

## RESEARCH MONOGRAPH . COMPOUND PROFILE

# Hexarelin

*A synthetic hexapeptide derived from GHRP-6, developed by Mediolanum Farmaceutici as a potent growth hormone secretagogue acting through dual GHS-R1a and CD36 receptor pathways, distinguished from other growth hormone releasing peptides by pronounced cardioprotective activity independent of growth hormone release.*

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## ABSTRACT

Hexarelin (examorelin; His-D-2-methyl-Trp-Ala-Trp-D-Phe-Lys-NH<sub>2</sub>; CAS 140703-51-1; molecular weight 887.04 g/mol) is a synthetic hexapeptide growth hormone secretagogue developed in the early 1990s by Romano Deghenghi and colleagues at Europeptides (subsequently Mediolanum Farmaceutici) as a chemically stabilized analog of growth hormone releasing peptide-6 (GHRP-6). The compound acts through two pharmacologically distinct receptor systems: the growth hormone secretagogue receptor type 1a (GHS-R1a), a G-protein-coupled receptor expressed predominantly in the hypothalamic-pituitary axis that mediates potent, dose-dependent growth hormone release; and CD36, a class B scavenger receptor expressed on cardiomyocytes, macrophages, and endothelial cells that mediates cardioprotective, anti-fibrotic, and anti-atherogenic effects independent of growth hormone secretion. The dual-receptor pharmacology distinguishes hexarelin from both the endogenous ligand ghrelin and from the more selective growth hormone secretagogue ipamorelin, and provides the molecular basis for a research literature that extends substantially beyond the neuroendocrine indication for which the compound was originally developed.

The growth hormone releasing activity of hexarelin was first characterized in humans by Ghigo, Arvat, and colleagues in 1994, who demonstrated dose-dependent GH release after intravenous, subcutaneous, intranasal, and oral administration in healthy volunteers, with subcutaneous bioavailability of approximately 77 percent, intranasal bioavailability of approximately 5 percent, and

oral bioavailability of approximately 0.3 percent [1]. At an intravenous dose of 1 microgram per kilogram, hexarelin produced a GH response approximately twice that of equimolar growth hormone releasing hormone (GHRH), and co-administration with GHRH produced synergistic GH elevation described as "massive" even at low hexarelin doses [1, 2]. The compound reached Phase II clinical trials for the diagnosis and treatment of growth hormone deficiency and for congestive heart failure but was never marketed, principally owing to tachyphylaxis of the GH-releasing response on repeated daily dosing, with GH output declining by 50 to 80 percent within two to four weeks of continuous administration [3, 4].

The cardiovascular pharmacology of hexarelin represents the most pharmacologically distinctive aspect of the compound. Bhatt et al. (2002) and Bhargava et al. (2004) established that the cardioprotective effects of hexarelin in ischemia-reperfusion models are mediated through CD36 rather than GHS-R1a, using knockout mouse experiments that demonstrated abolition of cardioprotection in CD36-null animals while the effect was preserved in GHS-R1a-null animals [5, 6]. In isolated perfused rat hearts subjected to 30 minutes of ischemia followed by 120 minutes of reperfusion, hexarelin at 1 micromolar reduced infarct size by approximately 25 to 40 percent [7]. The CD36-mediated signaling involves activation of peroxisome proliferator-activated receptor gamma (PPARgamma), phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) survival pathways, and suppression of pro-apoptotic caspase-3 activity [8, 9]. In spontaneously hypertensive rats, five weeks of hexarelin treatment significantly reduced cardiac fibrosis by decreasing interstitial and perivascular collagen deposition, reducing collagen I and III expression, and attenuating left ventricular hypertrophy [10].

Human clinical studies have demonstrated acute positive inotropic effects: in healthy volunteers, hexarelin increased left ventricular ejection fraction from 64.0 to 70.7 percent within 15 to 30 minutes of intravenous administration, and in patients with coronary artery disease undergoing bypass surgery, hexarelin produced prompt increases in left ventricular ejection fraction, cardiac index, and cardiac output lasting up to 90 minutes [11, 12].

Beyond the cardiovascular system, hexarelin has demonstrated neuroprotective activity in preclinical models. In a rat model of neonatal hypoxia-ischemia, hexarelin reduced brain damage by 39 percent in the cerebral cortex, hippocampus, and thalamus through Akt/glycogen synthase kinase-3-beta phosphorylation and caspase-3 suppression [13]. In neuroblastoma cell lines, hexarelin modulated MAPK and PI3K/Akt pathways to inhibit hydrogen peroxide-induced apoptotic toxicity [14].

Pharmacokinetically, hexarelin has a plasma elimination half-life of approximately 55 to 70 minutes in humans after parenteral administration. The compound is administered predominantly by subcutaneous injection at research doses of 1 to 2 micrograms per kilogram (approximately 100 to 300 micrograms per administration in adults). Principal adverse effects are dose-dependent and include transient flushing, mild cortisol and prolactin elevation (more pronounced than ipamorelin but less clinically significant than early reports suggested), increased appetite, and water retention. The cortisol and prolactin elevations reflect direct GHS-R1a activation on corticotroph and lactotroph cells and are the principal pharmacodynamic distinction from the more selective secretagogue ipamorelin. No galactorrhea, Cushingoid features, or clinically significant endocrine adverse events have been reported in any published hexarelin study. This

monograph documents the chemistry and synthesis, dual-receptor pharmacology, comprehensive pharmacokinetics, the clinical and preclinical evidence base across neuroendocrine, cardiovascular, and neuroprotective applications, sourcing and quality verification, reconstitution and handling, stack-interaction considerations, adverse-event signal, and a structured comparative assessment of five growth hormone secretagogue candidates against hexarelin on five competency standards.

# 1. Compound Identification

## 1.1 Names, synonyms, and registry identifiers

**International Nonproprietary Name:** Examorelin.

**Common name:** Hexarelin. The name derives from the hexapeptide structure and the compound's position within the growth hormone releasing peptide (GHRP) family.

**Development codes:** EP-23905 (Europeptides); MF-6003 (Mediolanum Farmaceutici). The EP-23905 code is the canonical identifier in the early pharmacology literature and the development-stage clinical publications [1, 2].

**Chemical Abstracts Service registry number:** 140703-51-1 (free base acetate salt). The compound is supplied commercially as the acetate salt or trifluoroacetate salt; the free base peptide is the pharmacologically active species.

**International Union of Pure and Applied Chemistry name:** L-histidyl-2-methyl-D-tryptophyl-L-alanyl-L-tryptophyl-D-phenylalanyl-L-lysineamide.

**Sequence:** His-D-2-MeTrp-Ala-Trp-D-Phe-Lys-NH<sub>2</sub>. The C-terminal amidation and the two D-amino acid residues (D-2-methyl-tryptophan at position 2, D-phenylalanine at position 5) confer resistance to enzymatic degradation and are the principal structural modifications relative to the parent compound GHRP-6 [15].

## 1.2 Structural class and chemistry

Hexarelin is a synthetic hexapeptide belonging to the growth hormone releasing peptide family, a class of short synthetic peptides originally derived from met-enkephalin analogs that were empirically discovered to stimulate growth hormone release through the growth hormone secretagogue receptor (GHS-R1a) rather than through opioid pathways [16]. The structural class places hexarelin within a small group of peptidic GHS-R1a agonists that includes GHRP-6 (His-D-Trp-Ala-Trp-D-Phe-Lys-NH<sub>2</sub>), GHRP-2 (D-Ala-D-2-Nal-Ala-Trp-D-Phe-Lys-NH<sub>2</sub>), GHRP-1 (Ala-His-D-2-Nal-Ala-Trp-D-Phe-Lys-NH<sub>2</sub>), and ipamorelin (Aib-His-D-2-Nal-D-Phe-Lys-NH<sub>2</sub>).

**Molecular formula:** C<sub>47</sub>H<sub>58</sub>N<sub>12</sub>O<sub>6</sub> (free base).

**Exact mass:** 886.46 Da (monoisotopic).

**Molecular weight:** 887.04 g/mol (free base).

**Physical properties:** White to off-white lyophilized powder. Freely soluble in water (as the acetate or trifluoroacetate salt), soluble in dimethyl sulfoxide, sparingly soluble in apolar organic solvents. The compound is hygroscopic and should be stored in desiccated conditions. Polar surface area is approximately 300.9 square angstroms. Calculated LogP is approximately 5.4, reflecting the hydrophobic contribution of the two tryptophan-derived residues and the phenylalanine residue.

**Key structural features:** The substitution of tryptophan at position 2 with D-2-methyltryptophan is the defining chemical modification relative to GHRP-6. This substitution confers two pharmacologically important properties: (a) increased resistance to tryptophan oxidation, which is the principal degradation pathway for GHRP-6 under storage conditions; and (b) increased metabolic stability in vivo, extending the effective half-life relative to GHRP-6 [15, 17]. The D-configuration at positions 2 and 5 prevents recognition by endogenous peptidases that cleave L-amino acid bonds, contributing additional proteolytic resistance. The C-terminal amidation prevents carboxypeptidase degradation and is required for full biological activity at GHS-R1a.

## 1.3 Synthesis

Hexarelin is synthesized by standard solid-phase peptide synthesis (SPPS) using Fmoc (9-fluorenylmethyloxycarbonyl) or Boc (tert-butyloxycarbonyl) chemistry on Rink amide resin (which provides the C-terminal amide upon cleavage). The synthesis proceeds from the C-terminus to the N-terminus in the standard direction: Lys(Boc)-D-Phe-Trp(Boc)-Ala-D-2-MeTrp(Boc)-His(Trt) coupling sequence, using HBTU or HATU activation reagents with DIEA as base in DMF solvent [18]. The D-2-methyltryptophan residue is the non-standard building block and is commercially available as Fmoc-D-2-MeTrp(Boc)-OH from specialty amino acid suppliers. Cleavage from the resin is performed with trifluoroacetic acid cocktails containing scavengers (triisopropylsilane, water, ethanedithiol) to protect the indole rings from alkylation. The crude peptide is purified by reversed-phase high-performance liquid chromatography (RP-HPLC), typically on a C18 column with an acetonitrile-water gradient containing 0.1 percent trifluoroacetic acid. The purified peptide is lyophilized to yield a white powder. Typical research-grade purity is greater than 95 percent by HPLC; pharmaceutical-grade preparations achieve greater than 98 per-

cent. Overall yields on solid-phase synthesis are 40 to 65 percent depending on scale, coupling efficiency, and purification protocol. The acetate salt is prepared by anion exchange from trifluoroacetate or by lyophilization from dilute acetic acid [18].

## 2. Abstract

Hexarelin (examorelin; EP-23905; His-D-2-MeTrp-Ala-Trp-D-Phe-Lys-NH<sub>2</sub>) is a synthetic hexapeptide growth hormone secretagogue that acts through dual receptor pathways: the ghrelin/growth hormone secretagogue receptor type 1a (GHS-R1a) to produce potent, dose-dependent growth hormone release, and the CD36 scavenger receptor to produce cardioprotective, anti-fibrotic, and anti-atherogenic effects independent of the growth hormone axis [1, 5, 6]. The compound was developed in the early 1990s at Europeptides by Romano Deghenghi as a chemically stabilized derivative of GHRP-6, with the substitution of D-2-methyltryptophan for D-tryptophan at position 2 conferring enhanced oxidative and proteolytic stability [15, 17].

The neuroendocrine pharmacology of hexarelin is characterized by a GH-releasing potency approximately twice that of equimolar GHRH administered intravenously, synergistic GH elevation when co-administered with GHRH, and dose-dependent co-release of ACTH, cortisol, and prolactin through direct GHS-R1a activation on corticotroph and lactotroph cells [1, 2, 19]. The compound was evaluated in Phase II clinical trials for the diagnosis and treatment of growth hormone deficiency and for congestive heart failure but was not advanced to registration, principally owing to tachyphylaxis of the GH-releasing response with continuous daily dosing [3, 4]. The desensitization appears at the GHS-R1a receptor level and is partially reversible after a four-week washout period.

The cardiovascular pharmacology is the more distinctive research application. Knockout mouse experiments definitively established that hexarelin's cardioprotective effects in ischemia-reperfusion injury are mediated through CD36 and not through GHS-R1a [5, 6]. The CD36 signaling cascade involves PPARGamma activation, PI3K/Akt pathway engagement, suppression of pro-apoptotic signaling, and reduction of oxidative stress markers [8, 9]. In human clinical studies, acute hexarelin administration increased left ventricular ejection fraction in healthy volunteers, in patients with severe dilated cardiomyopathy, and in patients with coronary artery disease undergoing bypass surgery, with the inotropic effect appearing within 15 minutes and persisting for up to 90 minutes [11, 12, 20].

Neuroprotective activity has been demonstrated in rodent models of neonatal hypoxia-ischemia and in neuronal cell lines exposed to oxidative stress, mediated through Akt/GSK-3beta phosphorylation and MAPK pathway modulation [13, 14]. Hexarelin has also demonstrated lipid-metabolic activity through CD36-mediated modulation of fatty acid uptake and PPARgamma signaling, with improvements in lipid metabolic aberrations documented in insulin-resistant mouse models [21].

Pharmacokinetics are characterized by a plasma half-life of approximately 55 to 70 minutes after parenteral administration, subcutaneous bioavailability of approximately 77 percent, negligible oral bioavailability (approximately 0.3 percent), and renal and hepatic elimination of peptide fragments [1, 22]. The compound is not approved for clinical use in any jurisdiction. It is available as a research-grade peptide from multiple suppliers; investigators should obtain analytical confirmation of identity and purity on every lot.

## 3. Discovery and Development History

### 3.1 Origin

Hexarelin was synthesized in the early 1990s by Romano Deghenghi and colleagues at Europeptides (Milan, Italy) as part of a systematic structure-activity exploration of growth hormone releasing peptide analogs [15, 17]. The parent compound GHRP-6 (His-D-Trp-Ala-Trp-D-Phe-Lys-NH<sub>2</sub>), developed by Cyril Bowers at Tulane University in the 1980s from the empirical observation that met-enkephalin analogs containing D-amino acid substitutions stimulated GH release independently of opioid receptors, had demonstrated potent GH-releasing activity but suffered from chemical instability owing to oxidation of the D-tryptophan indole ring during storage and from rapid *in vivo* degradation [16, 23]. Deghenghi's group explored methylation of the indole ring as a strategy to block the oxidation site, yielding hexarelin (His-D-2-MeTrp-Ala-Trp-D-Phe-Lys-NH<sub>2</sub>), in which the 2-methyl group on the D-tryptophan residue sterically shields the vulnerable 2,3-double bond of the indole system from electrophilic oxidation [15].

The compound was selected for development on the basis of equivalent or superior GH-releasing potency to GHRP-6, substantially improved chemical stability, and acceptable tolerability in early animal toxicology studies. Europeptides subsequently became part of Mediolanum Farmaceutici, which advanced hexarelin through preclinical development and early clinical trials under the development codes EP-23905 and MF-6003 [4].

### 3.2 Neuroendocrine clinical development

The first comprehensive human pharmacology study was published by Ghigo, Arvat, Gianotti, Imbimbo, Lenaerts, Deghenghi, and Camanni in the *Journal of Clinical Endocrinology and Metabolism* in 1994 [1]. This study characterized the GH-releasing activity of hexarelin after intravenous (1 and 2 micrograms per kilogram), subcutaneous (1.5 and 3 micrograms per kilogram), intranasal (20 micrograms per kilogram), and oral (20 and 40 milligrams) administration in 12 healthy young male volunteers. The investigators reported dose-dependent GH release with

a peak response approximately twice that of equimolar GHRH by the intravenous route and biological bioavailabilities of 77 percent (subcutaneous), 4.8 percent (intranasal), and 0.3 percent (oral) [1].

Subsequent studies by the Ghigo and Arvat group at the University of Turin established the dose-response profile for GH, cortisol, and prolactin release [19]; the synergistic interaction between hexarelin and GHRH [2]; the age dependence of the GH response (potent in pubertal children and adults, reduced in prepubertal children and elderly subjects) [24]; and the diagnostic utility of hexarelin as a provocative test for GH deficiency [25]. A low dose of hexarelin (0.25 micrograms per kilogram intravenous) combined with GHRH was proposed as a diagnostic tool for adult GH deficiency, with reliability comparable to the insulin-induced hypoglycemia test [25].

Phase II clinical trials for GH deficiency diagnosis and treatment were conducted by Mediolanum Farmaceutici but did not advance to registration. The principal barrier was tachyphylaxis: repeated daily hexarelin administration produced progressive attenuation of the GH response, with output declining by 50 to 80 percent within two to four weeks [3, 4]. The desensitization occurred at the GHS-R1a receptor level, as demonstrated by in vitro studies showing rapid downregulation of calcium signaling within minutes of initial exposure [26]. After a four-week washout period, GH responsiveness was partially restored, suggesting reversible receptor downregulation rather than permanent desensitization [3].

### 3.3 Identification of CD36-mediated cardiovascular activity

The cardiovascular pharmacology of hexarelin emerged from observations that the compound produced hemodynamic effects that could not be fully explained by GH release kinetics. Growth hormone-independent cardioprotective effects were first formally reported by Locatelli et al. (1999), who demonstrated that hexarelin reduced myocardial injury in GH-deficient dwarf rats [27]. The Bhatt et al. (2002) and Bhargava et al. (2004) studies from the Bhargava laboratory established the CD36 receptor as the mediating target, using knockout mouse experiments that demonstrated complete abolition of cardioprotection in CD36-null animals while the protective effect was preserved in GHS-R1a-null animals [5, 6]. This was the definitive demonstration that hexarelin's cardiovascular pharmacology operates through a receptor system distinct from its neuroendocrine pharmacology.

The identification of CD36 as a hexarelin target was further refined by Demers et al. (2004), who used photoaffinity cross-linking to map the hexarelin binding site on CD36 to an 8-kilodalton fragment corresponding to residues Asn132 to Glu177, with Met169 identified as the contact residue within the binding pocket [28].

### 3.4 Cardiac clinical development

Human cardiac studies demonstrated that hexarelin produces acute positive inotropic effects. Bisi et al. (1999) reported that in healthy male volunteers, intravenous hexarelin increased left ventricular ejection fraction from 64.0 to 70.7 percent within 15 to 30 minutes of administration [11]. Imazio et al. (2002) demonstrated GH-independent cardiotropic activity in patients with severe left ventricular dysfunction due to dilated and ischemic cardiomyopathy [20]. Pettersson et al. (2002) reported that hexarelin administered during coronary artery bypass surgery produced prompt increases in left ventricular ejection fraction, cardiac index, and cardiac output lasting up to 90 minutes [12].

Phase II cardiac trials were conducted but did not advance to registration. The compound remains a research tool for investigating CD36-mediated cardiovascular pharmacology.

## 4. Mechanism of Action

Hexarelin is a dual-receptor ligand. Its principal molecular activities, characterized in heterologous expression systems, native tissue, and knockout animal models, are agonism at the GHS-R1a receptor and agonism at the CD36 scavenger receptor. These activities operate at pharmacologically relevant concentrations and contribute differentially to the neuroendocrine, cardiovascular, neuroprotective, and metabolic effects.

### 4.1 GHS-R1a receptor agonism

The growth hormone secretagogue receptor type 1a (GHS-R1a) is a seven-transmembrane G-protein-coupled receptor of the rhodopsin family, expressed at high density in the anterior pituitary somatotrophs, the hypothalamic arcuate nucleus, and at lower density in the hippocampus, ventral tegmental area, and peripheral tissues including the heart, adrenal gland, and gastrointestinal tract [29]. The endogenous ligand is ghrelin, a 28-amino-acid acylated peptide produced predominantly by gastric oxyntic gland cells.

Hexarelin binds GHS-R1a at the orthosteric ghrelin binding site and activates phospholipase C-dependent signaling, producing inositol trisphosphate generation, intracellular calcium mobilization from endoplasmic reticulum stores, and protein kinase C activation [29, 30]. In pituitary somatotrophs, this signaling cascade triggers growth hormone vesicle exocytosis. The GH-releasing potency of hexarelin is approximately twice that of equimolar GHRH by the intravenous route, and co-administration produces synergistic effects, reflecting the complementary intracellular signaling of the two pathways: GHRH acts through adenylyl cyclase and cyclic AMP, while hexarelin acts through phospholipase C and calcium [1, 2].

Hexarelin also activates GHS-R1a on corticotroph cells (producing ACTH and cortisol release) and lactotroph cells (producing prolactin release) in a dose-dependent manner [19]. This off-target neuroendocrine stimulation distinguishes hexarelin from ipamorelin, which does not produce cortisol or prolactin elevation at GH-releasing doses, and from GHRH, which selectively stimulates somatotrophs [31]. Studies in patients with GHRH receptor deficiency demonstrated that hexarelin's GH-releasing activity is entirely dependent on intact GHRH signaling (no GH re-

sponse in GHRH receptor-deficient patients), while cortisol and prolactin release are independent of GHRH receptor status, confirming that these neuroendocrine effects arise from direct GHS-R1a activation on non-somatotroph pituitary cell populations [32].

## 4.2 CD36 scavenger receptor agonism

CD36 (cluster of differentiation 36, also known as fatty acid translocase, glycoprotein IV, or platelet glycoprotein IIb) is a class B scavenger receptor expressed on cardiomyocytes, monocytes/macrophages, endothelial microvasculature, adipocytes, skeletal muscle, and hepatocytes [33]. It is a transmembrane glycoprotein of approximately 88 kilodaltons with a large extracellular domain that binds diverse ligands including long-chain fatty acids, oxidized low-density lipoprotein (oxLDL), thrombospondin-1, and growth hormone secretagogue peptides.

Hexarelin binds CD36 at an extracellular site mapped by photoaffinity cross-linking to the region spanning residues Asn132 to Glu177, with Met169 as the principal contact residue [28]. The binding triggers intracellular signaling through multiple cascades:

**PI3K/Akt survival pathway:** Hexarelin-CD36 interaction activates phosphoinositide 3-kinase and downstream Akt phosphorylation, promoting cell survival through inhibition of pro-apoptotic mediators (Bad, caspase-9, caspase-3) and activation of anti-apoptotic mediators (Bcl-2) [8, 9].

**PPARgamma activation:** CD36-mediated signaling activates peroxisome proliferator-activated receptor gamma, producing anti-inflammatory gene expression, reduction of oxidative stress markers, and modulation of fatty acid metabolic pathways [9, 34].

**Src-family kinase and focal adhesion kinase (FAK) phosphorylation:** Hexarelin activates non-receptor tyrosine kinases downstream of CD36, contributing to cytoskeletal remodeling and cellular protection signals [6].

The cardiovascular consequences of CD36 activation include: reduced infarct size in ischemia-reperfusion models (25 to 40 percent reduction in infarct area) [7]; reduced cardiac fibrosis through decreased collagen I and III expression and increased matrix metalloproteinase (MMP)-2 and MMP-9 activity [10]; positive in-

otropic effects through enhanced calcium handling in cardiomyocytes [11, 12]; and potential anti-atherogenic effects through interference with CD36-mediated uptake of oxidized lipoproteins by macrophages [5, 6].

The definitive separation of the CD36 and GHS-R1a pathways was established by the Bhargava laboratory knockout experiments: cardioprotection was abolished in CD36-null mice but preserved in GHS-R1a-null mice, and GH release was abolished in GHS-R1a-null mice but preserved in CD36-null mice [5, 6].

### 4.3 Neuroprotective mechanisms

Hexarelin has demonstrated neuroprotective activity through mechanisms that overlap with but are not identical to its cardiovascular CD36 pharmacology. In a neonatal rat model of hypoxia-ischemia, hexarelin reduced brain injury by 39 percent, with protection observed in the cerebral cortex, hippocampus, and thalamus, accompanied by increased phosphorylation of Akt and glycogen synthase kinase-3-beta (GSK-3beta) and reduced caspase-3 activity [13]. In Neuro-2A neuroblastoma cells, hexarelin modulated MAPK (ERK1/2, JNK, p38) and PI3K/Akt pathways to inhibit hydrogen peroxide-induced apoptosis, reducing reactive oxygen species generation and preserving mitochondrial membrane potential [14]. In human neuroblastoma cells expressing the SOD1-G93A mutant protein (a model of amyotrophic lateral sclerosis), hexarelin and the analog JMV2894 demonstrated protective effects against oxidative stress-induced cell death [35].

The receptor mediating neuroprotection has not been definitively identified. Both GHS-R1a (expressed in the hippocampus and cortex) and CD36 (expressed on microglia and brain endothelium) are plausible candidates, and the neuroprotective signaling cascades (PI3K/Akt, MAPK modulation, caspase suppression) are consistent with either receptor system.

### 4.4 Composite pharmacology and selectivity

At research-grade subcutaneous doses (1 to 2 micrograms per kilogram), hexarelin produces plasma concentrations sufficient for full GHS-R1a activation (GH release, cortisol and prolactin co-release) and partial CD36 engagement. The neuroendocrine effects are immediate (GH peak at 15 to 30 minutes) and fully dose-depen-

dent. The cardiovascular effects, demonstrated in acute hemodynamic studies at higher doses (intravenous bolus), require local tissue concentrations that may exceed those achieved by standard subcutaneous research doses.

The selectivity profile of hexarelin across the GHRP class is: highest GH-releasing potency among the classical hexapeptide GHRPs; highest cortisol co-release among the GHRP class (approximately 100 to 140 percent above baseline at maximal GH-releasing doses); moderate prolactin co-release; and unique CD36-mediated cardioprotective activity that is not shared by ipamorelin and has not been extensively characterized for GHRP-2 and GHRP-6 [19, 31].

# 5. Pharmacokinetics and Routes of Administration

## 5.1 Absorption

The Ghigo et al. (1994) study systematically characterized the bioavailability of hexarelin across four routes of administration in healthy human volunteers [1]:

**Intravenous:** Immediate peak plasma concentrations with dose-dependent GH release. GH response to 1 microgram per kilogram intravenous hexarelin was approximately twice that of equimolar GHRH. Peak GH concentrations occurred at 15 to 30 minutes after injection.

**Subcutaneous:** Bioavailability approximately 77.0 percent (SD 10.5 percent) relative to intravenous administration. Peak GH response at 30 to 60 minutes. Subcutaneous administration is the standard route for research applications.

**Intranasal:** Bioavailability approximately 4.8 percent (SD 0.9 percent). Detectable GH elevation but substantially lower than parenteral routes. The low bioavailability reflects peptide degradation in the nasal mucosa and limited transmucosal absorption.

**Oral:** Bioavailability approximately 0.3 percent (SD 0.1 percent). Minimal GH elevation reflecting extensive first-pass gastrointestinal and hepatic proteolytic degradation. Deghenghi and colleagues pursued oral formulations on the basis of the 2-methyltryptophan stabilization, but the oral route was never clinically viable for the neuroendocrine indication [15, 17].

## 5.2 Distribution

Volume of distribution has not been precisely characterized in human pharmacokinetic studies, as most human investigations measured the pharmacodynamic response (GH release) rather than hexarelin plasma concentrations directly. In rats, the volume of distribution after intravenous bolus is approximately 1.2 liters per

kilogram, reflecting moderate tissue distribution [22]. Plasma protein binding has not been formally characterized but is expected to be moderate for a hexapeptide of this molecular weight and hydrophobicity.

Central nervous system penetration has been inferred from the compound's hypothalamic-pituitary effects and from the neuroprotective activity observed in neonatal hypoxia-ischemia models [13]. The compound is described as centrally penetrant [4], consistent with the lipophilicity imparted by the tryptophan and phenylalanine residues (calculated LogP approximately 5.4) and the relatively low molecular weight for a peptide (887 Da).

## 5.3 Metabolism

Hexarelin is metabolized by ubiquitous peptidases in plasma, liver, and kidney. The D-amino acid residues at positions 2 and 5 and the C-terminal amidation confer partial resistance to endopeptidases and exopeptidases, extending the effective half-life relative to unmodified L-amino acid peptides [15]. The 2-methyl group on the D-tryptophan residue additionally reduces indole ring oxidation, the principal chemical degradation pathway for GHRP-6 [17].

In rat pharmacokinetic studies, the terminal elimination half-life after intravenous injection was 75.9 minutes (SD 9.3 minutes); in dogs, the terminal half-life was approximately 120 minutes [22]. In humans, the plasma elimination half-life is estimated at approximately 55 to 70 minutes on the basis of the duration of the GH response and limited direct peptide measurement data.

## 5.4 Elimination

Elimination is through renal and hepatic clearance of peptide fragments. Less than 5 percent of the administered dose is excreted as intact peptide in urine. The metabolic clearance is predominantly peptidase-mediated proteolysis rather than cytochrome P450-dependent oxidative metabolism; hexarelin therefore has minimal CYP-mediated drug-drug interaction potential.

## 5.5 Routes of administration

Three routes have been characterized in human studies: intravenous bolus (for acute diagnostic and hemodynamic studies), subcutaneous injection (the standard research route), and intranasal (investigational). Oral administration has been characterized but is not viable for the neuroendocrine indication owing to less than 1 percent bioavailability. Interestingly, a single oral dose of hexarelin was reported to protect chronic cardiac function after myocardial infarction in a rodent model, suggesting that the CD36-mediated cardiovascular effects may be achievable at lower systemic exposures than required for GH release [36].

## 5.6 Special populations

The GH-releasing response to hexarelin is age-dependent. Studies by the Ghigo group demonstrated potent GH release in pubertal children and healthy adults, reduced response in prepubertal children, and substantially attenuated response in elderly subjects [24]. The age-related attenuation reflects declining GHS-R1a density and somatotroph reserve rather than altered hexarelin pharmacokinetics per se. Arginine and GHRH co-administration partially restored the blunted GH response in elderly subjects [37]. In obese subjects, the GH response to hexarelin is blunted relative to lean controls, consistent with the general observation that adiposity suppresses GH secretion through elevated free fatty acid and somatostatin tone [38].

## 6. Clinical Evidence Base

### 6.1 Growth hormone releasing activity in healthy volunteers

The foundational human pharmacology studies were conducted by the Ghigo, Arvat, and Camanni group at the University of Turin between 1994 and 2000. The 1994 Ghigo et al. study established the dose-response and route-of-administration profile [1]. Subsequent studies characterized the dose-response for GH, cortisol, and prolactin across a range of 0.25 to 2.0 micrograms per kilogram intravenous doses, demonstrating a GH dose-response plateau at approximately 1.0 microgram per kilogram with peak GH concentrations of approximately 140 mU/L and prolactin reaching a plateau of 180 percent above baseline at the same dose [19].

The synergistic interaction between hexarelin and GHRH was characterized in multiple studies [2, 37]. Co-administration of hexarelin with GHRH produced GH responses substantially exceeding the sum of individual responses, interpreted as evidence that the two compounds act through complementary intracellular signaling pathways (phospholipase C for hexarelin, adenylyl cyclase for GHRH) converging on a common final pathway of GH vesicle exocytosis.

### 6.2 Diagnostic applications in growth hormone deficiency

Hexarelin was evaluated as a provocative test for the diagnosis of adult GH deficiency, an application that leverages the compound's potent and reproducible GH-releasing activity. Gasperi et al. (1999) demonstrated that a low dose of hexarelin (0.25 micrograms per kilogram intravenous) combined with GHRH provided diagnostic reliability comparable to the insulin-induced hypoglycemia test, the established gold standard [25]. Patients with organic GH deficiency (pituitary stalk transection, pituitary surgery, cranial irradiation) showed absent or severely blunted GH responses to hexarelin, while patients with idiopathic GH deficiency showed partial responses.

In patients with GHRH receptor deficiency (a rare autosomal recessive cause of isolated GH deficiency), hexarelin produced no GH elevation despite normal cortisol and prolactin responses, establishing that hexarelin's somatotrophic effect re-

quires intact GHRH signaling at the pituitary level [32]. This finding was interpreted as evidence that hexarelin acts partly through hypothalamic GHRH neuron stimulation and partly through direct pituitary GHS-R1a activation that is functionally dependent on permissive GHRH receptor tone.

## 6.3 Cardiovascular studies in humans

Three principal human cardiovascular studies have been published:

**Bisi et al. (1999):** In 7 healthy male volunteers, intravenous hexarelin increased left ventricular ejection fraction from 64.0 percent (SD 1.5) to 70.7 percent (SD 3.0) without significant changes in mean blood pressure or heart rate. The ejection fraction increase appeared at 15 minutes, peaked at 30 minutes, and persisted up to 60 minutes, suggesting a short-lasting positive inotropic effect [11].

**Imazio et al. (2002):** In 8 patients with severe dilated cardiomyopathy (mean LVEF 16.7 percent) and 5 patients with ischemic cardiomyopathy (mean LVEF 22.6 percent), intravenous hexarelin produced significant improvements in left ventricular ejection fraction. The effect was independent of GH-releasing activity, supporting a direct cardiac mechanism mediated through CD36 [20].

**Pettersson et al. (2002):** In patients with coronary artery disease undergoing bypass surgery, hexarelin administered intraoperatively produced prompt increases in left ventricular ejection fraction, cardiac index, and cardiac output beginning at 10 minutes and persisting for up to 90 minutes, without changes in left ventricular end-diastolic volume [12]. The increase in cardiac index without volume loading is consistent with enhanced contractility (positive inotropy) rather than preload augmentation.

## 6.4 Tachyphylaxis and chronic dosing

The tachyphylaxis of the GH-releasing response represents the principal limitation that prevented hexarelin's clinical development for neuroendocrine indications. Studies by Imbimbo et al. and others documented progressive attenuation of the GH peak with daily subcutaneous hexarelin administration, with the response declining by approximately 50 percent within one to two weeks and by 80 percent within four weeks of continuous daily dosing [3, 4]. The mechanism involves GHS-

R1a receptor downregulation and desensitization at the second messenger level, with in vitro studies demonstrating marked attenuation of the calcium response within 2 to 5 minutes of initial hexarelin exposure [26].

The desensitization is partially reversible: after a four-week washout period, GH responsiveness was restored to approximately 70 to 80 percent of the initial response [3]. This observation supports the use of intermittent dosing protocols (typically 2 to 4 weeks on, 4 to 8 weeks off) in research applications where sustained GH-releasing activity is desired.

Notably, the cardiovascular effects mediated through CD36 have not been reported to undergo the same degree of tachyphylaxis, consistent with the different receptor system mediating these effects. The dissociation between GHS-R1a tachyphylaxis and preserved CD36 activity is a potential basis for chronic cardiovascular applications that do not depend on sustained GH release.

## 6.5 Neuroprotection

The neuroprotective evidence is preclinical. Brywe et al. (2005) demonstrated that hexarelin reduced neonatal brain injury by 39 percent in a rat model of hypoxia-ischemia, with protection in the cerebral cortex, hippocampus, and thalamus mediated through Akt/GSK-3beta phosphorylation and caspase-3 suppression [13]. Conte et al. (2021) demonstrated that hexarelin modulated MAPK and PI3K/Akt pathways to inhibit hydrogen peroxide-induced apoptosis in Neuro-2A cells [14]. Mao et al. (2023) reported protective effects of hexarelin and JMV2894 in human neuroblastoma cells expressing the SOD1-G93A amyotrophic lateral sclerosis mutant [35]. No human clinical studies of hexarelin for neuroprotective indications have been published.

## 6.6 Metabolic applications

Hexarelin has been investigated for metabolic applications on the basis of CD36's role in fatty acid metabolism. Ma et al. (2017) demonstrated that hexarelin improved lipid metabolic aberrations in the MKR mouse model of non-obese insulin resistance, through CD36-mediated modulation of PPARgamma signaling and fatty acid uptake [21]. These findings are preclinical and have not been extended to human metabolic studies.

# 7. Reported Research Dose Ranges

## 7.1 Neuroendocrine research doses

**Intravenous:** 1 to 2 micrograms per kilogram as a single bolus injection for acute GH-stimulation studies and diagnostic testing. The dose of 1 microgram per kilogram produces robust GH release approximately twice the magnitude of equimolar GHRH [1]. A lower dose of 0.25 micrograms per kilogram has been used in combination with GHRH for diagnostic testing [25].

**Subcutaneous:** 1 to 3 micrograms per kilogram per administration (approximately 70 to 200 micrograms per injection in a 70-kilogram adult). The subcutaneous route is the standard research route, with 77 percent bioavailability relative to intravenous [1]. Typical research protocols use 100 to 300 micrograms subcutaneously per administration, one to three times daily during on-cycle periods.

**Intranasal:** 20 micrograms per kilogram (approximately 1,400 micrograms in a 70-kilogram adult). The intranasal route has approximately 5 percent bioavailability and is not commonly used in current research protocols [1].

## 7.2 Cardiovascular research doses

Intravenous doses in human cardiac studies have ranged from 1 to 2 micrograms per kilogram as acute bolus injections. The Pettersson et al. (2002) bypass surgery study used a single intravenous administration at the time of surgery [12]. Chronic cardiovascular dosing protocols in animal models have used daily subcutaneous administration over periods of five to eight weeks.

## 7.3 Cycling considerations

Owing to GHS-R1a tachyphylaxis, research protocols targeting GH release typically employ cycling schedules of 2 to 4 weeks of daily administration followed by 4 to 8 weeks of washout to restore receptor sensitivity [3]. Research protocols targeting CD36-mediated cardiovascular effects may not require cycling, as CD36 desensitization has not been documented to the same degree.

## 8. Sourcing and Quality Verification

Hexarelin is not approved for clinical use in any jurisdiction and is not available as a pharmaceutical-grade marketed product. All current sources are research-grade peptide preparations supplied by chemical and peptide synthesis companies.

Research-grade hexarelin is supplied by multiple peptide synthesis and chemical research suppliers (Cayman Chemical, Sigma-Aldrich, Santa Cruz Biotechnology, Creative Peptides, Biosynth, Tocris, Bachem, and others) at greater than 95 percent purity by HPLC, typically as the acetate or trifluoroacetate salt. Supply is in milligram to multi-gram quantities. Pricing is moderate, in the range of \$30 to \$150 per 5 milligrams of research-grade material at the time of monograph preparation.

Investigators should confirm compound identity and purity on every research-grade lot through:

- High-performance liquid chromatography area-percent purity, ideally greater than 95 percent (greater than 98 percent for quantitative pharmacology studies)
- Mass spectrometry confirmation of molecular weight (887.04 Da for the free base;  $[M+H]^+$  expected at  $m/z$  888.05)
- Amino acid analysis or N-terminal sequencing to confirm the hexapeptide identity and the correct incorporation of D-2-methyltryptophan (as opposed to D-tryptophan, which would indicate GHRP-6 rather than hexarelin)
- Peptide content analysis (correcting for acetate, trifluoroacetate, and water content, which typically reduce the peptide content to 70 to 85 percent of the total mass for lyophilized peptide salts)
- Endotoxin testing (Limulus amebocyte lysate assay) for preparations intended for in vivo administration

Third-party analytical laboratories (Janoshik Analytical, Anabolic Lab, and others) provide independent confirmation of vendor certificates of analysis. The research-grade hexarelin market is well established, but lot-to-lot variability in peptide con-

tent and counterion composition is common with lyophilized peptide preparations. Investigators should not assume that the nominal mass on the vial label represents pure peptide content; peptide content correction is required for accurate dosing.

## 9. Reconstitution and Handling

Hexarelin is supplied as a white to off-white lyophilized powder. The compound is hygroscopic and should be stored desiccated at minus 20 degrees Celsius for long-term storage or at 2 to 8 degrees Celsius for short-term storage (up to 3 months).

**Reconstitution for subcutaneous administration:** Reconstitute in bacteriostatic water for injection (containing 0.9 percent benzyl alcohol) at the desired concentration. Typical reconstitution volumes are 1 to 2 milliliters per 5-milligram vial, yielding concentrations of 2.5 to 5 milligrams per milliliter. Allow the lyophilized peptide to reach room temperature before reconstitution to minimize precipitation. Direct the diluent gently against the side of the vial rather than onto the peptide cake; allow the peptide to dissolve without agitation (vigorous shaking can cause peptide aggregation and surface adsorption losses). Reconstituted solution should be clear and colorless; discard any preparation that is turbid or contains visible particulates.

**Reconstitution for in vitro research:** Prepare concentrated stock solutions (10 to 100 millimolar) in dimethyl sulfoxide (DMSO) for cell culture and receptor binding studies. Working dilutions are prepared in the appropriate assay buffer. DMSO stock solutions are stable at minus 20 degrees Celsius for at least 6 months.

**Storage after reconstitution:** Bacteriostatic water reconstitutions should be refrigerated (2 to 8 degrees Celsius) and used within 14 to 30 days. Sterile water reconstitutions (without preservative) should be used within 24 to 48 hours. Aliquoting of reconstituted solutions into single-use portions is recommended to avoid repeated freeze-thaw cycles, although the compound demonstrates reasonable stability through at least 3 to 5 freeze-thaw cycles without measurable potency loss.

**Compatibility:** Hexarelin is compatible with normal saline, bacteriostatic water, and sterile water. It is not compatible with strongly acidic (pH below 3) or strongly alkaline (pH above 9) solutions, which promote peptide bond hydrolysis. The compound should not be mixed with other peptides in the same vial unless compatibility has been verified for the specific combination.

## 10. Stack Interaction Considerations

Investigators conducting research with hexarelin in combination with other compounds should consider receptor-level interactions, pharmacodynamic interactions, and potential additive effects on the growth hormone and adrenal axes.

**Growth hormone releasing hormone (GHRH) and analogs (sermorelin, CJC-1295, tesamorelin):** Co-administration with GHRH or GHRH analogs produces synergistic GH release through complementary intracellular signaling (phospholipase C for hexarelin, adenylyl cyclase for GHRH) [2]. This combination is pharmacologically rational for maximizing GH output and is the basis for the hexarelin plus GHRH diagnostic test [25]. The synergy may also accelerate tachyphylaxis if used chronically.

**Other growth hormone secretagogues (GHRP-2, GHRP-6, ipamorelin, MK-677):** Concurrent use with another GHS-R1a agonist is pharmacologically redundant for the GH-releasing effect and may accelerate receptor desensitization. The combination would produce additive cortisol and prolactin co-release for non-selective GHRPs. Combination is not recommended except for receptor pharmacology studies comparing individual compound profiles.

**Somatostatin and analogs (octreotide, lanreotide):** Somatostatin suppresses GH release through Gi-coupled inhibition of adenylyl cyclase and direct inhibition of calcium channels in somatotrophs. Concurrent somatostatin analog administration blunts the GH response to hexarelin and is a useful research tool for dissecting GH-dependent from GH-independent effects of hexarelin [38].

**Insulin and insulin sensitizers:** GH release produces acute insulin resistance through lipolytic free fatty acid elevation. Concurrent hexarelin and insulin administration may partially antagonize insulin's glucose-lowering effect during the acute GH peak (2 to 4 hours post-injection). This interaction is clinically insignificant at standard research doses but should be considered in metabolic study designs.

**Glucocorticoids:** Hexarelin produces cortisol elevation through ACTH co-release. Concurrent exogenous glucocorticoid administration produces additive HPA axis suppression on chronic dosing. Investigators using hexarelin in subjects re-

ceiving chronic glucocorticoid therapy should consider the additive adrenal suppression potential.

**Cardiovascular agents:** The acute positive inotropic effect of hexarelin through CD36 signaling should be considered in subjects receiving concurrent beta-blockers (which may blunt the hemodynamic response), calcium channel blockers, or other positive inotropes (digoxin, dobutamine), where additive effects on cardiac contractility could be pharmacodynamically significant.

**Dopamine agonists (cabergoline, bromocriptine):** Hexarelin produces prolactin co-release. Concurrent dopamine agonist administration opposes this effect through D2 receptor-mediated lactotroph inhibition. The combination is not pharmacologically problematic but should be noted for prolactin monitoring purposes.

# 11. Adverse Events and Safety

## 11.1 Common adverse events

The principal adverse events at research doses are dose-dependent and include:

- Transient facial flushing and warmth (15 to 30 percent of subjects at standard subcutaneous doses; onset within 5 minutes, resolution within 30 minutes)
- Increased appetite (10 to 20 percent; reflects GHS-R1a activation in hypothalamic appetite circuits, though less pronounced than with GHRP-6)
- Mild water retention and peripheral edema (5 to 15 percent; reflects GH-mediated sodium and water retention, onset over days of dosing)
- Transient numbness or tingling in extremities (5 to 10 percent; reflects GH-mediated peripheral effects)
- Injection site reactions (mild pain, erythema; common with subcutaneous peptide administration)

These adverse-event rates are derived from the published human pharmacology studies at the University of Turin and from the Phase II clinical trial safety reports. All reported effects were dose-dependent, transient, and fully reversible on discontinuation.

## 11.2 Neuroendocrine effects

**Cortisol elevation:** Hexarelin produces the most pronounced cortisol co-release of any GHRP class member at equivalent GH-releasing doses, reflecting direct GHS-R1a activation on corticotroph cells [19]. At a maximal GH-releasing dose of 1 microgram per kilogram intravenous, cortisol increases of 100 to 140 percent above baseline have been reported. Despite these acute elevations, no Cushingoid features, adrenal suppression, or clinically significant HPA axis dysfunction have been observed in any published hexarelin study, including those with repeated dosing over weeks [3, 4].

**Prolactin elevation:** Dose-dependent prolactin release reaching approximately 180 percent of baseline at the maximal GH-releasing dose [19]. No galactorrhea or clinically significant hyperprolactinemia has been reported. The prolactin elevation is less than that produced by typical dopamine antagonists and is transient, resolving within 2 to 4 hours of dosing.

**IGF-1 elevation:** Chronic hexarelin dosing (prior to tachyphylaxis) produces sustained IGF-1 elevation reflecting chronic GH stimulation. The IGF-1 elevation is within the physiological range at standard research doses but should be monitored in chronic dosing protocols.

### 11.3 Cardiovascular safety

The acute positive inotropic effect of hexarelin has not been associated with arrhythmias, QT prolongation, or adverse cardiovascular events in published human studies. Bisi et al. (1999) reported no significant changes in mean blood pressure or heart rate during the ejection fraction increase [11]. No proarrhythmic signal has been identified in the published clinical or preclinical literature.

### 11.4 Tachyphylaxis as a safety consideration

The GHS-R1a desensitization that produces tachyphylaxis also limits the duration and magnitude of the off-target cortisol and prolactin effects on chronic dosing. In this sense, tachyphylaxis functions as a self-limiting safety mechanism: the neuroendocrine adverse effects attenuate in parallel with the therapeutic GH-releasing effect over the first two to four weeks of daily administration [3].

### 11.5 Contraindications and precautions

Hexarelin is not approved for clinical use and has no formal contraindication list. Based on pharmacological considerations, research use should proceed with caution in: individuals with active malignancy (GH and IGF-1 are mitogenic growth factors); individuals with diabetic retinopathy (GH-mediated progression risk); and individuals with known CD36 deficiency (a relatively common polymorphism in East Asian populations that could alter the cardiovascular pharmacology).

## 12. Comparable Compounds Assessment

This section presents a structured comparative assessment of five growth hormone secretagogue candidates against hexarelin, evaluated against five competency standards: novelty, effect size, promising potential (current clinical state and trajectory), side-effect profile (clinical or in vitro), and overall validation. The five candidates were selected as the principal GH-releasing peptides and peptidomimetics with substantive clinical or preclinical programs, with priority on those that share hexarelin's receptor pharmacology or research-clinical positioning.

### 12.1 GHRP-6 (Growth Hormone Releasing Peptide-6)

**Mechanism:** GHS-R1a agonist with additional appetite-stimulating activity through hypothalamic ghrelin pathway activation. Amino acid sequence: His-D-Trp-Ala-Trp-D-Phe-Lys-NH<sub>2</sub>. Hexarelin's direct structural parent, differing only in the absence of the 2-methyl group on D-tryptophan.

**Development trajectory:** Developed by Cyril Bowers at Tulane University in the 1980s as the prototype growth hormone releasing peptide [16, 23]. Extensively characterized in human pharmacology studies but never advanced to registration. Remains widely available as a research tool.

**Novelty:** Low. GHRP-6 is the historical reference compound for the hexapeptide GHS-R1a agonist class. Its chemistry and pharmacology are well established and have not produced novelty in the past decade.

**Effect size:** GH release is comparable to hexarelin on a molar basis but with greater inter-individual variability and more pronounced appetite stimulation [23]. The GH peak is approximately 80 to 120 percent above baseline at standard subcutaneous doses.

**Promising potential:** Low as a clinical candidate (no active development). Persistent as a reference research tool for GHS-R1a pharmacology.

**Side-effect profile:** More pronounced appetite stimulation than hexarelin, reflecting stronger hypothalamic ghrelin pathway activation. Moderate cortisol and prolactin co-release, similar to hexarelin. Chemical instability (D-tryptophan oxidation) is a practical limitation for storage and consistent dosing. No serious adverse events reported in published human studies.

**Overall validation:** Moderate. Extensive human pharmacology literature spanning four decades, but no clinical registration and declining use as hexarelin and ipamorelin have supplanted it for most research applications.

**Comparison to hexarelin:** Hexarelin was developed specifically to address GHRP-6's limitations: the 2-methyltryptophan substitution resolves the oxidative stability problem and confers modestly enhanced potency. Hexarelin also has the distinctive CD36-mediated cardiovascular pharmacology, which has not been systematically characterized for GHRP-6. GHRP-6's principal advantage over hexarelin is its longer track record and broader published literature in basic GH physiology research.

## 12.2 GHRP-2 (Pralmorelin)

**Mechanism:** GHS-R1a agonist. Amino acid sequence: D-Ala-D-2-Nal-Ala-Trp-D-Phe-Lys-NH<sub>2</sub>. A hexapeptide GHRP with a non-natural D-2-naphthylalanine substitution. Generally considered the most potent GH-releasing hexapeptide in competitive displacement assays [39].

**Development trajectory:** Developed by Bowers and colleagues, advanced to clinical registration in Japan for the diagnosis of GH deficiency under the International Nonproprietary Name pralmorelin (marketed as GH-test GHRP Ferring). This is the only growth hormone releasing peptide that has achieved any form of regulatory approval [39].

**Novelty:** Low. The mechanism is conventional GHS-R1a agonism within a well-characterized hexapeptide scaffold.

**Effect size:** Highest absolute GH-releasing potency among the classical hexapeptide GHRPs in competitive binding assays. Clinical GH release is potent and reproducible. Cortisol and prolactin co-release are the highest in the GHRP class, exceeding even hexarelin [19, 39].

**Promising potential:** Low for new indications; the compound is already marketed in Japan for diagnostic use but is not in active development for therapeutic applications.

**Side-effect profile:** Highest cortisol and prolactin co-release among the classical GHRPs [39]. Moderate appetite stimulation. No serious adverse events in published clinical studies. The higher hormonal side-effect burden relative to hexarelin and ipamorelin limits its utility for chronic research applications.

**Overall validation:** Moderate to high. The only GHRP with a regulatory approval (Japan, diagnostic indication), supported by an extensive human pharmacology literature.

**Comparison to hexarelin:** GHRP-2 has higher binding affinity at GHS-R1a and higher peak GH release but also higher off-target cortisol and prolactin stimulation. Hexarelin has the advantage of CD36-mediated cardiovascular activity and better chemical stability than GHRP-6. GHRP-2 has the operational advantage of regulatory approval in Japan.

## 12.3 Ipamorelin

**Mechanism:** Selective GHS-R1a agonist. Amino acid sequence: Aib-His-D-2-Nal-D-Phe-Lys-NH<sub>2</sub>. A pentapeptide growth hormone secretagogue distinguished by selectivity for GH release without co-release of cortisol or prolactin at GH-releasing doses [31].

**Development trajectory:** Developed by Novo Nordisk as a selective GH secretagogue that overcomes the cortisol and prolactin limitations of the earlier GHRPs. Characterized in clinical studies by Raun et al. (1998) as "the first selective growth hormone secretagogue" [31]. Advanced through Phase II clinical trials for postoperative ileus (by Helsinn under the development code NNR 0049) but did not achieve registration. The compound has become the dominant research-grade GH secretagogue owing to its selectivity profile.

**Novelty:** Moderate. The selectivity for GH release without cortisol and prolactin co-release was a meaningful advance over prior GHRPs at the time of discovery.

**Effect size:** GH release is approximately 60 to 80 percent of the peak achievable with hexarelin at equivalent doses, reflecting lower intrinsic activity at GHS-R1a [31]. The lower potency is partially offset by the ability to use higher doses without

cortisol and prolactin side effects.

**Promising potential:** Moderate. Not in active clinical development for any indication, but the dominant research-grade GH secretagogue owing to selectivity. Widely available and extensively used.

**Side-effect profile:** The principal advantage: no cortisol or prolactin co-release at GH-releasing doses. Minimal appetite stimulation. Adverse events limited to mild injection-site reactions and transient water retention. The selectivity profile makes ipamorelin the preferred GH secretagogue for chronic research protocols where hormonal side effects must be minimized.

**Overall validation:** Moderate. Extensive human pharmacology data. Phase II development for postoperative ileus did not succeed, but the compound's research utility is well established.

**Comparison to hexarelin:** Ipamorelin is the cleaner compound for pure GH-releasing applications, with minimal hormonal side effects. Hexarelin is the more potent GH releaser and has the unique CD36-mediated cardiovascular pharmacology that ipamorelin lacks. The choice between the two depends on the research objective: ipamorelin for chronic, selective GH-axis research; hexarelin for cardiovascular research, acute GH-stimulation studies, or research requiring maximum GH-releasing potency.

## 12.4 MK-677 (Ibutamoren)

**Mechanism:** Non-peptide, orally active GHS-R1a agonist (growth hormone secretagogue). A spiroindoline peptidomimetic that binds GHS-R1a at the same orthosteric site as ghrelin and hexarelin [40].

**Development trajectory:** Developed by Merck Research Laboratories. Advanced through extensive Phase II clinical trials for GH deficiency, sarcopenia, hip fracture recovery, and Alzheimer's disease-associated weight loss [40, 41]. A Phase II trial in elderly subjects demonstrated sustained GH and IGF-1 elevation over 12 months of daily oral dosing without the tachyphylaxis observed with hexarelin [41]. The compound has not achieved regulatory approval for any indication.

**Novelty:** High at the time of discovery. MK-677 was the first orally active, non-peptide GHS-R1a agonist with a pharmacokinetic profile suitable for chronic dosing.

**Effect size:** Oral dosing at 25 milligrams daily produces sustained IGF-1 elevation to the upper physiological range. GH pulsatility is enhanced rather than producing a single supraphysiological peak. The chronic IGF-1 elevation is more relevant to anabolic applications than the acute GH spike produced by hexarelin. Notably, the absence of tachyphylaxis on chronic dosing distinguishes MK-677 from hexarelin and the other peptide secretagogues [41].

**Promising potential:** Moderate. Not approved, but extensive clinical data across multiple indications. Widely used as a research compound and available from research-grade suppliers.

**Side-effect profile:** Increased appetite (20 to 40 percent of subjects; more pronounced than hexarelin), transient water retention and edema, mild blood glucose elevation (reflecting GH-mediated insulin resistance), drowsiness at higher doses. Cortisol elevation is modest and less pronounced than with hexarelin. The blood glucose elevation is the principal safety concern for chronic use in metabolic populations [40, 41].

**Overall validation:** Moderate to high. The most clinically advanced GH secretagogue (multiple Phase II trials, 12-month dosing data), but no registration.

**Comparison to hexarelin:** MK-677 has the critical advantage of oral bioavailability and absence of tachyphylaxis on chronic dosing, making it suitable for sustained IGF-1 elevation protocols that hexarelin cannot support. Hexarelin has higher peak GH-releasing potency and the unique CD36-mediated cardiovascular pharmacology. The two compounds occupy complementary niches: MK-677 for chronic oral GH-axis research, hexarelin for acute parenteral GH-stimulation studies and cardiovascular CD36 research.

## 12.5 Ghrelin (endogenous ligand)

**Mechanism:** Endogenous 28-amino-acid acylated peptide. GHS-R1a agonist. The octanoyl modification at Ser3 is required for GHS-R1a activation [29]. Additional activities include appetite stimulation through hypothalamic and brainstem circuits, gastric motility enhancement, and immunomodulatory effects.

**Development trajectory:** Identified by Kojima et al. (1999) at Kurume University as the endogenous ligand for the growth hormone secretagogue receptor [29]. Extensively studied as a research tool and as a potential therapeutic agent for cancer-related cachexia, anorexia nervosa, and gastroparesis. Clinical trials of syn-

thetic ghrelin and ghrelin analogs (anamorelin, a non-peptide ghrelin agonist) have been conducted for cancer-associated cachexia, with anamorelin achieving regulatory approval in Japan [42].

**Novelty:** High at time of discovery (1999). The identification of ghrelin as the endogenous GHS-R1a ligand was a landmark in endocrine physiology.

**Effect size:** GH release is potent but variable, reflecting rapid enzymatic deacylation in plasma (the des-acyl form is inactive at GHS-R1a). Appetite stimulation is more pronounced than with hexarelin. Chronic administration has not been extensively studied owing to the short plasma half-life (approximately 10 to 30 minutes for acyl-ghrelin).

**Promising potential:** Moderate as a direct therapeutic agent; high as the basis for derivative compounds (anamorelin, relamorelin). The short half-life and chemical instability of the octanoyl ester limit direct ghrelin's clinical utility.

**Side-effect profile:** Pronounced appetite stimulation and gastric acid secretion. GH, cortisol, and prolactin co-release. Rapid deacylation limits exposure duration and adverse-event magnitude.

**Overall validation:** High as a physiological reference; low as a clinical candidate (supplanted by stable analogs).

**Comparison to hexarelin:** Ghrelin is the endogenous physiological ligand; hexarelin is a synthetic high-potency agonist with superior pharmacokinetic properties (longer half-life, resistance to deacylation). Hexarelin produces higher and more reproducible GH peaks owing to its metabolic stability. Ghrelin has the broader physiological activity profile (appetite, gastric motility, immunomodulation) and the endogenous reference status. Hexarelin has the unique CD36 cardiovascular pharmacology that ghrelin has not been demonstrated to share at pharmacologically relevant concentrations.

## 12.6 Comparator summary

The five compounds occupy distinct positions in the growth hormone secretagogue pharmacology space. **GHRP-6** is the historical reference hexapeptide, supplanted by hexarelin for stability reasons. **GHRP-2** has the highest binding affinity and the only regulatory approval (Japan, diagnostic use) but the highest hormonal side-effect burden. **Ipamorelin** has the cleanest selectivity profile (no cortisol or prolactin

co-release) and is the preferred compound for chronic selective GH-axis research. **MK-677** has the critical advantage of oral bioavailability and absence of tachyphylaxis on chronic dosing. **Ghrelin** is the endogenous reference ligand with the broadest physiological activity profile.

Hexarelin occupies a unique position in this comparator landscape: it is the only compound with definitively characterized, GH-independent cardioprotective activity mediated through the CD36 scavenger receptor, the most potent peak GH releaser among the hexapeptide class, and the compound with the most extensive human cardiovascular pharmacology data. Its principal limitations are parenteral administration, GHS-R1a tachyphylaxis on chronic dosing, and higher cortisol and prolactin co-release relative to ipamorelin. The CD36 cardiovascular pharmacology is the distinctive research contribution that no other compound in this panel provides and is the primary basis for hexarelin's continued scientific relevance beyond the neuroendocrine applications for which it was originally developed.

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