WORLD ANTI-DOPING AGENCY
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Testing Guide for

GROWTH HORMONE RELEASING FACTORS (GHRFs)

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1.0 Substance Class Overview

Growth hormone-releasing factors (GHRFs) is a term used to describe a set of substances that have potent stimulatory effects on growth hormone (GH) secretion from the pituitary gland in humans and are described in three groups within the WADA Prohibited List (S2.2.3) including:

- Endogenous GH-releasing hormone (GHRH) and its analogues, e.g. CJC-1293, CJC-1295, sermorelin and tesamorelin;
- GH secretagogues that bind the GH secretagogue receptor, e.g. lenomorelin (ghrelin) and its mimetics, e.g. anamorelin, ipamorelin, macimorelin and tabimorelin; and
- Other synthetic GH releasing peptides (GHRPs), e.g. alexamorelin, GHRP-1, GHRP-2 (pralmorelin), GHRP-3, GHRP-4, GHRP-5, GHRP-6, and examorelin (hexarelin).

TABLE 1: Reported WADA Testing Statistics – GHRFs

	# of samples analyzed	# of Sports	# of TAs	AAFs
2018[1]	60,964	124	231	21
2017	57,869	119	218	19
2016	42,730	111	207	15
2015	21,654	88	145	14

2.0 Performance-Enhancing Benefits

Similar to the purported benefits of GH administration, GHRF use promotes beneficial effects such as decreased body fat, increased muscle, and increased strength and stamina, as well as image-enhancing properties. The GH-releasing activity of GHRFs is significant and dose-related after intravenous (IV), subcutaneous (SC), intranasal (IN), buccal and even oral administration. The systemic absorption and bioavailability of GHRFs via multiple routes of administration is possible due to their low molecular mass. Prolonged administration of GHRFs increases insulin-like growth factor 1 (IGF-1) levels both in animals and in humans and the amount of endogenous GH and IGF-1 has been shown to increase in a gender-independent, but age-related fashion. Besides their general ability to increase GH and presumably provide an advantage due to transiently elevated GH plasma levels, GHRFs reportedly possess the potential to transiently mask a recent injection of recombinant GH, when tested with the GH Isoforms method, by counteracting the suppressive effect of the injected GH on the natural production and secretion of GH. Therefore, GHRFs are used as an alternative to recombinant GH because of their potential to avoid detection by the currently applied GH isoforms test, their variety of administration routes and relatively low costs.

3.0 How Supplied

Peptides are comprised of amino acids, the building blocks of protein. Commonly supplied in multi-dose vials from internet peptide suppliers “for research purposes only “, peptides appear as a pure white powder or flakes that requires reconstitution with sterile diluent before injection. Quality can vary as to the purity of the product. Recently, mixtures of different peptides have been appearing

[1] Data to be confirmed once WADA 2018 Anti-Doping Testing Figures are published.

on the black market, and also GHRPs have been discovered in over-the-counter dietary supplements (in powder and liquid form), “bio-identical hormone” creams, and some claiming enhanced effects through “proprietary delivery systems”. Peptides can also be deceptively called “amino-acids” or marketed together with thyroid- and GH-stimulating substances. With the exception of GHRP-2 (pralmorelin, assessment of GH deficiency), macimorelin (assessment of GH deficiency), and tesamorelin (treatment of HIV-associated lipodystrophy), these substances represent non-approved pharmaceuticals; however, via internet providers, all compounds are readily available. Peptides and their mimetics are easily transported through the mail system. A survey of customs seizures in Switzerland demonstrated that in addition to hGH and hCG, GHRPs are a common PED distributed through mail packages. There is a lack of quality control as these substances are not produced in regulated environments. There are reported instances of commensal skin microorganisms in unregulated peptide samples which pose additional health concerns.

4.0 Cost

Cost is moderate to very expensive but GHRFs can easily be obtained on the internet or from black market providers. There are many suppliers, mainly in the US and Canada, making prices competitive and relatively low. Many companies are known to ship via post/courier internationally, or athletes may transport products with them over international borders.

5.0 Routes of Administration & Dosing Regimes

In general, GHRPs are used in doses of 50-200 µg/kg body weight IV, and orally up to 10 mg/day. However, dosage varies: “recommendations” of internet forums advise oral daily amounts of GHRP-2 range from 150µg to 10mg, and IV administration from 50-200 µg/kg body weight. For GHRHs, the dose may be between 50-1000 µg/day. Recent nasal preparations of GHRP-1, GHRP-2, GHRP-6, ipamorelin, and hexarelin were discovered. The effectiveness of the nasal administration shows that the peptides could be easily transported across the nasal epithelium and stimulate GH and IGF-1 production in healthy subjects. Nasal route seems effective for systemic delivery of peptides; therefore, it can be used by athletes as a non-invasive method of administration.

Little is known about doping in combination with other products, but the perception of getting caught among athletes may be diminished because of their short biological half-lives. The use of GHRPs and GHRH (analogs) in combination with rGH is suspected in doping practices. GHRP analogues have also been identified in black market products as Gly-GHRP-6, Gly-GHRP-2 and Gly-Ipamorelin, representing the corresponding GHRP extended by a N-terminal glycine residue which may be an attempt to circumvent detection methods.

6.0 Other considerations

Reasons for use are either assistance with recovery from injury or heavy training and enhancement of power, strength or muscular size. There seems to be no ideal timing to use GHRFs within a single day, but immediately post-workout or before bedtime has been reported purportedly to “synergize” with natural GH spikes which occur while sleeping. Proteases, enzymes which breakdown proteins, can be used to defeat detection of GHRFs, thus if proteins are undetectable in a urine sample, additional consideration should be given to possible tampering.

7.0 Detection Matrices and Methods

Much progress has been made in recent years on developing appropriately sensitive mass spectrometry (MS)-based methods for the detection of peptides and their corresponding metabolites in both urine and blood. Detection can be challenging because metabolism studies of some peptides have revealed detection windows of the parent compound of minutes, and 12-72 hours for metabolic products after a single therapeutic dose.

Low blood and/or urine concentrations combined with very short half-lives and potential combinations of GHRF types add significant detection challenges. The specific GHRF screens offered by WADA-accredited laboratories may vary. Degradation of GHRPs is possible due to proteases and/or other factors, therefore due to their general low urinary concentration, it's imperative that the B-sample analysis occurs within a short period of the time after the A-sample confirmation and AAF report.

In general, two different LC-MS/MS-based detection methods are applied, which are basically determined by the size (molecular weight) and structural complexity of the target molecule. A simpler method, based on solid-phase extraction followed by liquid chromatography (LC) and tandem mass spectrometry (MS/MS) can be applied to the detection of GH secretagogues and GHRPs, which are short peptides. This method is mandatory and therefore available in all WADA-accredited laboratories and includes the detection of other short target peptides such as gonadotophin releasing factors (buserelin, deslorelin, gonadorelin, goserelin, leuprorelin, nafarelin and triptorelin), hGH fragments (e.g. AOD-9604) and TB-500.

For detection of larger GHRHs (and also other prohibited substances such as insulins and IGF-1 analogs), a more complex and time consuming technique is used, including the immunopurification of the target analytes prior to the LC-MS/MS analysis. This method is not mandatory and therefore it is available only in some laboratories. As a consequence, laboratories may apply different price brackets for both methods.

GHRFs which may be detected by the WADA-accredited laboratories include: alexamorelin, anamorelin, AOD-9604, CJC-1293, CJC-1295, capromorelin, deslorelin, examorelin (hexarelin), GHRP-1, GHRP-2 (pralmorelin), GHRP-3, GHRP-4, GHRP-5, GHRP-6, hexarelin, hgh 176-191, ibutamoren, iox2, ipamorelin, lenomorelin (ghrelin), macimorelin, peforelin, sermorelin, tabimorelin and tesamorelin.

8.0 High Risk Sports

GHRF abuse has been reported in a wide variety of sports/disciplines, including strength (bodybuilding, football, powerlifting) and endurance (cycling, triathlon, tennis, athletics) or those requiring frequent and repetitive intense activity (sprint events, weightlifting, boxing).
For information on the minimum levels of GHRFs analysis required for sports/disciplines refer to Appendix 1 and Appendix 2 of the Technical Document for Sport Specific Analysis (TDSSA).

TABLE 2. GHRFs AAFs 2015-2018 in sports or disciplines listed in the TDSSA

Sport - Discipline	MLA	IC	OOC	GHRF AAFs
Athletics - Combined Events	15%		1	1
Athletics - Jumps	15%	1		1
Athletics - Long Distance 3000m or greater	5%	1	1	2
Athletics - Sprint 400m or less	15%		3	3
Athletics - Throws	15%		5	5
Bodybuilding - Bodybuilding	30%	4	1	5
Bodybuilding - Fitness	30%		1	1
Canoe/Kayak - Long Distance 1000m	5%		1	1
Cycling - Road	10%	5	8	13
Football - Football	10%	1	1	2
Judo - Judo	10%		1	1
Karate - Karate	10%		1	1
Para-Powerlifting - Para-Powerlifting	30%		1	1
Powerlifting - Bench Press	30%	1		1
Powerlifting - Powerlifting	30%	3	2	5
Rugby Union - Fifteens	10%	1	1	2
Tennis - Tennis	5%		1	1
Weightlifting - Weightlifting	30%	3	8	11
Grand Total		**20**	**37**	**57**

9.0 Suggested Testing Strategies

Integration of analytical and non-analytical intelligence strategies is a key to success for detection of peptides. Evidence discovered through tips and investigations is critical to selecting the right athletes and timing the testing to maximize chances of detection success.

GHRFs can be abused at any point during the competition or off-seasons. However, in the recent years, the majority of AAFs for GHRFs are the result of out-of-competition testing. Patterns of use may occur in areas where athletes believe testing is less likely or in hard to test locations for urine and/or blood collections.

- Prioritize a list of sports/disciplines as outlined in the TDSSA that require GHRFs and GH testing and determine the level and number of athletes to be tested.

- Obtain non-analytical information about key events for the season, as well as team information regarding training and selection camps/events. Monitor whereabouts for abnormal patterns or patterns of whereabouts changes for training partners.

- Work backwards from key events and schedule testing unpredictably on all athletes, paying special attention to collecting urine and blood at different times of the day and week, as well as samples at least two weeks before major events before athletes begin to taper their training.

- As natural GH levels spike soon after bed time, athletes may choose to take peptides in the evening, or after intense training.

- Testing early in the morning should be part of your testing strategy whilst ensuring other testing sessions are varied and unpredictable.

- Consider testing between 11pm-5am if suspicious results or other intelligence suggests usage of GHRFs/GH.

- Monitor athlete declarations for high risk dietary supplements which may have GHRFs or claims of GH boosting effects.

- Monitor multiple dilute urine collections on the same athlete in attempts to mask the presence of GHRFs.

- Consider screening all urine samples for GHRFs in high risk sports for a period of time as athletes may have the perception that GHRFs are undetectable.

- Consider interviewing any athletes with past adverse analytical findings (AAF) to learn about dosing and use patterns, and monitor athlete discussion boards to learn about evolution in GHRFs/GH abuse.

- Determine the controlled status of peptides in your country and work with law enforcement and customs to learn of seizures and related customer lists to target athletes or high risk sports. Non-analytical cases have also been successful.

- Consider testing of blood samples both for GH (isoforms and biomarkers test) as well as urine samples for GHRFs in order to optimize detection of abuse with these doping agents.

- Consider discussing with the laboratories for a 'pricing package' where multiple analytical methods are requested.

- Combining samples together (batching) for the same analytical method is often a more cost-effective option. However, this is subject to the laboratory's capacity and the timing requirements for reporting of results.

10.0 References

WADA 2017 Anti-Doping Testing Figures

WADA 2016 Anti-Doping Testing Figures

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