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# Recombinant Human GHRH(1-44)$NH_2$

*Clinical Utility and Therapeutic Development Program*

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**Growth hormone (GH) secretagogues are becoming increasingly attractive alternatives to GH or insulin-like growth factor-I (IGF-I) for the treatment of conditions that may benefit from activation of the GH/IGF-I axis. This stems from the realization that (1) GH secretagogues stimulate the pulsatile release of endogenous GH; (2) feedback control of endogenous GH and IGF-I is preserved, guarding against imbalances between GH and IGF-I levels; and (3) GH treatment is associated with adverse effects in the elderly. Of the GH secretagogues, growth hormone-releasing hormone (GHRH) remains the best characterized, in terms of identity of the ligand-receptor pair and its exclusive somatotropic activity at the level of the pituitary. Full-length natural GHRH (1-44) amide can now be produced by recombinant technologly on a commercially viable scale, and is currently being evaluated in early phase clinical trials. The purpose of these studies is to evaluate the efficacy and tolerability of chronic subcutaneous administration of GHRH over a range of doses in elderly subjects. Therapeutic areas that are being investigated in the elderly include congestive heart failure, osteoporosis, and improvements in body composition and function in the frail elderly.**

**Key Words:** GH secretagogues; heart failure; osteoporosis; frailty; body composition; IGF-I

## Introduction

It is now well established that the growth hormone/insulin-like growth factor-1 (GH/IGF-1) axis is critical not only for normal growth and development in childhood, but also for maintenance of the structure and function of adult tissues, particularly cardiac and skeletal muscle and bone *(1)*. It is generally accepted that these effects are achieved both by the direct actions of GH at peripheral tissues and via the endocrine, paracrine, and autocrine activities of IGF-1. The pituitary secretion of GH is episodic, or pulsatile, and is under the coordinate control of at least three messenger molecules: growth hormone–releasing hormone (also referred to as GH-releasing factor [GRF]), endogenous ligand for the GH secretagogue (GHS) system, and somatostatin *(2)*. In addition to complex and subtle interactions among these messenger molecules, GH secretion is under long feedback control by IGF-1 and short feedback control by GH. Also, the pulsatility of plasma GH levels likely contributes to its peripheral actions, although the mechanistic basis for this is not resolved. Thus, the GH/IGF-1 axis is an intricate endocrine cascade under complex neuroendocrine control and modulated by feedback inhibition *(2)*.

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## Therapeutic Activation of the GH/IGF-1 Axis

In principle, therapeutic interventions are possible at four levels: the hypothalamus (GHS agonist), the pituitary (GRF), the pituitary-peripheral axis (GH), and the peripheral tissues (IGF-1). If the hypothalamopituitary axis is intact, then, in principle at least, the use of one of the GHSs or of GRF should be preferable to either GH or IGF-1, for the following reasons:

1. Both GRF and the GHS agonists stimulate the pulsatile release of endogenous GH (assuming continuous or long-acting administration) *(2)*.
2. The levels of GH and IGF-1 will more likely be balanced and should approach the physiologic relationship. Administration of IGF-1 alone will lead to GH suppression, whereas direct administration of GH can lead to an abnormally high GH/IGF-1 ratio, especially in the setting of GH resistance *(3)*.
3. Levels of endogenous GH and IGF-1 are controlled by negative feedback loops *(2)*.

### *Relative Profiles of GRF and GHS*

If the two fundamental classes of secretagogues—GHS agonists and GRF—are compared, are there significant differences that may be clinically important? From a therapeutic perspective, both have class-specific disadvantages. In the case of the GHSs, these include receptor desensitization after long-term treatment; the requirement for an intact hypothalamopituitary axis; activation, albeit only moder-

ately, of the hypothalamo-pituitary-adrenal and prolactin axes and thus lack of specificity; and the expression of GHS receptors (GHS-Rs) widely in the brain outside of the hypothalamopituitary axis, including in the hippocampus and substantia nigra, further raising questions about their specificity *(4,5)*.

By contrast, the disadvantages of GRF are as follows: GRF is a short-acting, rapidly degraded peptide that requires repeated or continuous administration; its ability to stimulate GH secretion in the elderly is initially poor and is never as pronounced as after GHS treatment; there are peripheral GRF receptors of unknown function; and costs of production are potentially high *(2)*.

### *GRF: Key Properties*

Despite these disadvantages, several features make GRF an attractive therapeutic agent. First, GRF is a classic hypothalamic releasing factor whose role in the hypothalamopituitary axis is much better defined than that of the GHS agonists, whose natural counterpart still remains to be finally resolved. Second, GRF is both essential, in that GRF receptor mutations are associated with severe growth retardation, and potent, producing acromegaly when secreted in excess—these pathologic entities firmly establish the physiologic basis for the actions of GRF *(2)*, which cannot be said for the GHS agonists. Third, although the production of a 44 residue, C-terminally amidated peptide is a challenge, once the appropriate recombinant and downstream-processing technologies are in place, low-cost, efficient production becomes possible.

### *Recombinant Production of Human GRF(1-44)amide*

Efficient production requires, essentially, two key elements. First, it requires a recombinant expression system enabling high-yield fermentation. This involves tricks such as powerful low-temperature promoters and short leader sequences that promote high-level translation and precipitation of the peptide into inclusion bodies. Second, it requires a downstream processing technology that cleaves and removes leader and linker sequences and generates the C-terminal amide. This technology has now been developed to the level of pilot-scale commercial plants that can produce multiple kilograms of peptide per year suitable for use in clinical trials. Scale-up to 200 kg/yr and greater is expected to produce cGMP-grade peptide at an acceptable cost, making full-length natural human GRF(1-44)amide (rGRF) available for therapeutic use.

### *Therapeutic Indications for GRF*

In general, the therapeutic indications for GRF are the same as those for GH and IGF-1 *(6)*. These include the following:

1. GH deficiency in children and in adults with an intact hypothalamopituitary axis.
2. Functional GH deficiency in aging associated with poor body composition, loss of function, and frailty.
3. Chronic catabolic states, such as after surgery and trauma, or chronic wasting diseases with cachexia.
4. Dilated cardiomyopathy and congestive heart failure (CHF).
5. Osteoporosis.

Because of the properties noted earlier, especially the stimulation of the balanced secretion of both GH and IGF-1 and maintenance of negative feedback control, GRF may have an advantage in the frail, the elderly, and the severely ill, in terms of a lower potential for GH-related adverse effects *(3)*. An overview of some of the clinical trials currently in progress, the underlying rationale, and preliminary results, where available, are presented next.

### *Reactivation of GH/IGF-1 Axis in the Elderly*

Despite some contradictory data regarding the functional benefits resulting from GH therapy for the elderly without organic GH deficiency *(7)*, there remains considerable interest in reactivating the GH/IGF-1 axis in the frail elderly, primarily with a view to improving body composition and function. Early dose-ranging trials are currently in progress in which the safety and tolerability of twice daily sc injections of rGRF at doses up to 8 mg/d for up to 3 mo are being evaluated in older subjects. Early efficacy, in terms of extent of activation of the GH/IGF-1 axis, changes in body composition and functional improvements, gender-based differences in response, and influence of concurrent estrogen replacement, will be assessed. Further development of rGRF as a therapeutic for long-term use in this population will require clear evidence for enhancements in both body composition and function (which may only become apparent after 12 mo of continuous use) and an acceptable risk profile. The pivotal trials for regulatory approval will likely be lengthy and costly. Moreover, the development of a formulation that will facilitate long-term, continuous delivery, e.g., by depot or implantable pump, will be critical.

### *Anabolic Therapy for Osteoporosis: Combination of GRF and Parathyroid Hormone*

A second indication that is of interest is the development of GRF as an anabolic agent for the treatment of osteoporosis in combination with parathyroid hormone (PTH). Although GH has a clear role in bone metabolism, as evidenced by osteoporotic changes in adult GH deficiency, its use in the treatment of osteoporosis in non-GH-deficient adults has yielded largely disappointing results; however, this lack of efficacy may be more apparent than real *(8)*. The apparent early loss in bone mineral density (BMD) seen after GH therapy really reflects an increase in bone cross-sectional area, which initially exceeds a continuous increase in bone mineral content *(9)*. Many believe that the active bone remodeling induced by both PTH and GH results in stronger bone, even though BMD changes are not necessarily impressive.

The fact remains that anabolic therapies for osteoporosis are urgently needed, because the currently available antiresorptive agents have their limitations. For this reason, the combination with PTH is interesting because of a theoretical synergy: both GH and PTH stimulate bone turnover and remodeling, but GH typically stimulates resorption before formation, and builds cortical bone in the long bones *(9)*. In contrast, PTH rapidly stimulates bone formation first before resorption becomes prominent and has a much greater effect on cancellous bone in the axial skeleton *(10)*. Hence, there may be a very useful synergism between the actions of GH and PTH that may produce early, net gains in bone mass and strength, which has been shown in animal studies *(11)* but remains to be demonstrated in humans.

A short-term (3-mo) pilot study designed to evaluate the hypothesis that the bone turnover effects of GRF combined with PTH are synergistic has recently begun. The relative extent of bone formation versus bone resorption, as assessed by the biochemical markers bone-specific alkaline phosphatase, propeptide of type I procollagen, and osteocalcin (formation), and urinary N and C telopeptides (resorption), will be determined for each agent alone and for the combination in osteoporotic, postmenopausal subjects. This trial should provide safety data and an initial indication of whether there is indeed an early net increase in bone formation with this regimen.

### *GH Therapy for CHF*

CHF is a growing problem worldwide. Whereas the overall incidence of ischemic heart disease (IHD) in the Western world has stabilized or is declining, the incidence of CHF is rising *(12)*. This has been ascribed to several factors, including an aging population, long-term survival of patients with IHD who have suffered one or more episodes of myocardial infarction, and a rising incidence of adult-onset diabetes mellitus. Although there have been recent advances in the management of CHF, including successful reductions in mortality achieved with angiotensin-converting enzyme inhibitors, β-blockers, and aldosterone receptor antagonists, the development of inotropic agents that can safely enhance myocardial contractility, and hence improve overall cardiac performance and quality of life, has been disappointing *(13–15)*. In this context, the realization that GH (or IGF-1) may offer a novel approach to improving myocardial performance has received considerable attention in recent years *(16)*. Based on numerous clinical observations and animal experiments, several pilot clinical studies have been performed to evaluate the usefulness of stimulating the GH/IGF-1 axis in patients with CHF (reviewed in refs. *17–19*).

Two aspects are most striking: First, a series of small, open trials have largely yielded dramatic improvements in myocardial structure and performance and clinical status, which gave cause for considerable optimism. The most notable of these was the study by Fazio et al. *(20)*. Second, more recent larger trials that included controls and/or were double blind, placebo controlled largely failed to show a benefit and have thus had a dampening influence on the field *(21,22)*. How are these discrepancies to be resolved?

### *Clinical and Experimental Basis for Treating CHF with GH*

To explore the discrepancies in the trial data on GH therapy for CHF, it is useful to consider the basis for treating CHF with GH. Several clinical observations, all interconnected, have provided a rationale for the use of GH for the treatment of CHF, and these have been supported by animal studies. First, there is the acromegaly model, in which excess GH produces early increases in left ventricular performance *(18)*. Second, organic GH deficiency can lead to impaired contractility and eventual dilated cardiomyopathy, which is partially or completely reversed by GH treatment *(1,23)*. Third, several reports have noted that mean GH secretion is depressed in severe CHF *(24,25)*. Fourth, the incidence of CHF increases with age and is concordant with the age-related decline in the GH/IGF-1 axis *(23)*.

A large number of animal studies clearly reveal a benefit of GH or IGF-1 treatment in various models of ischemic or pacing-induced cardiomyopathy and CHF. In general, these studies have revealed that GH treatment increases cardiac growth, especially left ventricular mass; increases ventricular contractility and function, which may be owing to the increased left ventricular contractile mass; induces an acute inotropic effect via increased $Ca^{2+}$ transients (likely due to increased expression of sarcoplasmic $Ca^{2+}$-ATP-ases); and potently reduces the systemic vascular resistance (SVR) *(26–28)*. The latter effect may, in fact, account for most, and perhaps all, of the acute hemodynamic improvement observed during a GH infusion, as demonstrated in a clinical study by Giustina et al. *(29)* of a 24-h iv GH infusion. Most notable was the remarkable improvement in cardiac output and reductions in pulmonary arterial and left atrial pressures, which most likely could be accounted for by the dramatic reduction in SVR.

### *Cardiac Effect of GH in CHF*

From these various considerations has emerged a view of the cardiac actions of the GH/IGF-1 axis that can be summarized as follows. GH induces cardiac hypertrophy that appears to be of the physiologic type, which is similar to that induced by exercise, and is distinct from the concentric or eccentric types induced by pressure overload and dilated cardiomyopathy, respectively. Concomitantly, GH, via IGF-1, inhibits myocyte apoptosis, a major mechanism contributing to failure *(30)*.

How does hypertrophy benefit the patient with CHF? Left ventricular dilatation and failure are mainly the result of increased left ventricular wall stress, which in turn is directly related to the ratio of load and filling pressures to wall thickness *(16)*. As shown in Fig. 1, current therapies are overwhelmingly focused on reducing load and filling

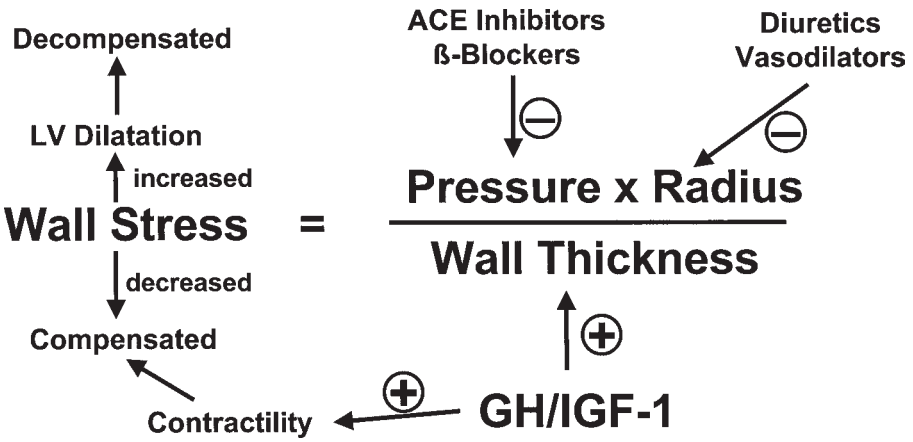


**Fig. 1.** Influence of medical interventions on the wall stress equation. Minus signs denote decreases, and plus signs denote increases. LV, left ventricle; ACE, angiotensin-converting enzyme.

pressures, whereas GH/IGF-1 provides a novel intervention by increasing wall thickness and thereby reducing wall stress. In addition, as already mentioned, there may be a direct inotropic effect that further augments contractility and left venticular performance.

### *Benefits of GH-Induced Cardiac Hypertrophy: The Acromegaly Paradox*

When treating CHF with GH, however, one has to consider what can be referred to as the acromegaly paradox. During the early phase of this disease, the beneficial effects of excess GH on cardiac function are observed: typically a hyperkinetic syndrome owing to increased cardiac output coupled with decreased SVR *(18)*. However, during the later phase, the heart decompensates, largely owing to increasing cardiac fibrosis, leading to nonphysiologic hypertrophy, increasing wall stress, CHF, and fatal arrhythmias *(31)*. Because GH therapy for CHF will induce a state of biochemical acromegaly, a careful balance will need to be maintained that avoids progression to the late phase of the disease. Moreover, it should also be remembered that even under ideal circumstances, GH therapy will not be a wonder drug for CHF. This was clearly seen in a pig model of dilated cardiomyopathy in which CHF was induced by 3 wk of rapid pacing. Although GH treatment improved some parameters—notably wall stress and fractional shortening—these were by no means normalized. Furthermore, a key hemodynamic parameter, left ventricular end-diastolic volume, was not affected at all *(26)*.

### *Stimulation of GH/IGF-1 Axis by GRF in the Elderly with CHF*

The treatment of CHF with GH or GHSs offers sufficient promise that exploratory trials are warranted. The balance of evidence with GH obtained to date suggests that there is a potential but that the effects of key variables such as age, extent of GH resistance, stage of disease, and etiology remain to be clarified. Moreover, for the reasons discussed earlier, treatment with GRF may offer some advantages over the use of either GH or IGF-1, especially in the elderly. Also, note that several animal and human studies have indicated that the GH-releasing peptide hexarelin (a GHS agonist) may have a direct action on the myocardium, distinct from its GH-releasing activity. This action is apparently mediated by GHS-Rs expressed in the heart *(32)*. Such a direct cardiac activity has not been demonstrated for GRF, and GRF receptors are not known to be expressed in the heart.

An initial evaluation of rGRF for the treatment of CHF in the elderly requires an assessment of the efficacy of rGRF to activate the GH/IGF-1 axis in this population. Unpublished preliminary data from a trial of rGRF treatment (up to 4 mg/d by sc injections given once or twice daily for 3 wk) in the healthy elderly (>65 yr) or the elderly with CHF indicate several trends. First, as is well established, elderly subjects have a poor initial GH response to rGRF injections when compared with young control subjects, but this response improves over the 3-wk treatment period and is dose dependent. Second, the increase in IGF-1 levels is modest over the 3-wk treatment period, reaching statistical significance only in males on twice daily injections; IGF-1 levels can be expected to increase further with a longer duration of treatment with rGRF. Third, the acute GH response to an iv rGRF bolus is impaired in the elderly with CHF, consistent with published reports *(25,33)*; however, although there is initially also a poor GH response to sc rGRF in these patients, the response improves after 21 d of treatment. These early data suggest that it may be possible to induce modest increases in GH and IGF-1 levels in elderly with CHF by treatment with sc rGRF. The extent to which these increases in GH and IGF-1 can significantly improve myocardial contractility and hemodynamics remains to be demonstrated. As noted, the clinical response may depend on age, gender, and etiology and severity of the CHF.

## Conclusion

Full-length, natural human GRF(1-44)amide has only recently become available in significant quantities by the application of modern recombinant technologies and innovations in downstream processing that facilitate high-yield production and C-terminal amidation. This has enabled the initiation of various human clinical trials aimed at evaluating the utility of rGRF in a variety of indications in which activation of the endogenous GH/IGF-1 axis may have therapeutic benefits. These trials are focused on the elderly presenting with conditions in which essentially there is loss of structure and function of muscle and bone: old age frailty, osteoporosis, and heart failure. Treatment with GH itself in the elderly has been associated with adverse events, occasionally serious, and there is a reasonable expectation that treatment with rGRF will reactivate the GH/IGF-1 axis in a more physiologic fashion that remains subject to feedback control. However, long-term tolerability and efficacy of rGRF for these indications remain to be established, and suitable delivery systems for chronic administration need to be developed.

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