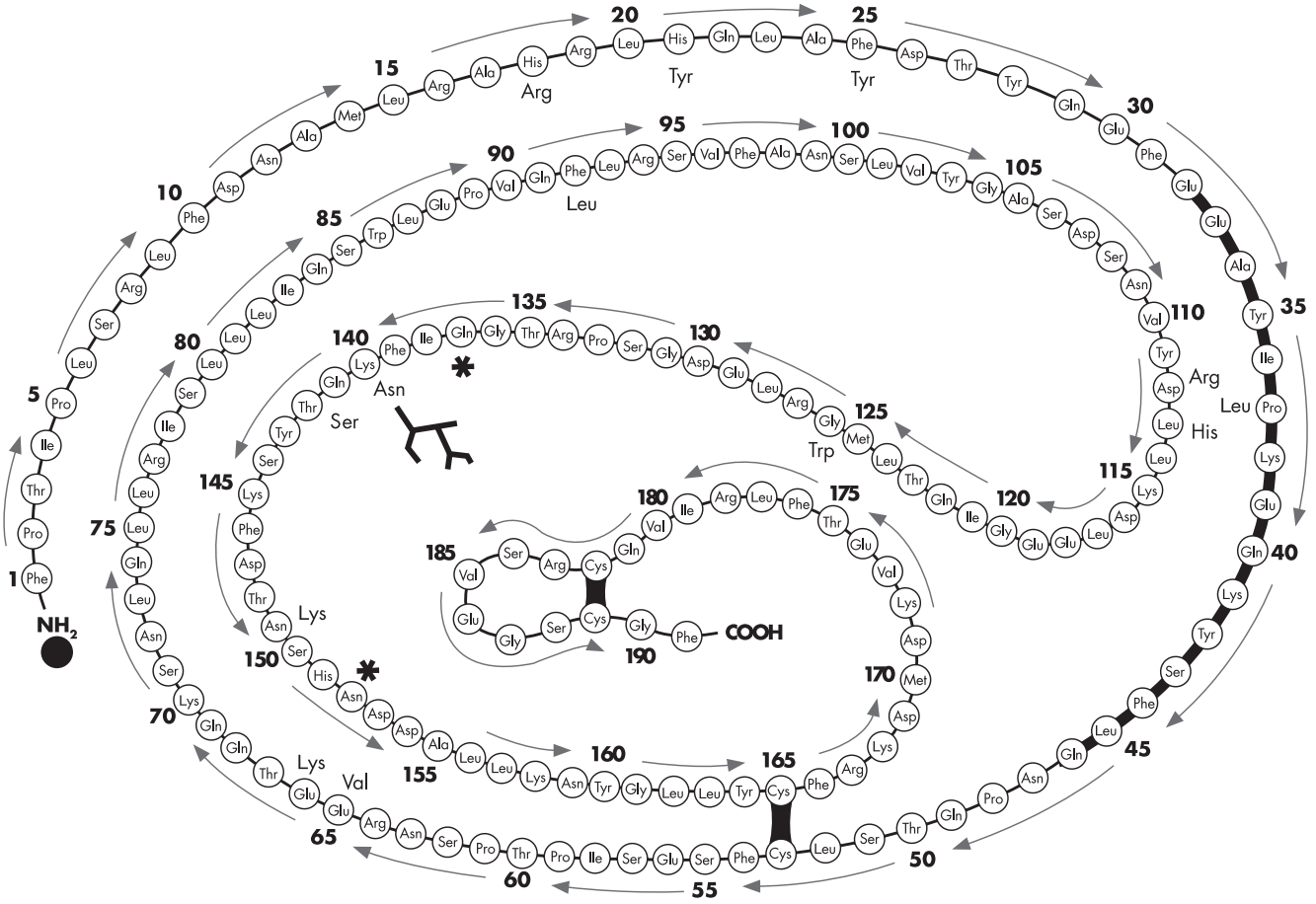

Figure 1. Primary structure of hGH and its isoforms. The main chain represents 22K-GH (GH-N). The sequence indicated by the *bold line* from residue 32 to 46 is deleted in 20K-GH. The *black dot* at the amino terminus denotes the acyl (probably acetyl) group in N-acylated GH. The *two asterisks* denote the deamidated residues in desamido-GH forms. The amino acid designations next to the main chain denote the residues that are changed in placental GH (GH-V). The *tree structure* at residue 140 indicates the glycosylation site in glycosylated GH-V. [Reproduced from G. Baumann: Growth hormone heterogeneity: genes, isohormones, variants, and binding proteins. *Endocr Rev* 12:424–449, 1991 (20), with permission. © The Endocrine Society.]

it is available for illicit use is currently unknown. Several posttranslationally modified monomeric GH forms exist; they include two deamidated forms (Asn^{137} and Asn^{152}), N_α-acylated, and glycosylated [an O-linked N-acetyl-hexosamine-hexose-(neuraminic acid)$_2$ glyco-moiety at Thr^{60} has been proposed] 22K-GH (24–27). Proteolytically cleaved GH forms are not considered native forms (20). GH isoforms also exist as an oligomeric series of at least up to pentameric GH, with both covalent (disulfide-linked) and noncovalently associated oligomers. Homo- as well as heterooligomers composed of the described monomeric forms have been described. Oligomers are present in the pituitary, are secreted as such, and circulate in blood (21, 22, 28, 29).

D. GH structure

The tertiary structure of monomeric 22K-GH (and 20K-GH) is a four-helix, antiparallel, twisted bundle characteristic of the cytokine family of proteins (30). Crystal structures have not been obtained for the other GH isoforms, but it is likely that they retain the same overall conformation. Part of helix 1 and the loop between helices 1 and 2 with its embedded minihelix are missing in 20K-GH (30).

E. The GH receptor (GHR)

GH action is initiated by its binding to the GHR in target tissues. The GHR is a plasma membrane-resident receptor of the cytokine receptor class I superfamily (31). It is expressed ubiquitously and is particularly abundant in the liver (32, 33). The GHR primary structure differs among species, and the species specificity of GH action is dictated by high-affinity interaction of GH with its cognate GHR. GH has two receptor binding epitopes on its surface; upon binding of a GHR to site 1, a second GHR

TABLE 2. Estimated average proportions for GH isoforms in human blood 15–30 min after a secretory pulse

Monomeric GH	
22K-GH	45%
20K-GH	5%
Acidic GH (desamido-, acylated, and glycosylated GH)	5%
Dimeric GH	
22K-GH dimers	
Noncovalent dimers	14%
Disulfide dimers	6%
Total 22K-GH dimers	20%
20K-GH dimers	
Noncovalent dimers	3%
Disulfide dimers	2%
Total 20K-GH dimers	5%
Acidic GH dimers (desamido-, acylated, and glycosylated GH)	
Noncovalent dimers	1.5%
Disulfide dimers	0.5%
Total acidic GH dimers	2%
Oligomeric GH (trimer-pentamer)	
22K-GH oligomers	
Noncovalent oligomers	7%
Disulfide oligomers	3%
Total 22K-GH oligomers	10%
20K-GH oligomers	
Noncovalent oligomers	1%
Disulfide oligomers	0.5%
Total 20K-GH oligomers	2%
Acidic GH oligomers (desamido-, acylated, and glycosylated GH)	
Noncovalent oligomers	1%
Disulfide oligomers	0.5%
Total acidic GH oligomers	2%

Adapted from G. Baumann: Growth hormone heterogeneity: genes, isohormones, variants, and binding proteins. *Endocr Rev* 12:424–449, 1991 (20), with permission. © The Endocrine Society.

binds to site 2, forming a 2:1 complex between GHR and GH (34). The two GHR exist in a predimerized form; binding of GH leads to a conformational change of the dimer followed by signal transduction (35–37). The GHR signals through several intracellular phosphorylation cascades, of which the JAK2-Stat5b pathway is particularly important for its growth-promoting activity (37, 38). The other pathways include the IRS-PI3K, SHC-MAPK, PIP-Akt, Stat 1 and 3, and other signaling cascades; their discussion goes beyond the scope of this review.

hGH also interacts with the prolactin receptor (39); it is unclear whether it can fully supplant the role of prolactin in lactation. Animal GH do not bind to the prolactin receptor, although in some species (*e.g.*, cow) GH promotes milk production through the GHR. This property is the basis for the commercial use of bovine GH in the dairy industry.

F. Biological activities of GH

Table 3 lists the principal biological activities of GH. Of particular interest to the athlete are its anabolic and lipolytic activities. From these properties alone it has been assumed that GH must be an ergogenic, performance-enhancing substance.

TABLE 3. Principal biological activities of human GH

Nitrogen retention
Amino acid transport into muscle
Promotion of somatic growth
Growth plate elongation
IGF-I generation
IGFBP3 generation
ALS generation
Lipolysis
Sodium retention
Phosphorus retention
Insulin antagonism
β-Cell hyperplasia
Early insulin-like effect
Lactogenesis
Modulation of immune function

The various GH isoforms have qualitatively similar bioactivities in humans (reviewed in Ref. 18). The reduced diabetogenic activity attributed to 20K-GH based on some rodent data has not been confirmed in human subjects (40). Among the monomeric forms, their *in vivo* bioactivity appears to be similar in both qualitative and quantitative terms. Oligomeric GH forms generally have reduced bioactivity compared with GH monomers as assessed by *in vitro* assays; there is only limited information about their bioactivity *in vivo* (18).

G. Regulation of GH secretion

GH is secreted from the pituitary gland in a pulsatile fashion under dual hypothalamic control by GHRH (stimulatory) and somatostatin (inhibitory). Ghrelin, derived from the stomach and possibly the hypothalamus, plays at best a minor role in physiological GH secretion. [In contrast, ghrelin and its synthetic congeners (GH secretagogues, GHS) or GH-releasing peptides (GHRP) are potent pharmacological stimuli for GH secretion when administered *in vivo*.] GH secretory pulses occur every 2–3 h and vary greatly in amplitude (41–43) (Figs. 2 and 3). The largest pulses generally occur at night and are associated with stage IV (slow wave) sleep, typically in the early phases of the sleep cycle. The ultradian pattern of GH secretion differs between the sexes, with women having generally higher secretion rates/serum levels, more erratic secretion patterns, and higher interpeak (basal) GH secretion/serum levels compared to men (Fig. 2). This difference is attributable to an estrogen effect (44).

The GH secretion rate peaks during adolescence and declines thereafter throughout life, with an approximately 15% decline per decade (45). Obesity attenuates GH secretion; undernutrition and physical fitness enhance it

Figure 2.

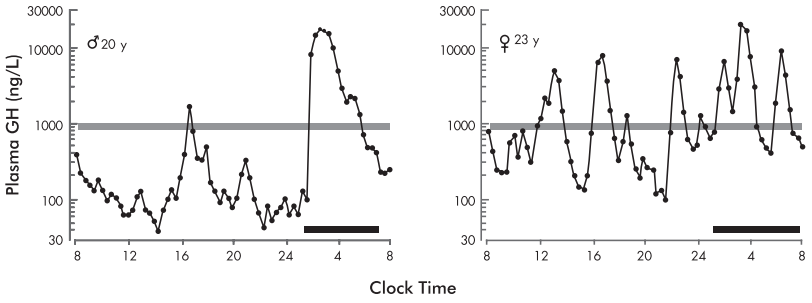

Figure 2. Diurnal profiles of plasma GH concentrations. Patterns representative for men (*left*) and women (*right*) are shown. Note the logarithmic ordinate, which serves to highlight the lower range of GH fluctuations. The *hatched bar* denotes the 1 ng/ml level commonly taken as the boundary between basal and stimulated GH levels. The *solid black bars* indicate sleep periods. Note the higher nadirs, higher peak averages, and generally "noisier" pattern characteristic of women. [Adapted from L. M. Winer *et al.*: Basal plasma growth hormone levels in man: new evidence for rhythmicity of growth hormone secretion. *J Clin Endocrinol Metab* 70:1678–1686, 1990 (41), with permission. © The Endocrine Society.]

(45–47). Acute physiological stimuli for GH release are sleep, exercise, stress, and fasting (46, 48–50). The GH response to exercise has been well-documented and reviewed in detail (51–55).

GH inhibits its own secretion through both short loop (autofeedback) (56, 57) and long loop (IGF-I-mediated) feedback (58, 59) (Fig. 4). Feedback regulation occurs both at the hypothalamic (principal site of GH autofeedback) and pituitary levels (main but not exclusive site of IGF-I feedback). Additional feedback regulation of GH secretion occurs through metabolic factors elicited by GH action (*e.g.*, free fatty acids, glucose).

With respect to GH isoform secretion, there is no evidence for differential regulation of isoforms. Rather, it appears that all isoforms are cosecreted during a secretory burst (60–63) (Fig. 3).

Figure 3.

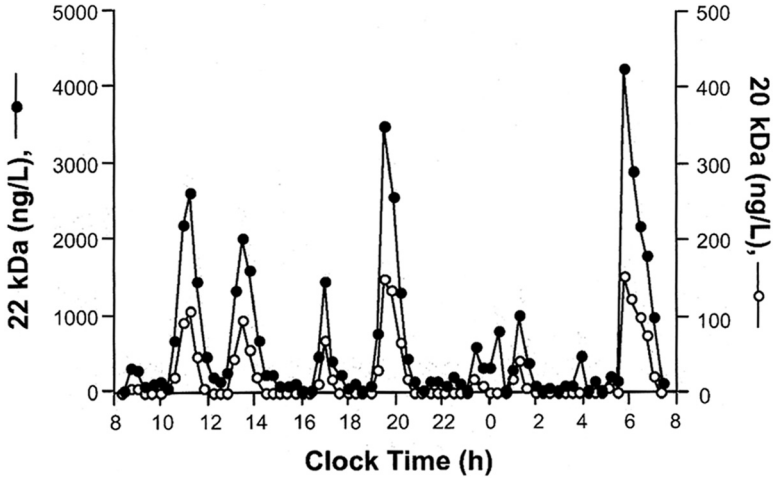

Figure 3. Cosecretion of GH isoforms. Diurnal profiles of 22K-GH and 20K-GH in serum. The temporal coincidence of 22K-GH and 20K-GH peaks is evident, indicating cosecretion of the two GH isoforms. [Reproduced from K. C. Leung *et al.*: Physiological and pharmacological regulation of 20-kDa growth hormone. *Am J Physiol Endocrinol Metab* 283:E836–E843, 2002 (62), with permission. © American Physiological Society.]

H. Metabolism and clearance

A major portion of the metabolic clearance of monomeric GH occurs in the kidney, with efficient glomerular filtration followed by extensive degradation in the proximal tubule (64–68). Only approximately 1/10,000th of glomerularly filtered GH is excreted in the final urine (69, 70). Other sites of metabolic clearance are the liver and other tissues, where GH is cleared via GHR-mediated cellular uptake and intracellular degradation. There is little quantitative information available on this process and how it is distributed among organs; the liver is thought to be an important site because the GHR is abundantly expressed in that organ.

The plasma half-life of total (free and GH-binding protein bound) GH is approximately 14–18 min (see *Section II.I* for discussion of GH-binding proteins) (67, 71). Estimates for free and bound GH are 11 and 27 min, respectively (72). The half-life of 20K-GH is somewhat longer (19–25 min) than that of 22K-GH (61, 62). It is not clear whether this property is due to its tendency for dimer formation, thereby slowing renal clearance, to its lower affinity for the GHR, or both. Similarly, the clearance of oligomeric GH forms is also slower than that of monomeric GH; with reported plasma half-lives of 19, 27, and 45 min for monomeric, dimeric, and oligomeric GH, respectively (73). The slower clearance of 20K-GH and oligomeric forms is reflected in the (compared with 22K-GH) longer half-life of "pituitary GH," which contains all these isoforms (61). Because of the differences in clearance rates, the relative proportions of GH isoforms in blood change over time, with relative accumulation of the more slowly cleared forms (61). This is the main reason for the observation that 20K-GH and oligomeric GH forms tend to be proportionately higher in blood than in the pituitary (74).

The pharmacokinetics of exogenous 22K-GH in healthy young volunteers after iv injection

Figure 4.

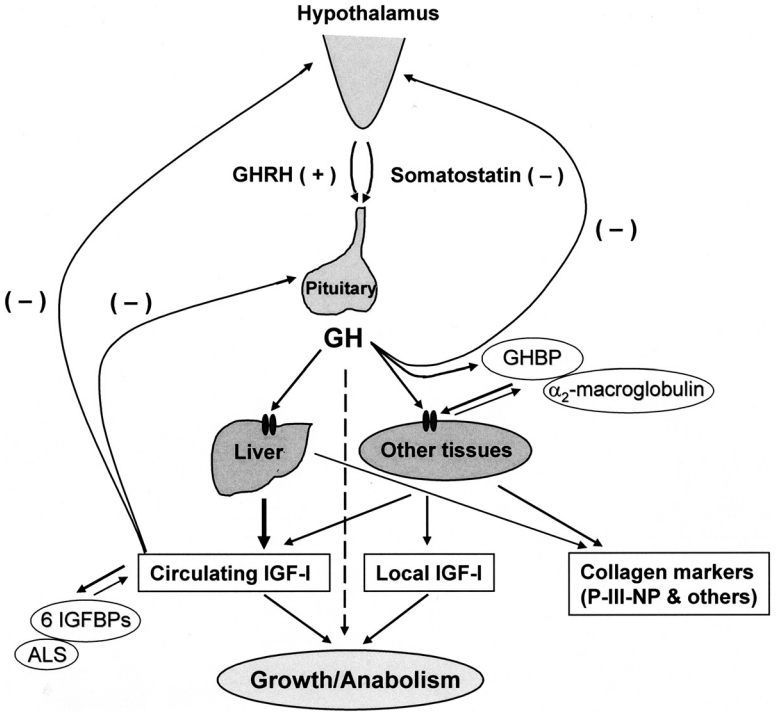

Figure 4. The GH-IGF-I axis. Schematic representation of the hypothalamic-pituitary-peripheral GH-IGF-I axis. *Minus signs* denote inhibitory action, the *plus sign* denotes stimulatory action. The *dashed line* indicates direct (non-IGF-I-mediated) GH action on tissues. Collagen markers produced by tissues in response to GH are added for the purposes of this review, although they are not strictly part of the GH-IGF axis. [Adapted from G. Baumann: Growth hormone binding proteins. In: The Endocrine System in Sports and Exercise, WJ Kraemer and AD Rogol, eds, 2005, with permission. © Wiley-Blackwell Publishing.]

show a plasma half-life of 22 min, a volume of distribution of 70 ml/kg, and a clearance rate of 135 ml/kg · h (75). After sc injection, a plasma peak is achieved at 4 h, the half-life is 3.8–4 h, the clearance rate is 179 ml/kg · h, and plasma GH stays elevated for at least 12 h (62, 75) (Fig. 5). After im injection, the values are similar to those after sc administration, except that the peak is reached earlier (at 2 h) and the half-life is 4.9 h (75). It should be noted that half-lives after sc or im administration are not true half-lives, but represent a combination of continued absorption and elimination kinetics. Absolute bioavailability is listed as 75% after sc and 63% after im injection (75). The pharmacokinetics of exogenous 20K-GH in healthy young subjects, as assessed in a single study using sc administration, showed a plasma peak time of 3.7 h and a half-life of 1.9–2.9 h (76).

Administration of either 22K-GH or 20K-GH suppresses endogenous GH secretion for at least 12 h, as evidenced by the absence of secretory pulses of 20K- or 22K-GH, respectively (62, 76) (Fig. 5).

I. GH in blood

After secretion, GH rapidly associates with two circulating GH binding proteins (GHBP) (Fig. 4). Binding to the main (high-affinity) GHBP is readily reversible and follows a dynamic equilibrium. The high-affinity GHBP is the ectodomain of the GHR, generated from the GHR by the action of the metalloproteinase TNF-α converting enzyme (see Ref. 77 for review). The low-affinity GHBP has been shown to correspond to the transformed form of α_2-macroglobulin (78). Under basal conditions (GH level <10 ng/ml), 45–55% of 22K-GH and ~25% of 20K-GH is bound to the high-affinity GHBP, and 5–7% is bound to the low-affinity GHBP. At higher GH levels (>20 ng/ml), the fraction of GH bound to the high-affinity GHBP declines due to saturation of the GHBP (79). The circulating complexes have mol wt of ~85,000 and > 150,000, respectively. GHBP protect GH from renal clearance and degradation; the complexes serve as a circulating GH pool, prolonging the bioavailability of GH. In addition, GHBP competes with GHR for GH binding and may inhibit signaling, thereby modulating GH bioactivity. The high-affinity GHBP can interfere with GH measurement in serum (see *Section II.L*).

Serum GH levels are conventionally reported as total (bound + free) GH. They fluctuate widely, reflecting the pulsatile secretion from the pituitary (Figs. 2 and 3). In the basal state (interpulse levels), GH levels range between 0.01 and 1 ng/ml. After a secretory pulse, they may range between 1 and 100 ng/ml. The boundary between a basal level and a small pulse is ill-defined and somewhat arbitrary. Pulse detection algorithms, such as cluster and deconvolution, can help define what constitutes a pulse. The highest serum GH peaks are typically seen at night (during slow-wave sleep) and generally reside in the 10–20 ng/ml range. Occasionally, peaks can be considerably higher. During the day, GH peaks are typically smaller, in the 2–10 ng/ml range. The spectrum of GH pulse amplitudes extends over at least two orders of magnitude, and peaks of widely varying height can occur at any time. Age, gender, body mass index/adiposity, physical activity, stress, time of day, and nutritional and metabolic status all influence GH secretion.

Most GH immunoassays do not fully discriminate between GH isoforms but may differ partially in their recognition of isoforms; this has implications for GH measurement by immunoassay (see *Section II.L*). Isoform-

Figure 5.

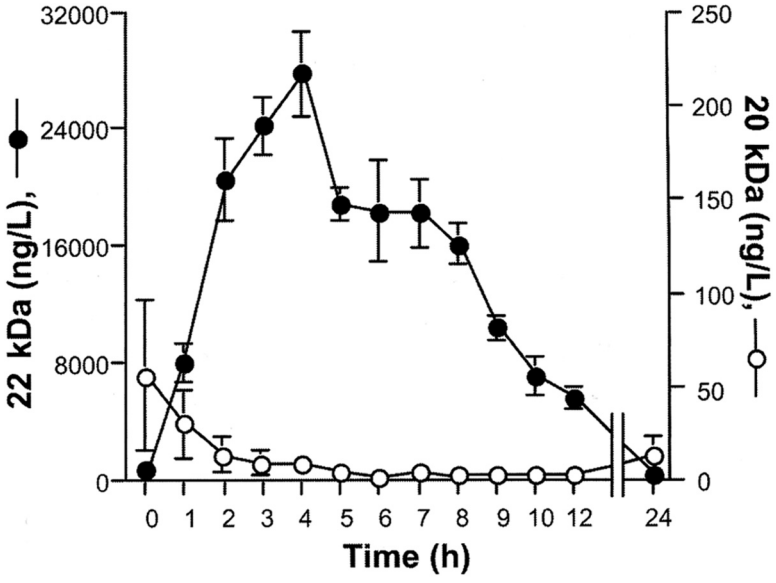

Figure 5. Response of serum 20K-GH to exogenous GH administration. The pharmacokinetic serum profile of sc injected recombinant 22K-GH is depicted in the *solid circles*. In response to the exogenous GH, endogenous 20K-GH is suppressed for a period between 12 and 24 h (*open circles*). [Reproduced from K. C. Leung *et al.*: Physiological and pharmacological regulation of 20-kDa growth hormone. *Am J Physiol Endocrinol Metab* 283:E836–E843, 2002 (62), with permission. © American Physiological Society.]

specific immunoassays have been developed for 22K-GH and 20K-GH (and placental GH). Using these assays, the proportion of 20K-GH as part of total serum GH ranges between 3 and 28%, with an average of 5–9%, and with no consistent differences between adults, children, genders, ages, or physiological states (61–63, 74, 80, 81). No specific assays exist for the other GH-N related isoforms; their proportions in serum (Table 2) are derived from physicochemical separation followed by polyvalent immunoassay (82–86).

The stability of GH in blood is high. GH is an inherently stable protein with a long shelf life when purified. Degradation within blood is minimized by the high concentration of protease inhibitors present in plasma (87). GH concentrations in serum or plasma stored at 4 C or at −20 C are not changing significantly over days to weeks (88) (G. Baumann, personal observation). Incubation of pituitary GH with human blood plasma at 37 C for up to 24 h has not revealed detectable degradation products (89). No statistically significant changes in serum immunoreactive GH concentrations were found after 24 h at room temperature, 2–7 d at 2−8 C, or 6 months at −15 C (90). Thus, GH is not subject to significant intravascular metabolism or degradation in blood plasma or serum stored *ex vivo*.

J. GH in urine

Small amounts of GH are excreted in the urine. Despite the fact that glomerular filtration is the main route of GH clearance, the uptake and degradation of filtered GH in the proximal nephron is so efficient as to leave only a minute fraction (~0.01%) to reach the final urine (69, 70). This process is mediated by the multispecific megalin-cubulin-amnionless receptor system, which leads to endocytosis of filtered proteins followed by their proteolytic digestion in lysosomes (see Ref. 91 for review). Thus, urinary GH excretion accounts for less than 0.005% of the GH secreted by the pituitary or administered exogenously (69, 70, 92, 93). Nevertheless, even these small amounts are readily measurable by modern immunoassays (94–100). In older, less sensitive assays requiring larger sample volumes, the high osmolality of urine caused interference and spuriously high readings (70, 92, 101).

With respect to urinary excretion of GH isoforms, there is only very limited information. Baumann and Abramson (70) showed evidence of the presence of monomeric 20K-GH and acidic GH forms in urine but found no evidence for dimeric or oligomeric GH. Similarly, Mauri *et al.* (102) reported only monomeric GH in urine. This would be expected based on molecular size restriction at the glomerular sieve. There are only two reports that show evidence for 20K-GH in the urine (70, 103).

The stability of GH in stored urine was evaluated by Main *et al.* (95), who showed stability at −20 C for 2 wk but a 25% loss over 7 months, whereas GH remained stable when stored at −80 C for the same period.

The amount of GH excreted in the urine is highly variable, both between subjects and within the same individual from day to day, with intraindividual coefficients of variation of 40–60% (95, 104, 105). Numerous studies in the 1990s evaluated the potential utility of 24-h urinary GH excretion as a diagnostic tool for disorders of GH secretion, such as hypopituitarism, GH deficiency, and acromegaly (94–98, 100, 104–109; only a few selected references are listed here, but a complete list is available from the author upon request). Urinary GH excretion rises after administration of exogenous GH (106, 110), but there is limited information and likely overlap with normal excretion rates. In all, over 3200 subjects have been evaluated, representing a robust database on urinary GH excretion. The results of these studies can be summarized as follows. 1) The amount of GH excreted in normal sub-

jects in a 24-h period ranges between 0.3 and 80 ng, a greater than 100-fold range, with the majority of values between 2 and 15 ng per 24 h. 2) Excreted amounts vary widely among subjects for reasons that are poorly understood. 3) On a population basis, urinary GH excretion roughly follows trends of plasma GH (*e.g.*, values are lowest in hypopituitarism, high during puberty, highest in acromegaly, *etc.*), but there is substantial overlap between these categories. 4) Among individuals, there is no correlation between 24-h integrated plasma GH levels and urinary GH excretion. 5) No correlation is found between urinary GH excretion and auxological measurements in children. 6) Day-to-day variation in excretion renders the interpretation of a single measurement unreliable. 7) The intra- and intersubject variability far exceeds that which can be attributed to analytical imprecision and disparities among assays. And 8) Individual urine GH measurements are too variable to be useful as a tool for clinical diagnosis, even in conditions at the extremes of the GH secretion spectrum (*i.e.*, hypopituitarism and acromegaly). GH excretion is also strongly impacted by renal factors, such as proteinuria of pathological or physiological origin (including exercise-induced proteinuria) (107, 108, 111). Renal insufficiency also leads to increased GH excretion (112). For all these reasons, the scientific literature on urinary GH excretion has largely fallen silent in the last decade. Two recent publications reported the use of isopropylacrylamide hydrogel particles loaded with Cibacron Blue to concentrate GH from urine before immunoassay (113, 114). The GH concentrations measured by that technique are lower (<1 pg/ml) than those by direct assay. Unfortunately, no recovery data were reported, and it appears likely that adsorptive losses may have contributed to incomplete recovery of GH from the particles. This would be expected at such low protein concentrations and would explain the lower values. No results were reported on isoforms extracted from urine. It is not clear whether concentration of GH from urine is advantageous over direct measurement using high-sensitivity assays.

K. GH in saliva

There is little information on the presence of GH in saliva. One study in normal subjects reported salivary GH levels to be 1000-fold lower than those in serum and a significant correlation between salivary and serum GH concentrations (115).

L. GH measurement

GH in biological fluids can be measured by *in vitro* bioassay, radioreceptor assay, or immunoassay. Bioassays and radioreceptor assays are not suitable for routine purposes, are highly vulnerable to interference from GHBP, and are generally used only in the research setting. Immunoassays are of either the single-site competitive type or the two-site sandwich type [radioimmunoassay (RIA), immunoradiometric assay (IRMA), enzyme immunoassay (EIA), enzyme-linked immunosorbent assay (ELISA)], and use radioactivity, colorimetry, fluorescence, or chemiluminescence as a readout. Modern immunoassays in clinical use are of the two-site immunometric design and are highly sensitive. Most antibodies recognize all GH isoforms, but a few isoform-specific assays exist for 22K-GH, 20K-GH, and placental GH. Disparities of results obtained by different assays of up to at least 100% have been reported, depending on reagents, epitope recognition among GH isoforms, assay design (equilibrium *vs.* nonequilibrium, incubation time, and temperature), and matrix effects. Important, but not exclusive, reasons for assay disparities are differential recognition of GH isoforms and interference by the high-affinity GHBP. Typically, modern monoclonal, nonequilibrium assays are more affected than older, polyclonal assays with longer incubation times. This topic has been reviewed in detail (116, 117). Efforts are under way to harmonize GH measurements in clinical chemistry laboratories as much as possible, and a recent workshop of the GH Research Society, the IGF Society, and the International Federation for Clinical Chemistry and Laboratory Medicine (IFCC) has addressed this issue (118). Although assay discrepancies present a significant problem in the clinical arena, they are less of a concern in the antidoping field because absolute levels of GH are not a major endpoint in detection of GH abuse.

Nonimmunological, mass-based measurements of GH in biological fluids [*e.g.*, mass spectrometry (MS)] are currently not used because of insufficient sensitivity of these methods at the GH levels prevailing in blood or urine. Efforts are being made to improve sensitivity with the goal to develop MS-based assays for GH in serum (119, 120).

III. Strategies for Detection of GH Abuse

The fact that exogenous GH is identical to the main form of endogenous GH (22K-GH) renders its detection challenging. Thus, conventional forensic identification methods for foreign substances are not applicable. Furthermore, the pulsatile secretion pattern of GH makes it difficult, if not impossible, to interpret a high serum GH level as evidence for GH doping. Two main strategies for detection have been developed: the GH isoform test and the biomarker test. Both are currently applicable only to blood samples.

A. The GH isoform test

The GH isoform test is a direct detection method (by itself not definitive, for reasons mentioned above) com-

bined with a biological response based on suppression of endogenous GH secretion by exogenous GH. This general strategy, first proposed by Wu *et al.* (121) and Momomura *et al.* (103), was further developed (90) and tested at the Olympic Games in 2004 (Athens), 2006 (Turin), and 2008 (Beijing); it is now in general use as a WADA-sanctioned test. In essence, the test consists of two GH immunoassays: one that is relatively specific for 22K-GH and another that is "permissive," that is it recognizes a number of pituitary isoforms in addition to 22K-GH. It is not known to what degree the various GH isoforms (except for 20K-GH; see *Section III.A*) are measured by the permissive test, but such knowledge is not critical for antidoping purposes. A dose of exogenous GH suppresses the endogenous forms, including 22K-GH, 20K-GH, and other isoforms (Fig. 5). Thus, the ratio between 22K-GH and pituitary GH increases because most of the measurable GH is of exogenous origin (90, 121). For validation purposes, WADA requires two independent assays, and thus two separate pairs of 22K-GH-specific (named "rec" for recombinant) and permissive (named "pit" for pituitary) antibodies are used in two independent assays (named A and B) (90) (Fig. 6). Using these assays, the normal rec/pit ratio has a median value of approximately 0.8 and ranges from 0.1 to 1.2. The median value of less than 1 reflects the fact that 22K-GH accounts for only 75–80% of the GH isoforms. The current rec/pit ratio cutoffs ("decision limits") used by WADA for evidence of doping is 1.81 for men and 1.46 for women (assay kit 1) and/or 1.68 for men and 1.55 for women (assay kit 2) (122). These values have been derived from the analysis of athlete samples obtained under real-world doping control conditions and are designed to yield a combined test specificity (between the two kits) of 99.99%. Of interest, none of the four assays in current use measures 20K-GH because the detection antibody used for signal generation does not recognize 20K-GH (90). Replacement of the detection antibody with one that also recognizes 20K-GH would probably be advantageous because 20K-GH is an important constituent of pituitary GH. It has been suggested that the permissive assay could/should be replaced by a specific 20K-GH assay, which would be chemically better defined and scientifically more rigorous (103, 123). Although correct, this idea would be disadvantageous if an athlete were to use a mixture of 22K-GH and 20K-GH in physiological proportions. It is unknown whether recombinant 20K-GH, which has been pharmaceutically produced but not marketed, is available on the black market. With the permissive assay, it is unlikely that an athlete could duplicate a normal pattern unless he or she were taking cadaveric GH or using a GH secretagogue. Thus, both specific assays and permissive assays have their unique advantages and disadvantages. It may be possible in the future to supplement the existing isoform test with one that specifically measures the 22K-GH/20K-GH ratio, keeping the above-mentioned relative ease of evading detection by such a test in mind. All four assays used in the GH isoform test show some cross-reactivity with GH-V (placental GH) (90), raising the question of applicability of the test in pregnant women. Interference by GH-V is negligible at levels below 10 ng/ml, which in normal pregnancy are not reached until the end of the second trimester (13, 14). Since most women in their third trimester are not likely to participate in competitive sports, and since pregnancy is usually obvious at that

Figure 6.

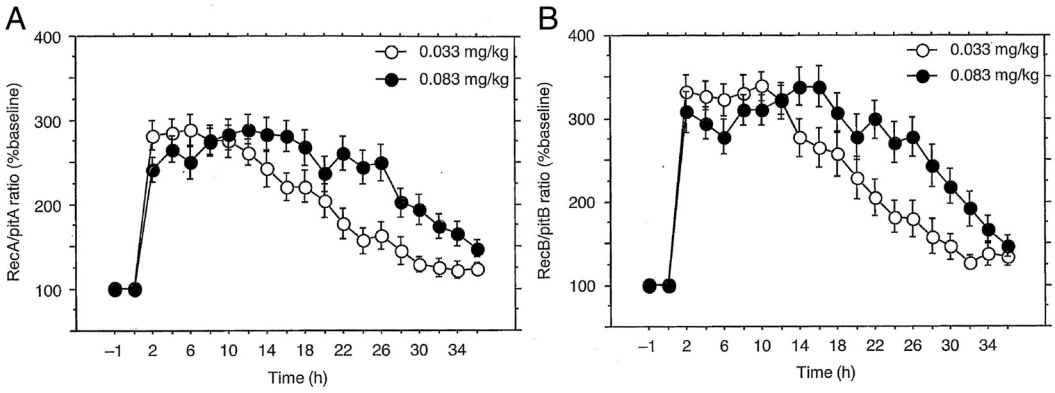

Figure 6. GH isoform test. The response of the rec/pit ratio to administration of exogenous GH (two dose levels) at time 0 in two different assays (assay A in *left panel*, assay B in *right panel*). The ratio rises to 250–350% over baseline and remains elevated for 24–36 h. The higher GH dose results in longer elevation of the ratio. For a 70-kg person, the GH doses listed correspond to 2.31 and 5.81 mg, respectively. [Reproduced from M. Bidlingmaier *et al.*: High-sensitivity chemiluminescence immunoassays for detection of growth hormone doping in sports. *Clin Chem* 55:445–453, 2009 (90), with permission. © American Association for Clinical Chemistry.]

stage, GH-V cross-reactivity in the GH isoform test is not a significant problem in practice.

The isoform test is an excellent strategy to detect GH doping, provided it is administered shortly after the last GH dose (within ~24–36 h, depending on the dose) (90), realistically probably within 12–24 h. A recent placebo-controlled study of the detection time window in young men after administration of recombinant GH (33 μg/kg, or ~2.3 mg for a 70-kg person), and using the currently employed WADA assays, procedures, and decision thresholds, showed a postinjection duration of test positivity of 14.5 ± 5.5 h (mean ± SD) (248). In the same study, repeated daily administration of the same dose of GH for 2 wk and sequential testing revealed that blood samples obtained 10 h after the preceding GH dose always tested positive, whereas samples taken 21 h after the preceding dose always tested negative. This short window of opportunity has been the Achilles heel of the isoform test, with the first positive result occurring only after more than 2 yr of general implementation encompassing more than 1500 tests (124). This experience is not indicative of presumed (and in some cases acknowledged) use of GH; it can be explained by the athletes stopping GH injections at least 1 d before an expected test. The test, therefore, is not well suited for in-competition testing. Its use in unannounced out-of-competition testing, however, should be more successful in catching GH abusers, and recently it has been used mostly in that setting. Despite that, at the time of this writing (November 2011), only eight positive findings have been recorded among over 3400 tests, one of them in an athlete possessing a therapeutic use exemption (248). One likely reason for this relatively low "yield" is the high decision threshold, designed to protect the athlete by minimizing false-positive results. As experience with the test and data for the normative range accrue, it is possible that the cutoff values for positivity can be set at a more stringent level, allowing better discrimination between users and nonusers without sacrificing the conservative nature of the test.

The strategy used for the blood isoform test would be theoretically applicable to a urine isoform test. Indeed, limited data have shown that urinary GH excretion rises after administration of exogenous GH (103, 106, 110), presumably representing the injected 22K-GH. Very little information is available about suppression of endogenous GH isoforms in urine; one publication showed no suppression or even slightly higher urinary 20K-GH levels after administration of GH, although the 20K/22K ratio was lower because of the elevated 22K-GH level (103). Reliable detection of minor isoforms in urine is a substantial challenge, given the low concentrations of total GH in urine. Additional difficulties would be those discussed in *Section II.J*, including lack of scientific background information about isoform handling by the kidney, distorted isoform profiles because of glomerular filtration cutoffs, nonspecific influences such as proteinuria, and most importantly, the same short window of opportunity that applies to blood testing.

B. The biomarker test

1. GH biomarkers and the biomarker test in blood

The GH biomarker test is an indirect test based on downstream biochemical changes resulting from GH action. Well-known effects of GH are the induction of IGF-I expression and promotion of collagen turnover in bone and connective tissues (125). Thus, IGF-I and procollagen type III amino-terminal propeptide (P-III-NP) have been selected as relatively specific GH-responsive biomarkers suitable for an antidoping test. [Other GH-dependent biomarkers considered but not ultimately selected for various reasons (discussed in *Section B.1*) were IGF-binding protein (IGFBP) 2 and IGFBP3, acid-labile subunit (ALS), and markers of bone turnover, such as procollagen type I amino-terminal propeptide (PINP) and carboxy-terminal propeptide (PICP), osteocalcin, and type I collagen carboxy-terminal cross-linked telopeptide (ICTP).] Major efforts have been made by the GH-2000/GH-2004 consortium, the Australian-Japanese consortium, and other groups to validate the biomarker test under various circumstances (age, gender and ethnicity, elite *vs.* recreational athletes *vs.* the general population, type of sport, effect of training, injury, anabolic steroid or erythropoietin use, *etc.*). A substantial database regarding these GH biomarkers and conditions has been accumulated over the past one to two decades. The history of the development of the biomarker test has been summarized by Sönksen (126) and Holt *et al.* (5).

IGF-I, a 70-amino acid peptide with three disulfide bridges and a mol wt of 7649, is an important mediator of many GH actions and exhibits mitogenic, anabolic, and insulin-like metabolic activities. It shares structural and functional features with insulin and acts through the type 1 IGF receptor (also known as IGF-1 receptor), which shares homology with the insulin receptor. IGF-I binds with high affinity to the IGF-1 receptor and with lower affinity to the insulin receptor. At physiological concentrations, most of IGF-I action is mediated through the IGF-1 receptor. GH is the principal regulator of IGF-I production in healthy individuals. IGF-I is synthesized and released into the bloodstream by the liver in response to GH; it is also produced as a paracrine/autocrine factor in many other GH-responsive tissues, with some spillover into the circulation (Fig. 4). The liver accounts for the

Figure 7.

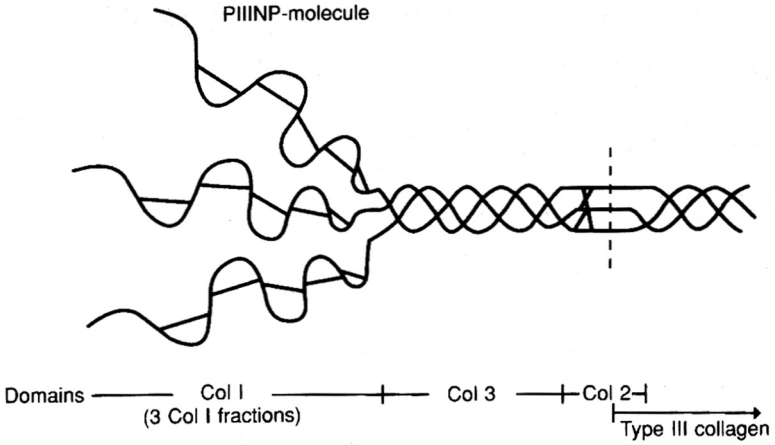

Figure 7. Structural organization of P-III-NP. The diagram represents the amino-terminal portion of the type III procollagen molecule. The triple helix of mature collagen is depicted/truncated on the *right*. The *dashed line* denotes the proteolytic cleavage site within the N-telopeptide region of procollagen that gives rise to P-III-NP. Triple helix formation of procollagen precedes P-III-NP cleavage, resulting in the latter being a homotrimer of three α1(III) linked chains, by two interchain disulfide bridges in the nonhelical Col 2 domain as well as stabilization in the triple-helical Col 3 domain. The globular Col 1 domain contains several intrachain disulfide bridges (indicated in the *graph*), the sulfate group(s), and the specific immunological epitopes. [Reproduced from K. D. Bentsen: Type III procollagen peptide: studies on the circulating peptide as a marker of fibrinogenesis with special reference to the liver. *Dan Med Bull* 40:235–246, 1993 (140), with permission. © The Danish Medical Association.]

majority (~75%) of circulating IGF-I (127). IGF-I in blood is bound to six IGFBP in ternary and binary complexes. Ternary complexes, formed with IGFBP3 and IGFBP5, also contain another GH-dependent protein, ALS, as the third component (Fig. 4). The majority of circulating IGF-I is bound in the IGFBP3/ALS/IGF-I complex. The protein-bound state of IGF-I in blood is responsible for its long circulating half-life.

P-III-NP, a protein with mol wt of approximately 40,000, is a by-product of type III collagen biosynthesis. Type III collagen is a constituent of numerous tissues, including the vasculature, skin, intestines, and other viscera; its distribution is ubiquitous as a component of blood vessels. Procollagen is secreted in soluble form into the extracellular space, where it undergoes condensation to a triple helix under the guidance of its C-terminal propeptide domain, which serves as a nucleation focus. After this, both the C-terminal and N-terminal propeptides are cleaved from procollagen by bone morphogenetic protein 1 and one or more metalloproteinases of the ADAMTS family, respectively, and released into the lymphatic system and bloodstream (see Ref. 128 for review). P-III-NP is a trimeric protein composed of three identical partial procollagen α1(III) polypeptide chains, which in humans contain 129 amino acids and have a mol wt of 13,116 each (129–132). P-III-NP consists of an amino-terminal globular region, a central triple helical region, and a carboxy-terminal telopeptide region; these domains are named Col 1, Col 3, and Col 2, respectively (133, 134) (Fig. 7). The trimer is stabilized by two interchain disulfide bridges near the carboxy-terminus and by a collagen-like triple helix formation in the central Col 3 domain (135). P-III-NP is a very acidic protein (pI- ~3) due to sulfation in the Col 1 domain; the precise residue(s) carrying sulfate has not been identified (130). A consensus sequence for N-linked glycosylation exists near the carboxy-terminus, but no glycosylated P-III-NP has been described. The globular Col 1 domain appears to be the principal epitope recognized by polyclonal antisera generated against P-III-NP (130). Human and bovine P-III-NP have 95% sequence identity (131), and human, bovine and porcine P-III-NP show complete cross-reactivity in polyclonal immunoassays (130, 136). The principal immunoreactive region resides in the Col 1 domain; this is a conformational epitope because the intact P-III-NP trimer is much more immunoreactive than the monomeric peptide (130, 137, 138). Monoclonal antibodies have been developed, but it has been difficult to define the exact epitopes recognized because of the complexities inherent in P-III-NP and its isoforms and degradation products (for review, see Ref. 139).

P-III-NP circulating in blood is heterogeneous and consists of at least four immunoreactive forms of different molecular size (see Refs. 134 and 140 for review) (Fig. 8). Intact P-III-NP is a minority component designated Peak III on gel filtration chromatography. Peak II, with molecular size about twice that of P-III-NP, is thought to be a P-III-NP dimer [*i.e.*, a hexamer of monomeric partial procollagen α1(III) chains]. Peak I is of high mol wt and remains largely uncharacterized. It may represent P-III-NP aggregates, P-III-NP bound to plasma proteins, or incompletely cleaved P-III-NP still attached to the rest of the collagen molecule (also known as pN-collagen type III). Peak IV has a smaller molecular size than P-III-NP and is assumed to be the Col 1 fragment and/or degradation product(s) of P-III-NP, or a species unrelated to procollagen III. To date, none of these interpretations of the nature of P-III-NP size variants has been corroborated by direct chemical analysis. Of importance, depending on the immunoassay, these different molecular species are recognized to different degrees. The two commercial assays cur-

Figure 8.

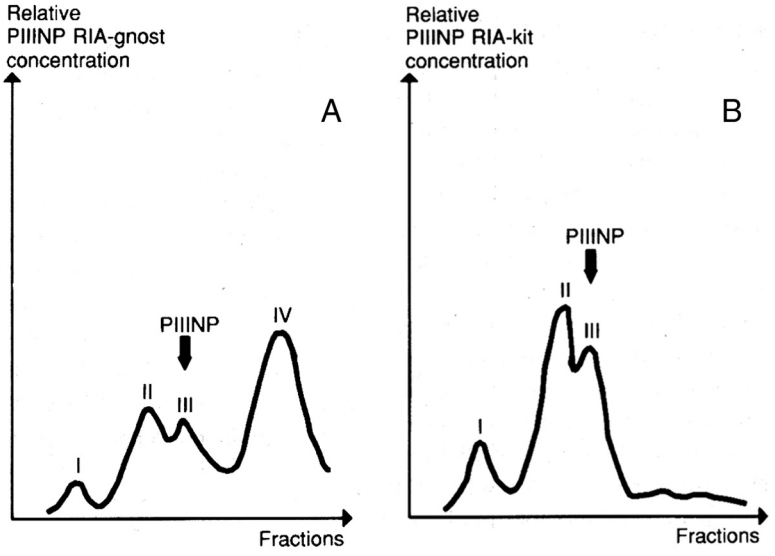

Figure 8. P-III-NP immunoreactivity in serum. Gel filtration profiles of serum P-III-NP immunoreactivity, as measured by two immunoassays. Four peaks of different molecular size are seen; the elution position of intact P-III-NP is indicated. A, Profile obtained with the CIS RIA-gnost assay. B, Profile obtained with an early version of the Orion UniQ assay. The different components are recognized to different degrees by the two assays. The precise molecular nature of the four peaks has not been determined. [Reproduced from K. D. Bentsen: Type III procollagen peptide: studies on the circulating peptide as a marker of fibrinogenesis with special reference to the liver. *Dan Med Bull* 40:235–246, 1993 (140), with permission. © The Danish Medical Association.]

rently in use (see *Section III.B.4*) are reportedly not sensitive to Col 1/peak IV material.

The blood levels of both IGF-I and P-III-NP increase in response to GH and disappear with reported half-lives of 90 and 700 h, respectively (141). These values are probably overestimates of true plasma half-lives, which are 14–18 h for IGF-I in man (142) and ~60 min for P-III-NP in the pig (143) (no human data on plasma P-III-NP half-life are available). The discrepancies may be explained by continued production of the biomarkers for some time after cessation of GH dosing and, in the case of P-III-NP, generation of high mol wt immunoreactive degradation products with long half-lives (143). In any case, serum immunoreactive IGF-I and P-III-NP remain elevated for about 4 d and 2–8 wk, respectively, depending on the GH dose (144–146) (Fig. 9). Unlike the pulsatile pattern of GH in blood, their serum levels remain relatively constant throughout the day and between days (147). Thus, although less specific than a direct test for GH, biomarkers have the practical advantage of a longer window of opportunity for detection. The biomarker test is also potentially applicable to the detection of IGF-I abuse, and studies to assess this possibility are in progress (see *Section V*).

Two concerns exist with respect to the biomarker test: 1) lack of specificity and vulnerability to factors not related to GH or IGF-I; and 2) limitations of available assays. In addition, test interpretations are complicated by age- and sex-dependent variation.

With respect to specificity of IGF-I, few if any conditions elevate IGF-I as consistently as GH. One possibility is obesity, which can result in mildly increased serum IGF-I, but most studies show no correlation between body mass index and serum IGF-I. Furthermore, obesity is not likely to be a major confounder in most sports, with the possible exception of Sumo wrestling. Neither exercise nor injury significantly affects IGF-I levels, although in some subjects exercise resulted in a mild and transient (<30 min) increase in serum IGF-I that may represent hemoconcentration (148, 149). Sports injuries have been shown to have either no effect or only a minimal effect on IGF-I (150, 151). Even after major injury (tibia fracture), the transient IGF-I response is much lower than that obtained with even a modest GH dose (15 μg/kg · d; ~1 mg/d) (151). Testosterone administration does not alter IGF-I levels or the response of IGF-I to GH (152). Similarly, erythropoietin has no effect on IGF-I levels (153). Thus, serum IGF-I is a very good biomarker for GH action; its only drawback is the relatively short duration of elevation (a few days).

The specificity of P-III-NP is not as narrow as that of IGF-I. Its plasma levels have been evaluated in the context of exercise and injury. Exercise increased P-III-NP levels in some studies but not others, and when present, the rise was much smaller than what is seen after GH administration (reviewed in Ref. 149). After an injury, collagen turnover is expected to be increased as part of the healing process, and indeed P-III-NP levels rise after sports injuries. After a soft tissue injury, they peak at 2 wk and are back to baseline after 7 wk; after a bony injury, they peak at 6 wk and return to baseline after 12 wk (150). However, the rise is relatively minor compared to that which occurs after GH administration (150). Similarly, other markers of collagen and bone turnover (small fragment of C-terminal cross-linked telopeptide of type I collagen (CTX) and osteocalcin) after tibial fracture show elevations that are substantially lower than those seen with GH treatment (151). Erythropoietin has no effect on P-III-NP levels (153), but testosterone administration mildly increases P-III-NP and enhances its response to GH (152). However, this effect appears insufficient to adversely affect the dis-

Figure 9.

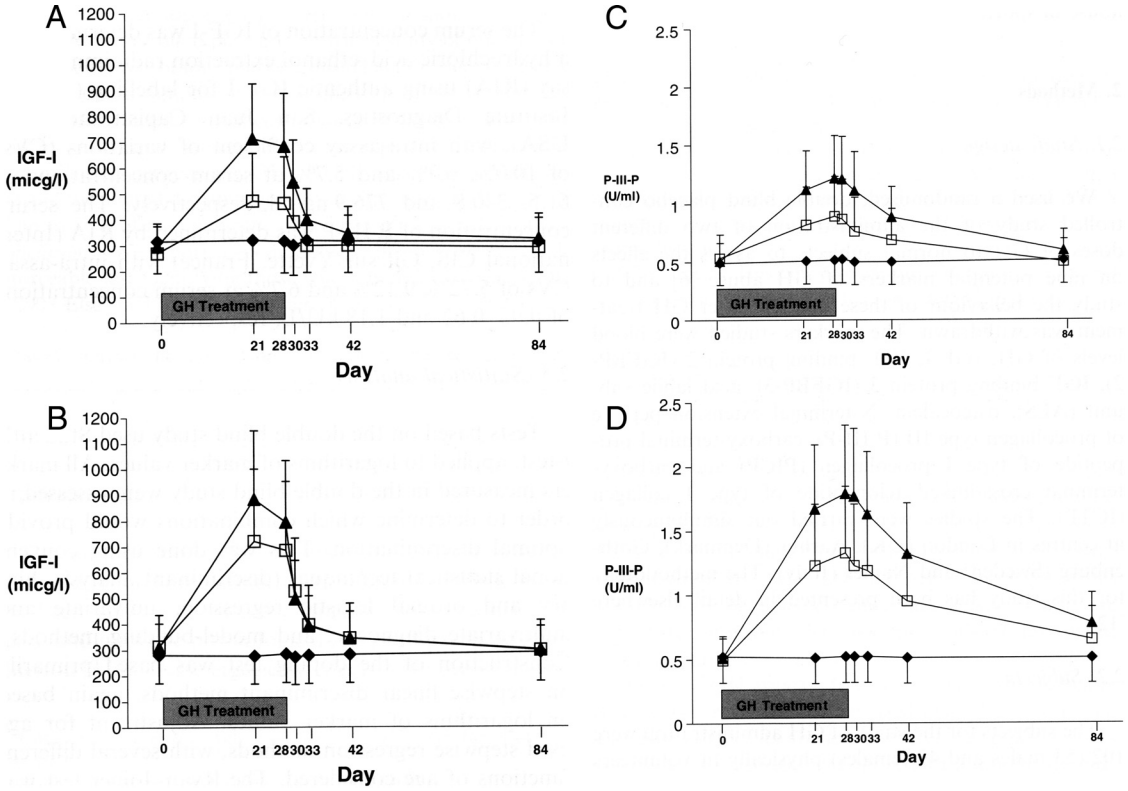

Figure 9. The biomarker test. Time course of changes in serum IGF-I (A and B) and P-III-NP (C and D) during and after cessation of GH treatment. A and C, Women; B and D, men. The period of GH treatment (28 d) is indicated by the *cross-hatched bar*. *Diamonds*, placebo; *squares*, low-dose GH (33 μg/kg · d; ~2.3 μg/d); *triangles*, high-dose GH (66 μg/kg · d; ~4.6 μg/d). Note the more exuberant responses for both biomarkers in men. [Reproduced from J. K. Powrie *et al.*: Detection of growth hormone abuse in sport. *Growth Horm IGF Res* 17:220–226, 2007 (146), with permission. © Elsevier B.V.]

criminant function (see below) used to distinguish GH abusers from nonusers (5).

Because both IGF-I and P-III-NP levels change as a function of age, with a peak in adolescence and a gradual, lifelong decline thereafter (paralleling the age-dependent changes in GH secretion), values must be interpreted against an age-appropriate normative range. Similarly, gender and possibly ethnicity affect these biomarkers, which requires interpretation against appropriate normative ranges. IGF-I levels tend to be higher in women, whereas collagen markers, including P-III-NP, are higher in men. Extensive study of these parameters in elite athletes, both immediately after a competitive event and at random times (representing out-of-competition conditions) have shown that age and gender are the major confounders, whereas ethnicity and sport type have only a minor influence (149, 154–156).

The dynamics of IGF-I and P-III-NP during and after GH treatment can be summarized as follows (152): IGF-I rises rapidly to near peak levels within 2 wk after starting GH, with P-III-NP following more slowly to near peak levels within 4–6 wk. After cessation of GH treatment, IGF-I falls most rapidly to reach baseline after 7 d, whereas P-III-NP declines more slowly toward near-baseline after 4 wk and fully to baseline by 6 wk. Thus, IGF-I is more useful as a detection tool in the early phases of both initiation and cessation of GH use, whereas P-III-NP is most useful for the later time points after cessation.

The combined values of IGF-I and P-III-NP have been used to devise a discriminant formula that separates GH users from nonusers and thus can be used as a practical GH doping detection test (146). The discriminant functions are different for men and women, they take age into account, and they are based on biomarker values obtained in specific commercial immunoassays (see *Section III.B.4* for comments on the latter). Using these formulae, a positive doping test score has been proposed at a threshold that is predicted to yield a false-positive reading in no more than 1 in 10,000 tests, *i.e.*, at a Z value of at least 3.72 (146, 157). Because of the above-mentioned dynamics, the test relies increasingly on P-III-NP as time elapses after stopping GH use, and P-III-NP is therefore given more weight.

For the biomarker test as currently designed, a window of opportunity of several days following cessation of GH exists. In normal volunteers given doses of approximately 2–4 mg of GH daily, the test remained positive following cessation of GH in 69–79% after 2 d, in 53–64% after 5 d, and in 20% after 14 d (146). The duration of this window of opportunity is dose-dependent, varies among individuals, and tends to be longer in men. A realistic estimate in practice may be 1 wk, although a window of up to 14 d has been suggested (5).

Intraindividual fluctuations in biomarkers over time have been examined, and coefficients of variation ranging from 14 to 20% for IGF-I and 7 to 18% for P-III-NP have been found (147, 158). This degree of variability did not interfere with the performance of the discriminant function (147). Both IGF-I and P-III-NP show the most rapid changes during adolescence under the influence of pubertal up-regulation of GH secretion. A study in adolescent elite athletes has confirmed that the discriminant function remains valid as a determinant of GH abuse even in this most demanding circumstance (159), but particular caution is probably advisable for the interpretation of results in adolescents.

GH biomarkers other than IGF-I and P-III-NP have also been investigated for their suitability for a detection test; they include several members of the IGF system and various markers of collagen and bone turnover. Most have been less extensively studied than IGF-I and P-III-NP, and some were not further pursued because they were judged to be less well-suited for a detection test than those two biomarkers. Among members of the IGF system, IGFBP3, IGFBP2, and ALS were shown to be less responsive to GH treatment than IGF-I (144, 152, 160). Among collagen and bone markers, osteocalcin, procollagen type I amino-terminal propeptide (PINP), carboxy-terminal propeptide (PICP), and type I collagen carboxy-terminal cross-linked telopeptide (ICTP) were found to respond less vigorously to GH and return to baseline more quickly after GH cessation than P-III-NP (141, 145, 160–162). Other considerations include the inherent variability of a biomarker within or between subjects, or as a function of gender and ethnicity, which from the standpoint of a detection test should be kept to a minimum (158, 163). The aggregate of all these observations led to the selection of IGF-I and P-III-NP as the currently most suitable biomarker pair for development of a GH doping detection test.

2. Biomarkers in urine

Biomarker testing is currently only applicable to blood; its potential use as a urine test faces significant obstacles. There is little information on how the kidney handles IGF-I or P-III-NP. Based on insulin excretion data (164), it can be assumed that (free) IGF-I is filtered at the glomerulus and extensively taken up and degraded in the proximal tubule, akin to the fate of GH described in *Section II.J*. A similar renal degradation process has been shown for P-III-NP (165). There is evidence that IGF-I is directly produced by the kidney and excreted in the urine (166, 167). Some studies have examined urinary IGF-I in clinical and antidoping contexts, whereas there is very little information on urinary P-III-NP. Tönshoff *et al.* (168) reported urinary IGF-I concentrations of 0.08 ± 0.07 ng/ml, which did not change after 3 d of GH treatment. Gill *et al.* (98) showed widely varying excretion rates (0–1350 ng/24 h) in normal adults and a similar range of values (0–950 ng/24 h) in matched patients with severe organic GH deficiency. Similarly, no difference in IGF-I excretion rates was shown between GH-deficient and GH-sufficient children and adolescents (169). Attempts to use urinary IGF-I as a diagnostic tool for IGF deficiency or excess states were abandoned when it was realized that urinary IGF-I does not correlate with serum IGF-I and does not reflect underlying GH secretion status (98). De Palo *et al.* (170, 171) compared IGF-I excretion in sedentary individuals and trained cyclists before and after strenuous exercise. They found a wide interindividual range of IGF-I excretion (0–350 ng/liter) and a 240% increase in excretion after exercise. A highly significant correlation existed between urinary total protein and IGF-I, but no correlation was found between plasma IGF-I and urinary IGF-I. A weak correlation was shown between urinary GH and urinary IGF-I excretion, but this was mostly dependent on a few outlier values. The urinary IGF-I/urinary GH molar ratio showed major differences between sedentary subjects, cyclists before exercise, and cyclists after exercise (means of 190, 15, and 577, respectively). The high variability of these findings may be explained by inherent variability of IGF-I (and GH) excretion, exercise-induced proteinuria (including IGF-I and GH), and renal production of IGF-I not reflective of plasma IGF-I. Pichini *et al.* (172) compared urinary IGF-I values in sedentary individuals and recreational and elite athletes and also found wide ranges and overlaps among the three groups, without consistent changes in response to training and competition. Uemasu *et al.* (173) examined the effect of exogenous GH administration on urinary IGF-I excretion and found that despite the expected increase in serum GH and IGF-I, urinary IGF-I output actually decreased significantly. Taken together, the available literature on urinary IGF-I can be summarized as follows. 1) Urinary IGF-I excretion is highly variable, ranging from undetectable to 1000 ng/24 h with an approximate mean of 130–450 ng/24 h, depending on the study. 2) Urinary IGF-I excretion does not reflect serum IGF-I or GH secretion rate in clinical studies.

3) Exercise increases urinary IGF-I, an effect that can be at least partially attributed to exercise-induced proteinuria. And 4) The administration of GH does not raise urinary IGF-I excretion. Thus, the biology of IGF-I in urine does not appear to be an index of GH status and is unlikely to be useful for detection of illicit GH use. Furthermore, because IGFBPs are also present in urine (98, 169, 172, 174), the use of urine does not avoid the problem of IGFBP interference in IGF-I measurements (see *Section III.B.4*), thereby not conferring an analytical advantage over the use of blood.

With respect to urinary P-III-NP, one publication listed a daily excretion of P-III-NP immunoreactivity of 30–110 μg, but indicated that this represented the Col 1 fragment rather than the intact peptide (175). Another recent publication reports a range of 2–110 ng/mmol creatinine (corresponding to an excretion rate of roughly 20–1430 ng/24 h) in subjects with normal renal function (176). The vast majority of this activity is kidney-derived, rather than blood-derived, and urinary P-III-NP is increased in patients with renal disease resulting in fibrosis (176). The scientific background information on urinary P-III-NP and its relation to GH is insufficient to permit contemplation of a detection test for GH abuse based on urine P-III-NP at this time. The nature of the limited data available raises doubts about the feasibility of a robust urine test.

Urinary excretion of other, small collagen biomarkers [N- and C-terminal cross-linked telopeptides of type I collagen (NTX and CTX), pyridinoline, deoxypyridinoline, and hydroxyproline] is known to be highly variable and subject to diurnal fluctuation, which necessitates 24-h urine collections (177). Because of these characteristics, their measurement has proven to be of limited diagnostic value in the assessment of clinical bone disorders. Although these urinary biomarkers have not been examined in an antidoping context, the experience in the clinic does not support their suitability for a reliable GH detection test.

3. Biomarkers in saliva

Saliva has also been considered as a biological fluid for IGF-I measurement. Limited data show that salivary IGF-I concentration is 40- to 200-fold lower than serum IGF-I, and that its source is at least in part derived from local synthesis in the salivary gland (178–181). Early suggestions of using saliva as a diagnostic tool to assess GH deficiency or excess states have not been adopted because salivary GH did not reliably identify such conditions (180). Antonelli *et al.* (182, 183) examined salivary IGF-I in athletes and found that they had lower levels than control subjects, and that after exercise IGF-I levels rose in saliva, but not in blood. No reports have yet appeared on the response of salivary IGF-I to exogenous GH. Taken together, the published data suggest that salivary IGF-I may bear a rough relationship to serum IGF-I and GH, but that its biology is poorly understood and its correlation with GH status is insufficient to yield a robust detection tool for GH abuse.

4. Analytical considerations and challenges

a. IGF-I measurement. Currently the measurement of IGF-I in blood is conducted by immunoassay. Its assay presents significant challenges, primarily because of interference by IGFBPs. The "gold standard" for IGF-I measurement in serum is acidification, which dissociates the complexes, followed by removal of binding proteins by acid gel filtration on a sizing column. This technique is laborious and not amenable to routine, high throughput use. Alternative methods are acid-ethanol precipitation, extraction of IGF-I on C_{18} Sep-Pak cartridges, dissociation of complexes with acid followed by blocking of rebinding with excess IGF-II, and conducting the assay in commercial "dissociation buffers." The latter likely contain IGF-II. None of these methods are successful in completely removing IGFBP interference. As a result, major disparities in results exist among assays. This issue has been recently reviewed in detail (118, 184).

An additional problem with IGF-I measurement lies with the international reference standard, against which assays are calibrated. The World Health Organization international reference reagent 87/518 is not pure and therefore has an artificially high weight assignment. In addition, its stocks are depleted, and a new, pure international reference reagent (02/254) has been adopted. Assays calibrated against the new standard will yield lower results, thereby rendering comparisons with earlier studies difficult. Efforts to harmonize IGF-I assay results are being undertaken after a recent workshop jointly sponsored by the GH Research Society, the IGF Society, and the IFCC (118), but the effect of residual IGFBP in assayed samples will remain a thorny problem.

The two IGF-I assays used for the GH-2004 project were the DSL-5600 IRMA and the Immunotech A15728 IRMA; their technical aspects have been reported in detail (185). To minimize IGFBP interference, the DSL assay uses acid-ethanol precipitation, whereas the Immunotech assay uses acidification and excess IGF-II to prevent rebinding of IGF-I. There is good correlation between the two assays, but there is a systematic bias in favor of the DSL assay, which yields values that are about 20% higher than those obtained by the Immunotech assay (185). The DSL assay is no longer available and has been replaced by the Siemens Immulite assay system, which does not use extraction. No back-to-back comparison between the Immunotech and Immulite assays has been published.

Preanalytical considerations are not of major concern because IGF-I is a stable peptide, and no special precautions are necessary during transport and storage of serum (118, 186).

Mass-based measurements of IGF-I are being developed and are beginning to approach the necessary sensitivity for measuring IGF-I concentrations in serum (187–190) and urine (191). Measurement by MS would alleviate some of the problems with immunoassays, although the issue of removal of (or accounting for) IGFBP would still be a challenge for accurate quantitation.

b. P-III-NP measurement. Serum P-III-NP is currently also measured by immunoassay. Unlike for IGF-I, where a number of commercial and in-house assays are widely employed, there are only two commercial assays in general use: the Orion UniQ RIA and the CIS Biointernational RIA-gnost IRMA. No international reference standard exists for P-III-NP, and there is no information given by the manufacturers regarding the exact nature or source of their standards (natural or recombinant, monomeric or trimeric, human, bovine, or porcine, *etc.*).

Knowledge about the specificity of either assay with respect to the different immunoreactive forms of circulating P-III-NP is limited. The Orion UniQ assay is described as measuring intact P-III-NP and its higher mol wt forms, but not smaller degradation products found in blood (assay kit instructional pamphlet). The CIS RIA-gnost assay is reported to measure P-III-NP Col 1–3 (intact P-III-NP), but not the Col 1 fragment (assay kit brochure and Ref. 139). These descriptions do not take into account the complexity of immunoreactive P-III-NP species in serum, nor do they identify the epitopes recognized. An early version of the RIA-gnost assay recognized peak IV material (Fig. 8A), which is thought to at least partially consist of the Col 1 fragment, suggesting that the assay reagents or conditions have changed over time.

The two assays are not directly comparable because they express results in different units (nanograms per milliliter and units per milliliter, respectively), but they show a good correlation (185, 192), suggesting that they measure a comparable substance(s). Moreover, when the conversion factor of 8 (provided in the RIA-gnost brochure) is used to convert units per milliliter to nanograms per milliliter, values for the normative ranges in the two assays are comparable. It is unclear why the RIA-gnost manufacturer does not use this conversion factor in expression of results. Technical details of the two commercial assays are summarized by Abellan *et al.* (192) and Cowan and Bartlett (185). The absence of a universal standard for P-III-NP is a major shortcoming that should be addressed by the antidoping and clinical chemistry communities.

The stability of P-III-NP during storage and transportation has been evaluated and found to be acceptable when serum was stored frozen or kept at 4 C for up to at least 5 d (186, 192). Two to three freeze-thaw cycles did not significantly affect assay results (192).

As with IGF-I and all other analytes, a mass-based measurement technique would be highly desirable. Currently there are no reports on attempts to develop MS analysis of P-III-NP in serum. Although its mol wt (~40,000) may act as a deterrent to such efforts, it should be realized that P-III-NP is a homotrimer of a protein that has a mol wt of only about 13,000. The molecular heterogeneity of circulating P-III-NP may be elucidated by MS. However, its low concentration in blood (subnanomolar) still presents a challenge for current MS technology.

c. Reagent availability. From the antidoping perspective, an additional significant problem with the vagaries of immunoassays is that the original reference values used to derive the discriminant functions are no longer representative for values obtained with the newer assays. For example, the Nichols IGF-I assay employed for accumulating the original large GH-2000 database is no longer available, and several subsequent efforts at securing a stable reagent supply were unsuccessful (see Ref. 5 for review). Similarly, the DSL IGF-I assay used for the GH-2004 project is no longer available. Adjustments in the form of correction factors can be made to allow comparison of newer values with historical results, and this has been successfully applied in clinical chemistry, including for IGF-I and P-III-NP (156, 157, 193, 194). Nevertheless, such correction maneuvers are not optimal because they do not represent primary data, and the need for assays that perform in a robust manner over long time periods is evident. History has shown that commercial immunoassays are probably not able to fulfill this requirement. Hence, the need for future assays that do not depend on biologics or commercial suppliers of unique reagents.

d. Implementation. The WADA code requires that a positive test result be confirmed by a second, independent method. Ideally, the two methods should be based on different analytical principles, but currently both IGF-I and P-III-NP measurements are limited to immunoassay measurement. In the case of immunoassays, the WADA code stipulates that the assay used for confirmation needs to recognize a different epitope(s) on the analyte than the original assay. Alternatively, a purification/separation method can be used before immunoassay to eliminate potential cross-reactivity. Precise epitope maps are not available for either IGF-I or P-III-NP, but indirect evidence suggests that the diverse antibodies used in the assays recognize different

aspects of the analytes. In addition, one of the IGF-I assays used incorporates a purification step. [It should be noted that for the GH isoform test described in *Section III.A*, the epitopes recognized by the four assays employed are well characterized (90).] These criteria may be sufficient to satisfy WADA requirements. Nevertheless, for the purpose of independent confirmatory testing, it would be highly desirable to have available mass spectroscopy-based methods that are unquestionably independent and distinct from immunologically based methods.

The biomarker test is poised for general implementation as a WADA-sanctioned test in the near future. It will serve as an important complementary test to the already implemented GH isoform test, providing independent confirmation and a longer window of opportunity. Because of the latter characteristic, it may be suitable for both in-competition and out-of-competition testing. It is important to note that thus far neither the isoform test nor the biomarker test has been scrutinized under legal challenge.

C. Novel approaches

Research is continuing to identify additional indicators for GH use that may be useful for antidoping purposes. In particular, genomic and proteomic approaches are being explored in an attempt to identify a "signature" that would be indicative of exogenous GH use. Mitchell *et al.* (195) examined transcriptome changes in peripheral blood leukocytes obtained from recreational athletes treated with GH (2 mg/d) for 8 wk. They identified induction or repression of several genes in GH-treated subjects, but the magnitude of transcript changes was small and within the variability range seen among different untreated subjects. None of the genes significantly up-regulated (*IGF2*, *MED18*, *PDK4*) or down-regulated (*AREG*, *ARG1*, *CYYR1*) are classical GH-responsive genes, and disparate responses have been found for some of them (*PDK4* and *AREG*) in different tissues or physiological states. The authors concluded that transcriptome analysis in leukocytes is unlikely to yield a viable antidoping test.

Proteomic approaches to detection of GH use have been employed using serum and peripheral blood leukocytes and either protein chip adsorption or two-dimensional electrophoresis followed by mass spectroscopy (196–200). These efforts have identified changes in unexpected proteins, such as free hemoglobin A1 chain, β-hemoglobin, transthyretin, apolipoprotein A_1, and fragments of albumin and Ig in serum, and calgranulins and DAMP (damage-associated molecular pattern) proinflammatory molecules in leukocytes. Some of the serum proteins are acute-phase reactants, and all proteins mentioned show considerable variability. Their physiological significance and potential biological link to GH remains to be established. It is evident from these preliminary data that a considerable amount of work will be required before proteomic approaches will become a realistic tool for antidoping purposes. It is currently not clear whether anonymous/comprehensive or GH-targeted proteomic or genomic inquiries will be more productive in yielding a GH-specific signature. It is also not clear whether the identification of many GH-responsive endpoints is superior to one or a few well-chosen endpoints, or whether it simply increases analytical noise.

Reports on new GH-responsive biochemical markers, such as mannan-binding lectin (201), will continue to appear in the literature. The specificity and sensitivity of such novel markers will have to be rigorously demonstrated before they are considered as an antidoping strategy. The experience with IGF-I and P-III-NP, two well-established GH biomarkers, suggests that development of a robust biomarker test is a time-consuming process.

IV. Secretagogues

GH secretagogues are peptides or nonpeptidic agents that act to release GH from the pituitary. There is evidence that they are being used by athletes as an indirect method for GH doping. Secretagogues include GHRH and its analogs, ghrelin analogs [known as GH-releasing peptides (GHRP) or GHS (GH secretagogues in a narrower sense), and amino acids (*e.g.*, arginine or ornithine). GHRH acts through the GHRH receptor; GHRP/GHS acts through the ghrelin receptor, also known as the GHS receptor 1a; both receptors are coupled to G proteins and signal primarily though $G_s\alpha$ and $G_{q/11}$ pathways, respectively. Arginine and ornithine have to be given in high doses (*e.g.*, 30 g iv); they are thought to stimulate GH secretion through suppression of somatostatin. General features of secretagogues are that their effect is short-lived and they provide a relatively weak boost in GH exposure compared with what can be achieved by direct GH administration. GH secretagogues are attractive to athletes who want to avoid detection because the GH released is endogenous and therefore not detectable by the GH isoform test.

A. GHRH and its analogs

GHRH is a 44- or 40-amino acid linear peptide secreted by the hypothalamus; it stimulates pituitary somatotroph proliferation and GH production (both synthesis and release). Its fully bioactive shorter version, GHRH(1–29) (sermorelin), was marketed in the 1980s to treat idiopathic GH deficiency in children and also for diagnostic purposes in pituitary disease. It was found to be largely ineffective as a growth promoter, and its use as a thera-

peutic agent was abandoned. It is no longer available on the U.S. market. It is unclear whether it exists on the black market for doping purposes. Bioactive GHRH has a very short half-life (~7 min) in blood, being rapidly degraded by dipeptidyl-aminopeptidase IV (202). Intravenous GHRH administration elicits a spike in plasma GH that peaks (at ~10–25 ng/ml) at 15–30 min and returns to baseline after 120 min. Studies conducted with GHRH in the elderly in an effort to reverse the somatopause have yielded varying degrees of mild elevation of serum IGF-I and changes in body composition, but little improvement in physical performance (203, 204). Based on its short duration, need for repeated administration, and limited efficacy in GH deficiency or GH insufficiency in the elderly, it is unlikely that GHRH provides significant GH doping "benefits" to the athlete.

There is currently no detection test for GHRH abuse. Its low dosing, short half-life, and structural similarity with endogenous GHRH (which is produced not only in the hypothalamus, but primarily in gut and other extraneural tissues) would present a substantial challenge to development of a detection test.

Newer, long-acting analogs of GHRH, such as tesamorelin and CJC-1295 have been developed; the former is approved by the Food and Drug Administration for treatment of HIV-associated lipodystrophy; the latter has undergone clinical trials. Strategies to increase half-life include amino acid substitutions and other modifications targeting the dipeptidyl-aminopeptidase IV cleavage site at position 2 and incorporating a linker with a reactive group that allows covalent linkage to albumin *in vivo* after injection. The plasma half-life of tesamorelin is ~30 min (205); and that of CJC-1295 is 6–8 d (206). Despite the relatively short half-life of tesamorelin, once a day administration results in enhanced GH pulsatility over 24 h and a mean IGF-I increase of 108–122% (205, 207). Treatment of HIV-associated lipodystrophy with tesamorelin resulted in an 18% loss of visceral fat, suggesting a GH-induced lipolytic effect (207). Administration of a single dose of CJC-1295 resulted in an elevation of plasma GH trough, but not peak levels, and an ~40% increase in IGF-I levels 1 wk later (208). Thus, it appears that these long-acting GHRH analogs have a moderate enhancing effect on GH secretion and its downstream biomarkers. There is evidence that these drugs have entered the black market (209).

Currently there is no published method to detect use of these GHRH analogs, but because they differ structurally from native GHRH, unequivocal detection methods should be feasible if sufficient sensitivity can be achieved.

B. Ghrelin mimetics

Ghrelin is an orexigenic peptide produced by the stomach. It is a 28-amino acid, linear peptide that exists both as a 3-octanoylated form and as a nonacylated form. The octanoylated form is bioactive; the biological role of the nonacylated form is currently a matter of debate. In the presence of an intact hypothalamo-pituitary system (*i.e.*, GHRH functionality), ghrelin is a potent secretagogue for GH *in vivo*. GHRH signaling is crucial for this pronounced ghrelin effect on GH release (210). Despite its efficacy as a pharmacological agent, the role of ghrelin in the physiological regulation of GH secretion is minor at best. Its main physiological role appears to lie in the area of appetite regulation. Discovery of ghrelin analogs (GHRP, GHS) as well as the ghrelin receptor preceded the identification of ghrelin by many years.

GHRPs are non-native hexapeptides originally derived from enkephalin, including GHRP-6, GHRP-2 (pralmorelin), and hexarelin; other GHS are modified peptides such as tabimorelin, and nonpeptide compounds such as MK-677, L-692,429, SM-130,686, and TZP-101. A considerable number of studies have evaluated the short-term and long-term effects of ghrelin mimetics on the GH-IGF axis. A typical GH response to an iv bolus of ghrelin or GHRP yields a peak serum GH of 70–110 ng/ml at 15–30 min, with return to baseline at 120–180 min. Long-term therapy has been attempted for idiopathic GH deficiency or short stature, frailty in the elderly, osteoporosis, and amyotrophic lateral sclerosis. A few representative studies will be cited. For example, a 2-yr study in children with idiopathic GH deficiency or short stature with intranasal GHRP-2 three times a day produced a modest gain in growth velocity but no change in serum IGF-I (211). Oral GHRP-2 in a similar study resulted in an approximate 2-fold increase in GH secretion, a modest increase in growth velocity, and again no change in serum IGF-I (212). Addition of GHRH to the GHRP regimen did not improve outcome (212). Tabimorelin treatment for 7 d in young, healthy male subjects yielded a 50% increase of serum IGF-I and an attenuation of the GH response over time (213). A 2-yr, randomized, double-blind trial of daily MK-677 treatment in elderly subjects showed a 1.8-fold increase in GH secretion and a 1.5-fold increase in serum IGF-I, an increase in both lean body mass/water and fat mass, little effect on bone mineral density, a smaller decline in muscle strength than in controls, and no effect on physical function and quality of life (214). A 1-yr trial of capromorelin in elderly subjects increased IGF-I by 60%, and some performance measures (stair climb and tandem walk) increased, whereas several others did not (215). The effect was considered insufficient to warrant continuation

of the trial or further development of the drug. The topic of ghrelin mimetic and GHRH therapy was reviewed by Hersch and Merriam (216).

Taken together, these and several other studies indicate that ghrelin mimetics have a moderate effect on GH secretion and IGF-I levels, but they have insufficient impact on growth or physical performance to be considered marketable at this time. The same side effects as those noted with GH treatment were observed, but at a lesser frequency and severity. This observation is congruent with the pharmacological concept that less effect is accompanied by fewer side effects. An undesirable and GH-unrelated side effect of all ghrelin mimetics is increased cortisol production and increased appetite/adipose weight gain—both intrinsic features of the ghrelin system.

Given the relatively mild effect of ghrelin mimetics on overall GH secretion and the uncertainty about the ergogenicity of even large GH doses (see *Section VI*), it appears unlikely that athletes would derive significant performance-enhancing benefits from abusing ghrelin mimetics or GHRH analogs.

There is evidence that ghrelin mimetics are being offered on the black market for doping purposes (217, 218). Methods to identify these non-native substances in urine have been developed (219–222).

C. Amino acids

Large iv doses of certain amino acids (arginine, ornithine, lysine) have GH-releasing activity and are in use diagnostically as a GH stimulation test (especially arginine, 30 g rapidly iv). Even at these large doses they are relatively weak stimuli unless given together with GHRH. These uses of amino acids have been extrapolated to mean that oral arginine supplements are GH stimulators, and the Internet is replete with arginine advertisements. There is no reason to believe that typical oral doses of arginine elicit significant GH release. This can be verified during a protein meal and has indeed been directly shown (223). Testing for abuse of amino acids is not currently feasible, but may also not be necessary in view of their limited efficacy.

V. IGF-I as a Doping Agent

Since many actions of GH are mediated through IGF-I, it is not surprising that IGF-I is also being abused for the purposes of performance enhancement. IGF-I appears on the WADA list of banned substances. IGF-I is commercially available for medical indications, such as genetic GH resistance and primary IGF-I deficiency. A preparation combining IGF-I with IGFBP3 is not marketed the United States, but clinical trials for certain neurological diseases (amyotrophic lateral sclerosis) are ongoing. The Internet is replete with advertisements for IGF-I and its more potent analogs— des(1–3)IGF-I, R^3-IGF-I, Long-R^3-IGF-I, and mechano-growth factor (IGF-IEc or a peptide derived from the E-domain of pro-IGF-I). The former three analogs have low affinity for IGFBPs due to modification of the amino terminus (deletion of residues 1–3, substitution of glutamic acid in position 3 with arginine, and a 13-amino acid amino-terminal extension in addition to the Glu^3-Arg modification, respectively). The latter is derived from an *IGF1* gene splice variant that includes a carboxy-terminal sequence encoded by a 3′-exon (exon 5) that is excluded from liver IGF-I transcripts. The physiological importance of IGF-IEc is controversial, and the existence of mechano-growth factor as a native peptide is not established (see Ref. 224 for review). IGF-I analogs have been discovered in supplement products sold on the black market (217). There is also evidence that even IGF-I products strictly intended for *in vitro* use have entered the supply stream available to athletes (225).

IGF-I is a mitogenic, anabolic, and metabolically active peptide generated in response to GH in most tissues, with the liver the predominant source. The bioactivity spectra of GH and IGF-I are overlapping but not identical. One prominent example where GH and IGF actions diverge is lipolysis: GH has a direct lipolytic activity, whereas IGF-I does not. The treatment of patients with GH resistance with IGF-I does not completely mimic the treatment of GH deficiency with GH, and the phenotypic features differ (226). Thus, it cannot be presumed that IGF-I abuse in sports has the identical effect as GH abuse. IGF-I does have anabolic action in numerous tissues, including muscle, and is an important mediator of GH action in muscle. This anabolic action cannot, however, be interpreted as necessarily indicating that IGF-I enhances athletic performance in healthy individuals (see *Section VI*). In contrast to GH, there is relatively little information on the possible performance-enhancing action of IGF-I in normal human subjects.

Currently, there is no established detection method for IGF-I abuse. Studies evaluating the biomarker approach outlined above for detection of IGF-I abuse are ongoing (227, 228).

VI. GH as an Ergogenic Substance

GH appears as the ideal ergogenic agent: it is the prototype master anabolic hormone, promoting nitrogen accretion, protein synthesis in numerous tissues including muscle, and physical growth. In addition, it has lipolytic activity,

causing adipose tissue to shrink and divert liberated calories toward carbohydrate fuel generation and protein synthesis. GH undeniably exhibits all these activities, which have been documented in numerous *in vitro* and *in vivo* studies. Based on these facts, the sports, body-building, and antiaging communities believe that GH must be beneficial for building musculature and therefore physical performance. This apparently reasonable assumption is, however, still a matter of debate as far as athletic performance is concerned, due to the difficulty in demonstrating ergogenicity of GH in scientific studies (229).

A fundamental principle in endocrinology states that the effect of a hormone is most evident when the hormone is replaced in an individual who is deficient in that hormone. Accordingly, the ergogenic effect of GH should be most obvious in GH-deficient patients when treated with GH. The scientific literature on this point is mixed: some studies show increased stamina, few show increased strength, and some show little effect on parameters related to physical performance. The complexity of GH status (deficiency or excess) on muscle morphology, metabolism, and function has been reviewed in detail by Woodhouse *et al.* (230). From that review, it appears that both a deficiency and an excess of GH are deleterious to muscle health. Assessment of physical function in GH deficiency and its response to GH replacement has been the subject of numerous studies, with less than consistent conclusions. The very fact that this is still a subject of investigation after two decades of study attests to the difficulty of settling this issue. A detailed discussion of the many studies addressing the ergogenic effects (or lack thereof) of GH replacement therapy in hypopituitarism is beyond the scope of this review. A recent meta-analysis of 11 randomized, double-blind, placebo-controlled studies concluded that GH replacement improved the exercise performance of GH-deficient patients (231). Another meta-analysis of muscle strength outcome in eight randomized, double-blind, placebo-controlled studies (some are the same as those included above) concluded that there was no improvement in muscle strength after 6–8 months of GH replacement (232). The authors point out that longer duration (years) of therapy might have resulted in increased strength. A recent study of cardiovascular function in patients given physiological replacement doses of GH (mean, 0.64 mg/d) found no improvement in exercise performance, in contrast to earlier studies using higher GH doses (233). Taken together, the studies in GH deficiency suggest that there probably is an overall improvement in physical function with GH replacement, but this is variable, complex in nature, less than compelling, and not universally accepted. Thus, even in the "ideal" setting of GH deficiency, it is difficult to unequivocally demonstrate an ergogenic effect of GH.

The question then arises whether ergogenicity can be shown in normal (*i.e.*, GH-replete) subjects, which includes both untrained individuals and athletes. Based on the above-mentioned endocrine/biological concept, it would be predicted that this may be more difficult. A number of studies examining the effect of GH on athletic performance have been conducted. A systematic literature review by Liu *et al.* (234) summarized the results of 27 randomized, controlled trials involving 303 young, lean, physically fit subjects receiving GH at an average dose of ~2.5 mg/d—a 5- to 10-fold excess over the physiological GH production rate. While there were the expected changes in body composition (increased lean body mass and marginally lower fat mass), there were no differences in strength or exercise capacity between those taking GH and those who did not. The authors point out the limitations of the studies in terms of duration and dose of GH, which may be less than what is typically used by athletes. Nevertheless, the typical side effects associated with GH administration (edema, arthralgias, carpal tunnel syndrome, sweating) were observed in 15–44% of the participants. A list of typical adverse effects seen with GH administration is given in Table 4.

There are relatively few controlled studies of GH effects in trained athletes. The Mitchell report (Ref. 4, pages 9–10) relates the impression of athletes that GH did not have a positive effect on their performance. Deyssig *et al.* (235) showed in a double-blind, placebo-controlled trial

TABLE 4. Adverse effects of GH administration

Sodium and fluid retention
Soft tissue swelling
Paresthesias
Nerve entrapment, carpal tunnel syndrome
Joint stiffness
Hypertension
Peripheral edema
Arthralgias
Myalgias
Insulin resistance
Carbohydrate intolerance
Diabetes mellitus
Gynecomastia
Acromegalic changes expected with prolonged, high-dose GH
Acral enlargement
Bone remodeling
Arthritis
Bone spurs
Frontal bossing
Dental malocclusion
Spinal stenosis
Disfigurement
Cardiovascular changes
Cardiac dysfunction

Adverse effects are dose-dependent, treatment duration-dependent, and age-dependent. Susceptibility varies among individuals. Older people are more prone to side effects even at low doses.

that GH [30 μg/kg · d (or ~2–2.5 mg/d) for 6 wk] had no effect on muscle strength. Lange *et al.* (236) showed that acute GH administration (2.5 mg 4 h before exercise) did not increase bicycling performance measured as speed or VO_2 but had a deleterious effect in two cyclists. Meinhardt *et al.* (237), in a double-blind, placebo-controlled study of recreational athletes, showed that GH (2 mg/d for 8 wk) had no effect on muscle strength (dead lift), power (jump height), or endurance (VO_2max) but did improve sprint capacity by 5.5% in men but not women (by 2.5%; not significant). Testosterone coadministration in men enhanced the effect of GH. Adverse effects typical for GH administration were seen in a significant number of the treated subjects. This isolated improvement in anaerobic muscle performance during sprinting is somewhat unexpected, especially because other anaerobic muscle functions were not affected. The authors state that athletic significance of this finding is uncertain, but they also speculate that the improvement might translate into a 0.4-sec advantage in a 100-m sprint.

In view of the overall scientific literature, the evidence for GH as an ergogenic substance in healthy humans is weak. Yet athletes continue GH abuse in the belief that it improves their performance. Numerous reasons can be given why the scientific literature does not reflect GH use in the sports arena: GH doses are too low; duration of treatment is not long enough; GH in conjunction with anabolic steroids, insulin, and other doping agents may have greater ergogenicity than when given alone; GH in combination with exercise is particularly potent; athletes react to GH in a different manner than nonathletes, *etc.* While all of these arguments have some validity and should not be readily dismissed, perhaps the most pertinent are those regarding dose and duration. Dosages and injection patterns among athletes are difficult to assess because of the lack of documentation and prevailing secrecy. Ehrnborg *et al.* (2) mention doses of 3–8 mg three to four times a week but state that the mean daily dose is mostly ~1.3 mg. Saugy *et al.* (238) estimate that, based on underground information, athletes inject 3–8 mg three to four times a week. The reliability of such underground information is uncertain because athletes themselves (or their trainers) may not know in detail what or how much is being administered. Nature has provided for an excellent model of the effects of high-dose, long-duration GH exposure: the patient with acromegaly. Acromegaly is caused by excess production of GH, usually by a benign pituitary tumor. It is a disease with insidious onset; the delay between onset and diagnosis is estimated as at least 7–10 yr (239). Acromegaly has a high morbidity and carries a 1.5- to 3-fold increase in mortality, with a direct relationship between GH levels and risk of premature death (240); it adversely affects many tissues and functions critical to physical performance (joints, heart and skeletal muscle, connective tissue, nerve entrapment, hypertension, metabolic derangements, diabetes mellitus, *etc.*) (241). Physical performance is clearly impaired in established acromegaly due to numerous physical and metabolic reasons. Therefore, high doses of GH over a long time are not performance enhancing. It could be argued that there may be an early phase of acromegaly, before the establishment of physical and metabolic derangements, when the high prevailing GH levels are ergogenic. This would be akin to the athlete abusing GH. However, with the exception of one spectacular case self-report (242), there is no evidence that patients with acromegaly experience such a phase of enhanced physical functioning. Rather, the disease progresses in silence for years before disfigurement leads to the diagnosis. Of interest, the patient in the cited case report was still growing at age 22, with a height increase of 6 inches (15.2 cm) over the preceding 4 yr. Given his pituitary tumor, this phenomenon is best explained by hypogonadism, calling into question the contribution of high endogenous testosterone levels to the postulated ergogenic effect high GH levels. Despite this report, the general lesson taught by acromegaly does not support the notion that high-dose GH is more ergogenic than low-dose GH.

Another reason sometimes given for the use of GH by athletes is the belief that GH accelerates recovery from injury. There is only limited information about this issue in the scientific literature. Involvement of GH in healing may be postulated based on the fact that collagen turnover increases after GH administration (see *Section III.B.1*). However, it is unknown whether after an injury GH plays a role in this response or whether local factors operating at the injury site are responsible. Furthermore, it is unknown whether supraphysiological GH concentrations confer any advantage over the normal physiological response of the GH-IGF system. One recent study examined collagen synthesis in patellar tendon and quadriceps muscle in response to 14 d of high-dose GH treatment (33–50 μg/kg · d, ~2–4 mg/d) in noninjured young male volunteers; GH treatment increased collagen protein synthesis 1.3-fold over placebo (significant) in tendon and 5.8-fold (not significant) in muscle (243). Another study examined the effect of three doses of GH (15, 30, and 60 μg/kg · d, ~1, 2, and 4 mg/d) on tibial fracture healing (244). At the highest dose, GH accelerated fracture healing by 29% in patients with closed fractures, but had no significant effect on the healing of open fractures. The two lower doses of GH had no discernible effect on fracture healing (244). These findings need to be corroborated and expanded before firm conclusions can be drawn about the effect of GH

on recovery from injury. Furthermore, the response of different types of injury and tissues to GH treatment, as well as the doses required need to be investigated before the significance of GH for recovery from athletic injuries becomes clear. It is of interest that promising early trials of GH therapy for burns (245, 246) have not been widely accepted as a therapeutic modality. The currently available evidence is insufficient to warrant the use of GH to promote healing of sports injuries.

A third reason for GH misuse is the belief by athletes that the lipolytic activity of GH results in weight loss, which they believe to be beneficial to athletic performance. However, GH administration does not typically result in a net weight change because the loss of fat is compensated for by a gain in lean body mass [of which a substantial part (50–80%) represents retained fluid]. This is true in the GH-deficient patient on GH replacement therapy as well as in normal subjects, including athletes, taking GH (237, 247).

The conviction in athletic circles that GH is a performance-enhancing substance appears to run deep, despite assertions to the contrary cited in the Mitchell report (4). An athlete's personal sense of what makes him or her perform better should not be easily dismissed. Neither should the powers of a placebo effect, hearsay, peer/coach pressure, and advertising be underestimated. It should also be remembered in this context that an ergogenic effect of androgens was questioned by the scientific community for years before their performance-enhancing potency was proven. In the final analysis, even with the best scientific evidence it will be impossible to prove a negative, namely that GH does not have an ergogenic effect. Nevertheless, given what is known, the burden of proof lies with those who advocate GH use in the belief that it enhances physical performance in healthy humans. Education of athletes, trainers and other sports personnel about the facts known regarding GH effects on performance should be undertaken by sports organizations as part of their antidoping strategy. This education should also include information about the short-term and long-term side effects of high-dose GH use. Given the dearth of scientific evidence for ergogenicity and the potential serious adverse effects on health, it seems ill-advised to use GH for uncertain performance enhancement in healthy individuals.

VII. Summary and Conclusions

GH is reported to be widely abused by athletes in many types of sport. The attractiveness of GH lies in its anabolic and lipolytic activities, combined with an aura of "undetectability." GH abuse extends beyond professional sports and is also present among adolescents engaged in sports in schools. This widespread use presents a public health problem because GH use is accompanied by adverse effects, and long-term use can lead to serious morbidity.

Because GH is secreted in a pulsatile manner and therefore fluctuates widely in blood, a high serum GH cannot be interpreted as evidence for exogenous GH administration.

GH used for doping purposes is said to be like a natural substance and therefore not detectable. However, pituitary GH consists of a number of molecular variants (isoforms), whereas recombinant GH corresponds to only one (the most prevalent) isoform, 22K-GH. This difference forms the basis of a detection test, where the ratio between 22K-GH and pituitary GH (a mixture of isoforms) is used as the endpoint. The isoform detection test performs well, but has a limited window of opportunity (12–24 h after the last GH injection). It has been a WADA-sanctioned and generally implemented test for over 2 yr; positive results have been few, presumably because of the short window of detection opportunity. The isoform test can be circumvented by using cadaveric GH (with attendant risk of acquiring Creutzfeld-Jakob disease) or using GH secretagogues (resulting in only mild GH stimulation).

A second detection test for GH abuse, the biomarker test, is based on measurement of biochemical effects of GH administration. Serum levels of IGF-I and P-III-NP rise after GH administration and remain elevated for several days to weeks after a GH dose. They are not completely specific for GH, but extensive validation studies have resulted in a discriminant formula that allows distinction of GH-induced elevation from most if not all nonspecific stimuli. The biomarker test has a window of opportunity of several days—realistically probably 5–7 d. It is scheduled to be implemented by WADA in time for the London 2012 Olympiad.

The immunoassays currently used for IGF-I and P-III-NP are somewhat problematic because of the lack of consistency over time and notorious interference by IGFBPs (IGF-I), and insufficient standardization due to absence of an international reference standard (P-III-NP). The development of mass-based identification and quantification methods that do not depend on antibodies or poorly defined/impure reference standards is highly recommended as MS-based technology becomes feasible.

Detection of doping substances (such as anabolic steroids) in urine has been a time-honored and successful tradition in sports. This methodology is not easily applicable to polypeptides such as GH, IGF-I, or P-III-NP. The reasons are multiple and include extremely low levels of residual peptide in urine, renal factors impinging on excretion, lack of evidence that urinary peptide concentra-

tion reflects plasma concentration or overall production/dose, and insufficient scientific background information on how peptide excretion is regulated. The fact that for the polypeptides under consideration here, quantitative differences rather than qualitative differences (such as *e.g.*, a foreign substance or an abnormal glycosylation pattern) are determined presents an additional challenge to urine testing. For all these reasons, it is probably unwise to pursue the elusive goal of a urine test for detection of GH abuse. It is likely more productive to expend efforts to convince athletes that blood testing is necessary for these substances and to develop ultrasensitive methods that permit minimization of the required blood volume. The former should not be as difficult as is assumed because blood testing is already well-accepted in the blood doping field.

Saliva is under consideration as an ideal biological fluid that would allow noninvasive testing for doping substances. However, obstacles similar to those mentioned for urine would have to be overcome. The limited data available about salivary GH and IGF-I do not appear encouraging.

GH secretagogues of the GHRH and particularly GHRP/GHS variety are likely being used as doping agents in an effort to boost endogenous GH production, while at the same time evading detection by the GH isoform test. The boost in GH levels is far smaller than what can be achieved with direct GH administration, and the ergogenic effect (if any) would be significantly less. Amino acids (arginine, ornithine, lysine) are ineffective in boosting GH secretion unless they are given as large iv bolus doses. Urine tests are being developed for GHRP/GHS and possibly GHRH analogs; this is feasible because these compounds are structurally different from their endogenous counterparts.

Doping with IGF-I is the newest form of "GH doping;" there is forensic evidence that IGF-I and its congeners are being used. To date, no test to detect IGF-I abuse is available, but the above-mentioned GH biomarker test (see *Section III.B.1*) and variations thereof are being studied as a detection strategy. Obviously, the GH isoform test would not be applicable, nor would urinary IGF-I testing be suitable for the reasons outlined above.

The question regarding the ergogenicity of GH has been asked for many years. The notion that GH is a performance-enhancing substance is based on its known anabolic action, amplified by its lipolytic action. Countless studies have documented the effect of GH on muscle mass, muscle architecture, metabolism, and function *in vitro*, and there is no doubt about the anabolic effects of GH, especially in the context of GH deficiency. The picture is less clear in GH sufficiency. Furthermore, the link between muscle mass, muscle function, and physical performance *in vivo* is less than straightforward. Accumulating scientific evidence in normal humans (including athletes) has for the most part failed to demonstrate a significant ergogenic effect of GH in supraphysiological doses, although perhaps in lower doses than those speculated to be used by athletes. The best model of high-dose, long-term GH "administration," acromegaly, also fails to support an ergogenic effect. There is anecdotal evidence [from the Mitchell report (4)] that athletes recognize that GH does not enhance their performance. Given this overall evidence, the burden of proof that GH is ergogenic lies with those advocating its use. Sports organizations should educate athletes and trainers about these facts in an effort to combat GH doping, an expensive, probably poorly effective, medically hazardous form of unfair behavior.

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References

1. **Rickert VI, Pawlak-Morello C, Sheppard V, Jay MS** 1992 Human growth hormone: a new substance of abuse among adolescents? Clin Pediatr (Phila) 31:723–726
2. **Ehrnborg C, Bengtsson BA, Rosén T** 2000 Growth hormone abuse. Baillieres Best Pract Res Clin Endocrinol Metab 14:71–77
3. **Buzzini SR** 2007 Abuse of growth hormone among young athletes. Pediatr Clin North Am 54:823–843, xiii
4. **Mitchell G** 2007 Report to the commissioner of baseball of an independent investigation into the illegal use of steroids and other performance enhancing substances by players in major league baseball. http://files.mlb.com/mitchrpt.pdf
5. **Holt RI, Erotokritou-Mulligan I, Sönksen PH** 2009 The history of doping and growth hormone abuse in sport. Growth Horm IGF Res 19:320–326
6. **Beck JC, McGarry EE, Dyrenfurth I, Venning EH** 1957 Metabolic effects of human and monkey growth hormone in man. Science 125:884–885
7. **Raben MS** 1958 Treatment of a pituitary dwarf with human growth hormone. J Clin Endocrinol Metab 18:901–903

8. **Chen EY, Liao YC, Smith DH, Barrera-Saldaña HA, Gelinas RE, Seeburg PH** 1989 The human growth hormone locus: nucleotide sequence, biology, and evolution. Genomics 4:479–497
9. **DeNoto FM, Moore DD, Goodman HM** 1981 Human growth hormone DNA sequence and mRNA structure: possible alternative splicing. Nucleic Acids Res 9:3719–3730
10. **Lecomte CM, Renard A, Martial JA** 1987 A new natural hGH variant—17.5 kd—produced by alternative splicing. An additional consensus sequence which might play a role in branchpoint selection. Nucleic Acids Res 15:6331–6348
11. **Cooke NE, Ray J, Watson MA, Estes PA, Kuo BA, Liebhaber SA** 1988 Human growth hormone gene and the highly homologous growth hormone variant gene display different splicing patterns. J Clin Invest 82:270–275
12. **Palmetshofer A, Zechner D, Luger TA, Barta A** 1995 Splicing variants of the human growth hormone mRNA: detection in pituitary, mononuclear cells and dermal fibroblasts. Mol Cell Endocrinol 113:225–234
13. **Chellakooty M, Vangsgaard K, Larsen T, Scheike T, Falck-Larsen J, Legarth J, Andersson AM, Main KM, Skakkebaek NE, Juul A** 2004 A longitudinal study of intrauterine growth and the placental growth hormone (GH)-insulin-like growth factor I axis in maternal circulation: association between placental GH and fetal growth. J Clin Endocrinol Metab 89:384–391
14. **Wu Z, Bidlingmaier M, Friess SC, Kirk SE, Buchinger P, Schiessl B, Strasburger CJ** 2003 A new nonisotopic, highly sensitive assay for the measurement of human placental growth hormone: development and clinical implications. J Clin Endocrinol Metab 88:804–811
15. **MacLeod JN, Worsley I, Ray J, Friesen HG, Liebhaber SA, Cooke NE** 1991 Human growth hormone-variant is a biologically active somatogen and lactogen. Endocrinology 128:1298–1302
16. **Baumann G, Dávila N, Shaw MA, Ray J, Liebhaber SA, Cooke NE** 1991 Binding of human growth hormone (GH)-variant (placental GH) to GH-binding proteins in human plasma. J Clin Endocrinol Metab 73:1175–1179
17. **Igout A, Frankenne F, L'Hermite-Balériaux M, Martin A, Hennen G** 1995 Somatogenic and lactogenic activity of the recombinant 22 kDa isoform of human placental growth hormone. Growth Regul 5:60–65
18. **Baumann GP** 2009 Growth hormone isoforms. Growth Horm IGF Res 19:333–340
19. **Lewis UJ, Dunn JT, Bonewald LF, Seavey BK, Vanderlaan WP** 1978 A naturally occurring structural variant of human growth hormone. J Biol Chem 253:2679–2687
20. **Baumann G** 1991 Growth hormone heterogeneity: genes, isohormones, variants, and binding proteins. Endocr Rev 12:424–449
21. **Chapman GE, Rogers KM, Brittain T, Bradshaw RA, Bates OJ, Turner C, Cary PD, Crane-Robinson C** 1981 The 20,000 molecular weight variant of human growth hormone. Preparation and some physical and chemical properties. J Biol Chem 256:2395–2401
22. **Stolar MW, Amburn K, Baumann G** 1984 Plasma "big" and "big-big" growth hormone (GH) in man: an oligomeric series composed of structurally diverse GH monomers. J Clin Endocrinol Metab 59:212–218
23. **Uchida H, Naito N, Asada N, Wada M, Ikeda M, Kobayashi H, Asanagi M, Mori K, Fujita Y, Konda K, Kusuhara N, Kamioka T, Nakashima K, Honjo M** 1997 Secretion of authentic 20-kDa human growth hormone (20K hGH) in *Escherichia coli* and properties of the purified product. J Biotechnol 55:101–112
24. **Lewis UJ, Singh RN, Bonewald LF, Lewis LJ, Vanderlaan WP** 1979 Human growth hormone: additional members of the complex. Endocrinology 104:1256–1265
25. **Lewis UJ, Singh RN, Bonewald LF, Seavey BK** 1981 Altered proteolytic cleavage of human growth hormone as a result of deamidation. J Biol Chem 256:11645–11650
26. **Haro LS, Lewis UJ, Garcia M, Bustamante J, Martinez AO, Ling NC** 1996 Glycosylated human growth hormone (hGH): a novel 24 kDa hGH-N variant. Biochem Biophys Res Commun 228:549–556
27. **Kohler M, Thomas A, Püschel K, Schänzer W, Thevis M** 2009 Identification of human pituitary growth hormone variants by mass spectrometry. J Proteome Res 8:1071–1076
28. **Lewis UJ, Peterson SM, Bonewald LF, Seavey BK, Vanderlaan WP** 1977 An interchain disulfide dimer of human growth hormone. J Biol Chem 252:3697–3702
29. **Brostedt P, Roos P** 1989 Isolation of dimeric forms of human pituitary growth hormone. Prep Biochem 19:217–229
30. **Ultsch MH, Somers W, Kossiakoff AA, de Vos AM** 1994 The crystal structure of affinity-matured human growth hormone at 2 A resolution. J Mol Biol 236:286–299
31. **Brooks AJ, Waters MJ** 2010 The growth hormone receptor: mechanism of activation and clinical implications. Nat Rev Endocrinol 6:515–525
32. **Mercado M, DáVila N, McLeod JF, Baumann G** 1994 Distribution of growth hormone receptor messenger ribonucleic acid containing and lacking exon 3 in human tissues. J Clin Endocrinol Metab 78:731–735
33. **Mertani HC, Delehaye-Zervas MC, Martini JF, Postel-Vinay MC, Morel G** 1995 Localization of growth hormone receptor messenger RNA in human tissues. Endocrine 3:135–142
34. **de Vos AM, Ultsch M, Kossiakoff AA** 1992 Human growth hormone and extracellular domain of its receptor: crystal structure of the complex. Science 255:306–312
35. **Ross RJ, Leung KC, Maamra M, Bennett W, Doyle N, Waters MJ, Ho KK** 2001 Binding and functional studies with the growth hormone receptor antagonist, B2036-PEG (pegvisomant), reveal effects of pegylation and evidence that it binds to a receptor dimer. J Clin Endocrinol Metab 86:1716–1723
36. **Gent J, van Kerkhof P, Roza M, Bu G, Strous GJ** 2002 Ligand-independent growth hormone receptor dimerization occurs in the endoplasmic reticulum and is required for ubiquitin system-dependent endocytosis. Proc Natl Acad Sci USA 99:9858–9863
37. **Brooks AJ, Wooh JW, Tunny KA, Waters MJ** 2008 Growth hormone receptor; mechanism of action. Int J Biochem Cell Biol 40:1984–1989
38. **Lanning NJ, Carter-Su C** 2006 Recent advances in growth hormone signaling. Rev Endocr Metab Disord 7:225–235
39. **Cunningham BC, Bass S, Fuh G, Wells JA** 1990 Zinc me-

diation of the binding of human growth hormone to the human prolactin receptor. Science 250:1709–1712
40. **Hayakawa M, Shimazaki Y, Tsushima T, Kato Y, Takano K, Chihara K, Shimatsu A, Irie M** 2004 Metabolic effects of 20-kilodalton human growth hormone (20K-hGH) for adults with growth hormone deficiency: results of an exploratory uncontrolled multicenter clinical trial of 20K-hGH. J Clin Endocrinol Metab 89:1562–1571
41. **Winer LM, Shaw MA, Baumann G** 1990 Basal plasma growth hormone levels in man: new evidence for rhythmicity of growth hormone secretion. J Clin Endocrinol Metab 70:1678–1686
42. **Hartman ML, Faria AC, Vance ML, Johnson ML, Thorner MO, Veldhuis JD** 1991 Temporal structure of in vivo growth hormone secretory events in humans. Am J Physiol 260:E101–E110
43. **Maheshwari HG, Pezzoli SS, Rahim A, Shalet SM, Thorner MO, Baumann G** 2002 Pulsatile growth hormone secretion persists in genetic growth hormone-releasing hormone resistance. Am J Physiol Endocrinol Metab 282:E943–E951
44. **Ho KY, Evans WS, Blizzard RM, Veldhuis JD, Merriam GR, Samojlik E, Furlanetto R, Rogol AD, Kaiser DL, Thorner MO** 1987 Effects of sex and age on the 24-hour profile of growth hormone secretion in man: importance of endogenous estradiol concentrations. J Clin Endocrinol Metab 64:51–58
45. **Iranmanesh A, Lizarralde G, Veldhuis JD** 1991 Age and relative adiposity are specific negative determinants of the frequency and amplitude of growth hormone (GH) secretory bursts and the half-life of endogenous GH in healthy men. J Clin Endocrinol Metab 73:1081–1088
46. **Ho KY, Veldhuis JD, Johnson ML, Furlanetto R, Evans WS, Alberti KG, Thorner MO** 1988 Fasting enhances growth hormone secretion and amplifies the complex rhythms of growth hormone secretion in man. J Clin Invest 81:968–975
47. **Weltman A, Weltman JY, Hartman ML, Abbott RD, Rogol AD, Evans WS, Veldhuis JD** 1994 Relationship between age, percentage body fat, fitness, and 24-hour growth hormone release in healthy young adults: effects of gender. J Clin Endocrinol Metab 78:543–548
48. **Van Cauter E** 2000 Slow wave sleep and release of growth hormone. JAMA 284:2717–2718
49. **Pritzlaff CJ, Wideman L, Weltman JY, Abbott RD, Gutgesell ME, Hartman ML, Veldhuis JD, Weltman A** 1999 Impact of acute exercise intensity on pulsatile growth hormone release in men. J Appl Physiol 87:498–504
50. **Vigas M, Malatinský J, Németh S, Jurcovicová J** 1977 α-Adrenergic control of growth hormone release during surgical stress in man. Metabolism 26:399–402
51. **Vanhelder WP, Goode RC, Radomski MW** 1984 Effect of anaerobic and aerobic exercise of equal duration and work expenditure on plasma growth hormone levels. Eur J Appl Physiol Occup Physiol 52:255–257
52. **Felsing NE, Brasel JA, Cooper DM** 1992 Effect of low and high intensity exercise on circulating growth hormone in men. J Clin Endocrinol Metab 75:157–162
53. **Weltman A, Weltman JY, Schurrer R, Evans WS, Veldhuis JD, Rogol AD** 1992 Endurance training amplifies the pulsatile release of growth hormone: effects of training intensity. J Appl Physiol 72:2188–2196
54. **Godfrey RJ, Madgwick Z, Whyte GP** 2003 The exercise-induced growth hormone response in athletes. Sports Med 33:599–613
55. **Kraemer WJ, Dunn-Lewis C, Comstock BA, Thomas GA, Clark JE, Nindl BC** 2010 Growth hormone, exercise, and athletic performance: a continued evolution of complexity. Curr Sports Med Rep 9:242–252
56. **Rosenthal SM, Hulse JA, Kaplan SL, Grumbach MM** 1986 Exogenous growth hormone inhibits growth hormone-releasing factor-induced growth hormone secretion in normal men. J Clin Invest 77:176–180
57. **Ross RJ, Borges F, Grossman A, Smith R, Ngahfoong L, Rees LH, Savage MO, Besser GM** 1987 Growth hormone pretreatment in man blocks the response to growth hormone-releasing hormone; evidence for a direct effect of growth hormone. Clin Endocrinol (Oxf) 26:117–123
58. **Hartman ML, Clayton PE, Johnson ML, Celniker A, Perlman AJ, Alberti KG, Thorner MO** 1993 A low dose euglycemic infusion of recombinant human insulin-like growth factor I rapidly suppresses fasting-enhanced pulsatile growth hormone secretion in humans. J Clin Invest 91:2453–2462
59. **Bermann M, Jaffe CA, Tsai W, DeMott-Friberg R, Barkan AL** 1994 Negative feedback regulation of pulsatile growth hormone secretion by insulin-like growth factor I. Involvement of hypothalamic somatostatin. J Clin Invest 94:138–145
60. **Baumann G, Stolar MW** 1986 Molecular forms of human growth hormone secreted *in vivo*: nonspecificity of secretory stimuli. J Clin Endocrinol Metab 62:789–790
61. **Wallace JD, Cuneo RC, Bidlingmaier M, Lundberg PA, Carlsson L, Boguszewski CL, Hay J, Healy ML, Napoli R, Dall R, Rosén T, Strasburger CJ** 2001 The response of molecular isoforms of growth hormone to acute exercise in trained adult males. J Clin Endocrinol Metab 86:200–206
62. **Leung KC, Howe C, Gui LY, Trout G, Veldhuis JD, Ho KK** 2002 Physiological and pharmacological regulation of 20-kDa growth hormone. Am J Physiol Endocrinol Metab 283:E836–E843
63. **Wu Z, Devany E, Balarini G, Junnila R, Bidlingmaier M, Strasburger CJ** 2010 Specific monoclonal antibodies and ultrasensitive immunoassays for 20K and 22K human growth hormone. Growth Horm IGF Res 20:239–244
64. **Johnson V, Maack T** 1977 Renal extraction, filtration, absorption, and catabolism of growth hormone. Am J Physiol 233:F185–F196
65. **Kau ST, Maack T** 1986 Mechanism of tubular uptake on human growth hormone in perfused rat kidneys. J Pharmacol Exp Ther 236:596–601
66. **Rabkin R, Gottheiner TI, Fang VS** 1981 Removal and excretion of immunoreactive rat growth hormone by the isolated kidney. Am J Physiol 240:F282–F287
67. **García-Mayor RV, Pérez AJ, Gandara A, Andrade A, Mallo F, Casanueva FF** 1993 Metabolic clearance rate of biosynthetic growth hormone after endogenous growth hormone suppression with a somatostatin analogue in chronic renal failure patients and control subjects. Clin Endocrinol (Oxf) 39:337–343
68. **Haffner D, Schaefer F, Girard J, Ritz E, Mehls O** 1994

Metabolic clearance of recombinant human growth hormone in health and chronic renal failure. J Clin Invest 93: 1163–1171
69. **Hanssen KF** 1972 Immunoreactive growth hormone in human urine. Acta Endocrinol (Copenh) 71:665–676
70. **Baumann G, Abramson EC** 1983 Urinary growth hormone in man: evidence for multiple molecular forms. J Clin Endocrinol Metab 56:305–311
71. **Faria AC, Veldhuis JD, Thorner MO, Vance ML** 1989 Half-time of endogenous growth hormone (GH) disappearance in normal man after stimulation of GH secretion by GH-releasing hormone and suppression with somatostatin. J Clin Endocrinol Metab 68:535–541
72. **Veldhuis JD, Johnson ML, Faunt LM, Mercado M, Baumann G** 1993 Influence of the high-affinity growth hormone (GH)-binding protein on plasma profiles of free and bound GH and on the apparent half-life of GH. Modeling analysis and clinical applications. J Clin Invest 91:629–641
73. **Hendricks CM, Eastman RC, Takeda S, Asakawa K, Gorden P** 1985 Plasma clearance of intravenously administered pituitary human growth hormone: gel filtration studies of heterogeneous components. J Clin Endocrinol Metab 60:864–867
74. **Murakami Y, Mori T, Koshimura K, Kurosaki M, Takenobu A, Hashimoto Y, Kato Y** 2000 Twenty-kilodalton human growth hormone (20K hGH) secretion from growth hormone-secreting pituitary adenoma cells in vitro. Endocr J 47:563–568
75. **Product insert** 2010 Humatrope. Indianapolis, IN: Eli Lilly & Co.
76. **Hashimoto Y, Kamioka T, Hosaka M, Mabuchi K, Mizuchi A, Shimazaki Y, Tsunoo M, Tanaka T** 2000 Exogenous 20K growth hormone (GH) suppresses endogenous 22K GH secretion in normal men. J Clin Endocrinol Metab 85:601–606
77. **Baumann G, Frank SJ** 2002 Metalloproteinases and the modulation of growth hormone signalling. J Endocrinol 174:361–368
78. **Kratzsch J, Selisko T, Birkenmeier G** 1995 Identification of transformed α2-macroglobulin as a growth hormone-binding protein in human blood. J Clin Endocrinol Metab 80:585–590
79. **Baumann G, Amburn K, Shaw MA** 1988 The circulating growth hormone (GH)-binding protein complex: a major constituent of plasma GH in man. Endocrinology 122: 976–984
80. **Ishikawa M, Yokoya S, Tachibana K, Hasegawa Y, Yasuda T, Tokuhiro E, Hashimoto Y, Tanaka T** 1999 Serum levels of 20-kilodalton human growth hormone (GH) are parallel those of 22-kilodalton human GH in normal and short children. J Clin Endocrinol Metab 84:98–104
81. **Tsushima T, Katoh Y, Miyachi Y, Chihara K, Teramoto A, Irie M, Hashimoto Y** 1999 Serum concentration of 20K human growth hormone (20K hGH) measured by a specific enzyme-linked immunosorbent assay. Study Group of 20K hGH. J Clin Endocrinol Metab 84:317–322
82. **Baumann G, MacCart JG** 1982 Growth hormone production by human pituitary glands in organ culture: evidence for predominant secretion of the single-chain 22,000 molecular weight form (isohormone B). J Clin Endocrinol Metab 55:611–618
83. **Baumann G, MacCart JG, Amburn K** 1983 The molecular nature of circulating growth hormone in normal and acromegalic man: evidence for a principal and minor monomeric forms. J Clin Endocrinol Metab 56:946–952
84. **Stolar MW, Baumann G, Vance ML, Thorner MO** 1984 Circulating growth hormone forms after stimulation of pituitary secretion with growth hormone-releasing factor in man. J Clin Endocrinol Metab 59:235–239
85. **Baumann G, Stolar MW, Amburn K** 1985 Molecular forms of circulating growth hormone during spontaneous secretory episodes and in the basal state. J Clin Endocrinol Metab 60:1216–1220
86. **Stolar MW, Baumann G** 1986 Big growth hormone forms in human plasma: immunochemical evidence for their pituitary origin. Metabolism 35:75–77
87. **Norman PS** 1958 Studies of the plasmin system. II. Inhibition of plasmin by serum or plasma. J Exp Med 108: 53–68
88. **Derr RL, Cameron SJ, Golden SH** 2006 Pre-analytic considerations for the proper assessment of hormones of the hypothalamic-pituitary axis in epidemiological research. Eur J Epidemiol 21:217–226
89. **Baumann G** 1976 Failure of endogenous plasmin to convert human growth hormone to its "activated" isohormones. J Clin Endocrinol Metab 43:222–225
90. **Bidlingmaier M, Suhr J, Ernst A, Wu Z, Keller A, Strasburger CJ, Bergmann A** 2009 High-sensitivity chemiluminescence immunoassays for detection of growth hormone doping in sports. Clin Chem 55:445–453
91. **Christensen EI, Verroust PJ, Nielsen R** 2009 Receptor-mediated endocytosis in renal proximal tubule. Pflugers Arch 458:1039–1048
92. **Bala RM, Beck JC** 1971 Human growth hormone in urine. J Clin Endocrinol Metab 33:799–806
93. **Owens D, Srivastava MC, Tompkins CV, Nabarro JD, Sönksen PH** 1973 Studies on the metabolic clearance rate, apparent distribution space and plasma half-disappearance time of unlabelled human growth hormone in normal subjects and in patients with liver disease, renal disease, thyroid disease and diabetes mellitus. Eur J Clin Invest 3:284–294
94. **Main K, Philips M, Jørgensen M, Skakkebaek NE** 1991 Urinary growth hormone excretion in 657 healthy children and adults: normal values, inter- and intraindividual variations. Horm Res 36:174–182
95. **Main KM, Jarden M, Angelo L, Dinesen B, Hertel NT, Juul A, Müller J, Skakkebaek NE** 1994 The impact of gender and puberty on reference values for urinary growth hormone excretion: a study of 3 morning urine samples in 517 healthy children and adults. J Clin Endocrinol Metab 79: 865–871
96. **Pirazzoli P, Mandini M, Zucchini S, Gualandi S, Vignutelli L, Capelli M, Cacciari E** 1996 Urinary growth hormone estimation in diagnosing severe growth hormone deficiency. Arch Dis Child 75:228–231
97. **Georges P, Liefooghe J, Ponchaux D, Forzy G, Di Nicola L, Chaussain JL** 1997 Urinary growth hormone excretion: results of a multicenter study in France. Horm Res 47: 30–37

98. **Gill MS, Toogood AA, O'Neill PA, Thorner MO, Shalet SM, Clayton PE** 1998 Urinary growth hormone (GH), insulin-like growth factor I (IGF-I), and IGF-binding protein-3 measurements in the diagnosis of adult GH deficiency. J Clin Endocrinol Metab 83:2562–2565
99. **Cappellin E, Gatti R, De Palo EF** 1999 Influence of gender in growth hormone status in adults: role of urinary growth hormone. Clin Chem 45:443–444
100. **Bona G, Petri A, Rapa A, Conti A, Sartorio A** 1999 The impact of gender, puberty and body mass on reference values for urinary growth hormone (GH) excretion in normally growing non-obese and obese children. Clin Endocrinol (Oxf) 50:775–781
101. **Girard J, Greenwood FC** 1968 The absence of intact growth hormone in urine as judged by radioimmunoassay. J Endocrinol 40:493–503
102. **Mauri M, Picó AM, Alfayate R, Dominguez JR, Cámara R, Miralles C** 1993 Usefulness of urinary growth hormone (GH) measurement for evaluating endogenous GH secretion in acromegaly. Horm Res 39:13–18
103. **Momomura S, Hashimoto Y, Shimazaki Y, Irie M** 2000 Detection of exogenous growth hormone (GH) administration by monitoring ratio of 20 kDa- and 22 kDa-GH in serum and urine. Endocr J 47:97–101
104. **Winer LM, Shaw MA, Baumann G** 1989 Urinary growth hormone excretion rates in normal and acromegalic man: a critical appraisal of its potential clinical utility. J Endocrinol Invest 12:461–467
105. **Léger J, Reverchon C, Porquet D, Noël M, Czernichow P** 1995 The wide variation in urinary excretion of human growth hormone in normal growing and growth hormone-deficient children limits its clinical usefulness. Horm Res 44:57–63
106. **Skinner AM, Clayton PE, Addison GM, Price DA** 1995 Nocturnal urinary growth hormone excretion in growth hormone-deficient children on and off growth hormone treatment. Horm Res 44:147–151
107. **Turner G, Coates P, Warren S, Woodhead JS, Peters JR** 1997 Proximal tubular reabsorption of growth hormone and sodium/fluid in normo- and microalbuminuric insulin-dependent diabetes mellitus. Acta Diabetol 34:27–32
108. **Flanagan DE, Taylor MC, Parfitt V, Mardell R, Wood PJ, Leatherdale BA** 1997 Urinary growth hormone following exercise to assess growth hormone production in adults. Clin Endocrinol (Oxf) 46:425–429
109. **Fall CH, Clark PM, Hindmarsh PC, Clayton PE, Shiell AW, Law CM** 2000 Urinary GH and IGF-I excretion in nine year-old children: relation to sex, current size and size at birth. Clin Endocrinol (Oxf) 53:69–76
110. **Saugy M, Cardis C, Schweizer C, Veuthey JL, Rivier L** 1996 Detection of human growth hormone doping in urine: out of competition tests are necessary. J Chromatogr B Biomed Appl 687:201–211
111. **Turner G, Coates P, Porter S, Peters JR, Woodhead JS** 1993 Urinary growth hormone measurements as a marker of renal tubular function in diabetes mellitus. Clin Chim Acta 220:19–30
112. **Hattori N, Kato Y, Murakami Y, Hashida S, Ishikawa E, Mohri Z, Imura H** 1988 Urinary growth hormone levels measured by ultrasensitive enzyme immunoassay in patients with renal insufficiency. J Clin Endocrinol Metab 66:727–732
113. **Fredolini C, Meani F, Reeder KA, Rucker S, Patanarut A, Botterell PJ, Bishop B, Longo C, Espina V, Petricoin 3rd EF, Liotta LA, Luchini A** 2008 Concentration and preservation of very low abundance biomarkers in urine, such as human growth hormone (hGH), by Cibacron Blue F3G-A loaded hydrogel particles. Nano Res 1:502–518
114. **Fredolini C, Tamburro D, Gambara G, Lepene BS, Espina V, Petricoin 3rd EF, Liotta LA, Luchini A** 2009 Nanoparticle technology: amplifying the effective sensitivity of biomarker detection to create a urine test for hGH. Drug Test Anal 1:447–454
115. **Rantonen PJ, Penttilä I, Meurman JH, Savolainen K, Närvänen S, Helenius T** 2000 Growth hormone and cortisol in serum and saliva. Acta Odontol Scand 58:299–303
116. **Popii V, Baumann G** 2004 Laboratory measurement of growth hormone. Clin Chim Acta 350:1–16
117. **Bidlingmaier M, Freda PU** 2010 Measurement of human growth hormone by immunoassays: current status, unsolved problems and clinical consequences. Growth Horm IGF Res 20:19–25
118. **Clemmons DR** 2011 Consensus statement on the standardization and evaluation of growth hormone and insulin-like growth factor assays. Clin Chem 57:555–559
119. **Thevis M, Bredehöft M, Kohler M, Schänzer W** 2010 Mass spectrometry-based analysis of IGF-1 and hGH. Handb Exp Pharmacol 195:201–207
120. **Arsene CG, Henrion A, Diekmann N, Manolopoulou J, Bidlingmaier M** 2010 Quantification of growth hormone in serum by isotope dilution mass spectrometry. Anal Biochem 401:228–235
121. **Wu Z, Bidlingmaier M, Dall R, Strasburger CJ** 1999 Detection of doping with human growth hormone. Lancet 353:895
122. 2010 World Anti-Doping Agency Guidelines: hGH isoform differential immunoassays for anti-doping analyses, version 1.0, June 2010. http://www.wada-ama.org/Documents/Science_Medicine/Anti-Doping-Labs-Guidelines/WADA_SCGuidelines_hGH%20Differential%20Immunoassays_EN_June10.pdf
123. **Irie M, Ueki M, Kishikawa Y, Nishii M, Kawahara T** 2009 20K-GH and its use in detecting GH abuse. Growth Horm IGF Res 19:352–356
124. **Travis J** 2010 Pharmacology. Growth hormone test finally nabs first doper. Science 327:1185
125. **Kassem M, Brixen K, Blum WF, Mosekilde L, Eriksen EF** 1994 Normal osteoclastic and osteoblastic responses to exogenous growth hormone in patients with postmenopausal spinal osteoporosis. J Bone Miner Res 9:1365–1370
126. **Sönksen P** 2009 The International Olympic Committee (IOC) and GH-2000. Growth Horm IGF Res 19:341–345
127. **Sjögren K, Liu JL, Blad K, Skrtic S, Vidal O, Wallenius V, LeRoith D, Törnell J, Isaksson OG, Jansson JO, Ohlsson C** 1999 Liver-derived insulin-like growth factor I (IGF-I) is the principal source of IGF-I in blood but is not required for postnatal body growth in mice. Proc Natl Acad Sci USA 96:7088–7092
128. **Greenspan SL** 2005 Biosynthetic processing of collagen molecules. Top Curr Chem 247:149–183
129. **Brandt A, Glanville RW, Hörlein D, Bruckner P, Timpl R,**

Fietzek PP, Kühn K 1984 Complete amino acid sequence of the N-terminal extension of calf skin type III procollagen. Biochem J 219:625–634

130. **Niemelä O, Risteli L, Parkkinen J, Risteli J** 1985 Purification and characterization of the N-terminal propeptide of human type III procollagen. Biochem J 232:145–150
131. **Ala-Kokko L, Kontusaari S, Baldwin CT, Kuivaniemi H, Prockop DJ** 1989 Structure of cDNA clones coding for the entire prepro α1 (III) chain of human type III procollagen. Differences in protein structure from type I procollagen and conservation of codon preferences. Biochem J 260: 509–516
132. **Bulleid NJ, Wilson R, Lees JF** 1996 Type-III procollagen assembly in semi-intact cells: chain association, nucleation and triple-helix folding do not require formation of interchain disulphide bonds but triple-helix nucleation does require hydroxylation. Biochem J 317:195–202
133. **Nowack H, Olsen BR, Timpl R** 1976 Characterization of the amino-terminal segment in type III procollagen. Eur J Biochem 70:205–216
134. **Jensen LT** 1997 The aminoterminal propeptide of type III procollagen. Studies on physiology and pathophysiology. Dan Med Bull 44:70–78
135. **Kühn K, Wiestner M, Krieg T, Müller PK** 1982 Structure and function of the amino terminal propeptide of type I and III collagen. Connect Tissue Res 10:43–50
136. **Jensen LT, Risteli J, Nielsen MD, Henriksen JH, Olesen HP, Risteli L, Lorenzen I** 1992 Purification of porcine aminoterminal propeptide of type III procollagen from lymph and use for lymphatic clearance studies in pigs. Matrix 12:73–79
137. **Rohde H, Bruckner P, Timpl R** 1983 Immunochemical properties of the aminopropeptide of procollagen type III. Eur J Biochem 135:197–202
138. **Risteli J, Niemi S, Trivedi P, Mäentausta O, Mowat AP, Risteli L** 1988 Rapid equilibrium radioimmunoassay for the amino-terminal propeptide of human type III procollagen. Clin Chem 34:715–718
139. **Burchardt ER, Kroll W, Gehrmann M, Schröder W** 2009 Monoclonal antibody and assay for detecting PIIINP. U.S. Patent no. 7,541,149 B1
140. **Bentsen KD** 1993 Type III procollagen peptide: studies on the circulating peptide as a marker of fibrinogenesis with special reference to the liver. Dan Med Bull 40:235–246
141. **Wallace JD, Cuneo RC, Lundberg PA, Rosén T, Jørgensen JO, Longobardi S, Keay N, Sacca L, Christiansen JS, Bengtsson BA, Sönksen PH** 2000 Responses of markers of bone and collagen turnover to exercise, growth hormone (GH) administration, and GH withdrawal in trained adult males. J Clin Endocrinol Metab 85:124–133
142. **Guler HP, Zapf J, Schmid C, Froesch ER** 1989 Insulin-like growth factors I and II in healthy man. Estimations of half-lives and production rates. Acta Endocrinol (Copenh) 121: 753–758
143. **Jensen LT, Henriksen JH, Risteli J, Olesen HP, Nielsen MD, Lorenzen I** 1993 Fate of circulating amino-terminal propeptide of type III procollagen in conscious pigs. Am J Physiol 265:R139–R145
144. **Dall R, Longobardi S, Ehrnborg C, Keay N, Rosén T, Jørgensen JO, Cuneo RC, Boroujerdi MA, Cittadini A, Napoli R, Christiansen JS, Bengtsson BA, Sacca L, Baxter RC, Basset EE, Sönksen PH** 2000 The effect of four weeks of supraphysiological growth hormone administration on the insulin-like growth factor axis in women and men. GH-2000 Study Group. J Clin Endocrinol Metab 85:4193–4200
145. **Longobardi S, Keay N, Ehrnborg C, Cittadini A, Rosén T, Dall R, Boroujerdi MA, Bassett EE, Healy ML, Pentecost C, Wallace JD, Powrie J, Jørgensen JO, Saccà L** 2000 Growth hormone (GH) effects on bone and collagen turnover in healthy adults and its potential as a marker of GH abuse in sports: a double blind, placebo-controlled study. The GH-2000 Study Group. J Clin Endocrinol Metab 85: 1505–1512
146. **Powrie JK, Bassett EE, Rosen T, Jørgensen JO, Napoli R, Sacca L, Christiansen JS, Bengtsson BA, Sönksen PH** 2007 Detection of growth hormone abuse in sport. Growth Horm IGF Res 17:220–226
147. **Erotokritou-Mulligan I, Eryl Bassett E, Cowan DA, Bartlett C, Milward P, Sartorio A, Sönksen PH, Holt RI** 2010 The use of growth hormone (GH)-dependent markers in the detection of GH abuse in sport: physiological intra-individual variation of IGF-I, type 3 pro-collagen (P-III-P) and the GH-2000 detection score. Clin Endocrinol (Oxf) 72:520–526
148. **Wallace JD, Cuneo RC, Baxter R, Orskov H, Keay N, Pentecost C, Dall R, Rosén T, Jørgensen JO, Cittadini A, Longobardi S, Sacca L, Christiansen JS, Bengtsson BA, Sönksen PH** 1999 Responses of the growth hormone (GH) and insulin-like growth factor axis to exercise, GH administration, and GH withdrawal in trained adult males: a potential test for GH abuse in sport. J Clin Endocrinol Metab 84:3591–3601
149. **Abellan R, Ventura R, Pichini S, Di Giovannandrea R, Bellver M, Olive R, Pacifici R, Pascual JA, Zuccaro P, Segura J** 2006 Effect of physical fitness and endurance exercise on indirect biomarkers of recombinant growth hormone misuse: insulin-like growth factor I and procollagen type III peptide. Int J Sports Med 27:976–983
150. **Erotokritou-Mulligan I, Bassett EE, Bartlett C, Cowan D, McHugh C, Seah R, Curtis B, Wells V, Harrison K, Sönksen PH, Holt RI** 2008 The effect of sports injury on insulin-like growth factor-I and type 3 procollagen: implications for detection of growth hormone abuse in athletes. J Clin Endocrinol Metab 93:2760–2763
151. **Krusenstjerna-Hafstrøm T, Rasmussen MH, Raschke M, Govender S, Madsen J, Christiansen JS** 2011 Biochemical markers of bone turnover in tibia fracture patients randomly assigned to growth hormone (GH) or placebo injections: implications for detection of GH abuse. Growth Horm IGF Res 21:331–335
152. **Nelson AE, Meinhardt U, Hansen JL, Walker IH, Stone G, Howe CJ, Leung KC, Seibel MJ, Baxter RC, Handelsman DJ, Kazlauskas R, Ho KK** 2008 Pharmacodynamics of growth hormone abuse biomarkers and the influence of gender and testosterone: a randomized double-blind placebo-controlled study in young recreational athletes. J Clin Endocrinol Metab 93:2213–2222
153. **Nelson AE, Howe CJ, Nguyen TV, Seibel MJ, Baxter RC, Handelsman DJ, Kazlauskas R, Ho KK** 2005 Erythropoietin administration does not influence the GH–IGF axis or

makers of bone turnover in recreational athletes. Clin Endocrinol (Oxf) 63:305–309
154. **Healy ML, Dall R, Gibney J, Bassett E, Ehrnborg C, Pentecost C, Rosen T, Cittadini A, Baxter RC, Sönksen PH** 2005 Toward the development of a test for growth hormone (GH) abuse: a study of extreme physiological ranges of GH-dependent markers in 813 elite athletes in the post-competition setting. J Clin Endocrinol Metab 90:641–649
155. **Nelson AE, Ho KK** 2009 Demographic factors influencing the GH system: implications for the detection of GH doping in sport. Growth Horm IGF Res 19:327–332
156. **Erotokritou-Mulligan I, Bassett EE, Cowan DA, Bartlett C, McHugh C, Sönksen PH, Holt RI** 2009 Influence of ethnicity on IGF-I and procollagen III peptide (P-III-P) in elite athletes and its effect on the ability to detect GH abuse. Clin Endocrinol (Oxf) 70:161–168
157. **Erotokritou-Mulligan I, Bassett EE, Kniess A, Sönksen PH, Holt RI** 2007 Validation of the growth hormone (GH)-dependent marker method of detecting GH abuse in sport through the use of independent data sets. Growth Horm IGF Res 17:416–423
158. **Nguyen TV, Nelson AE, Howe CJ, Seibel MJ, Baxter RC, Handelsman DJ, Kazlauskas R, Ho KK** 2008 Within-subject variability and analytic imprecision of insulin-like growth factor axis and collagen markers: implications for clinical diagnosis and doping tests. Clin Chem 54:1268–1276
159. **Guha N, Erotokritou-Mulligan I, Burford C, Strobridge G, Brigg J, Drake T, Bassett EE, Cowan D, Bartlett C, Sönksen PH, Holt RI** 2010 Serum insulin-like growth factor-I and pro-collagen type III N-terminal peptide in adolescent elite athletes: implications for the detection of growth hormone abuse in sport. J Clin Endocrinol Metab 95:2969–2976
160. **Kniess A, Ziegler E, Kratzsch J, Thieme D, Müller RK** 2003 Potential parameters for the detection of hGH doping. Anal Bioanal Chem 376:696–700
161. **Sartorio A, Agosti F, Marazzi N, Maffiuletti NA, Cella SG, Rigamonti AE, Guidetti L, Di Luigi L, Muller EE** 2004 Combined evaluation of resting IGF-I, N-terminal propeptide of type III procollagen (PIIINP) and C-terminal cross-linked telopeptide of type I collagen (ICTP) levels might be useful for detecting inappropriate GH administration in athletes: a preliminary report. Clin Endocrinol (Oxf) 61:487–493
162. **Di Luigi L, Rigamonti AE, Agosti F, Mencarelli M, Sgrò P, Marazzi N, Cella SG, Müller EE, Sartorio A** 2009 Combined evaluation of resting IGF1, N-terminal propeptide of type III procollagen and C-terminal cross-linked telopeptide of type I collagen levels might be useful for detecting inappropriate GH administration in female athletes. Eur J Endocrinol 160:753–758
163. **Nelson AE, Howe CJ, Nguyen TV, Leung KC, Trout GJ, Seibel MJ, Baxter RC, Handelsman DJ, Kazlauskas R, Ho KK** 2006 Influence of demographic factors and sport type on growth hormone-responsive markers in elite athletes. J Clin Endocrinol Metab 91:4424–4432
164. **Maack T** 1975 Renal handling of low molecular weight proteins. Am J Med 58:57–64
165. **Jensen LT, Blaehr H, Andersen CB, Risteli J, Lorenzen I** 1992 Metabolism of the aminoterminal propeptide of type III procollagen in cultures of human proximal tubular cells. Scand J Clin Lab Invest 52:1–8
166. **Aron DC, Rosenzweig JL, Abboud HE** 1989 Synthesis and binding of insulin-like growth factor I by human glomerular mesangial cells. J Clin Endocrinol Metab 68:585–591
167. **Aron DC, Saadi HF, Nye CN, Douglas JG** 1991 Secretion of insulin-like growth factor I and its binding proteins by collecting duct cells. Kidney Int 39:27–32
168. **Tönshoff B, Blum WF, Vickers M, Kurilenko S, Mehls O, Ritz E** 1995 Quantification of urinary insulin-like growth factors (IGFs) and IGF binding protein 3 in healthy volunteers before and after stimulation with recombinant human growth hormone. Eur J Endocrinol 132:433–437
169. **Gill MS, Whatmore AJ, Tillman V, White A, Addison GM, Price DA, Clayton PE** 1997 Urinary IGF and IGF binding protein-3 in children with disordered growth. The North West Paediatric Endocrine Group. Clin Endocrinol (Oxf) 46:483–492
170. **De Palo EF, Gatti R, Lancerin F, Cappellin E, Soldà G, De Palo CB, Spinella P** 2002 Urinary insulin-like growth factor I in athletes, before and after physical exercise, and in sedentary subjects. Clin Chim Acta 322:51–57
171. **De Palo EF, Gatti R, Lancerin F, Cappellin E, De Palo CB, Spinella P** 2005 Urinary insulin-like growth factor-I measurement in an actual sport competition, an additional approach in laboratory antidoping tests. Clin Chim Acta 351:73–78
172. **Pichini S, Ventura R, Palmi I, di Carlo S, Bacosi A, Langohr K, Abellan R, Pascual JA, Pacifici R, Segura J, Zuccaro P** 2010 Effect of physical fitness and endurance exercise on indirect biomarkers of growth hormone and insulin misuse: immunoassay-based measurement in urine samples. J Pharm Biomed Anal 53:1003–1010
173. **Uemasu J, Fujihara M, Kawasaki H** 1996 Effects of low dose of recombinant human growth hormone on renal excretion of insulin-like growth factor-I (IGF-I) in humans: an inverse relation between serum and urinary IGF-I level. Clin Nephrol 46:1–5
174. **Gargosky SE, Hasegawa T, Tapanainen P, MacGillivray M, Hasegawa Y, Rosenfeld RG** 1993 Urinary insulin-like growth factors (IGF) and IGF-binding proteins in normal subjects, growth hormone deficiency, and renal disease. J Clin Endocrinol Metab 76:1631–1637
175. **Rohde H, Langer I, Krieg T, Timpl R** 1983 Serum and urine analysis of the aminoterminal procollagen peptide type III by radioimmunoassay with antibody Fab fragments. Coll Relat Res 3:371–379
176. **Ghoul BE, Squalli T, Servais A, Elie C, Meas-Yedid V, Trivint C, Vanmassenhove J, Grünfeld JP, Olivo-Marin JC, Thervet E, Noël LH, Prié D, Fakhouri F** 2010 Urinary procollagen III aminoterminal propeptide (PIIINP): a fibrotest for the nephrologist. Clin J Am Soc Nephrol 5:205–210
177. **Civitelli R, Armamento-Villareal R, Napoli N** 2009 Bone turnover markers: understanding their value in clinical trials and clinical practice. Osteoporos Int 20:843–851
178. **Costigan DC, Guyda HJ, Posner BI** 1988 Free insulin-like growth factor I (IGF-I) and IGF-II in human saliva. J Clin Endocrinol Metab 66:1014–1018
179. **Ryan J, Mantle T, Costigan DC** 1992 A normal population study of human salivary insulin-like growth factor 1 (IGF

1) concentrations from birth through puberty. J Clin Endocrinol Metab 74:774–778
180. **Halimi S, Tepavceviæ D, Suchanek D, Giljeviæ Z, Palvsiæ V, Korsiæ M** 1994 Measurement of salivary insulin-like growth factor-I in acromegaly: comparison with serum insulin-like growth factor-I and growth hormone concentrations. Eur J Clin Chem Clin Biochem 32:705–707
181. **Ryan J, Mantle T, McQuaid S, Costigan DC** 1992 Salivary insulin-like growth factor-I originates from local synthesis. J Endocrinol 135:85–90
182. **Antonelli G, Cappellin E, Gatti R, Chiappin S, Spinella P, De Palo EF** 2007 Measurement of free IGF-I saliva levels: perspectives in the detection of GH/IGF axis in athletes. Clin Biochem 40:545–550
183. **Antonelli G, Gatti R, Prearo M, De Palo EF** 2009 Salivary free insulin-like growth factor-I levels: effects of an acute physical exercise in athletes. J Endocrinol Invest 32:1–5
184. **Frystyk J, Freda P, Clemmons DR** 2010 The current status of IGF-I assays—a 2009 update. Growth Horm IGF Res 20:8–18
185. **Cowan DA, Bartlett C** 2009 Laboratory issues in the implementation of the marker method. Growth Horm IGF Res 19:357–360
186. **Holt RI, Erotokritou-Mulligan I, Ridley SA, McHugh CM, Bassett EE, Cowan DA, Bartlett C, Sönksen PH** 2009 A determination of the pre-analytical storage conditions for insulin like growth factor-I and type III procollagen peptide. Growth Horm IGF Res 19:43–50
187. **Kirsch S, Widart J, Louette J, Focant JF, De Pauw E** 2007 Development of an absolute quantification method targeting growth hormone biomarkers using liquid chromatography coupled to isotope dilution mass spectrometry. J Chromatogr A 1153:300–306
188. **Bredehöft M, Schänzer W, Thevis M** 2008 Quantification of human insulin-like growth factor-1 and qualitative detection of its analogues in plasma using liquid chromatography/electrospray ionisation tandem mass spectrometry. Rapid Commun Mass Spectrom 22:477–485
189. **Kay RG, Barton C, Velloso CP, Brown PR, Bartlett C, Blazevich AJ, Godfrey RJ, Goldspink G, Rees R, Ball GR, Cowan DA, Harridge SD, Roberts J, Teale P, Creaser CS** 2009 High-throughput ultra-high-performance liquid chromatography/tandem mass spectrometry quantitation of insulin-like growth factor-I and leucine-rich α-2-glycoprotein in serum as biomarkers of recombinant human growth hormone administration. Rapid Commun Mass Spectrom 23:3173–3182
190. **Barton C, Kay RG, Gentzer W, Vitzthum F, Pleasance S** 2010 Development of high-throughput chemical extraction techniques and quantitative HPLC-MS/MS (SRM) assays for clinically relevant plasma proteins. J Proteome Res 9:333–340
191. **Thomas A, Kohler M, Schänzer W, Delahaut P, Thevis M** 2011 Determination of IGF-1 and IGF-2, their degradation products and synthetic analogues in urine by LC-MS/MS. Analyst 136:1003–1012
192. **Abellan R, Ventura R, Pichini S, Pascual JA, Pacifici R, Di Carlo S, Bacosi A, Segura J, Zuccaro P** 2005 Evaluation of immunoassays for the measurement of insulin-like growth factor-I and procollagen type III peptide, indirect biomarkers of recombinant human growth hormone misuse in sport. Clin Chem Lab Med 43:75–85
193. **Holt RI, Bassett EE, Erotokritou-Mulligan I, McHugh C, Cowan D, Bartlett C, Sönksen PH** 2009 Moving one step closer to catching the GH cheats: the GH-2004 experience. Growth Horm IGF Res 19:346–351
194. **Bassett EE, Erotokritou-Mulligan I** 2009 Statistical issues in implementing the marker method. Growth Horm IGF Res 19:361–365
195. **Mitchell CJ, Nelson AE, Cowley MJ, Kaplan W, Stone G, Sutton SK, Lau A, Lee CM, Ho KK** 2009 Detection of growth hormone doping by gene expression profiling of peripheral blood. J Clin Endocrinol Metab 94:4703–4709
196. **Chung L, Clifford D, Buckley M, Baxter RC** 2006 Novel biomarkers of human growth hormone action from serum proteomic profiling using protein chip mass spectrometry. J Clin Endocrinol Metab 91:671–677
197. **Chung L, Nelson AE, Ho KK, Baxter RC** 2009 Proteomic profiling of growth hormone-responsive proteins in human peripheral blood leukocytes. J Clin Endocrinol Metab 94:3038–3043
198. **Chung L, Baxter RC** 2009 Detection of growth hormone responsive proteins using SELDI-TOF mass spectrometry. Growth Horm IGF Res 19:383–387
199. **Sackmann-Sala L, Ding J, Frohman LA, Kopchick JJ** 2009 Activation of the GH/IGF-1 axis by CJC-1295, a long-acting GHRH analog, results in serum protein profile changes in normal adult subjects. Growth Horm IGF Res 19:471–477
200. **Ding J, Okada S, Jørgensen JO, Kopchick JJ** 2011 Novel serum protein biomarkers indicative of growth hormone doping in healthy human subjects. Proteomics 11:3565–3571
201. **Such-Sanmartín G, Bosch J, Segura J, Gutiérrez-Gallego R** 2011 Growth hormone abuse and biological passport: is mannan-binding lectin a complementary candidate? Clin J Sport Med 21:441–443
202. **Frohman LA, Downs TR, Williams TC, Heimer EP, Pan YC, Felix AM** 1986 Rapid enzymatic degradation of growth hormone-releasing hormone by plasma in vitro and in vivo to a biologically inactive product cleaved at the NH2 terminus. J Clin Invest 78:906–913
203. **Corpas E, Harman SM, Piñeyro MA, Roberson R, Blackman MR** 1993 Continuous subcutaneous infusions of growth hormone (GH) releasing hormone 1–44 for 14 days increase GH and insulin-like growth factor-I levels in old men. J Clin Endocrinol Metab 76:134–138
204. **Veldhuis JD, Patrie JT, Frick K, Weltman JY, Weltman A** 2004 Sustained growth hormone (GH) and insulin-like growth factor I responses to prolonged high-dose twice-daily GH-releasing hormone stimulation in middle-aged and older men. J Clin Endocrinol Metab 89:6325–6330
205. **Stanley TL, Chen CY, Branch KL, Makimura H, Grinspoon SK** 2011 Effects of a growth hormone-releasing hormone analog on endogenous GH pulsatility and insulin sensitivity in healthy men. J Clin Endocrinol Metab 96: 150–158
206. **Teichman SL, Neale A, Lawrence B, Gagnon C, Castaigne JP, Frohman LA** 2006 Prolonged stimulation of growth hormone (GH) and insulin-like growth factor I secretion by

CJC-1295, a long-acting analog of GH-releasing hormone, in healthy adults. J Clin Endocrinol Metab 91:799–805
207. **Falutz J, Mamputu JC, Potvin D, Moyle G, Soulban G, Loughrey H, Marsolais C, Turner R, Grinspoon S** 2010 Effects of tesamorelin (TH9507), a growth hormone-releasing factor analog, in human immunodeficiency virus-infected patients with excess abdominal fat: a pooled analysis of two multicenter, double-blind placebo-controlled phase 3 trials with safety extension data. J Clin Endocrinol Metab 95:4291–4304
208. **Ionescu M, Frohman LA** 2006 Pulsatile secretion of growth hormone (GH) persists during continuous stimulation by CJC-1295, a long-acting GH-releasing hormone analog. J Clin Endocrinol Metab 91:4792–4797
209. **Henninge J, Pepaj M, Hullstein I, Hemmersbach P** 2010 Identification of CJC-1295, a growth-hormone-releasing peptide, in an unknown pharmaceutical preparation. Drug Test Anal 2:647–650
210. **Maheshwari HG, Rahim A, Shalet SM, Baumann G** 1999 Selective lack of growth hormone (GH) response to the GH-releasing peptide hexarelin in patients with GH-releasing hormone receptor deficiency. J Clin Endocrinol Metab 84:956–959
211. **Pihoker C, Badger TM, Reynolds GA, Bowers CY** 1997 Treatment effects of intranasal growth hormone releasing peptide-2 in children with short stature. J Endocrinol 155: 79–86
212. **Mericq V, Cassorla F, Salazar T, Avila A, Iñiguez G, Bowers CY, Merriam GR** 1998 Effects of eight months treatment with graded doses of a growth hormone (GH)-releasing peptide in GH-deficient children. J Clin Endocrinol Metab 83: 2355–2360
213. **Zdravkovic M, Christiansen T, Eliot L, Agersoe H, Thomsen MS, Falch JF, Søgaard B, Ynddal L, Ilondo MM** 2001 The pharmacokinetics, pharmacodynamics, safety and tolerability following 7 days daily oral treatment with NN703 in healthy male subjects. Growth Horm IGF Res 11:41–48
214. **Nass R, Pezzoli SS, Oliveri MC, Patrie JT, Harrell Jr FE, Clasey JL, Heymsfield SB, Bach MA, Vance ML, Thorner MO** 2008 Effects of an oral ghrelin mimetic on body composition and clinical outcomes in healthy older adults: a randomized trial. Ann Intern Med 149:601–611
215. **White HK, Petrie CD, Landschulz W, MacLean D, Taylor A, Lyles K, Wei JY, Hoffman AR, Salvatori R, Ettinger MP, Morey MC, Blackman MR, Merriam GR** 2009 Effects of an oral growth hormone secretagogue in older adults. J Clin Endocrinol Metab 94:1198–1206
216. **Hersch EC, Merriam GR** 2008 Growth hormone (GH)-releasing hormone and GH secretagogues in normal aging: Fountain of Youth or Pool of Tantalus? Clin Interv Aging 3:121–129
217. **Kohler M, Thomas A, Geyer H, Petrou M, Schänzer W, Thevis M** 2010 Confiscated black market products and nutritional supplements with non-approved ingredients analyzed in the Cologne Doping Control Laboratory 2009. Drug Test Anal 2:533–537
218. **Thomas A, Kohler M, Mester J, Geyer H, Schänzer W, Petrou M, Thevis M** 2010 Identification of the growth-hormone-releasing peptide-2 (GHRP-2) in a nutritional supplement. Drug Test Anal 2:144–148
219. **Thevis M, Wilkens F, Geyer H, Schänzer W** 2006 Determination of therapeutics with growth-hormone secretagogue activity in human urine for doping control purposes. Rapid Commun Mass Spectrom 20:3393–3402
220. **Okano M, Sato M, Ikekita A, Kageyama S** 2010 Determination of growth hormone secretagogue pralmorelin (GHRP-2) and its metabolite in human urine by liquid chromatography/electrospray ionization tandem mass spectrometry. Rapid Commun Mass Spectrom 24:2046–2056
221. **Okano M, Nishitani Y, Sato M, Ikekita A, Kageyama S** 2010 Influence of intravenous administration of growth hormone releasing peptide-2 (GHRP-2) on detection of growth hormone doping: growth hormone isoform profiles in Japanese male subjects. Drug Test Anal 2:548–556
222. **Thomas A, Höppner S, Geyer H, Schänzer W, Petrou M, Kwiatkowska D, Pokrywka A, Thevis M** 2011 Determination of growth hormone releasing peptides (GHRP) and their major metabolites in human urine for doping controls by means of liquid chromatography mass spectrometry. Anal Bioanal Chem 401:507–516
223. **Corpas E, Blackman MR, Roberson R, Scholfield D, Harman SM** 1993 Oral arginine-lysine does not increase growth hormone or insulin-like growth factor-I in old men. J Gerontol 48:M128–M133
224. **Matheny Jr RW, Nindl BC, Adamo ML** 2010 Minireview: mechano-growth factor: a putative product of IGF-I gene expression involved in tissue repair and regeneration. Endocrinology 151:865–875
225. **Kohler M, Thomas A, Walpurgis K, Terlouw K, Schänzer W, Thevis M** 2010 Detection of His-tagged Long-R(3)-IGF-I in a black market product. Growth Horm IGF Res 20:386–390
226. **Chernausek SD, Backeljauw PF, Frane J, Kuntze J, Underwood LE** 2007 Long-term treatment with recombinant insulin-like growth factor (IGF)-I in children with severe IGF-I deficiency due to growth hormone insensitivity. J Clin Endocrinol Metab 92:902–910
227. **Guha N, Dashwood A, Thomas NJ, Skingle AJ, Sönksen PH, Holt RI** 2009 IGF-I abuse in sport. Curr Drug Abuse Rev 2:263–272
228. **Erotokritou-Mulligan I, Holt RI** 2010 Insulin-like growth factor I and insulin and their abuse in sport. Endocrinol Metab Clin North Am 39:33–43, viii
229. **Birzniece V, Nelson AE, Ho KK** 2011 Growth hormone and physical performance. Trends Endocrinol Metab 22: 171–178
230. **Woodhouse LJ, Mukherjee A, Shalet SM, Ezzat S** 2006 The influence of growth hormone status on physical impairments, functional limitations, and health-related quality of life in adults. Endocr Rev 27:287–317
231. **Widdowson WM, Gibney J** 2008 The effect of growth hormone replacement on exercise capacity in patients with GH deficiency: a metaanalysis. J Clin Endocrinol Metab 93: 4413–4417
232. **Widdowson WM, Gibney J** 2010 The effect of growth hormone (GH) replacement on muscle strength in patients with GH-deficiency: a meta-analysis. Clin Endocrinol (Oxf) 72:787–792
233. **Newman CB, Frisch KA, Rosenzweig B, Roubenoff R, Rey M, Kidder T, Kong Y, Pursnani A, Sedlis SP, Schwartzbard A, Kleinberg DL** 2011 Moderate doses of

hGH (0.64 mg/d) improve lipids but not cardiovascular function in GH-deficient adults with normal baseline cardiac function. J Clin Endocrinol Metab 96:122–132
234. **Liu H, Bravata DM, Olkin I, Friedlander A, Liu V, Roberts B, Bendavid E, Saynina O, Salpeter SR, Garber AM, Hoffman AR** 2008 Systematic review: the effects of growth hormone on athletic performance. Ann Intern Med 148: 747–758
235. **Deyssig R, Frisch H, Blum WF, Waldhör T** 1993 Effect of growth hormone treatment on hormonal parameters, body composition and strength in athletes. Acta Endocrinol (Copenh) 128:313–318
236. **Lange KH, Larsson B, Flyvbjerg A, Dall R, Bennekou M, Rasmussen MH, Ørskov H, Kjaer M** 2002 Acute growth hormone administration causes exaggerated increases in plasma lactate and glycerol during moderate to high intensity bicycling in trained young men. J Clin Endocrinol Metab 87:4966–4975
237. **Meinhardt U, Nelson AE, Hansen JL, Birzniece V, Clifford D, Leung KC, Graham K, Ho KK** 2010 The effects of growth hormone on body composition and physical performance in recreational athletes: a randomized trial. Ann Intern Med 152:568–577
238. **Saugy M, Robinson N, Saudan C, Baume N, Avois L, Mangin P** 2006 Human growth hormone doping in sport. Br J Sports Med 40(Suppl 1):i35–i39
239. **Holdaway IM, Rajasoorya C** 1999 Epidemiology of acromegaly. Pituitary 2:29–41
240. **Holdaway IM** 2007 Excess mortality in acromegaly. Horm Res 68(Suppl 5):166–172
241. **Melmed S** 2006 Medical progress: acromegaly. N Engl J Med 355:2558–2573
242. **Sharp RJ** 2009 Land of the giants. Growth Horm IGF Res 19:291–293
243. **Doessing S, Heinemeier KM, Holm L, Mackey AL, Schjerling P, Rennie M, Smith K, Reitelseder S, Kappelgaard AM, Rasmussen MH, Flyvbjerg A, Kjaer M** 2010 Growth hormone stimulates the collagen synthesis in human tendon and skeletal muscle without affecting myofibrillar protein synthesis. J Physiol 588:341–351
244. **Raschke M, Rasmussen MH, Govender S, Segal D, Suntum M, Christiansen JS** 2007 Effects of growth hormone in patients with tibial fracture: a randomised, double-blind, placebo-controlled clinical trial. Eur J Endocrinol 156: 341–351
245. **Wilmore DW, Moylan Jr JA, Bristow BF, Mason Jr AD, Pruitt Jr BA** 1974 Anabolic effects of human growth hormone and high caloric feedings following thermal injury. Surg Gynecol Obstet 138:875–884
246. **Ramirez RJ, Wolf SE, Herndon DN** 1998 Is there a role for growth hormone in the clinical management of burn injuries? Growth Horm IGF Res 8(Suppl B):99–105
247. **Salomon F, Cuneo RC, Hesp R, Sönksen PH** 1989 The effects of treatment with recombinant human growth hormone on body composition and metabolism in adults with growth hormone deficiency. N Engl J Med 321:1797–1803
248. **Jing J, Yang S, Zhou X, Zhang L, Xu Y, Xie M, Wu M** Detection of doping with rhGH-Detection time with WADA approved kits. Program of the 10th Annual USADA Symposium on Anti-Doping Science, London, UK, 2011 p 8–10