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- (71) Applicant: **THE GENERAL HOSPITAL CORPORATION** [US/US]; 55 Fruit Street, Boston, Massachusetts 02114 (US).
- (72) Inventor: **GRINSPOON, Steven K.**; 255 Westerly Road, Weston, Massachusetts 02493 (US).
- (74) Agent: **BERNSTEIN, Mitchell**; Fish & Richardson P.C., P.O. Box 1022, Minneapolis, Minnesota 55440-1022 (US).
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(54) Title: GH-RELEASING AGENTS IN THE TREATMENT OF VASCULAR STENOSIS AND ASSOCIATED CONDITIONS

(57) Abstract: A method for preventing or treating vascular stenosis and/or associated conditions, such as atherosclerosis, in a growth hormone-deficient (GHD) obese subject by administration of an effective amount of a growth hormone (GH)-releasing agent, such as a growth hormone-releasing factor (GRF) or a GRF analog, is disclosed.

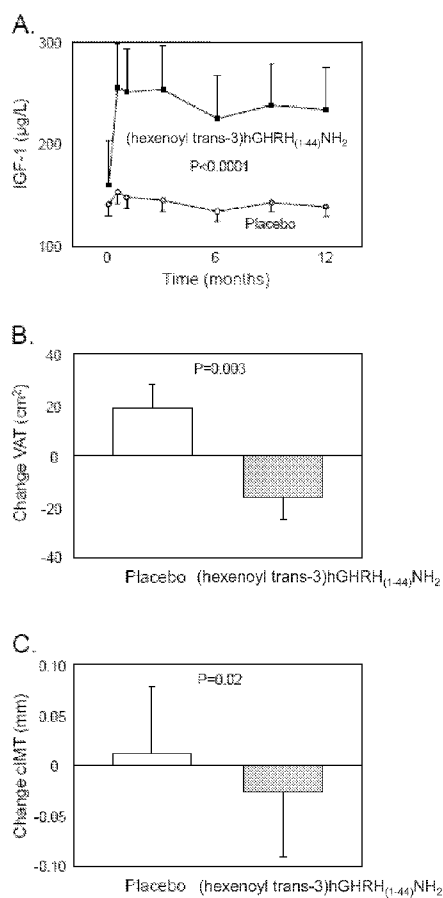


FIG. 2



MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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GH-RELEASING AGENTS IN THE TREATMENT OF VASCULAR STENOSIS AND ASSOCIATED CONDITIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of United States Provisional Application serial No. 61/663,017 filed on June 22, 2012, the content of which is incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with Government support under NIH Grant Nos. R01HL085268, K23DK087857, K24DK064545, M01RR01066 and UL1RR025758. The Government has certain rights in this invention.

TECHNICAL FIELD

The present invention generally relates to the treatment of vascular stenosis and associated conditions, and more particularly carotid vascular stenosis and atherosclerosis.

BACKGROUND ART

Vascular stenosis is a condition resulting from the narrowing of blood vessels, which may occur for example as a result of atherosclerosis inside arteries, the vessels which carry blood from the heart to supply the body.

Atherosclerosis, or hardening of the arteries, is a process in which fat, cells and other substances build up inside artery walls forming what are called plaques. These atheromatous plaques make the walls of an artery harder and thicker, and as a result the blood vessel becomes narrower and less flexible, making it more difficult for blood to flow through. Vascular stenosis typically affects arteries in the brain, heart and legs and, when severe, causes tissue death, resulting in conditions such as strokes and heart attacks. Atherosclerosis is the leading cause of illness and death in the United States and most developed countries. It is estimated that cardiovascular disease, primarily due to coronary artery disease and stroke, affects at least 22 million individuals in the United States, and causes nearly 900,000 deaths each year.

The abnormal narrowing of arteries caused by atherosclerosis can affect arteries in the legs, particularly those supplying the calf muscles. This can lead to a condition known as intermittent claudication, where, in the early stages, cramping pains are felt in the calves after walking a certain distance. The pain goes away after resting for a few minutes. If the disease progresses, the pain is experienced even while resting and leg ulcers, or even gangrene of the toes and feet, may occur.

Vascular stenosis of arteries in the heart can lead to a condition called angina, where pain is felt in the chest during exercise. Again, resting usually causes the pain to go away within a short time, although there is the potential for an artery to become completely blocked. In this case the pain becomes heavy and persistent, possibly spreading to the arm or neck, and a heart attack takes place, in which an area of heart muscle dies off.

When arteries in the brain are affected by vascular stenosis, this can shut off the blood supply to part of the brain tissue or lead to the rupture of a small blood vessel, with bleeding into the brain. Either of these events results in a stroke, where an area of brain tissue dies off, causing a range of possible symptoms including paralysis, numbness, and problems with speech, sight, movement and balance. Carotid artery stenosis, which is narrowing of the large arteries in the neck, may also reduce the brain's blood supply and lead to a stroke.

Vascular stenosis and associated conditions such as atherosclerosis can therefore result in very serious cardiovascular pathologies such as infarction, peripheral vascular disease, stroke, sudden death, cardiac decompensation, cerebral vascular accidents and the like. Often, by the time that heart problems are detected, the underlying cause, atherosclerosis, is usually quite advanced, having progressed for decades.

Despite widespread use of current therapies including cholesterol lowering and platelet inhibiting therapies, as well as angioplasty and surgical bypass procedures, vascular stenosis and associated conditions such as atherosclerosis remain the largest cause of morbidity and mortality in developed countries.

Thus there continues to be an unmet need for additional effective therapies for the prevention and/or treatment of vascular stenosis and associated conditions.

The present description refers to a number of documents, the content of which is herein incorporated by reference in their entirety.

SUMMARY OF THE INVENTION

In a first aspect, the present invention provides a method for preventing or treating vascular stenosis and/or associated conditions in a growth hormone-deficient (GHD) obese subject, said method comprising administering to a subject in need thereof an effective amount of a growth hormone (GH)-releasing agent.

In another aspect, the present invention provides a growth hormone (GH)-releasing agent for use in preventing or treating vascular stenosis and/or associated conditions in a growth hormone-deficient (GHD) obese subject.

In another aspect, the present invention provides a growth hormone (GH)-releasing agent for the manufacture of a medicament for use in preventing or treating vascular stenosis and/or associated conditions in a growth hormone-deficient (GHD) obese subject.

In another aspect, the present invention provides the use of a growth hormone (GH)-releasing agent for preventing or treating vascular stenosis and/or associated conditions in a growth hormone-deficient (GHD) obese subject.

5 In another aspect, the present invention provides the use of a growth hormone (GH)-releasing agent for the manufacture of a medicament for preventing or treating vascular stenosis and/or associated conditions in a growth hormone-deficient obese (GHD) subject.

In an embodiment, the above-mentioned GH-releasing agent is a growth hormone-releasing factor (GRF) or a GRF analog.

10 In a further embodiment, the above-mentioned GRF analog is a GRF analog of formula A:



wherein;

the GRF peptide is a peptide of formula B:

15 A1-A2-Asp-Ala-Ile-Phe-Thr-A8-Ser-Tyr-Arg-Lys-A13-Leu-A15-Gln-Leu-A18-Ala-Arg-Lys-Leu-Leu-A24-A25-Ile-A27-A28-Arg-A30-R0 (B) (SEQ ID NO:1)

wherein,

A1 is Tyr or His;

A2 is Val or Ala;

A8 is Asn or Ser;

20 A13 is Val or Ile;

A15 is Ala or Gly;

A18 is Ser or Tyr;

A24 is Gln or His;

A25 is Asp or Glu;

25 A27 is Met, Ile or Nle

A28 is Ser or Asn;

A30 is a bond or amino acid sequence of 1 up to 15 residues; and

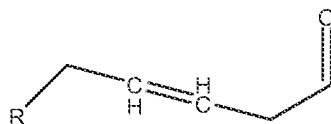
R0 is NH_2 or $\text{NH}-(\text{CH}_2)_n-\text{CONH}_2$, with $n=1$ to 12; and

30 X is a hydrophobic tail anchored via an amide bond to the N-terminus of the GRF peptide and the hydrophobic tail comprising (i) a backbone of 5 to 9 atoms, wherein the backbone can be substituted by C_{1-6} alkyl, C_{3-6} cycloalkyl, or C_{6-12} aryl and (ii) at least one rigidifying moiety connected to at least two atoms of the backbone; said moiety is a double bond, triple bond, saturated or unsaturated C_{3-9} cycloalkyl, or C_{6-12} aryl, or a pharmaceutically acceptable salt thereof.

35 In a further embodiment, the above-mentioned X is:

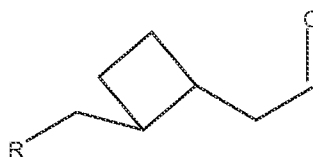
(i)

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wherein R is H, CH₃ or CH₂CH₃, and the double bond is *cis* or *trans*;

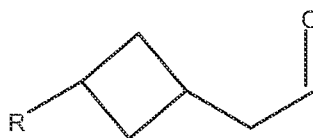
(ii)



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wherein R is H, CH₃ or CH₂CH₃, wherein X is in a *cis* or *trans* configuration, and wherein said GRF analog is a racemic mixture or a pure enantiomer;

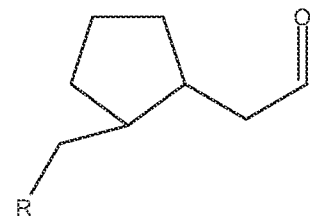
(iii)



10

wherein R is H, CH₃ or CH₂CH₃, and wherein when R is CH₃ or CH₂CH₃, X is in a *cis* or *trans* configuration;

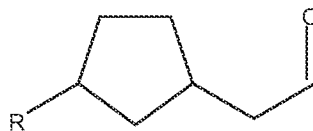
(iv)



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wherein R is H, CH₃ or CH₂CH₃, wherein X is in a *cis* or *trans* configuration, and wherein said GRF analog is a racemic mixture or a pure enantiomer;

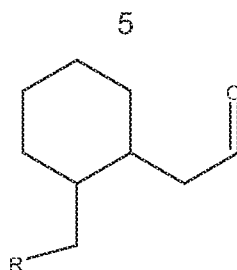
(v)



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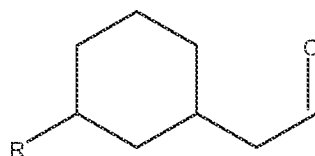
wherein R is H, CH₃ or CH₂CH₃, wherein when R is CH₃ or CH₂CH₃, X is in a *cis* or *trans* configuration, and wherein said GRF analog is a racemic mixture or a pure enantiomer;

(vi)



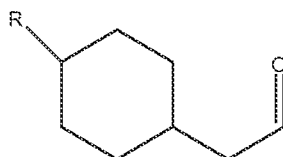
wherein R is H, CH₃ or CH₂CH₃, wherein X is in a *cis* or *trans* configuration, and wherein said GRF analog is a racemic mixture or a pure enantiomer;

5 (vii)



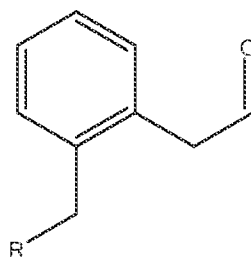
wherein R is H, CH₃ or CH₂CH₃, wherein when R is CH₃ or CH₂CH₃, X is in a *cis* or *trans* configuration, and wherein said GRF analog is a racemic mixture or a pure enantiomer;

10 (viii)



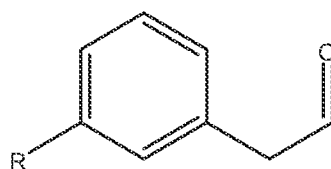
wherein R is H, CH₃ or CH₂CH₃, and wherein when R is CH₃ or CH₂CH₃, X is in a *cis* or *trans* configuration;

15 (ix)



wherein R is H, CH₃ or CH₂CH₃;

(x)

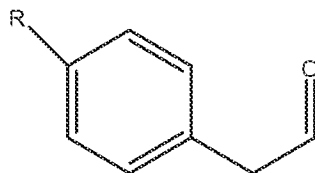


20

wherein R is H, CH₃ or CH₂CH₃;

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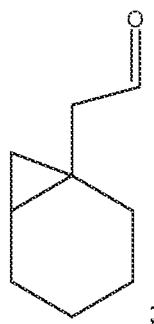
(xi)



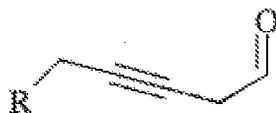
wherein R is H, CH₃ or CH₂CH₃;

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(xii)



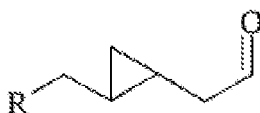
(xiii)



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wherein R is H, CH₃ or CH₂CH₃; or

(xiv)

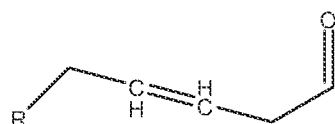


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wherein R is H, CH₃ or CH₂CH₃, wherein X is in a *cis* or *trans* configuration, and wherein said GRF analog is a racemic mixture or a pure enantiomer.

In a further embodiment, the above-mentioned X is:

(i)



20

wherein R is CH₃ and the double bond is *trans*.

In an embodiment, the above-mentioned A30 is: (a) a bond; (b) an amino acid sequence corresponding to positions 30-44 of a natural GRF peptide; and (c) said amino acid sequence of (b), having a 1-14 amino acid deletion from its C-terminus.

In a further embodiment, the above-mentioned GRF analog is (hexenoyl trans-3)hGHRH₍₁₋₄₄₎NH₂ (SEQ ID NO: 16).

In an embodiment, the above-mentioned vascular stenosis is carotid stenosis.

In an embodiment, the above-mentioned vascular stenosis and/or associated condition is atherosclerosis.

Other objects, advantages and features of the present invention will become more apparent upon reading of the following non-restrictive description of specific embodiments thereof, given by way of example only with reference to the accompanying drawings.

BRIEF DESCRIPTION OF DRAWINGS

In the appended drawings:

FIG. 1 shows the enrollment and outcomes of the subjects studied; and

FIG. 2 shows the effects of (hexenoyl trans-3)hGHRH₍₁₋₄₄₎NH₂ versus placebo on A) IGF-1; B) abdominal visceral adipose tissue (VAT) area; C) carotid intima-media thickness (cIMT). Panel A demonstrates the change in IGF-1 from baseline over time in (hexenoyl trans-3)hGHRH₍₁₋₄₄₎NH₂ versus placebo. Each point represents mean $\pm$ SEM of IGF-1 in each study group at baseline, 2-weeks, 1-month, 3-month, 6-month, 9-month and 12-month. While IGF-1 levels at baseline were similar between the two groups ($P=0.17$), IGF-1 levels at all subsequent time points were significantly different between each group ($P<0.05$ at each time point) with overall $P < 0.0001$ by longitudinal linear mixed effects modeling. Panel B demonstrates the difference in VAT between treatment groups with (hexenoyl trans-3)hGHRH₍₁₋₄₄₎NH₂ demonstrating a net -19% improvement in VAT compared to placebo. Panel C demonstrates the differences in cIMT between treatment groups with (hexenoyl trans-3)hGHRH₍₁₋₄₄₎NH₂ demonstrating a net -6% improvement in cIMT compared to placebo. The "T" bars on panel B and C denote the standard error. Statistical significance was determined by longitudinal linear mixed effects modeling with last value carried forward for both panels B and C.

DISCLOSURE OF INVENTION

In the studies described herein, it is demonstrated that administration of the GH-releasing agent (hexenoyl trans-3)hGHRH₍₁₋₄₄₎NH₂ to obese subjects with relative reductions in GH secretory capacity leads to a significant improvement (decrease) of carotid Intima-Media Thickness (cIMT), a direct measure of carotid atherosclerosis.

Accordingly, in a first aspect, the present invention provides a method for preventing or treating vascular stenosis and/or associated conditions in a growth hormone-deficient (GHD)

obese subject, the method comprising administering to a subject in need thereof an effective amount of a growth hormone (GH)-releasing agent.

In another aspect, the present invention provides a method for preventing, or reducing the risk of developing, a condition related to vascular stenosis in a growth hormone-deficient (GHD) obese subject, the method comprising administering to a subject in need thereof an effective amount of a growth hormone (GH)-releasing agent.

In another aspect, the present invention provides a method for reducing vascular stenosis in a growth hormone-deficient (GHD) obese subject, the method comprising administering to a subject in need thereof an effective amount of a growth hormone (GH)-releasing agent.

The term "growth hormone (GH)-releasing agent" as used herein refers to an agent that induces the physiological release and increase in plasma levels of endogenous GH in a subject. It excludes GH itself as well as GH variants, fragments, analogues, nucleic acids encoding GH, GH variants or GH fragments.

Numerous compounds have been developed which stimulate the physiological release of endogenous growth hormone.

In an embodiment, the GH-releasing agent is a GH secretagogue. Peptides and compounds which are known to stimulate the release of endogenous growth hormone include the growth hormone releasing peptides, ghrelin, GHRP-6 and GHRP-1 (described in U.S. Patent No. 4,411,890; International Patent Application Publication No. WO 89/07110; U.S. Patent No. 5,534,494 and International Patent Application Publication No. WO 89/07111), and GHRP-2 (described in International Patent Application Publication No. WO 93/04081 and U.S. Patent No. 5,663,146), as well as hexarelin (J. Endocrinol. Invest., 15 (Suppl. 4): 45 (1992)), and analogs thereof. Representative growth hormone secretagogues are disclosed in: U.S. Pat. No. 3,239,345; U.S. Pat. No. 4,036,979; U.S. Pat. No. 4,411,890; U.S. Pat. No. 5,206,235; U.S. Pat. No. 5,283,241; U.S. Pat. No. 5,284,841; U.S. Pat. No. 5,310,737; U.S. Pat. No. 5,317,017; U.S. Pat. No. 5,374,721; U.S. Pat. No. 5,430,144; U.S. Pat. No. 5,434,261; U.S. Pat. No. 5,438,136; U.S. Pat. No. 5,494,919; U.S. Pat. No. 5,494,920; U.S. Pat. No. 5,492,916; U.S. Pat. No. 5,536,716; U.S. Pat. No. 6, 194, 402; U.S. Patent Application Publication No. 2002/0028838; EPO Patent Pub. No. 0,144,230; EPO Patent Pub. No. 0,513,974; PCT Patent Pub. No. WO 89/07110; PCT Patent Pub. No. WO 89/07111; PCT Patent Pub. No. WO 93/04081; PCT Patent Pub. No. WO 94/07486; PCT Patent Pub. No. WO 94/08583; PCT Patent Pub. No. WO 94/11012; PCT Patent Pub. No. WO 94/13696; PCT Patent Pub. No. WO 94/19367; PCT Patent Pub. No. WO 95/03289; PCT Patent Pub. No. WO 95/03290; PCT Patent Pub. No. WO 95/09633; PCT Patent Pub. No. WO 95/11029; PCT Patent Pub. No. WO 95/12598; PCT Patent Pub. No. WO 95/13069; PCT Patent Pub. No. WO 95/14666; PCT Patent Pub. No. WO 95/16675; PCT Patent Pub. No. WO 95/16692; PCT Patent Pub. No. WO

95/17422; PCT Patent Pub. No. WO 95/17423; PCT Patent Pub. No. WO 95/34311; PCT Patent Pub. No. WO 96/02530; PCT Patent Pub. No. WO 96/05195; PCT Patent Pub. No. WO 96/15148; PCT Patent Pub. No. WO 96/22782; PCT Patent Pub. No. WO 96/22997; PCT Patent Pub. No. WO 96/24580; PCT Patent Pub. No. WO 96/24587; PCT Patent Pub. No. WO 96/35713; PCT Patent Pub. No. WO 96/38471; PCT Patent Pub. No. WO 97/00894; PCT Patent Pub. No. WO 97/06803; PCT Patent Pub. No. WO 97/07117; PCT Patent Pub. No. WO/08/145749, PCT Patent Pub. No. WO/09/150214, J. Endocrinol Invest., 15(Suppl 4), 45 (1992)); Science 260, 1640-1643 (Jun. 11, 1993); Ann. Rep. Med. Chem., 28, 177-186 (1993); Bioorg. Med. Chem. Ltrs., 4(22), 2709-2714 (1994); and Proc. Natl. Acad. Sci. USA 92, 7001-7005 (July 1995).

Some representative GH secretagogue compounds include, for example:

- (1) N-[1(R)-[(1,2-Dihydro-1-methanesulfonylspiro [3 H-indole-3,4'-piperidin] - 1'-yl)carbonyl]-2-(1H-indol-3-yl)ethyl]-2-amino-2-methylpropanamide;
- (2) N-[1(R)-[(1,2-Dihydro-1-methanecarbonylspiro [3 H-indole-3,4'-piperidin]- 1'-yl)carbonyl]-2-(1H-indol-3-yl)ethyl]-2-amino-2-methylpropanamide;
- (3) N-[1(R)-[(1,2-Dihydro-1-benzenesulfonylspiro [3 H-indole-3,4'-piperidin] - 1'-yl)carbonyl]-2-(1H-indol-3-yl)ethyl]-2-amino-2-methylpropanamide;
- (4) N-[1(R)-[(3,4-Dihydro-spiro[2H-1-benzopyran-2,4'-piperidin]-1'-yl)carbonyl]-2-(1H-indol-3-yl)ethyl]-2-amino-2-methylpropanamide;
- (5) N-[1(R)-[(2-Acetyl-1,2,3,4-tetrahydrospiro[isoquinolin-4,4'-piperidin]- 1'-yl)carbonyl]-2-(indol-3-yl)ethyl]-2-amino-2-methylpropanamide;
- (6) N-[1(R)-[(1,2-Dihydro-1-methanesulfonylspiro [3 H-indole-3,4'-piperidin] - 1'-yl)carbonyl]-2-(phenylmethoxy)ethyl]-2-amino-2-methylpropanamide;
- (7) N-[1(R)-[(1,2-Dihydro-1-methanesulfonylspiro [3 H-indole-3,4'-piperidin] - 1'-yl)carbonyl]-2-(phenylmethoxy)ethyl]-2-amino-2-methylpropanamide mesylate salt;
- (8) N-[1(R)-[(1,2-Dihydro-1-methanesulfonylspiro [3 H-indole-3,4'-piperidin] - 1'-yl)carbonyl]-2-(2',6'-difluorophenylmethoxy)ethyl]-2-amino-2-methylpropanamide;
- (9) N-[1(R)-[(1,2-Dihydro-1-methanesulfonyl-5-fluorospiro[3H-indole-3,4'-piperidin]-1'-yl)carbonyl]-2-(phenylmethoxy)ethyl]-2-amino-2-methylpropanamide ;
- (10) N-[1(S)-[(1,2-Dihydro-1-methanesulfonylspiro[3 H-indole-3,4'-piperidin] - 1'-yl)carbonyl]-2-(phenylmethylthio)ethyl]-2-amino-2-methylpropanamide;
- (11) N-[1(R)-[(1,2-Dihydro-1-methanesulfonylspiro [3 H-indole-3,4'-piperidin] - 1'-yl)carbonyl]-3-phenylpropyl]-2-amino-2-methylpropanamide;
- (12) N-[1(R)-[(1,2-Dihydro-1-methanesulfonylspiro [3 H-indole-3,4'-piperidin] - 1'-yl)carbonyl]-3-cyclohexylpropyl]-2-amino-2-methylpropanamide;
- (13) N-[1(R)-[(1,2-Dihydro-1-methanesulfonylspiro [3 H-indole-3,4'-piperidin] - 1'-yl)carbonyl]-4-phenylbutyl]-2-amino-2-methylpropanamide;

(14) N-[1(R)-[(1,2-Dihydro-1-methanesulfonylspiro[3H-indole-3,4'-piperidin]-1'-yl)carbonyl]-2-(5-fluoro-1H-indol-3-yl)ethyl]-2-amino-2-methylpropanamide;

(15) N-[1(R)-[(1,2-Dihydro-1-methanesulfonyl-5-fluorospiro[3H-indole-3,4'-piperidin]-1'-yl)carbonyl]-2-(5-fluoro-1H-indol-3-yl)ethyl]-2-amino-2-methylpropanamide;

5 (16) N-[1(R)-[(1,2-Dihydro-1-(2-ethoxycarbonyl)methylsulfonylspiro[3H-indole-3,4'-piperidin]-1'-yl)carbonyl]-1-(1H-indol-3-yl)ethyl]-2-amino-2-methylpropanamide;

(17) N-[1(R)-[(1,2-Dihydro-1,1-dioxospiro[3H-benzothiophene-3,4'-piperidin]-1'-yl)carbonyl]-2-(phenylmethyloxy)ethyl]-2-amino-2-methylpropanamide;

and pharmaceutically acceptable salts thereof.

10 In addition, the following growth hormone secretagogues are known in the art: MK-0677, L-162752 and L-163022 (Merck); NN703 and ipamorelin (Novo Nordisk); hexarelin (Pharmacia); GPA-748 (KP102, GHRP-2) (American Home Products); and LY444711 (Eli Lilly). Any ligand or agonist of the Growth Hormone (GH) Secretagogue Receptor (e.g., GHS-R1a) may be used in the method of the present invention.

15 In another embodiment, the GH-releasing agent is more specific to the GH axis, and does not significantly affect (increase) appetite, blood glucose and/or cortisol levels. Accordingly, in another embodiment, the GH-releasing agent is GRF or a GRF analog. GRF (also referred to as GH-releasing hormone or GHRH) is a 44 amino acid peptide secreted by the hypothalamus that regulates the expression, synthesis and release of GH from the somatotroph
20 cells of the anterior pituitary (Frohman LA *et al. Endocrine Reviews* 1986, 7: 223-253). A peptide consisting of the first 29 amino acids of human GRF (hGRF₍₁₋₂₉₎; sermorelin) retains the biological activity of the full-length peptide (Lance, V.A. *et al., Biochemical and Biophysical Research Communications* 1984, 119: 265-272).

Native human GRF is a peptide of 44 amino acids having the following structure: Tyr-
25 Ala-Asp-Ala-Ile-Phe-Thr-Asn-Ser-Tyr-Arg-Lys-Val-Leu-Gly-Gln-Leu-Ser-Ala-Arg-Lys-Leu-Leu-Gln-Asp-Ile-Met-Ser-Arg-Gln-Gln-Gly-Glu-Ser-Asn-Gln-Glu-Arg-Gly-Ala-Arg-Ala-Arg-Leu (SEQ ID NO:2).

The 29 amino acid N-terminal fragment of GRF₍₁₋₄₄₎, known as GRF₍₁₋₂₉₎, has been shown to exhibit biological activity and potency that is similar to GRF₍₁₋₄₄₎. GRF₍₁₋₂₉₎ has the
30 following sequence: Tyr-Ala-Asp-Ala-Ile-Phe-Thr-Asn-Ser-Tyr-Arg-Lys-Val-Leu-Gly-Gln-Leu-Ser-Ala-Arg-Lys-Leu-Leu-Gln-Asp-Ile-Met-Ser-Arg (SEQ ID NO:3).

Intermediates forms (in length) between GRF₍₁₋₂₉₎ and GRF₍₁₋₄₄₎, i.e., having the sequence of GRF₍₁₋₂₉₎ at the C-terminus of which 1-14 amino acids are added which correspond to residues 30-43 of native human GRF (or other residues), also possess GRF activity. Further,
35 certain GRF variants having one or more amino acid substitutions in the native sequence are known to possess GRF activity.

The term "GRF or GRF analog" as used in the context of the present invention includes, without limitation, human native GRF₍₁₋₄₄₎ and fragments (1-40), (1-29), fragments ranging between 1-29 and the 1-44 sequence, and any other fragments; GRF from other species and fragments thereof; GRF variants containing amino acid(s) substitution(s), addition(s) and/or deletion(s) such that the amino acid sequence of the variant has at least about 90% of identity with the native amino acid sequence, in an embodiment at least about 95% of identity with the native amino acid sequence. In an embodiment, the above-mentioned fragments/variants retain at least about 10% of the activity of stimulating GH secretion as compared to the native GRF; derivatives or analogs of GRF or fragments or variants thereof having for example an organic group or a moiety coupled to the GRF amino acid sequence at the N-terminus, the C-terminus or on the side-chain (see, e.g., WO2002/062844, US Patent No. 5,792,747); and salts of GRF (human or from other species), as well as salts of GRF fragments, variants, analogs and derivatives. The GRF or GRF analog also encompass the GRF molecules currently known in the art, including, without limitation, albumin-conjugated GRF (U.S. Patent No. 7,268,113); pegylated GRF peptide (U.S. Patent Nos. 7,256,258 and 6,528,485); porcine GRF (1-40) (U.S. Patent No. 6,551,996); canine GRF (U.S. patent application no. 2005/0064554); GRF variants of 1-29 to 1-44 amino acid length (U.S. Patent Nos. 5,846,936, 5,696,089, 5,756,458 and 5,416,073, and U.S. patent application Nos. 2006/0128615 and 2004/0192593); and Pro^o-GHRH peptide and variants thereof (U.S. Patent No. 5,137,872). GRF analogs are also disclosed in PCT publications Nos. WO2004/027064, WO2009/009727, WO2011/153491 and WO2012/037519. Pharmaceutically acceptable salts of GRF and GRF analogs are also included.

The GRF analogs also include those described in U.S. Patent Nos. 5,681,379 and 5,939,386, which also describe their method of synthesis. More particularly, these GHRH analogs are defined by the following formula A:

X-GRF peptide (A)

wherein;

the GRF peptide is a peptide of formula B:

A1-A2-Asp-Ala-Ile-Phe-Thr-A8-Ser-Tyr-Arg-Lys-A13-Leu-A15-Gln-Leu-A18-Ala-Arg-Lys-Leu-Leu-A24-A25-Ile-A27-A28-Arg-A30-R0 (B) (SEQ ID NO:1)

wherein,

A1 is Tyr or His;

A2 is Val or Ala;

A8 is Asn or Ser;

A13 is Val or Ile;

A15 is Ala or Gly;

A18 is Ser or Tyr;

A24 is Gln or His;

A25 is Asp or Glu;

A27 is Met, Ile or Nle

A28 is Ser or Asn;

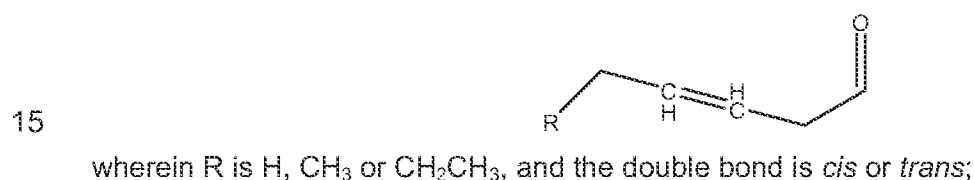
5 A30 is absent or is amino acid sequence of 1 up to 15 residues; and

R0 is NH_2 or $\text{NH}-(\text{CH}_2)_n-\text{CONH}_2$, with $n=1$ to 12; and

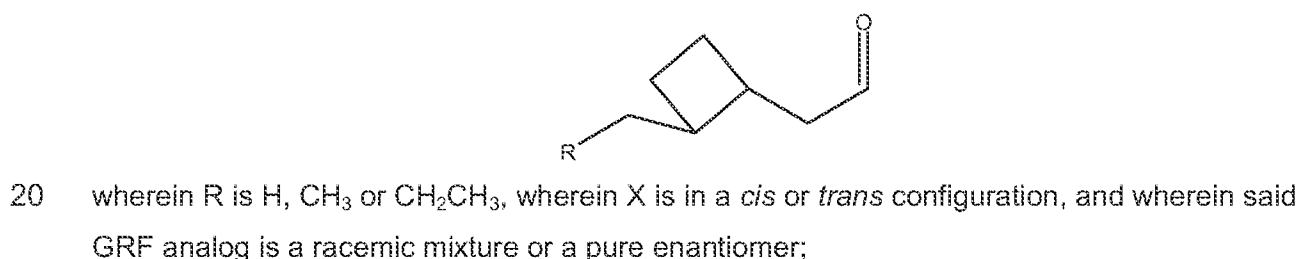
X is a hydrophobic tail anchored via an amide bond to the N-terminus of the GRF peptide and the hydrophobic tail comprising (i) a backbone of 5 to 9 atoms, wherein the backbone can be substituted by C_{1-6} alkyl, C_{3-6} cycloalkyl, or C_{6-12} aryl and (ii) at least one rigidifying moiety
10 connected to at least two atoms of the backbone; said moiety is a double bond, triple bond, saturated or unsaturated C_{3-9} cycloalkyl, or C_{6-12} aryl, or a pharmaceutically acceptable salt thereof.

In an embodiment, X is:

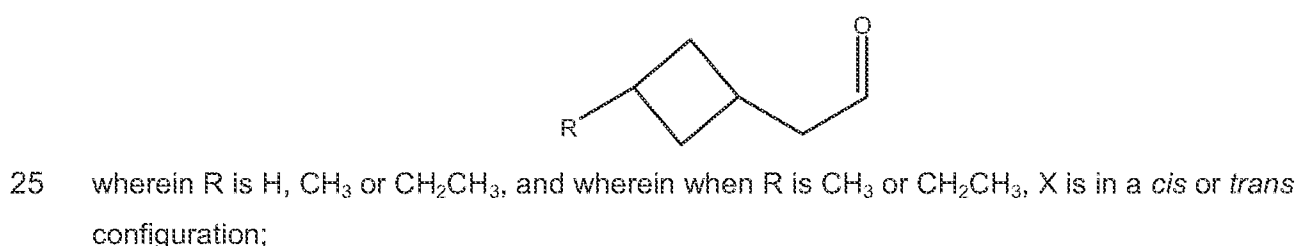
(i)



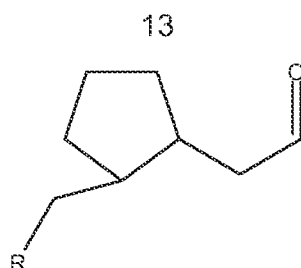
(ii)



(iii)

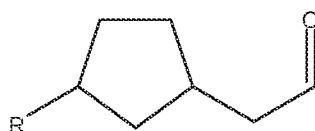


(iv)



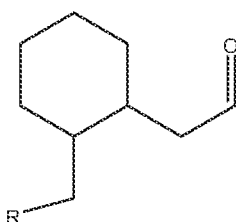
wherein R is H, CH_3 or CH_2CH_3 , wherein X is in a *cis* or *trans* configuration, and wherein said GRF analog is a racemic mixture or a pure enantiomer;

5 (v)



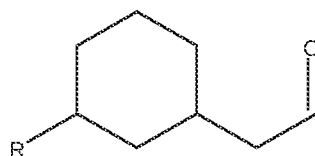
wherein R is H, CH_3 or CH_2CH_3 , wherein when R is CH_3 or CH_2CH_3 , X is in a *cis* or *trans* configuration, and wherein said GRF analog is a racemic mixture or a pure enantiomer;

10 (vi)



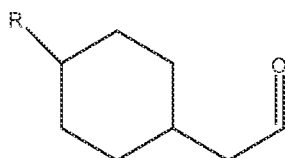
wherein R is H, CH_3 or CH_2CH_3 , wherein X is in a *cis* or *trans* configuration, and wherein said GRF analog is a racemic mixture or a pure enantiomer;

15 (vii)



wherein R is H, CH_3 or CH_2CH_3 , wherein when R is CH_3 or CH_2CH_3 , X is in a *cis* or *trans* configuration, and wherein said GRF analog is a racemic mixture or a pure enantiomer;

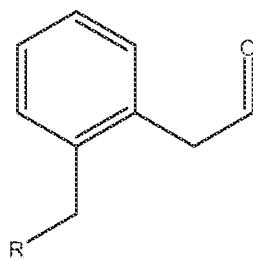
20 (viii)



wherein R is H, CH_3 or CH_2CH_3 , and wherein when R is CH_3 or CH_2CH_3 , X is in a *cis* or *trans* configuration;

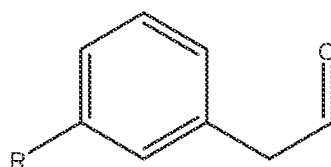
14

(ix)

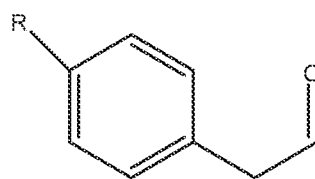
wherein R is H, CH_3 or CH_2CH_3 ;

5

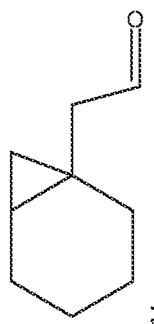
(x)

wherein R is H, CH_3 or CH_2CH_3 ;

10 (xi)

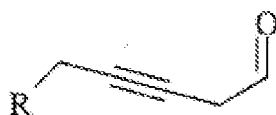
wherein R is H, CH_3 or CH_2CH_3 ;

(xii)



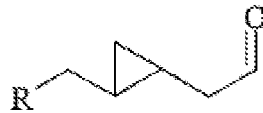
15

(xiii)



wherein R is H, CH₃ or CH₂CH₃; or

(xiv)



- 5 wherein R is H, CH₃ or CH₂CH₃, wherein X is in a *cis* or *trans* configuration, and wherein said GRF analog is a racemic mixture or a pure enantiomer.

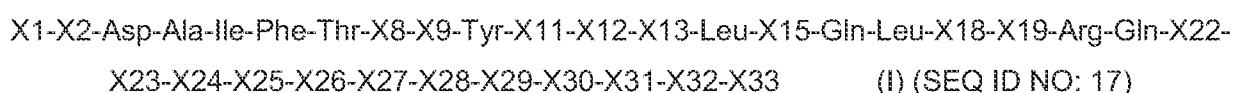
In an embodiment, A30 is: (a) absent; (b) an amino acid sequence corresponding to positions 30-44 of a natural (e.g., human) GHRH peptide; or (c) the amino acid sequence of (b), having a 1-14 amino acid deletion from its C-terminus.

- 10 In a further embodiment, A30 is one of the following amino acid sequences (i) to (xv):

(i) Gln; (ii) Gln-Gln; (iii) Gln-Gln-Gly; (iv) Gln-Gln-Gly-Glu (SEQ ID NO:4); (v) Gln-Gln-Gly-Glu-Ser (SEQ ID NO:5); (vi) Gln-Gln-Gly-Glu-Ser-Asn (SEQ ID NO:6); (vii) Gln-Gln-Gly-Glu-Ser-Asn-Gln (SEQ ID NO:7); (viii) Gln-Gln-Gly-Glu-Ser-Asn-Gln-Glu (SEQ ID NO:8); (ix) Gln-Gln-Gly-Glu-Ser-Asn-Gln-Glu-Arg (SEQ ID NO:9); (x) Gln-Gln-Gly-Glu-Ser-Asn-Gln-Glu-Arg-Gly (SEQ ID NO:10); (xi) Gln-Gln-Gly-Glu-Ser-Asn-Gln-Glu-Arg-Gly-Ala (SEQ ID NO:11); (xii) Gln-Gln-Gly-Glu-Ser-Asn-Gln-Glu-Arg-Gly-Ala-Arg (SEQ ID NO:12); (xiii) Gln-Gln-Gly-Glu-Ser-Asn-Gln-Glu-Arg-Gly-Ala-Arg-Ala (SEQ ID NO:13); (xiv) Gln-Gln-Gly-Glu-Ser-Asn-Gln-Glu-Arg-Gly-Ala-Arg-Ala-Arg (SEQ ID NO:14); or (xv) Gln-Gln-Gly-Glu-Ser-Asn-Gln-Glu-Arg-Gly-Ala-Arg-Ala-Arg-Leu (SEQ ID NO:15).

- 20 In an embodiment, the hydrophobic tail X is a *trans*-CH₃-CH₂-CH=CH-CH₂-CO moiety (or a *trans*-3-hexenoyl moiety). In a further embodiment, the above-mentioned GRF analog is (hexenoyl *trans*-3)hGHRH₍₁₋₄₄₎NH₂ (SEQ ID NO: 16).

Other GRF analogs exhibiting agonist properties toward the GRF receptor are disclosed in PCT application No. PCT/CA2012/050242 and U.S. patent No. 8,361,964. These GRF analogs are variants of GRF and active fragments and/or variants thereof, comprising a 3 amino acid residue deletion at positions corresponding to residues 21 to 23 (Lys-Leu-Leu in the sequences depicted above) of the native human GRF₍₁₋₄₄₎, its N-terminal fragment GRF₍₁₋₂₉₎, or the intermediate forms noted above. The GRF analog of the invention may further comprise an alteration of the serine (Ser) corresponding to position 18, preferably a substitution with Lys, L-Ornithine, L-2,4-diaminobutyric acid or L-2,3-diaminopropionic acid, of the native human GRF₍₁₋₄₄₎, its N-terminal fragment GRF₍₁₋₂₉₎, or the intermediate forms noted above. These GRF analogs comprise a domain of formula (I):



- 35 wherein:

X1 is any amino acid, in an embodiment Tyr or His, in a further embodiment Tyr;

X2 is any amino acid, in an embodiment Ala, D-Ala, Ser, Leu, α -aminoisobutyric acid (Aib), Val or Gly, in a further embodiment Ala or D-Ala;

X8 is any amino acid, in an embodiment Asn, Asp, Ala, Gln, Ser or Aib, in a further embodiment Ala or Asp, in a further embodiment Asp;

X9 is any amino acid, in an embodiment Ser, Asp or Ala, in a further embodiment Ser;

X11 is any amino acid, in an embodiment Arg or L-Homoarginine, in a further embodiment Arg;

X12 is any amino acid, in an embodiment Lys, L-Ornithine or L-homoarginine, in a further embodiment Lys or L-homoarginine, in yet a further embodiment Lys;

X13 is any amino acid, in an embodiment Val or Ala, in a further embodiment Val;

X15 is any amino acid, in an embodiment Gly or Ala, in a further embodiment Ala;

X18 is any amino acid, in an embodiment Lys, L-Ornithine, L-2,4-diaminobutyric acid or L-2,3-diaminopropionic acid, in a further embodiment Lys or L-Ornithine, in yet a further embodiment Lys;

X19 is Ala or Leu;

X22 is any amino acid, in an embodiment Asp or Glu, in a further embodiment Asp;

X23 is any amino acid, in an embodiment Ile or Leu, in a further embodiment Ile;

X24 is any amino acid, in an embodiment Met, Ile, Nle or Leu, in a further embodiment Met or Leu, in a further embodiment Leu;

X25 is any amino acid, in an embodiment Ser, Asn, Aib or Ala, in a further embodiment Ala or Ser, in yet a further embodiment Ala;

X26 is any amino acid, in an embodiment Arg, D-Arg, L-Homoarginine or Lys, in a further embodiment Arg or D-Arg, in yet a further embodiment Arg;

X27 is any amino acid, in an embodiment Ala, or is absent;

X28 is any amino acid, in an embodiment Ala, or is absent;

X29 is any amino acid, in an embodiment Arg, or is absent;

X30 is any amino acid, in an embodiment Ala, or is absent;

X31 is any amino acid, in an embodiment Ala or is absent;

X32 is any amino acid, in an embodiment Arg, or is absent; and

X33 is any amino acid, in an embodiment HoSer, or is absent;

and wherein if X18 is Ser, X27 to X32 or X27 to X33 are present,

or a pharmaceutically acceptable salt thereof.

Representative domains or GRF analogs of formula I include those having the following sequences:

Y-A-D-A-I-F-T-D-S-Y-R-K-V-L-A-Q-L-K-A-R-Q-D-I-L-A-R (SEQ ID NO: 18);

Y-a-D-A-I-F-T-D-S-Y-R-K-V-L-A-Q-L-K-A-R-Q-D-I-L-S-r (SEQ ID NO: 19);

- Y-a-D-A-I-F-T-D-S-Y-R-K-V-L-A-Q-L-K-A-R-Q-D-I-L-aib-R (SEQ ID NO: 20);
 Y-a-D-A-I-F-T-D-S-Y-R-K-V-L-A-Q-L-O-A-R-Q-D-I-L-S-r (SEQ ID NO: 21);
 Y-A-D-A-I-F-T-D-S-Y-R-K-V-L-A-Q-L-O-A-R-Q-D-I-L-S-r (SEQ ID NO: 22);
 Y-a-D-A-I-F-T-D-S-Y-R-K-V-L-A-Q-L-K-A-R-Q-D-L-L-S-r (SEQ ID NO: 23);
 5 Y-A-D-A-I-F-T-D-S-Y-R-K-V-L-A-Q-L-K-A-R-Q-D-I-L-A-R-A-A-R (SEQ ID NO: 24);
 Y-A-D-A-I-F-T-D-S-Y-R-K-V-L-A-Q-L-S-A-R-Q-D-I-L-A-R-A-A-R-A-A-R (SEQ ID NO: 25);
 Y-a-D-A-I-F-T-D-S-Y-R-K-V-L-A-Q-L-K-A-R-Q-D-I-L-A-R-A-A-R-A-A-R (SEQ ID NO: 26);
 Y-a-D-A-I-F-T-D-S-Y-R-K-V-L-A-Q-L-K-L-R-Q-D-I-L-A-R-A-A-R-A-A-R (SEQ ID NO: 27);
 Y-a-D-A-I-F-T-D-S-Y-R-K-V-L-A-Q-L-K-A-R-Q-D-L-L-A-R-A-A-R-A-A-R (SEQ ID NO: 28);
 10 Y-A-D-A-I-F-T-D-S-Y-R-K-V-L-A-Q-L-K-A-R-Q-D-I-L-A-R-A-A-R-A-A-R (SEQ ID NO: 29);
 Y-A-D-A-I-F-T-D-S-Y-R-K-V-L-A-Q-L-K-A-R-Q-D-L-L-A-R-A-A-R-A-A-R (SEQ ID NO: 30);
 Y-S-D-A-I-F-T-D-S-Y-R-K-V-L-A-Q-L-K-A-R-Q-D-I-L-A-R-A-A-R-A-A-R (SEQ ID NO: 31);
 Y-G-D-A-I-F-T-D-S-Y-R-K-V-L-A-Q-L-K-A-R-Q-D-I-L-A-R-A-A-R-A-A-R (SEQ ID NO: 32);
 Y-L-D-A-I-F-T-D-S-Y-R-K-V-L-A-Q-L-K-A-R-Q-D-I-L-A-R-A-A-R-A-A-R (SEQ ID NO: 33);
 15 Y-G-D-A-I-F-T-D-S-Y-R-K-V-L-A-Q-L-K-A-R-Q-D-I-L-A-R-A-A-R-A-A-R-HoSer (SEQ ID NO: 34);
 or
 Y-a-D-A-I-F-T-D-S-Y-R-K-V-L-A-Q-L-K-A-R-Q-D-I-L-A-R-A-A-R-A-A-R-HoSer (SEQ ID NO: 35);
 wherein a = D-Ala, O= L-Ornithine, Aib = α -aminoisobutyric acid, HoSer = L-homoserine and r = D-Arg.
- 20 Such GRF analogs may comprise N- and/or C-terminal modifications. The amino-terminal modification may be a C₁-C₁₆ or C₃-C₁₆ acyl group (linear or branched, saturated or unsaturated), in a further embodiment, a saturated C₁-C₆ acyl group (linear or branched) or an unsaturated C₃-C₆ acyl group (linear or branched), in a further embodiment an acetyl group (CH₃-CO-, Ac). The amino-terminal modification may also be a hydrophobic tail X as defined
- 25 above, for example a *trans*-CH₃-CH₂-CH=CH-CH₂-CO moiety (or *trans*-3-hexenoyl moiety). The carboxy-terminal modification may be an amidation, for example with an NH₂ residue.
- Representative GRF analogs include those having the following structure:
- Trans*-3-hexenoyl-Y-A-D-A-I-F-T-D-S-Y-R-K-V-L-A-Q-L-K-A-R-Q-D-I-L-A-R-NH₂ (SEQ ID NO: 36);
 30 Y-a-D-A-I-F-T-D-S-Y-R-K-V-L-A-Q-L-K-A-R-Q-D-I-L-S-r-NH₂ (SEQ ID NO: 37);
 Y-a-D-A-I-F-T-D-S-Y-R-K-V-L-A-Q-L-K-A-R-Q-D-I-L-aib-R-NH₂ (SEQ ID NO: 38);
 Y-a-D-A-I-F-T-D-S-Y-R-K-V-L-A-Q-L-O-A-R-Q-D-I-L-S-r-NH₂ (SEQ ID NO: 39);
Trans-3-hexenoyl-Y-A-D-A-I-F-T-D-S-Y-R-K-V-L-A-Q-L-O-A-R-Q-D-I-L-S-r-NH₂ (SEQ ID NO: 40);
 35 Y-a-D-A-I-F-T-D-S-Y-R-K-V-L-A-Q-L-K-A-R-Q-D-L-L-S-r-NH₂ (SEQ ID NO: 41);
Trans-3-hexenoyl-Y-A-D-A-I-F-T-D-S-Y-R-K-V-L-A-Q-L-K-A-R-Q-D-I-L-A-R-A-A-R-NH₂ (SEQ ID NO: 42);

Trans-3-hexenoyl-Y-A-D-A-I-F-T-D-S-Y-R-K-V-L-A-Q-L-S-A-R-Q-D-I-L-A-R-A-A-R-A-A-R-NH₂
(SEQ ID NO: 43);

Y-a-D-A-I-F-T-D-S-Y-R-K-V-L-A-Q-L-K-A-R-Q-D-I-L-A-R-A-A-R-A-A-R-NH₂ (SEQ ID NO: 44);

Y-a-D-A-I-F-T-D-S-Y-R-K-V-L-A-Q-L-K-L-R-Q-D-I-L-A-R-A-A-R-A-A-R-NH₂ (SEQ ID NO: 45);

5 Y-a-D-A-I-F-T-D-S-Y-R-K-V-L-A-Q-L-K-A-R-Q-D-L-L-A-R-A-A-R-A-A-R-NH₂ (SEQ ID NO: 46);

Trans-3-hexenoyl-Y-A-D-A-I-F-T-D-S-Y-R-K-V-L-A-Q-L-K-A-R-Q-D-I-L-A-R-A-A-R-A-A-R-NH₂
(SEQ ID NO: 47);

Trans-3-hexenoyl-Y-A-D-A-I-F-T-D-S-Y-R-K-V-L-A-Q-L-K-A-R-Q-D-L-L-A-R-A-A-R-A-A-R-NH₂
(SEQ ID NO: 48);

10 Y-S-D-A-I-F-T-D-S-Y-R-K-V-L-A-Q-L-K-A-R-Q-D-I-L-A-R-A-A-R-A-A-R-NH₂ (SEQ ID NO: 49);

Y-G-D-A-I-F-T-D-S-Y-R-K-V-L-A-Q-L-K-A-R-Q-D-I-L-A-R-A-A-R-A-A-R-NH₂ (SEQ ID NO: 50);

Y-L-D-A-I-F-T-D-S-Y-R-K-V-L-A-Q-L-K-A-R-Q-D-I-L-A-R-A-A-R-A-A-R-NH₂ (SEQ ID NO: 51);

Y-G-D-A-I-F-T-D-S-Y-R-K-V-L-A-Q-L-K-A-R-Q-D-I-L-A-R-A-A-R-A-A-R-HoSer-NH₂ (SEQ ID NO:
52); or

15 Y-a-D-A-I-F-T-D-S-Y-R-K-V-L-A-Q-L-K-A-R-Q-D-I-L-A-R-A-A-R-A-A-R-HoSer-NH₂ (SEQ ID NO:
53);

wherein a = D-Ala, O = L-Ornithine, Aib = α-aminoisobutyric acid, r = D-Arg, and HoSer
= L-homoserine.

As used herein the term "pharmaceutically acceptable salt" refers to salts of
20 compounds that retain the biological activity of the parent compound, and which are not
biologically or otherwise undesirable. Such salts can be prepared *in situ* during the final isolation
and purification of the analog, or may be prepared separately by reacting a free base function
with a suitable acid. Many of the GRF analogs disclosed herein are capable of forming acid
and/or base salts by virtue of the presence of amino and/or carboxyl groups or groups similar
25 thereto.

Pharmaceutically acceptable acid addition salts may be prepared from inorganic and
organic acids. Representative acid addition salts include, but are not limited to acetate, adipate,
alginate, citrate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, camphorate,
camphor sulfonate, decanoate, digluconate, glycerophosphate, hemisulfate, heptanoate,
30 hexanoate, fumarate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethansulfonate
(isothionate), lactate, maleate, methane sulfonate, nicotinate, 2-naphthalene sulfonate,
octanoate, oxalate, palmitoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate,
propionate, succinate, tartrate, thiocyanate, phosphate, glutamate, bicarbonate, p-
toluenesulfonate, and undecanoate. Salts derived from inorganic acids include hydrochloric
35 acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like. Salts derived from
organic acids include acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic
acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid,

cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluene-sulfonic acid, salicylic acid, and the like. Examples of acids which can be employed to form pharmaceutically acceptable acid addition salts include, for example, an inorganic acid, e.g., hydrochloric acid, hydrobromic acid, sulphuric acid, and phosphoric acid, and an organic acid, e.g., oxalic acid, maleic acid, succinic acid, and citric acid. In an embodiment, the pharmaceutically acceptable salt is acetate.

Basic addition salts also can be prepared by reacting a carboxylic acid-containing moiety with a suitable base such as the hydroxide, carbonate, or bicarbonate of a pharmaceutically acceptable metal cation or with ammonia or an organic primary, secondary, or tertiary amine. Pharmaceutically acceptable salts include, but are not limited to, cations based on alkali metals or alkaline earth metals such as lithium, sodium, potassium, calcium, magnesium, and aluminum salts, and the like, and nontoxic quaternary ammonia and amine cations including ammonium, tetramethylammonium, tetraethylammonium, methylammonium, dimethylammonium, trimethylammonium, triethylammonium, diethylammonium, and ethylammonium, amongst others. Other representative organic amines useful for the formation of base addition salts include, for example, ethylenediamine, ethanolamine, diethanolamine, piperidine, piperazine, and the like. Salts derived from organic bases include, but are not limited to, salts of primary, secondary and tertiary amines.

The GH-releasing agent may be formulated in a composition (e.g., a pharmaceutical composition). In an embodiment, the composition further comprises one or more pharmaceutically acceptable carriers, excipient, and/or diluents.

As used herein, "pharmaceutically acceptable" (or "biologically acceptable") refers to materials characterized by the absence of (or limited) toxic or adverse biological effects *in vivo*. It refers to those compounds, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the biological fluids and/or tissues and/or organs of a subject (e. g., human, animal) without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The term "pharmaceutically acceptable carriers, excipient, and/or diluents" refers to additives commonly used in the preparation of pharmaceutical compositions and includes, for example, solvents, dispersion media, saline solutions, surfactants, solubilizing agents, lubricants, emulsifiers, coatings, antibacterial and antifungal agents, chelating agents, pH-modifiers, soothing agents, buffers, reducing agents, antioxidants, isotonic agents, absorption delaying agents or the like (see, e.g., Rowe *et al.*, *Handbook of Pharmaceutical Excipients*, Pharmaceutical Press; 6th edition, 2009).

The GH-releasing agent may be formulated for administration via any conventional route, such as intravenous, oral, transdermal, intraperitoneal, subcutaneous, mucosal, intramuscular, intranasal, intrapulmonary, parenteral or topical administration. The preparation

of such formulations is well known in the art (see, *e.g.*, *Remington: The Science and Practice of Pharmacy*, Lippincott Williams & Wilkins; 21st edition, 2005).

As used herein, the term "vascular stenosis" refers to any conditions resulting from the narrowing of blood vessels, for example atherosclerosis.

5 The term "associated conditions" used in reference to "vascular stenosis" or "atherosclerosis" means a condition that is associated with, caused by and/or results from the vascular stenosis (*e.g.*, atherosclerosis). Such conditions include, for example, thrombosis, high blood pressure, aortic aneurysm, cerebrovascular disease (*e.g.*, cerebrovascular accident (CVA)), transient ischemic attack (TIA), acute myocardial ischemia, myocardial infarction, 10 stroke, limb ischemia (*e.g.*, critical limb ischemia), arterial disease of lower extremities, arterial occlusive diseases (*e.g.*, peripheral artery occlusive diseases), acute coronary syndromes and angina. Acute coronary syndromes can be caused by acute destabilization of atherosclerotic plaques (*e.g.*, plaque rupture) that results in acute myocardial ischemia. Acute myocardial ischemia is chest pain due to insufficient blood supply to the heart muscle that results from 15 coronary artery disease (also called coronary heart disease). The rupture of a soft plaque causes the formation of a blood clot (*e.g.*, thrombus) that will rapidly slow or stop blood flow, *e.g.* 5 minutes, leading to death of the tissues fed by the artery. A common endpoint of coronary thrombosis of a coronary artery is a myocardial infarction (*i.e.*, a heart attack). "Thrombosis" and "thrombosis-related disorder" refer to abnormal thrombus formation that causes obstruction of 20 blood vessels and conditions associated with such obstruction. In an embodiment, the method is for preventing or treating vascular stenosis. In another embodiment, the above-mentioned method is for preventing or treating one or more of the following vascular stenosis-related conditions in a GHD subject: thrombosis, high blood pressure, aortic aneurysm, cerebrovascular disease (*e.g.*, cerebrovascular accident (CVA)), transient ischemic attack (TIA), acute 25 myocardial ischemia, myocardial infarction, stroke, limb ischemia (*e.g.*, critical limb ischemia), arterial disease of lower extremities, acute coronary syndromes or angina. In an embodiment, the above-mentioned method is for preventing or treating thrombosis in a GHD subject. In an embodiment, the above-mentioned method is for preventing or treating high blood pressure in a GHD subject. In an embodiment, the above-mentioned method is for preventing or treating 30 aortic aneurysm in a GHD subject. In an embodiment, the above-mentioned method is for preventing or treating a cerebrovascular disease (*e.g.*, cerebrovascular accident (CVA)) in a GHD subject. In an embodiment, the above-mentioned method is for preventing or treating a transient ischemic attack (TIA) in a GHD subject. In an embodiment, the above-mentioned method is for preventing or treating acute myocardial ischemia in a GHD subject. In an 35 embodiment, the above-mentioned method is for preventing or treating myocardial infarction in a GHD subject. In an embodiment, the above-mentioned method is for preventing or treating a stroke in a GHD subject. In an embodiment, the above-mentioned method is for preventing or

treating limb ischemia (e.g., critical limb ischemia) in a GHD subject. In an embodiment, the above-mentioned method is for preventing or treating arterial disease of lower extremities in a GHD subject. In an embodiment, the above-mentioned method is for preventing or treating a vascular (arterial) occlusive disease in a GHD subject. In an embodiment, the above-mentioned method is for preventing or treating acute coronary syndromes in a GHD subject. In an embodiment, the above-mentioned method is for preventing or treating angina in a GHD subject. In an embodiment, the above method is for preventing vascular stenosis and/or associated conditions (e.g., one or more of the conditions noted above). In another embodiment, the above method is for treating vascular stenosis and/or associated conditions (e.g., one or more of the conditions noted above).

In an embodiment, the above-mentioned method comprises, prior to the administration, identifying or selecting a subject suffering from vascular stenosis and/or an associated condition, or at risk of suffering from vascular stenosis and/or an associated condition. Indicators of atherosclerosis include, for example, the development of plaques in the arteries, their calcification, the extent of which can be determined by Sudan IV staining, or the development of foam cells in arteries. The narrowing of the arteries (vascular stenosis) can be determined by coronary angioplasty, ultrafast CT, or ultrasound, for example.

Subjects suitable for treatment according to the methods described herein include those who a medical practitioner has diagnosed as having one or more symptoms of vascular stenosis or atherosclerosis, and particularly those patients who have had or are at risk of an atherosclerotic disease event. Diagnosis may be done by any suitable means known in the art. Methods for diagnosing atherosclerosis are well known in the art, e.g., by measuring systemic atherosclerotic or inflammatory markers such as C-reactive protein, homocysteine, fibrinogen, lipoprotein (a), IL-6, IL-8, or IL-17. Diagnosis and monitoring can also employ an electrocardiogram, chest X-ray, cardiac catheterization, ultrasound (for the measurement of vessel wall thickness), or measurement of blood levels of CPK, CPK-MB, myoglobin, troponin, homocysteine, or C-reactive protein. A subject is diagnosed using computed tomography to detect calcium in the coronary arteries, which is an indicator of plaque progression.

A patient at risk of development of a vascular stenosis-related condition (e.g., an atherosclerotic related condition) may have been subjected to the same tests (electrocardiogram, chest X-ray, etc.) or may have been identified, without examination, as one at high risk due to the presence of one or more risk factors (e.g., family history, hypertension, diabetes mellitus, high cholesterol levels, smoking, etc.). Individuals in risk populations can be monitored more rigorously and treatment of vascular stenosis can be started as soon as alterations in the markers or other physiological symptoms can be detected.

Typically, vascular stenosis such as atherosclerosis does not produce symptoms until it narrows the interior of an artery by more than 70%. Symptoms depend on location of the

narrowing or blockage, which can occur almost anywhere in the body. Symptoms occur because as an artery is narrowed, the tissues supplied by the artery may not receive enough blood and oxygen. The first symptom of a narrowing artery may be pain or cramps at times when blood flow cannot keep up with the tissues' need for oxygen. Typically, symptoms develop gradually as the stenosis (e.g., atheroma) slowly narrows an artery. However, sometimes the first symptoms occur suddenly because the blockage occurs suddenly, for example, when a blood clot lodges in an artery narrowed by a stenosis (e.g., atheroma), causing a heart attack or stroke.

As used herein, the term "subject" is intended to mean a human or other mammal, exhibiting, or at risk of developing, vascular stenosis (e.g., atherosclerosis) and/or an associated condition, an inflammatory condition or thrombosis. Such an individual can have, or be at risk of developing, for example, vascular stenosis (e.g., atherosclerosis) associated with conditions such as thrombosis, coronary heart disease, high blood pressure, myocardial infarction, stroke, critical limb ischemia, angina, peripheral artery disease and the like.

The term "growth hormone-deficient (GHD) subject" as used herein refers to a subject having a blunted or decreased GH response (e.g., elevation of GH levels in the blood) to stimulation by a GRF or GRF analog, for example GHRH-arginine. The decreased GH response is determined by comparison to the GH response (GH levels) measured in one or more control healthy subjects, or relative to a pre-determined control GH response (e.g., pre-determined "threshold" GH levels in the blood). In an embodiment, the GH deficiency is measured using a GHRH-arginine stimulation test. In an embodiment, the GHD subject exhibits a peak blood GH value of about 10 ng/mL (10 µg/L) or less. In another embodiment, the GHD subject exhibits a peak blood GH value of about 9 ng/mL (9 µg/L) or less. In another embodiment, the GHD subject exhibits a peak blood GH value of about 8 ng/mL (8 µg/L) or less. In another embodiment, the GHD subject exhibits a peak blood GH value of about 7 ng/mL (7 µg/L) or less. In another embodiment, the GHD subject exhibits a peak blood GH value of about 6 ng/mL (6 µg/L) or less. In another embodiment, the GHD subject exhibits a peak blood GH value of about 5 ng/mL (5 µg/L) or less. In another embodiment, the GHD subject exhibits a peak blood GH value of about 4 ng/mL (4 µg/L) or less. In an embodiment, the above-mentioned method further comprises identifying an obese subject having GH deficiency, i.e. exhibiting a peak blood GH value according to one or more of the thresholds defined above.

The term "obese subject" as used herein refers to a subject having a body mass index (BMI) of about 30 kg/m² or more. In an embodiment, the obese subject has a BMI of about 31 kg/m² or more. In another embodiment, the obese subject has a BMI of about 32 kg/m² or more. In another embodiment, the obese subject has a BMI of about 33 kg/m² or more. In another embodiment, the obese subject has a BMI of about 34 kg/m² or more. In another embodiment, the obese subject has a BMI of about 35 kg/m² or more. In another embodiment, the obese

subject has a BMI of about 36 kg/m² or more. In another embodiment, the obese subject has a BMI of about 37 kg/m² or more. In another embodiment, the obese subject has a BMI of about 38 kg/m² or more. In another embodiment, the obese subject has a BMI of about 39 kg/m² or more. In another embodiment, the obese subject has a BMI of about 40 kg/m² or more. In another embodiment, the subject is a moderately obese (class I) subject having a BMI of about 30 to about 35 kg/m². In another embodiment, the subject is a severely obese (class II) subject having a BMI of about 35 to about 40 kg/m². In another embodiment, the subject is a very severely obese (class III) subject having a BMI of more than about 40 kg/m².

In an embodiment, the subject has abdominal obesity. In a further embodiment, the subject is a man and has a waist circumference of about 102 cm (about 40 inches) or more. In another embodiment, the subject is a woman and has a waist circumference of about 88 cm (about 35 inches) or more.

In other embodiments, the subject has one or more of the following features/characteristics: (i) a hemoglobin concentration of about 12 g/dL or more, (ii) Serum Glutamylpyruvate Transferase (SGPT) levels less than about 2.5 times normal, (iii) Serum Glutamyloxaloacetate Transferase (SGOT) levels less than about 2.5 times normal, and (iv) creatine levels less than about 1.5 mg/dL. In a further embodiment, the subject has all the features (i) to (iv).

In other embodiments, the subject does not exhibit one or more of the following features: 1) obesity due to a known secondary cause (Cushing's syndrome, hypothyroidism, etc.) or a history of gastric bypass procedure; 2) known history of diabetes or use of any anti-diabetic drugs; 3) use of any weight lowering drugs; 4) use of estrogen, hormone replacement therapy, oral contraceptives, testosterone, glucocorticoids, anabolic steroids, GHRH, GH or IGF-1 within 3 months; 5) changes in lipid lowering or anti-hypertensive regimen within 3 months; 6) chronic illness including HIV, anemia, chronic kidney disease and liver disease; 7) history of malignancy (except surgically cured basal or squamous cell skin cancers) or history of abnormalities on age appropriate malignancy screen including mammography, colonoscopy, positive fecal occult blood testing, and prostate exam or PSA > 5 ng/ml; 8) history of hypopituitarism, pituitary surgery, pituitary/brain radiation or traumatic brain injury or other condition known to affect the GH axis; 9) history of any recent cardiovascular event including myocardial infarction (MI), cerebrovascular accident (CVA) or transient ischemic attack (TIA) within 3 months, unstable angina pectoris, or severe pulmonary disease; 10) recent alcohol or substance abuse (< 1 year); 11) pregnant or lactating females; and 12) FSH >20 IU/L (women).

In an embodiment, the growth hormone deficiency is not due to hypopituitarism.

In an embodiment, the subject exhibits increased carotid intima-media thickness (cIMT) relative to a healthy control subject, or relative to a cIMT value measured at an earlier time point

in the subject. In another embodiment, the subject is at risk of having an increase in carotid intima-media thickness (cIMT).

In another aspect, the present invention provides a method for improving or decreasing cIMT in a growth hormone-deficient (GHD) obese subject, the method comprising administering
5 to a subject in need thereof an effective amount of a growth hormone (GH)-releasing agent.

In an embodiment, the decrease is relative to the cIMT measured prior to administration/treatment). In embodiments, the decrease is of about 0.01 mm or more, in a further embodiment about 0.02 mm or more, (e.g. about 0.03 mm or more). In an embodiment, the decrease is of about 0.01 to 0.05 mm, in a further embodiment of about 0.02 to 0.04 mm,
10 (e.g. about 0.03 mm).

In another embodiment, the decrease in cIMT is relative to a subject (e.g., age and/or gender-matched subject) to whom a GH-releasing agent has not been administered (e.g., a subject treated with a placebo). In embodiments, the decrease in cIMT is of about 0.01 mm or more, in a further embodiment about 0.02 , 0.03 or 0.04 mm or more, relative to a subject (e.g.,
15 age and/or gender-matched subject) not administered with a GH-releasing agent (e.g., administered with a placebo). In an embodiment, the decrease in cIMT is of about 0.01 to 0.07 mm, in further embodiments of about 0.02 to 0.06 mm (e.g. about 0.03 mm), relative to a subject (e.g., age and/or gender-matched subject) not administered with a GH-releasing agent (e.g., administered with a placebo). In an embodiment, the decrease in cIMT is of about 0.003
20 to 0.09 mm, in further embodiments of about 0.01 to 0.09 mm (e.g. about 0.05 mm), relative to a subject (e.g., age and/or gender-matched subject) not administered with a GH-releasing agent (e.g., administered with a placebo).

In another embodiment, the decrease in cIMT is of at least about 3%, 4%, 5% or 6%, in further embodiment, from about 3% to about 10%, for example from about 4% to about 8% or
25 about 5% to about 7%.

In another aspect, the present invention provides a method for preventing an increase, over time, in cIMT in a growth hormone-deficient (GHD) obese subject, the method comprising administering to a subject in need thereof an effective amount of a growth hormone (GH)-releasing agent. In an embodiment, the prevention of the increase is relative to a subject (e.g.,
30 age and/or gender-matched subject) not administered with a GH-releasing agent (e.g., administered with a placebo).

cIMT may be measured using methods well known in the art, for example ultrasound-based methods (see, e.g., Stein JH *et al.*, Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the
35 American Society of Echocardiography Carotid Intima-Media Thickness Task Force endorsed by the Society for Vascular Medicine." *J Am Soc Echocardiogr.* 2008 Feb;21(2):93-111; quiz 189-90).

In embodiments, the administration of growth hormone (GH)-releasing agent leads to a significant decrease in certain body composition parameters including abdominal visceral adipose tissue (VAT), waist circumference, trunk fat, and total fat, with no significant change in subcutaneous adipose tissue (SAT) (*e.g.*, abdominal SAT), in the subject.

5 In other embodiments, the administration of growth hormone (GH)-releasing agent prevents the increase in certain body composition parameters including abdominal visceral adipose tissue (VAT), waist circumference, trunk fat, and total fat, with no significant change in subcutaneous adipose tissue (SAT) (*e.g.*, abdominal SAT), in the subject.

Accordingly, in another aspect, the present invention provides a method for:

- 10 (a) preventing or treating vascular stenosis and/or associated conditions; and
(b) decreasing, and/or preventing the increase of, at least one of the following body composition parameters: abdominal visceral adipose tissue (VAT), waist circumference, trunk fat, total fat, and the VAT to subcutaneous adipose tissue (SAT) ratio (VAT/SAT), without significantly decreasing subcutaneous adipose
15 tissue;

in a growth hormone-deficient (GHD) obese subject, the method comprising administering to a subject in need thereof an effective amount of a growth hormone (GH)-releasing agent.

In another aspect, the present invention provides a method for:

- (a) preventing, or reducing the risk of developing, a condition related to vascular
20 stenosis; and
(b) decreasing, and/or preventing the increase of, at least one of the following body composition parameters: abdominal VAT, waist circumference, trunk fat, total fat, and the VAT/SAT ratio, without significantly decreasing SAT (*e.g.*, abdominal SAT);

25 in a growth hormone-deficient (GHD) obese subject, the method comprising administering to a subject in need thereof an effective amount of a growth hormone (GH)-releasing agent.

In another aspect, the present invention further provides a method for decreasing, and/or preventing the increase of, at least one of the following body composition parameters: abdominal VAT, waist circumference, trunk fat, total fat, and the VAT/SAT ratio, without
30 significantly decreasing SAT (*e.g.*, abdominal SAT), in a growth hormone-deficient (GHD) obese subject, the method comprising administering to a subject in need thereof an effective amount of a growth hormone (GH)-releasing agent. In an embodiment, the GHD obese subject is a GHD abdominally obese subject.

In an embodiment, the body composition parameter is abdominal VAT or the VAT/SAT
35 ratio. In a further embodiment, the body composition parameter is abdominal VAT. In another embodiment, the body composition parameter is the VAT/SAT ratio.

The term "without significantly decreasing SAT" as used herein means that the change in SAT between the subject and a control subject or a group of control subjects not administered with a GH-releasing agent, e.g., administered with a placebo, over a given period of time, is not statistically significant as measured by conventional statistical methods, for example those described in the examples below.

In an embodiment, the decrease in the body composition parameter is an absolute decrease in the subject (i.e. relative to the body composition parameter measured prior to administration/treatment). In an embodiment, the body composition parameter is VAT. In a further embodiment, the decrease in VAT is of about 7 to about 23 cm², for example from about 10 to about 20 cm² or from about 15 to about 17 cm² (e.g., about 16 cm²). In another embodiment, the body composition parameter is waist circumference (WC). In a further embodiment, the decrease in WC is of about 1 to about 3 cm, for example about 2 cm.

In another embodiment, the decrease in the body composition parameter is a decrease relative to a control subject or a group of control subjects not administered with a GH-releasing agent, e.g., administered with a placebo, over a given period of time. In an embodiment, the body composition parameter is VAT. In a further embodiment, the decrease in VAT is a decrease of about 15% or more, for example of about 16%, 17%, 18%, 19%, 20% or more relative to a control subject or a group of control subjects. In an embodiment, the decrease in VAT is a decrease of about 12 cm² or more, for example about 35 cm² or about 40 cm² or more, or a decrease of more than 40 cm² relative to a control subject or a group of control subjects. In an embodiment, the decrease in VAT is a decrease of from about 12 cm² to about 58 cm², for example from about 20 cm² to about 50 cm², from about 25 cm² to about 45 cm², from about 30 cm² to about 40 cm² (e.g., about 35 cm²), from about 35 cm² to about 58 cm² or about 40 cm² to about 58 cm², of more than 40 to about 58 cm², relative to a control subject or a group of control subjects. In other embodiments, the decrease in VAT is a decrease of about 2 cm² or more relative to a control subject or a group of control subjects. In an embodiment, the decrease in VAT is a decrease of from about 2 cm² to about 63 cm², for example from about 15 cm² to about 50 cm², from about 25 cm² to about 40 cm², from about 28 cm² to about 36 cm² (e.g., about 32 cm²), relative to a control subject or a group of control subjects.

In another embodiment, the body composition parameter is waist circumference (WC). In a further embodiment, the decrease in WC is of about 0.3 to about 5 or 6 cm, in further embodiments of about 1 to about 5 cm, about 2 to about 4 cm (e.g., about 3 cm). In an embodiment, the decrease in WC is achieved exclusively on reductions in VAT (abdominal VAT), without any significant reduction in SAT (abdominal SAT).

In embodiments, the administration of growth hormone (GH)-releasing agent also leads to a significant increase in lean body mass.

In other embodiments, the administration of growth hormone (GH)-releasing agent prevents the decrease in lean body mass, in the subject.

Accordingly, in another aspect, the present invention provides a method for:

- (a) preventing or treating vascular stenosis and/or associated conditions; and
- (b) increasing, and/or preventing the decrease of lean body mass

in a growth hormone-deficient (GHD) obese subject, the method comprising administering to a subject in need thereof an effective amount of a growth hormone (GH)-releasing agent.

In another aspect, the present invention provides a method for:

- (c) preventing, or reducing the risk of developing, a condition related to vascular stenosis; and
- (d) increasing, and/or preventing the decrease of lean body mass;

in a growth hormone-deficient (GHD) obese subject, the method comprising administering to a subject in need thereof an effective amount of a growth hormone (GH)-releasing agent.

In another aspect, the present invention further provides a method for increasing, and/or preventing the decrease of lean body mass, in a growth hormone-deficient (GHD) obese subject, the method comprising administering to a subject in need thereof an effective amount of a growth hormone (GH)-releasing agent. In an embodiment, the GHD obese subject is a GHD abdominally obese subject.

In embodiments, the administration of growth hormone (GH)-releasing agent leads to a significant decrease in certain biochemical parameters including triglycerides and C-reactive protein (CRP) in the subject. Accordingly, the above-mentioned methods further comprise decreasing, and/or preventing the increase of, triglycerides and/or C-reactive protein levels in the subject.

In an embodiment, the decrease in triglycerides and/or logCRP is relative to the levels measured in the subject prior to administration of the GH-releasing agent. In a further embodiment, the decrease in triglycerides is of about 10 to about 42 mg/dL, for example of about 15 to about 37 mg/dL or about 20 to about 32 mg/dL (e.g., about 26 mg/dL). In another embodiment, the decrease in CRP, determined based on the log-transformed value of C-reactive protein concentration in serum, logCRP, is of about 0.13 to about 0.21, for example of about 0.15 to about 0.19 (e.g., about 0.17).

In another embodiment, the decrease is relative to a control subject or a group of control subjects (e.g., aged and/or gender-matched) not administered with a GH-releasing agent, e.g., administered with a placebo, over a given period of time. In an embodiment, the decrease in triglycerides is of about 10 to about 30%, for example from about 15 to about 25%, about 17% to about 23% (e.g., about 20%), relative to a control subject or a group of control subjects. In another embodiment, the decrease in triglycerides is of about 5 to about 67 to 80 mg/dL, for example of about 20 to about 55-60 mg/dL, about 30 to about 45 mg/dL or about 35

to about 40 mg/dL (e.g., about 37 mg/dL), relative to a control subject or a group of control subjects.

In an embodiment, the decrease in logCRP is of about 10 to about 35%, for example from about 15 to about 30%, about 20% to about 28% (e.g., about 24%), relative to a control subject or a group of control subjects. In another embodiment, the decrease in logCRP is of about 0.01 to about 0.30, for example of about 0.1 to about 0.2, about 0.13 to about 0.17 (e.g., about 0.15), relative to a control subject or a group of control subjects.

In embodiments, the above-mentioned administration/treatment results in an increase in IGF-1 levels. In an embodiment, the increase in IGF-1 levels is relative to the levels measured in the subject prior to administration of the GH-releasing agent. In an embodiment, the increase in IGF-1 levels is of about 60 to about 110 µg/L, in embodiments of about 65 to about 107 µg/L, about 75 to about 100 µg/L, about 80 to about 95 µg/L or about 80 to about 90 µg/L (e.g. about 86 µg/L). In an embodiment, the increase is relative to a control subject or a group of control subjects (e.g., aged and/or gender-matched) not administered with a GH-releasing agent, e.g., administered with a placebo, over a given period of time. In an embodiment, the increase in IGF-1 levels of about 52-54 to about 157 µg/L, for example 52 to 132 µg/L, in embodiments of about 70 to about 120 µg/L, about 80 to about 110 µg/L, about 92 to about 105 µg/L.

In an embodiment, the administration/treatment is associated with no significant change in blood glucose control in the subject. The term "no significant change in blood glucose control" as used herein means that (i) the difference in blood glucose levels (as measured by conventional assays, e.g. those described in the examples below) between the subject and a control subject or a group of control subjects to whom a GH-releasing agent has not been administered (e.g., administered with a placebo), over a given period of time, is not statistically significant as measured by conventional statistical methods, for example those described in the examples below; and/or (ii) that the administration/treatment is not associated with a statistically significant increase (relative to no treatment or placebo) in the frequency of patients developing conditions related to impaired glucose control such as insulin resistance, glucose intolerance and/or diabetes (i.e. no clinically significant effects).

The term "treatment" or "treating" as used herein, is defined as the application or administration of the above-mentioned GH-releasing agent or a composition comprising same to a subject, or application or administration of the above-mentioned GH-releasing agent or a composition comprising same to an isolated tissue or cell line from a subject, who has a disorder, a disease, a symptom of disorder or disease, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, reduce the progression or affect the disorder/disease and/or the symptoms of disorder/disease.

The term "prevention" or "preventing" as used herein, is defined as the application or administration of the above-mentioned GH-releasing agent or a composition comprising same to a subject, or application or administration of the above-mentioned GH-releasing agent or a composition comprising same to an isolated tissue or cell line from a subject, who has a predisposition toward a disorder/disease or who is at risk of developing the disorder/disease, with the purpose to prevent or delay the onset of the disease/disorder or of the symptoms, or reduce the severity of the disease/disorder or of the symptoms, when administered prior to the onset/appearance of the disease/disorder or of the symptoms.

An "effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired biological activity and/or the prophylactic/therapeutic result (e.g., prevention and/or treatment of the diseases/disorders noted above). A "therapeutically effective amount" refers to an effective amount in the context of therapy; a "prophylactically effective amount" refers to an effective amount in the context of prophylaxis. An effective amount of a compound of the invention may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the compound to elicit a desired response in the individual. Dosage regimens may be adjusted to provide the optimum prophylactic/therapeutic response. An effective amount is also one in which any toxic or detrimental effects of the compound are outweighed by the prophylactic/therapeutic beneficial effects. For any particular subject, specific dosage regimens may be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions. In an embodiment, the GH-releasing agent is administered at a daily dose of about 0.01 mg to about 30 mg, in a further embodiment of about 0.1 mg to about 20 or 25 mg, in a further embodiment of about 0.5 mg to about 20 mg, in a further embodiment at a daily dose of about 1 mg to about 20 mg, for example at a dose of about 1 to about 2 mg (e.g., 1 mg, 1.5 mg or 2 mg).

The GH-releasing agent or composition comprising same may be administered, or may be for administration, by any conventional route, such as intravenous, oral, transdermal, intraperitoneal, subcutaneous, mucosal, intramuscular, intranasal, intrapulmonary, parenteral or topical. In an embodiment, the GRF analog or pharmaceutically acceptable salt thereof is administered or is for administration by a subcutaneous route. In another embodiment, the GH-releasing agent or composition comprising same is administered or is for administration by a transdermal route (e.g., using transdermal delivery systems such as transdermal patches). Accordingly, in another aspect, the present invention provides a transdermal delivery system, such as a transdermal patch, comprising the above-mentioned GH-releasing agent or composition comprising same. Methods and systems for transdermal delivery are well known in the art and are described, for example, in *Transdermal Drug Delivery*, Second Edition, Marcel Dekker Inc., New York, 2003 and *Transdermal and Topical Drug Delivery*, Adrian C Williams,

Pharmaceutical Press, 2003. In another embodiment, the GH-releasing agent or composition comprising same is administered or is for administration by the oral route. In an embodiment, the GH-releasing agent or composition comprising same is administered or is for administration by a nasal route.

5 In an embodiment, the above-mentioned prevention and/or treatment comprises administration of the above-mentioned GH-releasing agent or composition comprising same, in combination with one or more additional active/therapeutic agents. The combination of prophylactic/therapeutic agents and/or compositions may be administered or co-administered (e.g., consecutively, simultaneously, at different times) in any conventional dosage form. Co-
10 administration in the context of the present invention refers to the administration of more than one therapeutic in the course of a coordinated treatment to achieve an improved clinical outcome. Such co-administration may also be coextensive, that is, occurring during overlapping periods of time. For example, a first agent may be administered to a patient before, concomitantly, before and after, or after a second active agent is administered. The agents may
15 in an embodiment be combined/formulated in a single composition and thus administered at the same time. In an embodiment, the one or more active agent(s) of the present invention is used/administered in combination with one or more agent(s) currently used to prevent or treat the disorder in question. In embodiments, the GH-releasing agent or composition comprising same may be used in combination with drugs used in the treatment of vascular stenosis such as
20 atherosclerosis and associated conditions including anti-atherosclerosis drugs, anti-thrombosis drugs, anti-inflammatory drugs, ACE Inhibitors, cholesterol lowering drugs (e.g., statins), drugs used in the prevention or treatment of cardiovascular diseases such as peripheral antiadrenergic drugs, antihypertensive drugs, drugs affecting renin-angiotensin system, antianginal drugs, cardiac glycosides, inodilators (e.g., aminone, milrinone, enoximone, fenoximone, imazodan, sulmazole), antidysrhythmic drugs, calcium entry blockers, ranitine,
25 bosentan, and rezulin.

In an embodiment, the above-mentioned administration/treatment is for a period of at least 3 months, in further embodiment of at least 6 months, 9 months or 12 months.

MODE(S) FOR CARRYING OUT THE INVENTION

30 The present invention is illustrated in further details by the following non-limiting examples.

Example 1: Methods

Subjects:

Subjects were recruited at Massachusetts General Hospital between June 2008 and
35 November 2010. Eligibility criteria were: 1) 18-55 year old men and women; 2) BMI ≥ 30 kg/m²; 3) waist circumference ≥ 102 cm (men) and 88 cm (women); 4) peak stimulated GH ≤ 9 μ g/L on

standardized GH releasing hormone (GHRH)-arginine stimulation test; 5) hemoglobin >12.0 g/dL, SGOT and SGPT < 2.5 times normal, and creatinine < 1.5 mg/dL.

Exclusion criteria for the study were: 1) obesity due to a known secondary cause (Cushing's syndrome, hypothyroidism, etc.) or a history of gastric bypass procedure; 2) known history of diabetes or use of any anti-diabetic drugs; 3) current use of any weight lowering drugs; 4) use of estrogen, hormone replacement therapy, oral contraceptives, testosterone, glucocorticoids, anabolic steroids, GHRH, GH or IGF-1 within 3 months of enrollment; 5) changes in lipid lowering or anti-hypertensive regimen within 3 months of screening; 6) chronic illness including HIV, anemia, chronic kidney disease and liver disease; 7) history of malignancy (except surgically cured basal or squamous cell skin cancers) or history of abnormalities on age appropriate malignancy screen including mammography, colonoscopy, positive fecal occult blood testing, and prostate exam or PSA > 5 ng/ml; 8) history of hypopituitarism, pituitary surgery, pituitary/brain radiation or traumatic brain injury or other condition known to affect the GH axis; 9) history of any recent cardiovascular event including MI, CVA or TIA within 3 months of screening, unstable angina pectoris, or severe pulmonary disease; 10) recent alcohol or substance abuse (< 1 year); 11) pregnant or lactating females; 12) FSH >20 IU/L (women).

Study Design and Interventions:

Subjects were randomized in a 1:1 fashion to receive 2 mg of the GRF analog (hexenoyl trans-3)hGHRH₍₁₋₄₄₎NH₂ or matching placebo. (Hexenoyl trans-3)hGHRH₍₁₋₄₄₎NH₂ and matching placebo were supplied as a lyophilized powder and subjects were trained to reconstitute and self-inject the medication subcutaneously daily for one year. The randomization was stratified by gender. Investigators and subjects were blinded to the treatment randomization. Assessment for the primary end point of abdominal VAT and CVD risk factors were performed at baseline, 26 weeks and 52 weeks. Safety parameters including fasting glucose and IGF-1 were also measured at 2 weeks, 4 weeks, 12 weeks and 36 weeks. Subjects with a fasting glucose greater than 125 mg/dl were discontinued from the study. IGF-1 levels were monitored by a physician independent from the study team. A dose algorithm was incorporated into the protocol such that if a subject's IGF-1 level increased above the age-adjusted upper limit of normal then the subject (and a matched placebo-treated subject) would be instructed to decrease the study drug dose to 1 mg once daily. Subjects with a repeatedly elevated age-adjusted IGF-1, despite dose adjustment, were discontinued from the study (FIG. 1). (Hexenoyl trans-3)hGHRH₍₁₋₄₄₎NH₂ was used under an investigator initiated IND (IND#:73,329).

Assessments:

GH Stimulation Test:

Standardized GHRH-arginine stimulation testing was performed after an overnight fast.

Measurement of Visceral Adiposity:

- 5 Abdominal visceral (VAT) and subcutaneous adipose tissue (SAT) were assessed using a single cross-sectional slice from non-contrast computed tomography (CT) at the L4 level (Borkan GA, *et al.*, Am J Clin Nutr 1982;36:172-7).

Carotid Intima-Media Thickness (cIMT):

- 10 cIMT was measured via ultrasound as previously described (Chan R, *et al.*, Computers in Cardiology 2000;27:37-40; Makimura H, *et al.*, J Clin Endocrinol Metab 2009;94:5131-8). The average cIMT over the length of the measured segments on the right carotid artery is reported.

Body Composition:

- 15 Body composition was assessed by dual energy x-ray absorptiometry (DXA, previously DEXA) (Hologic Discover™ A densitometer, software version APEX 3.1).

Laboratory Assessments:

- 20 GH was assessed using a chemiluminescent immunoassay (Beckman Coulter). IGF-1 was measured using the Immulite™ 2000 automated immuno-analyzer (Siemens Diagnostics). Lipid profile, glucose and high sensitivity CRP were assessed at the Massachusetts General Hospital (MGH) laboratory using standard methods (Falutz J, *et al.*, 2007 *N Engl J Med* 357:2359–2370; Falutz J *et al.*, 2010 *J Acquir Immune Defic Syndr* 53:311-322).

25 Compliance:

Adherence to the study drug was monitored via subject self-reported injection log, as well as a vial count of returned vials at each visit.

Statistical Methods:

- 30 The endpoints in this study included change in VAT from baseline, changes in cIMT, body composition, IGF-1, lipid profile, glucose, HbA1c and other safety parameters. Assuming evaluable post-baseline data on 48 patients, the study was powered at 80% to detect a difference of 32 cm² in VAT between the groups, with $\alpha=0.05$ and an estimated treatment SD of 38.6 cm² from prior studies of (hexenoyl trans-3)hGHRH₍₁₋₄₄₎NH₂ in lipodystrophy (Falutz J, *et al.* N Engl J Med 2007;357:2359-70).
- 35

Baseline variables were compared by chi-square test for non-continuous variables, t-test for continuous variables that were normally distributed and Wilcoxon's rank sum test for

continuous variables that were not normally distributed. Efficacy end points were analyzed using longitudinal linear mixed effects modeling with all available data and the last value carried forward for patients who discontinued and had missing data. A secondary analysis was performed using longitudinal linear mixed effects modeling with all available data, without carry forward. The reported treatment effect represents estimated differences between (hexenoyl trans-3)hGHRH₍₁₋₄₄₎NH₂ and placebo treatment over 12 months in the model. The P value was determined from the time x randomization effect in the model. Statistical analyses were performed using SAS and JMP 9.0 (SAS Institute). All reported P values are two-sided.

Sensitivity analyses were performed excluding data from subjects with elevated IGF-1 (n=4). These analyses were conducted to explore whether the effect on VAT and other endpoints was seen if the analysis was limited to patients in whom IGF-1 was within normal limits for the study duration. In addition, sensitivity analyses were performed including use of lipid lowering and antihypertensive therapies in the mixed effects modeling for cIMT and use of lipid lowering therapy for triglyceride.

Example 2: Results

Subjects:

148 obese patients were screened and 60 obese subjects with reduced GH secretion were enrolled and randomized (FIG. 1). Baseline demographics were similar between the groups including age, race and gender (Table 1). No patient had known coronary artery disease. No significant differences in VAT, other body composition parameters, cIMT or IGF-1 were noted between (hexenoyl trans-3)hGHRH₍₁₋₄₄₎NH₂ and the placebo group. The two groups did not differ for use of lipid lowering medications or anti-hypertensive medication use. The (hexenoyl trans-3)hGHRH₍₁₋₄₄₎NH₂ group had higher mean triglycerides at baseline (Table 2).

Two subjects completed the baseline visit but discontinued prior to initiation of study drug, resulting in 58 subjects who initiated treatment with (hexenoyl trans-3)hGHRH₍₁₋₄₄₎NH₂ (n=29) and placebo (n=29). Five subjects in each arm discontinued before the 6-month visit for a retention rate of 83% at 6 months in each arm. Seventeen subjects in the placebo group and 19 subjects in the (hexenoyl trans-3)hGHRH₍₁₋₄₄₎NH₂ group completed the 1 year study, for an overall retention rate of 62%, which was not different between the groups (FIG. 1).

Table 1: Demographic and baseline clinical characteristics of the patients enrolled in the study stratified by treatment group.

	(hexenoyl trans-3)hGHRH ₍₁₋₄₄₎ NH ₂ (N= 31)	Placebo (N= 29)	P value
Demographics			
Age (years)	42.5 ± 1.3	39.9 ± 1.8	0.25
Gender-M/F (%)	20/11 (65.5%)	19/10 (64.5%)	0.94

male)			
Race (% Caucasian)	16 (51.6%)	19 (65.5%)	0.28
Current or Past Tobacco Use	18 (58%)	13 (45%)	0.31
Medication Use*			
HMG Co-A Reductase Inhibitor (%)	3 (10 %)	1 (3%)	0.33
Lipid lowering therapy†(%)	8 (26%)	12 (41%)	0.20
Anti-hypertensive (%)	10 (32%)	5 (17%)	0.18
Body Composition			
Weight (kg)	115.7 ± 3.8	115.8 ± 3.3	0.99
Body Mass Index (kg/m ²)	[37.5 (34, 41.4)]	[38.3 (34.4, 40.5)]	0.81
Waist Circumference (cm)	121 ± 2	121 ± 2	0.94
VAT (cm ²)	209 ± 13	193 ± 14	0.39
Growth Hormone Parameters			
Fasting GH (μg/L)	[0.04 (0.02, 0.13)]	[0.05 (0.02, 0.15)]	0.41
Peak stimulated GH on GHRH-arginine stimulation test (μg/L)	4.22 ± 0.46	5.19 ± 0.47	0.15
IGF-1 (μg/L)	[101 (79, 145)]	[131 (107, 172)]	0.17

Non-continuous variables are compared using χ^2 test. Results are presented as the mean ± SEM for normally distributed data and analyzed by Student's t-test. For data that is not normally distributed, results are presented as median with interquartile range (25%, 75%) and analyzed using the Wilcoxon rank sum test. One patient in the (hexenoyl trans-3)hGHRH₍₁₋₄₄₎NH₂ group discontinued the study prior to obtaining baseline body composition data. *Medication use includes baseline data as well as initiation of new medications during the course of the study. †Lipid lowering medication use includes subjects using HMG Co-A reductase inhibitors, niacin and fish oil.

Abbreviations: HMG Co-A: 3-hydroxy-3-methyl-glutaryl-CoA reductase; VAT: abdominal visceral adipose tissue area (measured via abdominal computed tomography (CT) scan); GH: Growth hormone; GHRH: Growth hormone releasing hormone; IGF-1: Insulin-like growth factor-1.

Body Composition:

VAT (-16±9 vs. 19±9 cm², (hexenoyl trans-3)hGHRH₍₁₋₄₄₎NH₂ vs. placebo; net treatment effect vs. placebo [95% CI]: -35 [-58,-12]cm²; P=0.003) (Table 2 and FIG. 2) improved significantly in the (hexenoyl trans-3)hGHRH₍₁₋₄₄₎NH₂ group versus placebo. Other body composition parameters including waist circumference (WC) (-2±1 vs. 1±1cm; -3[-5,-0.3]cm; P=0.03), trunk fat (-0.6±0.4 vs. 0.7±0.4kg; -1.4 [-2.4, -0.3]kg; P=0.01), total fat (-0.7±0.7 vs.

1.0±0.7kg; -1.7 [-3.4, -0.1]kg; P=0.04), and lean body mass (1.0±0.5 vs. -0.4±0.4kg; 1.4 [0.2, 2.6]kg; P=0.03) improved in the (hexenoyl trans-3)hGHRH₍₁₋₄₄₎NH₂ versus placebo groups, whereas no significant effects on abdominal SAT (-6±6 vs. 3±11cm²; -10 [-32,13]cm²; P=0.40) or weight (0.1±1.1 vs. 0.9±1.1kg; -0.8 [-3.2, 1.6]kg, P=0.52) were seen. The changes in VAT
 5 amounted to a net treatment effect versus placebo of -19%. Results remained significant adjusting for gender (P=0.01) without any gender effect seen in the model. Similar results were seen in an analysis performed without carrying forward for missing data (Table 3).

Carotid IMT:

10 Treatment with (hexenoyl trans-3)hGHRH₍₁₋₄₄₎NH₂ for 12 months resulted in a significant reduction in cIMT compared to placebo (-0.03±0.01 vs. 0.01±0.01mm; -0.04 [-0.07,-0.01]mm; P= 0.02) for a net difference in mean percentage change of -6% (Table 2 and FIG. 2). Results remained significant in sensitivity analyses performed without carrying forward missing data (Table 3).

Biochemical Indices:

15 Treatment with (hexenoyl trans-3)hGHRH₍₁₋₄₄₎NH₂ resulted in increased IGF-1 (86±21 vs. -6±8µg/L; 92 [52, 132]µg/L; P<0.0001) (FIG. 2), decreased triglycerides (-26±16 vs. 12±8mg/dL; -37 [-67, -7]mg/dL; P=0.02) and decreased logCRP (-0.17±0.04 vs. -0.03±0.05mg/L; -0.15 [-0.30,-0.01]mg/L, P=0.04) compared to placebo. In terms of overall percent change, (hexenoyl trans-3)hGHRH₍₁₋₄₄₎NH₂ increased IGF-1 by 90% and decreased triglycerides by 20% and logCRP by 24% compared to placebo. (Hexenoyl trans-3)hGHRH₍₁₋₄₄₎NH₂ did not affect total cholesterol, HDL or LDL cholesterol (Table 2).

Dietary and Physical Activity Measurements:

25 There were no differences in dietary intake or physical activity between groups (all P>0.10) (Tables 4 and 6).

Sensitivity Analyses:

30 Similar results were seen, with significant effects of (hexenoyl trans-3)hGHRH₍₁₋₄₄₎NH₂ compared to placebo on VAT (-32 [-56, -9]cm²; P=0.008), cIMT (-0.05 [-0.08, -0.02]mm; P=0.004), triglyceride (-36 [-68, -4]mg/dL; P=0.03), logCRP (-0.15 [-0.30, 0]mg/L; P=0.05) and IGF-1 (78 [44, 111]µg/L; P<0.0001) in the sensitivity analyses excluding patients with dose reductions and elevated IGF-1. The effects of (hexenoyl trans-3)hGHRH₍₁₋₄₄₎NH₂ remained
 35 significant for cIMT and triglyceride in longitudinal linear mixed effect models including use of lipid lowering and antihypertensive therapies.

IGF-1 (µg/L)	160±43	142±12	0.17	225±42	134±9	234±41	139±10
Total Chol (mg/dL)	176±5	168±5	0.28	186±5	175±5	180±6	174±6
Triglycerides (mg/dL)	196±26	132±11	0.03	166±17	133±10	170±18	141±13
HDL Chol (mg/dl)	35±1	36±2	0.61	40±2	40±2	39±2	39±2
LDL Chol (mg/dL)	114±5	117±6	0.72	125±6	119±6	118±5	117±6
Log CRP*	0.51±0.15	0.66±0.09	0.89	0.42±0.15	0.65±0.08	0.35±0.15	0.64±0.08
Carotid IMT (mm)	0.68±0.02	0.66±0.04	0.18	0.68±0.02	0.67±0.04	0.66±0.02	0.67±0.04
Safety							
Systolic BP (mm Hg)	121±2	120±2	0.89	120±2	124±2	120±2	123±2
Diastolic BP (mm Hg)	78±2	76±1	0.54	79±1	79±1	75±2	77±2
Fasting glucose (mg/dL)	95±3	92±2	0.79	95±5	88±2	98±5	92±3
2-hour glucose (mg/dL)	138±7	134±6	0.71	126±9	136±9	127±9	136±10
Insulin (µIU/mL)	9.3±1.1	8.6±0.9	0.78	12.7±3.3	9.3±1.0	13.3±3.3	10.4±1.2
HOMA-IR	2.25±0.29	1.98±0.21	0.69	3.42±1.08	2.03±0.22	3.59±1.08	2.47±0.33
HbA1c (%)	5.8±0.1	5.6±0.1	0.26	6.1±0.1	5.8±0.1	6.0±0.1	5.8±0.1
AST (U/L)	25±3	25±2	0.52	25±3	29±3	25±3	26±3
ALT (U/L)	29±3	30±4	0.61	29±5	34±4	27±4	29±3

	Change		Effect Size for GRF vs. Placebo	
	GRF	Placebo	Effect Size (95% CI)	P value
Body Composition				
VAT (cm ²)	-16±9	19±9	-35 (-58, -12)	0.003
SAT (cm ²)	-6±6	3±11	-10 (-32, 13)	0.40
Weight (kg)	0.1±1.1	0.9±1.1	-0.8 (-3.2, 1.6)	0.52
BMI (kg/m ²)	-0.1±0.4	0.6±0.4	-0.6 (-1.4, 0.2)	0.14
Waist circ (cm)	-2±1	1±1	-3 (-5, -0.3)	0.03
Lean mass (kg)	1.0±0.5	-0.4±0.4	1.4 (0.2, 2.6)	0.03
Fat mass (kg)	-0.7±0.7	1.0±0.7	-1.7 (-3.4, -0.1)	0.04
Trunk fat (kg)	-0.6±0.4	0.7±0.4	-1.4 (-2.4, -0.3)	0.01
Biochemical				
IGF-1 (µg/L)	86±21	-6±8	92 (52, 132)	<0.0001
Total Chol	4±4	7±5	-2	0.69

(mg/dL)			(-13, 9)	
Triglycerides (mg/dL)	-26±16	12±8	-37 (-67, -7)	0.02
HDL Chol (mg/dL)	4±1	3±1	1 (-3, 5)	0.66
LDL Chol (mg/dL)	4±4	1±4	3 (-8, 14)	0.60
Log CRP*	-0.17±0.04	-0.03±0.05	-0.15 (-0.30, -0.01)	0.04
Carotid IMT (mm)	-0.03±0.01	0.01±0.01	-0.04 (-0.07, -0.01)	0.02
Safety				
Systolic BP (mm Hg)	-1±3	3±2	-5 (-11, 2)	0.15
Diastolic BP (mm Hg)	-3±2	1±1	-3 (-7, 1)	0.12
Fasting glucose (mg/dL)	2±3	1±2	1 (-5, 8)	0.73
2-hour glucose (mg/dL)	-11±7	-3±8	-10 (-27, 7)	0.25
Insulin (μIU/ml)	4.0±3.3	1.8±1.0	-0.3 (-7.5, 7.0)	0.94
HOMA-IR	1.46±1.08	0.50±0.26	-0.11 (-2.41, 2.19)	0.93
HbA1c (%)	0.2±0.1	0.2±0.1	-0.002 (-0.2, 0.2)	0.98
AST (U/L)	-1±2	1±2	-1 (-8, 5)	0.71
ALT (U/L)	-2±2	-1±2	-1 (-8, 5)	0.69

Results are presented as mean ± SEM. P value from baseline data are obtained by Student's t-test for normally distributed variables and Wilcoxon rank sum for non-normally distributed samples. Effects size and P values were obtained by longitudinal linear mixed effects modeling for each parameter over 12 months with last value carried forward. One patient in the (hexenoyl trans-3)hGHRH₍₁₋₄₄₎NH₂ group discontinued the study prior to obtaining baseline body composition and biochemical data. *For CRP, the final value in one patient was 5 SD above all other data points and over 100 points above all her prior data points. This patient had skin manifestations and swelling from multiple insect bites while camping immediately prior to the final visit. Therefore this point data was excluded as an outlier. Instead data was assessed from her 9 month safety visit, prior to the insect bites, and used in the analysis.

Abbreviations: VAT: visceral abdominal adipose tissue area; SAT: sub-cutaneous abdominal adipose tissue area; BMI: body mass index; IMT: intima-media thickness; HbA1c: Hemoglobin A1c; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GRF: (hexenoyl trans-3)hGHRH₍₁₋₄₄₎NH₂; P: P value.

Table 3. Effect of treatment with (hexenoyl trans-3)hGHRH₍₁₋₄₄₎NH₂ versus placebo on body composition, biochemical indices, carotid intima media thickness, and safety parameters analyzed by longitudinal linear mixed effects modeling without carrying forward missing data.

	Baseline			6-Month		12-Month	
	GRF	Placebo	P	GRF	Placebo	GRF	Placebo
Body Composition							
VAT (cm ²)	209±13	193±14	0.39	192±16	198±16	198±20	193±16

SAT (cm ²)	480±26	535±28	0.15	468±27	537±31	451±29	512±42
Weight (kg)	115.7±3.8	115.8±3.3	0.99	114.8±4.1	116.8±4.0	117.2±5.1	111.9±4.4
BMI (kg/m ²)	38.2±0.9	37.9±0.7	0.81	38.1±1.0	38.4±0.8	38.5±1.1	37.9±1.1
Waist circ (cm)	121±2	121±2	0.94	119±3	121±3	119±3	121±3
Lean mass (kg)	71.8±2.5	70.7±2.2	0.74	72.0±2.8	70.2±2.6	73.8±3.4	67.6±2.7
Fat mass (kg)	42.6±1.9	44.0±2.0	0.26	41.7±2.3	44.7±2.6	42.2±2.8	43.4±3.5
Trunk fat (kg)	23.3±1.3	23.3±1.1	0.53	22.5±1.5	23.5±1.4	22.6±1.7	23.3±2.0
Biochemical							
IGF-1 (µg/L)	160±43	142±12	0.17	203±23	135±11	216±20	144±14
Total Chol (mg/dl)	176±5	168±5	0.28	187±6	176±6	179±8	170±8
Triglycerides (mg/dl)	196±26	132±11	0.03	171±19	126±10	153±16	140±19
HDL Chol (mg/dl)	35±1	36±2	0.61	40±2	41±3	39±2	40±3
LDL Chol (mg/dl)	114±5	117±6	0.72	126±6	121±6	121±7	115±7
Log CRP*	0.51±0.15	0.66±0.09	0.89	0.60±0.07	0.62±0.09	0.42±0.09	0.54±0.11
Carotid IMT (mm)	0.68±0.02	0.66±0.04	0.18	0.68±0.03	0.67±0.04	0.67±0.03	0.70±0.06
Safety							
Systolic BP (mm Hg)	121±2	120±2	0.89	120±3	124±2	122±3	123±2
Diastolic BP (mm Hg)	78±2	76±1	0.54	78±2	79±1	73±3	76±2
Fasting glucose (mg/dl)	95±3	92±2	0.79	95±5	87±2	93±3	93±4
2-hour glucose (mg/dl)	138±7	134±6	0.71	129±10	134±10	121±9	134±14
Insulin (µIU/ml)	9.3±1.1	8.6±0.9	0.78	13.5±4.0	9.1±1.2	9.1±1.4	11.1±1.9
HOMA-IR	2.25±0.29	1.98±0.21	0.69	3.68±1.29	1.97±0.25	2.10±0.35	2.71±0.55
HbA1c (%)	5.8±0.1	5.6±0.1	0.26	6.2±0.1	5.8±0.1	5.9±0.2	5.9±0.1
AST (U/L)	25±3	25±2	0.52	26±3	30±3	27±4	22±2
ALT (U/L)	29±3	30±4	0.61	31±5	34±4	29±5	26±3

Effect Size for GRF vs. Placebo

40

	Effect Size (95% CI)	P value
Body Composition		
VAT (cm ²)	-32 (-63, -2)	0.04
SAT (cm ²)	-10 (-40, 21)	0.53
Weight (kg)	-0.4 (-3.4, 2.7)	0.81
BMI (kg/m ²)	-0.6 (-1.6, 0.5)	0.27
Waist circ (cm)	-3 (-6, 0)	0.06
Lean mass (kg)	2.0 (0.2, 3.7)	0.03
Fat mass (kg)	-2.0 (-4.4, 0.3)	0.09
Trunk fat (kg)	-1.6 (-3.1, -0.2)	0.03
Biochemical		
IGF-1 (µg/L)	105 (54, 157)	0.0001
Total Chol (mg/dl)	-3 (-18, 12)	0.72
Triglycerides (mg/dl)	-40 (-80, 0)	0.05
HDL Chol (mg/dl)	0 (-5, 5)	0.99
LDL Chol (mg/dl)	3 (-12, 18)	0.66
Log CRP*	-0.15 (-0.30, 0.00)	0.04
Carotid IMT (mm)	-0.05 (-0.09, -0.003)	0.04
Safety		
Systolic BP (mm Hg)	-3 (-12, 6)	0.50
Diastolic BP (mm Hg)	-5 (-10, 1)	0.10
Fasting glucose (mg/dl)	-1 (-9, 8)	0.90
2-hour glucose (mg/dl)	-10 (-34, 14)	0.40
Insulin (µIU/ml)	-0.3 (-7.5, 7.0)	0.94
HOMA-IR	-0.11 (-2.41, 2.19)	0.93

HbA1c (%)	0.0 (-0.3, 0.2)	0.77
AST (U/L)	1 (-7, 10)	0.77
ALT (U/L)	0 (-9, 10)	0.97

Results are presented as mean $\pm$ SEM. P value from baseline data are obtained by Student's t-test for normally distributed variables and Wilcoxon rank sum for non-normally distributed samples. One patient in the (hexenoyl trans-3)hGHRH₍₁₋₄₄₎NH₂ group discontinued the study prior to obtaining baseline body composition and biochemical data. *For CRP, the final value in one patient was 5 SD above all other data points and over 100 points above all her prior data points. This patient had skin manifestations and swelling from multiple insect bites while camping immediately prior to the final visit. Therefore this point data was excluded as an outlier. Instead data was assessed from her 9 month safety visit, prior to the insect bites, and used in the analysis. Abbreviations: VAT: visceral abdominal adipose tissue area; SAT: sub-cutaneous abdominal adipose tissue area; BMI: body mass index; IMT: intima-media thickness; HbA1c: Hemoglobin A1c; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GRF: (hexenoyl trans-3)hGHRH₍₁₋₄₄₎NH₂; P: P value.

Table 4: Effect of treatment with (hexenoyl trans-3)hGHRH₍₁₋₄₄₎NH₂ versus placebo on dietary parameters as assessed by 4-day food record.

	Baseline			6-Month		12-Month	
	(hexenoyl trans-3)hGHRH ₍₁₋₄₄₎ NH ₂	Placebo	P	(hexenoyl trans-3)hGHRH ₍₁₋₄₄₎ NH ₂	Placebo	(hexenoyl trans-3)hGHRH ₍₁₋₄₄₎ NH ₂	Placebo
Total caloric intake (kcal/day)	2233 $\pm$ 214	2213 $\pm$ 213	0.94	2309 $\pm$ 211	2278 $\pm$ 226	2265 $\pm$ 218	2054 $\pm$ 219
Total carbohydrate intake (g/day)	270 $\pm$ 23	256 $\pm$ 25	0.69	278 $\pm$ 29	262 $\pm$ 29	264 $\pm$ 24	212 $\pm$ 23
Total protein intake (g/day)	101 $\pm$ 14	99 $\pm$ 9	0.70	98 $\pm$ 9	95 $\pm$ 10	106 $\pm$ 13	100 $\pm$ 11
Total fat intake (g/day)	82 $\pm$ 10	89 $\pm$ 12	0.72	91 $\pm$ 10	94 $\pm$ 11	87 $\pm$ 11	88 $\pm$ 11

	Effect Size for (hexenoyl trans-3)hGHRH ₍₁₋₄₄₎ NH ₂ vs. Placebo	
	Effect Size (95% CI)	P value
Total caloric intake (kcal/day)	-15 (-584, 554)	0.96
Total carbohydrate intake (g/day)	7 (-64, 78)	0.83
Total protein intake (g/day)	-2 (-32, 28)	0.90
Total fat intake (g/day)	4 (-27, 35)	0.81

Results are presented as mean $\pm$ SEM. P value from baseline data are obtained by Student's t-test for normally distributed variables and Wilcoxon rank sum for non-normally distributed samples. Effects size and P values for overall effect was obtained by longitudinal linear mixed effects modeling for each parameter over 12 months.

5

Table 5: Effects of treatment with (hexenoyl trans-3)hGHRH₍₁₋₄₄₎NH₂ versus placebo on fasting plasma glucose.

	Baseline	2-Weeks	1-Month	3-Month	6-Month	9-Month	12-Month
(hexenoyl trans-3)hGHRH ₍₁₋₄₄₎ NH ₂	95 $\pm$ 3	97 $\pm$ 2	95 $\pm$ 2	96 $\pm$ 3	95 $\pm$ 5	96 $\pm$ 3	93 $\pm$ 3
Placebo	92 $\pm$ 2	93 $\pm$ 3	92 $\pm$ 2	93 $\pm$ 2	87 $\pm$ 2	94 $\pm$ 3	93 $\pm$ 4
P value	0.69	0.60	0.65	0.60	0.18	0.74	0.90

Results are presented as mean $\pm$ SEM. P value for each visit was obtained by longitudinal linear mixed effects modeling.

10

Table 6: Effect of treatment with (hexenoyl trans-3)hGHRH₍₁₋₄₄₎NH₂ versus placebo on physical activity as assessed by modified activity questionnaire.

	Baseline			6-Month		12-Month	
	(hexenoyl trans-3)hGHRH ₍₁₋₄₄₎ NH ₂	Placebo	P	(hexenoyl trans-3)hGHRH ₍₁₋₄₄₎ NH ₂	Placebo	(hexenoyl trans-3)hGHRH ₍₁₋₄₄₎ NH ₂	Placebo
Total activity (Met-hours/week)	76 $\pm$ 20	76 $\pm$ 18	0.98	64 $\pm$ 12	63 $\pm$ 18	70 $\pm$ 20	80 $\pm$ 28
Total television viewing time (hours/day)	3.3 $\pm$ 0.4	3.0 $\pm$ 0.6	0.25	3.2 $\pm$ 0.5	3.1 $\pm$ 0.7	3.3 $\pm$ 0.5	2.1 $\pm$ 0.5

Results are presented as mean $\pm$ SEM. P value from baseline data are obtained by Student's t-test for normally distributed variables and Wilcoxon rank sum for non-normally distributed samples. Effects size and P values for overall effect was obtained by longitudinal linear mixed effects modeling for each parameter over 12 months.

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Table 7: Adverse events

Event	(hexenoyl trans-3)hGHRH ₍₁₋₄₄₎ NH ₂ Number (%)	Placebo Number (%)	P value
Any adverse event	28 (90.3%)	26 (89.7%)	0.93
Related to treatment	22 (75.9%)	21 (80.8%)	0.66
Resulted in	4 (13.8%)	1 (3.9%)	0.20

discontinuation from study			
Serious adverse event	0 (0%)	0 (0%)	N/A
Discontinued prior to 6 months	5 (17.2%)	5 (17.2%)	1.00
Discontinued after 6 months	5 (16.1%)	7 (24.1%)	0.44
Dose adjustment prior to 6 months	3 (9.7%)	0 (0%)	N/A
Dose adjustment after 6 months	0 (0%)	0 (0%)	N/A
Adverse events potentially related to (hexenoyl trans-3)hGHRH₍₁₋₄₄₎NH₂			
Injection site bruising	17 (54.8%)	21 (72.4%)	0.16
Hypertension	8 (29.6%)	5 (17.2%)	0.27
Injection-site bleeding	4 (12.9%)	5 (17.2%)	0.64
Injection-site pain	3 (9.7%)	2 (6.9%)	0.70
Hyperglycemia	3 (9.7%)	2 (6.9%)	0.70
Tingling/paresthesia	3 (9.7%)	1 (3.5%)	0.33
Peripheral edema	3 (9.7%)	1 (3.5%)	0.33
Injection-site pruritis	2 (6.5%)	1 (3.5%)	0.59
Injection-site reaction	2 (6.5%)	0 (0%)	0.16
Arthralgia	2 (6.5%)	0 (0%)	0.16
Injection site erythema	2 (6.5%)	0 (0%)	0.16
Carpal tunnel syndrome	1 (3.2%)	0 (0%)	0.33
Hypersensitivity Reaction	1 (3.2%)	0 (0%)	0.33
Malignancy	0 (0%)	0 (0%)	N/A
Adverse events occurring in >5% of subjects			
Anxiety	1 (3.2%)	3 (10.3%)	0.27
Abdominal pain	2 (6.5%)	3 (10.3%)	0.59
Atypical chest pain	2 (6.5%)	2 (6.9%)	0.95
Back pain	4 (12.9%)	4 (13.8%)	0.92
Constipation	2 (6.5%)	0 (0%)	0.16
Depression	0 (0%)	2 (6.9%)	0.14
Dermatitis	3 (9.7%)	1 (3.5%)	0.33
Diarrhea	3 (9.7%)	1 (3.5%)	0.33
Fatigue	2 (6.5%)	1 (3.5%)	0.59
Fungal infection	0 (0%)	3 (10.3%)	0.07
Gastroenteritis	2 (6.5%)	1 (3.5%)	0.59
GERD	0 (0%)	2 (6.9%)	0.14
Headache	2 (6.5%)	5 (17.2%)	0.19
Infection	0 (0%)	2 (6.9%)	0.14
Mechanical injury	0 (0%)	3 (10.3%)	0.07

Musculoskeletal injury	5 (16.1%)	5 (17.2%)	0.91
Musculoskeletal pain	4 (12.9%)	5 (17.2%)	0.64
Myalgia	2 (6.5%)	3 (10.3%)	0.59
Nasopharyngitis	0 (0%)	2 (6.9%)	0.14
Nausea	3 (9.7%)	0 (0%)	0.09
Sinusitis	2 (6.5%)	6 (20.7%)	0.11
Tendonitis	2 (6.5%)	0 (0%)	0.16
Toothache	2 (6.5%)	0 (0%)	0.16
URI	5 (16.1%)	7 (24.1%)	0.44

No serious adverse events were reported during the study. There were no significant differences in the rate of adverse events between study groups.

Abbreviations: GERD: gastroesophageal reflux disease; URI: upper respiratory tract infection.

- 5 Although the present invention has been described hereinabove by way of specific embodiments thereof, it can be modified, without departing from the spirit and nature of the subject invention as defined in the appended claims. In the claims, the word "comprising" is used as an open-ended term, substantially equivalent to the phrase "including, but not limited to". The singular forms "a", "an" and "the" include corresponding plural references unless the
- 10 context clearly dictates otherwise.

WHAT IS CLAIMED IS:

1. A method for preventing or treating vascular stenosis and/or associated conditions in a growth hormone-deficient (GHD) obese subject, said method comprising administering to a subject in need thereof an effective amount of a growth hormone (GH)-releasing agent.

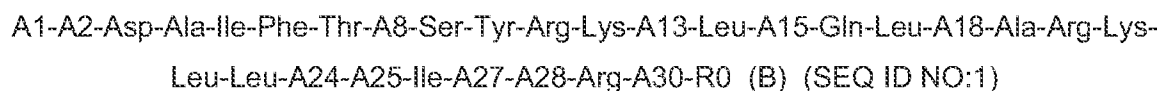
5 2. The method of claim 1, wherein said GH-releasing agent is a growth hormone-releasing factor (GRF) or a GRF analog.

3. The method of claim 2, wherein said GRF analog is a GRF analog of formula A:



wherein;

10 the GRF peptide is a peptide of formula B:



wherein,

A1 is Tyr or His;

15 A2 is Val or Ala;

A8 is Asn or Ser;

A13 is Val or Ile;

A15 is Ala or Gly;

A18 is Ser or Tyr;

20 A24 is Gln or His;

A25 is Asp or Glu;

A27 is Met, Ile or Nle

A28 is Ser or Asn;

A30 is a bond or amino acid sequence of 1 up to 15 residues; and

25 R0 is NH_2 or $\text{NH}-(\text{CH}_2)_n-\text{CONH}_2$, with $n=1$ to 12; and

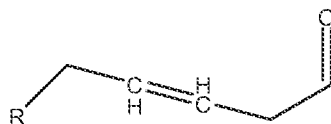
X is a hydrophobic tail anchored via an amide bond to the N-terminus of the GRF peptide and the hydrophobic tail comprising (i) a backbone of 5 to 9 atoms, wherein the backbone can be substituted by C_{1-6} alkyl, C_{3-6} cycloalkyl, or C_{6-12} aryl and (ii) at least one rigidifying moiety connected to at least two atoms of the backbone; said moiety is a double bond, triple bond, saturated or unsaturated C_{3-9} cycloalkyl, or C_{6-12} aryl, or a pharmaceutically acceptable salt thereof.

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4. The method of claim 3, wherein X is:

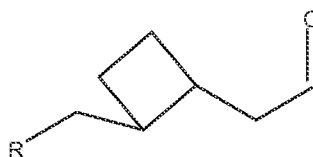
(i)

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wherein R is H, CH₃ or CH₂CH₃, and the double bond is *cis* or *trans*;

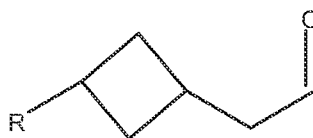
(ii)



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wherein R is H, CH₃ or CH₂CH₃, wherein X is in a *cis* or *trans* configuration, and wherein said GRF analog is a racemic mixture or a pure enantiomer;

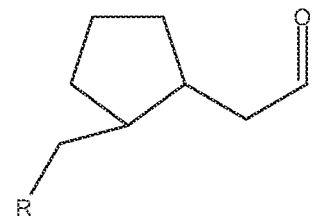
(iii)



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wherein R is H, CH₃ or CH₂CH₃, and wherein when R is CH₃ or CH₂CH₃, X is in a *cis* or *trans* configuration;

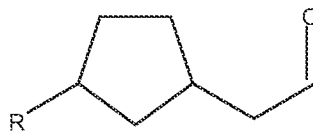
(iv)



15

wherein R is H, CH₃ or CH₂CH₃, wherein X is in a *cis* or *trans* configuration, and wherein said GRF analog is a racemic mixture or a pure enantiomer;

(v)

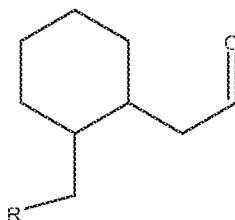


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wherein R is H, CH₃ or CH₂CH₃, wherein when R is CH₃ or CH₂CH₃, X is in a *cis* or *trans* configuration, and wherein said GRF analog is a racemic mixture or a pure enantiomer;

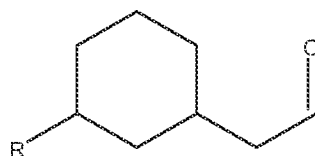
(vi)

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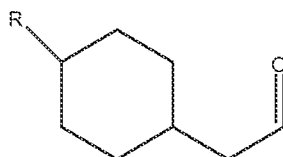
wherein R is H, CH₃ or CH₂CH₃, wherein X is in a *cis* or *trans* configuration, and wherein said GRF analog is a racemic mixture or a pure enantiomer;

5 (vii)



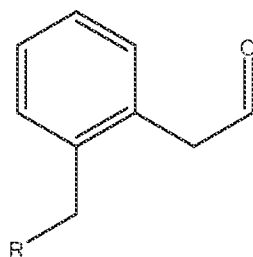
wherein R is H, CH₃ or CH₂CH₃, wherein when R is CH₃ or CH₂CH₃, X is in a *cis* or *trans* configuration, and wherein said GRF analog is a racemic mixture or a pure enantiomer;

10 (viii)



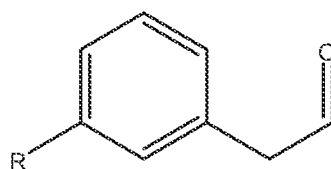
wherein R is H, CH₃ or CH₂CH₃, and wherein when R is CH₃ or CH₂CH₃, X is in a *cis* or *trans* configuration;

15 (ix)



wherein R is H, CH₃ or CH₂CH₃;

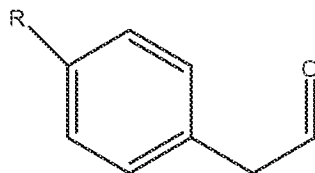
(x)



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wherein R is H, CH₃ or CH₂CH₃;

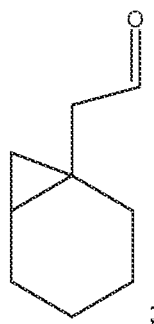
(xi)



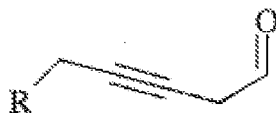
wherein R is H, CH₃ or CH₂CH₃;

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(xii)



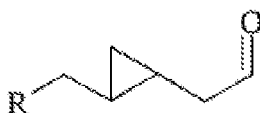
(xiii)



10

wherein R is H, CH₃ or CH₂CH₃; or

(xiv)

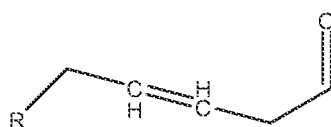


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wherein R is H, CH₃ or CH₂CH₃, wherein X is in a *cis* or *trans* configuration, and wherein said GRF analog is a racemic mixture or a pure enantiomer.

5. The method of claim 4, wherein X is :

(i)



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wherein R is CH₃ and the double bond is *trans*.

6. The method of any one of claims 3 to 5, wherein A30 is:

- (a) a bond;
- (b) an amino acid sequence corresponding to positions 30-44 of a natural GRF peptide; and
- (c) said amino acid sequence of (b), having a 1-14 amino acid deletion from its C-terminus.

7. The method of any one of claims 2 to 6, wherein said GRF analog is (hexenoyl trans-3)hGHRH₍₁₋₄₄₎NH₂ (SEQ ID NO:16).

8. The method of any one of claims 1 to 7, wherein said vascular stenosis is carotid stenosis.

9. The method of any one of claims 1 to 8, wherein said vascular stenosis and/or associated conditions is atherosclerosis.

10. A growth hormone (GH)-releasing agent for use in preventing or treating vascular stenosis and/or associated conditions in a growth hormone-deficient (GHD) obese subject.

11. A growth hormone (GH)-releasing agent for the manufacture of a medicament for preventing or treating vascular stenosis and/or associated conditions in a growth hormone-deficient (GHD) obese subject.

12. The GH-releasing agent of claim 10 or 11, wherein said GH-releasing agent is the GRF or GRF analog defined in any one of claims 2 to 7.

13. The GH-releasing agent of any one of claims 10 to 12, wherein said vascular stenosis is carotid stenosis.

14. The GH-releasing agent of any one of claims 10 to 13, wherein said vascular stenosis and/or associated conditions is atherosclerosis.

15. Use of a growth hormone (GH)-releasing agent for preventing or treating vascular stenosis and/or associated conditions in a growth hormone-deficient (GHD) obese subject.

16. Use of a growth hormone (GH)-releasing agent for the manufacture of a medicament for preventing or treating vascular stenosis and/or associated conditions in a growth hormone-deficient obese (GHD) subject.

17. The use of claim 15 or 16, wherein said GH-releasing agent is the GRF or GRF analog defined in any one of claims 2 to 7.

18. The use of any one of claims 15 to 17, wherein said vascular stenosis is carotid stenosis.
19. The use of any one of claims 15 to 18, wherein said vascular stenosis and/or associated conditions is atherosclerosis.

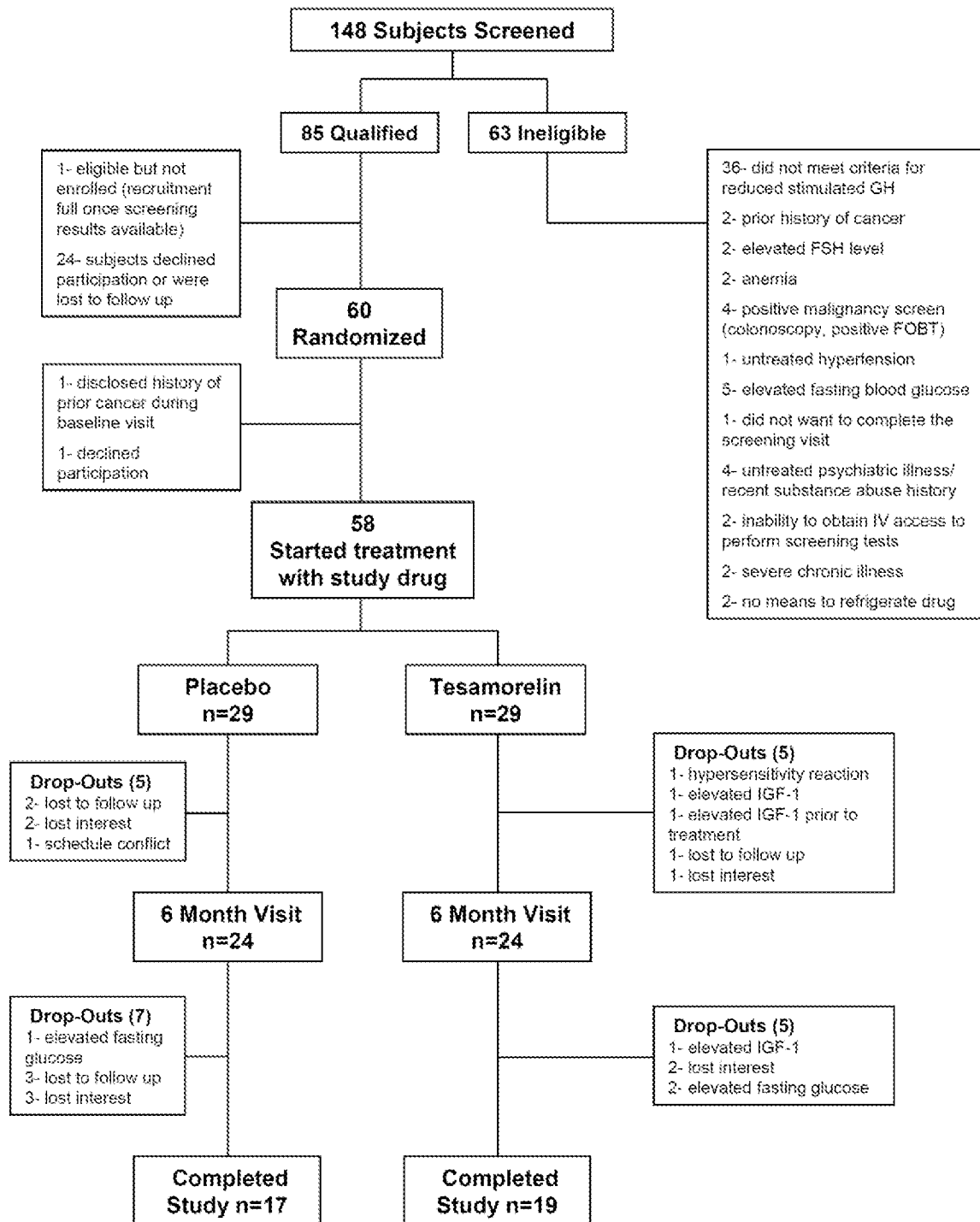
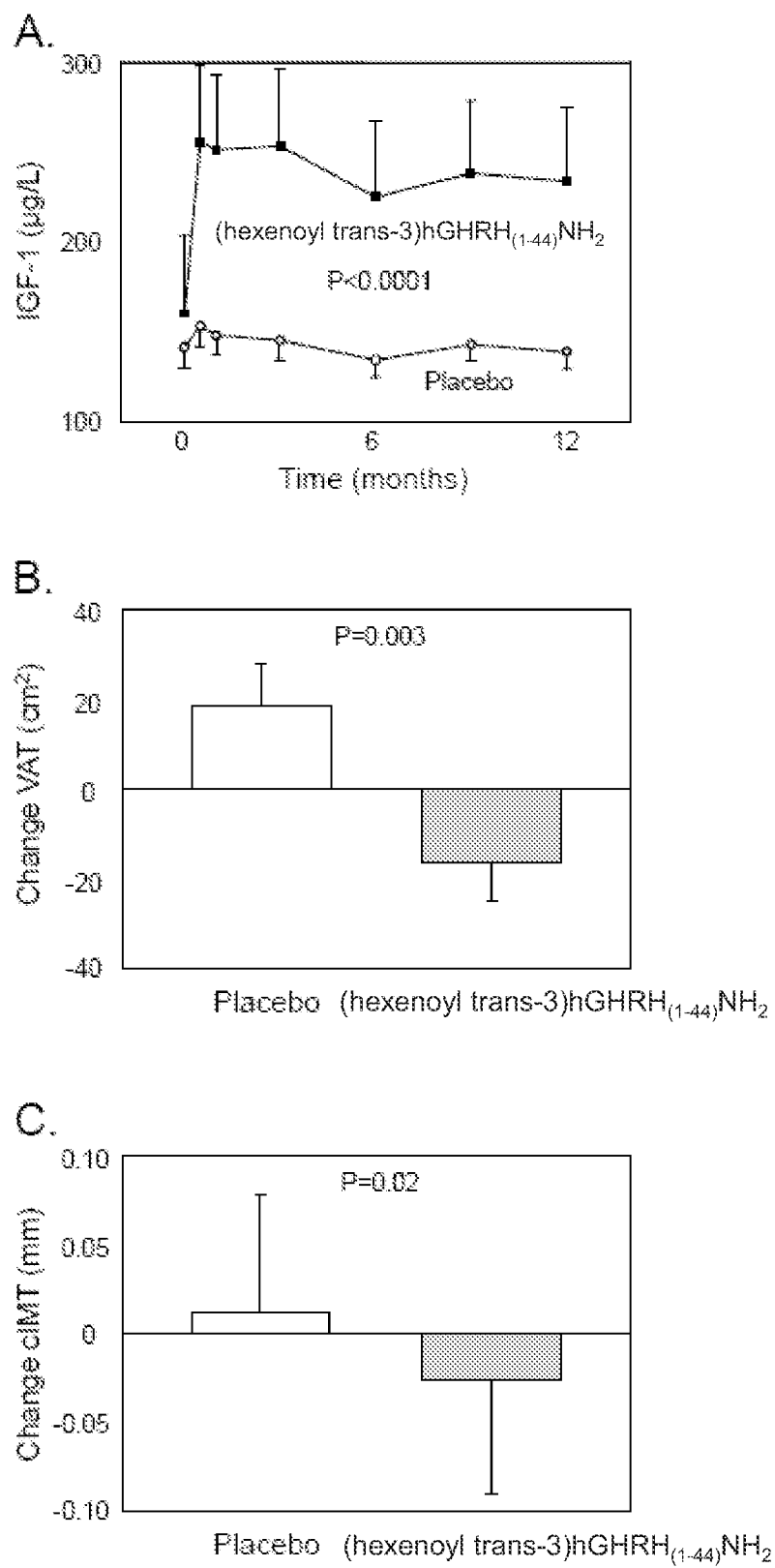


FIG. 1

2/2

**FIG. 2**