TITLE OF INVENTION

LOW-DOSE PHARMACEUTICAL COMPOSITIONS OF GHRH ANALOGS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of U.S. provisional patent application No. 63/048,167 filed on July 5, 2020, which is incorporated herein by reference in its entirety.

SEQUENCE LISTING

[0002] This application contains a Sequence Listing in computer readable form entitled "G11718_409_SeqList.txt", created on June 29, 2021 and having a size of about 5 KB. The computer readable form is incorporated herein by reference in its entirety.

TECHNICAL FIELD

[0003] The present disclosure generally relates to the field of growth hormone (GH) secretagogues, and more specifically to formulations of Growth Hormone-Releasing Hormone (GHRH) analogs such as tesamorelin and methods of administration thereof.

BACKGROUND ART

[0004] Tesamorelin (*trans*-3-hexenoyl-$GHRH_{(1-44)}$-NH_2, **FIG. 1**) is a stabilized synthetic peptide analogue of the hypothalamic peptide GHRH indicated for the reduction of excess abdominal fat in HIV-infected patients with lipodystrophy. It mediates its effect by acting on the pituitary somatotroph cells to stimulate the synthesis and pulsatile release of endogenous GH, which is both anabolic and lipolytic. Tesamorelin exerts its therapeutic effects by binding to, and being an agonist of GHRHr on pituitary somatotrophs; the triggered release GH in turn acts on a variety of target cells, including chondrocytes, osteoblasts, myocytes, hepatocytes and adipocytes, resulting in a host of pharmacodynamic effects, which are primarily mediated by insulin-like growth factor 1 (IGF-1) produced in the liver and in peripheral tissues.

[0005] The approved daily dosage of tesamorelin for the reduction of excess abdominal fat in HIV-infected patients with lipodystrophy is 2 mg administered by subcutaneous injection of 2 ml of a 1 mg/mL tesamorelin solution into abdominal skin. It is currently supplied to patients in two vials each comprising 1 mg of lyophilized tesamorelin. The patients must resuspend the lyophilized tesamorelin in the first vial with 2.2 mL of sterile water using a syringe with a first mixing needle, collect the prepared tesamorelin solution from the first vial, change the needle, add the prepared tesamorelin solution to the second vial with the second mixing needle, collect the prepared tesamorelin solution from the second vial, replace the second mixing needle with an injection needle, and subcutaneously inject 2 mL of the prepared tesamorelin solution. This

relatively complicated process for preparing the injectable tesamorelin solution is not very convenient for patients, and increases the risk of error, contaminations and improper handling of the tesamorelin solution. Furthermore, the volume of solution that must be subcutaneously injected to provide the suitable plasmatic tesamorelin levels to the patients is relatively large (2 ml), which may be associated with pain at the injection site (Usach *et al*., *Adv Ther* (2019) 36:2986-2996). Further, it would be more convenient for patients to have for example a single vial containing a dosage for treatment, or even a multidose vial containing multiple dosages for treatment (e.g., for multiple days of treatment).

[0006] There is thus a need for a more simple and convenient method of administration of tesamorelin.

[0007] The present description refers to a number of documents, the content of which is herein incorporated by reference in their entirety.

SUMMARY OF THE DISCLOSURE

[0008] The present disclosure generally relates to formulations of Growth Hormone-Releasing Hormone (GHRH) analogs, such as tesamorelin, and methods of administration thereof.

[0009] In an aspect, the present disclosure provides a pharmaceutical composition comprising a GHRH molecule or a pharmaceutically acceptable salt thereof (e.g., *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or a pharmaceutically acceptable salt thereof) and at least one pharmaceutically acceptable excipient.

[0010] In various aspects and embodiments, the present disclosure further provides the following items:

1. A pharmaceutical composition comprising (i) about 1.23 to about 1.32 mg of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or a pharmaceutically acceptable salt thereof at a concentration of about 7.5 mg/mL or more; and (ii) at least one pharmaceutically acceptable excipient.
2. The pharmaceutical composition of item 1, comprising about 1.25 to about 1.30 mg of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or pharmaceutically acceptable salt thereof.
3. The pharmaceutical composition of item 1 or 2, comprising about 1.27 to about 1.29 mg of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or pharmaceutically acceptable salt thereof.
4. The pharmaceutical composition of any one of items 1 to 3, comprising about 1.28 *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or pharmaceutically acceptable salt thereof.
5. The pharmaceutical composition of any one of items 1 to 4, wherein the *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or pharmaceutically acceptable salt thereof is at a concentration of about 7.5 to about 8.5 mg/mL.
6. The pharmaceutical composition of any one of items 1 to 5, wherein the *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or pharmaceutically acceptable salt thereof is at a concentration of about 8 mg/mL.

7. The pharmaceutical composition of any one of items 1 to 6, wherein the at least one pharmaceutically acceptable excipient comprises a diluent.
8. The pharmaceutical composition of any one of items 1 to 7, wherein the at least one pharmaceutically acceptable excipient comprises a bulking agent.
9. The pharmaceutical composition of item 8, wherein the bulking agent is mannitol.
10. The pharmaceutical composition of any one of items 1 to 9, wherein the at least one pharmaceutically acceptable excipient comprises a stabilizer.
11. The pharmaceutical composition of item 10, wherein the stabilizer is sucrose.
12. The pharmaceutical composition of any one of items 1 to 11, wherein the at least one pharmaceutically acceptable excipient comprises a surfactant.
13. The pharmaceutical composition of item 12, wherein the surfactant is polysorbate 20.
14. The pharmaceutical composition of any one of items 1 to 13, wherein the at least one pharmaceutically acceptable excipient comprises a buffering agent.
15. The pharmaceutical composition of item 14, wherein the buffering agent is histidine.
16. The pharmaceutical composition of any one of items 1 to 15, wherein the at least one pharmaceutically acceptable excipient comprises a cyclodextrin.
17. The pharmaceutical composition of item 16, wherein the cyclodextrin is a β-cyclodextrin.
18. The pharmaceutical composition of any one of items 1 to 17, wherein the pharmaceutically acceptable salt of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 is an acetate salt.
19. A method of administering *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or a pharmaceutically acceptable salt thereof to a subject to obtain plasmatic levels of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or a pharmaceutically acceptable salt thereof that are bioequivalent to administration of 2 mg of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 at a concentration of 1 mg/mL, the method comprising administering to the subject about 1.23 to about 1.32 mg of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or a pharmaceutically acceptable salt thereof at a concentration of about 7.5 mg/mL or more.
20. The method of item 19, comprising administering about 1.28 mg of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or a pharmaceutically acceptable salt thereof.
21. The method of item 19 or 20, wherein the *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or pharmaceutically acceptable salt thereof is at a concentration of about 7.5 to about 8.5 mg/mL.
22. The method of any one of items 19 to 21, wherein the pharmaceutically acceptable salt of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 is an acetate salt.
23. The method of any one of items 19 to 22, wherein the *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or pharmaceutically acceptable salt thereof is administered by subcutaneous injection.
24. The method of any one of items 19 to 23, further comprising

    resuspending lyophilized *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or pharmaceutically acceptable salt thereof in a suitable amount of a pharmaceutically acceptable diluent to

obtain a *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 salt solution at a concentration of about 7.5 mg/mL or more;

wherein a suitable volume of the *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 salt solution is administered so that about 1.23 to about 1.32 mg of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or pharmaceutically acceptable salt thereof is administered to the subject.

25. A method of administering *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or a pharmaceutically acceptable salt thereof to a human subject to obtain:

(i) a maximum plasmatic concentration (C_{max}) of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or a pharmaceutically acceptable salt thereof of about 1500 to about 4500 pg/mL in the subject; and/or

(ii) an area under the plasma concentration time curve extrapolated to infinity ($AUC_{0-\infty}$) of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or a pharmaceutically acceptable salt thereof of about 300 to about 1400 pg·h/mL in the subject;

the method comprising administering to the subject about 1.23 to about 1.32 mg of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or a pharmaceutically acceptable salt thereof at a concentration of about 7.5 mg/mL or more.

26. The method of item 25, comprising administering about 1.25 to about 1.30 mg of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or a pharmaceutically acceptable salt thereof.

28. 27. The method of item 25 or item 26, comprising administering about 1.28 mg of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or a pharmaceutically acceptable salt thereof. The method of any one of items 25 to 28, wherein the *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or pharmaceutically acceptable salt thereof is at a concentration of about 7.5 to about 8.5 mg/mL.

29. The method of any one of items 25 to 28, wherein the pharmaceutically acceptable salt of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 is an acetate salt.

30. The method of any one of items 25 to 29, wherein the *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or pharmaceutically acceptable salt thereof is administered by subcutaneous injection.

31. The method of any one of items 25 to 30, further comprising:

resuspending lyophilized *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or pharmaceutically acceptable salt thereof in a suitable amount of a pharmaceutically acceptable diluent to obtain a *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 salt solution at a concentration of about 7.5 mg/mL or more;

wherein a suitable volume of the *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 salt solution is administered so that about 1.23 to about 1.32 mg of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or pharmaceutically acceptable salt thereof is administered to the subject.

32. The method of any one of items 19 to 31, wherein the subject suffers from HIV-associated lipodystrophy.

33. A kit comprising:

(a) a first container comprising at least about 1.23 to about 1.32 mg of lyophilized *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or a pharmaceutically acceptable salt thereof;

(b) a second container comprising a pharmaceutically acceptable diluent;

(c) instructions setting forth the method of item 25; and optionally

(d) at least one syringe.

34. A pharmaceutical composition comprising *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or a pharmaceutically acceptable salt thereof for use in producing plasmatic levels of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or a pharmaceutically acceptable salt thereof in a subject that are bioequivalent to administration of 2 mg of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 at a concentration of 1 mg/mL, wherein the pharmaceutical composition is for administration of about 1.3 to about 1.6 mg or about 1.23 to about 1.32 mg of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or a pharmaceutically acceptable salt thereof at a concentration of about 7.5 mg/mL or more, to the subject.

35. A pharmaceutical composition comprising *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or a pharmaceutically acceptable salt thereof for use in administration to a human subject for producing:

(i) a maximum plasmatic concentration (C_{max}) of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or a pharmaceutically acceptable salt thereof of about 1500 to about 4500 pg/mL in the subject; and/or

(ii) an area under the plasma concentration time curve extrapolated to infinity ($AUC_{0-\infty}$) of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or a pharmaceutically acceptable salt thereof of about 300 to about 1400 pg·h/mL in the subject;

wherein the pharmaceutical composition is for administration of about 1.3 to about 1.6 mg or about 1.23 to about 1.32 mg of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or a pharmaceutically acceptable salt thereof at a concentration of about 7.5 mg/mL or more, to the subject.

36. The pharmaceutical composition for use of item 34 or 35, wherein the pharmaceutical composition is for administration of about 1.25 to about 1.30 mg of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or a pharmaceutically acceptable salt thereof.

37. The pharmaceutical composition for use of any one of items 34 to 36, wherein the pharmaceutical composition is for administration of the *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or pharmaceutically acceptable salt thereof at a concentration of about 7.5 to about 8.5 mg/mL, preferably about 8.0 mg/mL.

38. The pharmaceutical composition for use of any one of items 34 to 37, wherein the pharmaceutically acceptable salt of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 is an acetate salt.

39. The pharmaceutical composition for use of any one of items 34 to 38, wherein the subject suffers from HIV-associated lipodystrophy.

40. The pharmaceutical composition for use of any one of items 34 to 39, wherein the *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or pharmaceutically acceptable salt thereof is for administration by subcutaneous injection.

41. The pharmaceutical composition for use of any one of items 34 to 40, further comprising resuspending lyophilized *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or pharmaceutically acceptable salt thereof in a suitable amount of a pharmaceutically acceptable diluent to obtain a *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ salt solution at a concentration of about 7.5 mg/mL or more; thereby to provide the pharmaceutical composition for administration.

42. Use of a pharmaceutical composition comprising *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or a pharmaceutically acceptable salt thereof for producing plasmatic levels of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or a pharmaceutically acceptable salt thereof in a subject that are bioequivalent to administration of 2 mg of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ at a concentration of 1 mg/mL, wherein the pharmaceutical composition is for administration of about 1.23 to about 1.32 mg of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or a pharmaceutically acceptable salt thereof at a concentration of about 7.5 mg/mL or more, to the subject.

43. Use of a pharmaceutical composition comprising *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or a pharmaceutically acceptable salt thereof for use in administration to a human subject for producing:

    (i) a maximum plasmatic concentration (C_{max}) of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or a pharmaceutically acceptable salt thereof of about 1500 to about 4500 pg/mL in the subject; and/or

    (ii) an area under the plasma concentration time curve extrapolated to infinity ($AUC_{0-\infty}$) of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or a pharmaceutically acceptable salt thereof of about 300 to about 1400 pg·h/mL in the subject;

    wherein the pharmaceutical composition is for administration of about 1.23 to about 1.32 mg of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or a pharmaceutically acceptable salt thereof at a concentration of about 3.5 mg/mL or more, to the subject.

44. Use of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or a pharmaceutically acceptable salt thereof for the preparation of a pharmaceutical composition for producing plasmatic levels of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or a pharmaceutically acceptable salt thereof in a subject that are bioequivalent to administration of 2 mg of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ at a concentration of 1 mg/mL, wherein the pharmaceutical composition is for administration of about 1.23 to about 1.32 mg of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or a pharmaceutically acceptable salt thereof at a concentration of about 7.5 mg/mL or more, to the subject.

45. Use of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or a pharmaceutically acceptable salt thereof for the preparation of a pharmaceutical composition for administration to a human subject for producing:
    (i) a maximum plasmatic concentration (C_{max}) of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or a pharmaceutically acceptable salt thereof of about 1500 to about 4500 pg/mL in the subject; and/or
    (ii) an area under the plasma concentration time curve extrapolated to infinity ($AUC_{0-\infty}$) of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or a pharmaceutically acceptable salt thereof of about 300 to about 1400 pg·h/mL in the subject;
    wherein the pharmaceutical composition is for administration of about 1.3 to about 1.6 mg or about 1.23 to about 1.32 mg of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or a pharmaceutically acceptable salt thereof at a concentration of about 7.5 mg/mL or more, to the subject.
46. The use of any one of items 42 to 45, wherein the pharmaceutical composition is for administration of about 1.28 mg of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or a pharmaceutically acceptable salt thereof.
47. The use of any one of items 42 to 46, wherein the pharmaceutical composition is for administration of the *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or pharmaceutically acceptable salt thereof at a concentration of about 8 mg/mL.
48. The use of any one of items 42 to 47, wherein the pharmaceutically acceptable salt of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 is an acetate salt.
49. The use of any one of items 42 to 48, wherein the subject suffers from HIV-associated lipodystrophy.
50. The use of any one of items 42 to 49, wherein the *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or pharmaceutically acceptable salt thereof is for administration by subcutaneous injection.
51. The use of any one of items 42 to 50, further comprising resuspending lyophilized *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or pharmaceutically acceptable salt thereof in a suitable amount of a pharmaceutically acceptable diluent to obtain a *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 or *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2 salt solution at a concentration of about 7.5 mg/mL or more; thereby to provide the formulation for administration.

[0011] Other objects, advantages and features of the present disclosure 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

[0012] In the appended drawings:

[0013] FIG. 1 shows the structure of tesamorelin (*trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH_2; SEQ ID NO:

1).

DETAILED DESCRIPTION

[0014] The use of the terms "a" and "an" and "the" and similar referents in the context of describing the subject matter (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context.

[0015] The terms "comprising", "having", "including", and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to") unless otherwise noted.

[0016] Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All subsets of values within the ranges are also incorporated into the specification as if they were individually recited herein.

[0017] Similarly, herein a general chemical structure with various substituents and various radicals enumerated for these substituents is intended to serve as a shorthand method of referring individually to each and every molecule obtained by the combination of any of the radicals for any of the substituents. Each individual molecule is incorporated into the specification as if it were individually recited herein. Further, all subsets of molecules within the general chemical structures are also incorporated into the specification as if they were individually recited herein.

[0018] All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context.

[0019] The use of any and all examples, or exemplary language (“*e.g.*”, "such as", etc.) provided herein, is intended merely to better illustrate the disclosure and does not pose a limitation on the scope of the disclosure unless otherwise claimed.

[0020] No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the disclosure.

[0021] Herein, the term "about" has its ordinary meaning. The term “about” is used to indicate that a value includes an inherent variation of error for the device or the method being employed to determine the value, or encompass values close to the recited values, for example within 10% of the recited values (or range of values).

[0022] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs.

[0023] In the studies described herein, the present inventors have shown that tesamorelin formulated at 8 mg/mL is more bioavailable that corresponding tesamorelin formulations at 1 mg/mL. Pharmacokinetic (PK) studies in human subjects have shown that administration of 1.28

mg of tesamorelin formulated at 8 mg/mL is bioequivalent to administration of 2 mg of a 1 mg/mL tesamorelin formulation (*e.g.*, the Egrifta™ formulation), the approved daily dosage of tesamorelin (EGRIFTA®). Doses of 1.2 mg and 1.36 mg (at 8 mg/ml) were found to be slightly too low or slightly too high, respectively, to obtain bioequivalence with the Egrifta™ formulation. Thus, it was found that the amount of tesamorelin administered to the subject should be reduced by about 36% (i.e. 1.28 mg *vs*. 2 mg) to obtain bioequivalence in the subjects. This advantageously reduces the volume of administration (0.16 mL vs. 2 mL), and renders the preparation and handling of the formulation more user-friendly as it may be provided in a single vial instead of two, thereby reducing the risk of error and contaminations/infections.

[0024] Accordingly, in a first aspect, the present disclosure provides a pharmaceutical composition comprising (i) more than 1.2 mg and less than 1.36 mg, for example about 1.21 to about 1.35, about 1.22 to about 1.33 or 1.34, or about 1.23 to about 1.32 mg of a GHRH molecule or a pharmaceutically acceptable salt thereof, preferably *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or a pharmaceutically acceptable salt thereof, at a concentration of about 7.5 mg/mL or more; and (ii) at least one pharmaceutically acceptable excipient.

[0025] In embodiments, the pharmaceutical composition comprises from about 1.21, 1.22, 1.23, 1.24, 1.25, 1.26, or 1.27 mg to about 1.29, 1.30, 1.31, 1.32, 1.33, 1.34 or 1.35 mg of the GHRH molecule, preferably *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or a pharmaceutically acceptable salt thereof. In a further embodiment, the pharmaceutical composition comprises from about 1.24 to about 1.31 mg of the GHRH molecule, preferably *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or a pharmaceutically acceptable salt thereof. In a further embodiment, the pharmaceutical composition comprises from about 1.25 to about 1.30 mg of the GHRH molecule, preferably *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or a pharmaceutically acceptable salt thereof. In a further embodiment, the pharmaceutical composition comprises from about 1.26 to about 1.29 mg of the GHRH molecule, preferably *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or a pharmaceutically acceptable salt thereof. In a further embodiment, the pharmaceutical composition comprises from about 1.27 to about 1.29 mg of the GHRH molecule, preferably *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or a pharmaceutically acceptable salt thereof. In a further embodiment, the pharmaceutical composition comprises from about 1.28 mg of the GHRH molecule, preferably *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or a pharmaceutically acceptable salt thereof.

[0026] In an embodiment, the GHRH molecule, preferably *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or a pharmaceutically acceptable salt thereof, is at a concentration of about 12, 10 or 8 mg/mL or less. In embodiments, the GHRH molecule, preferably *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or a pharmaceutically acceptable salt thereof, is at a concentration of about 7.5 to about 10, 9, 8.5 or 8 mg/mL, for example a concentration of about 7.5 to about 8.5 mg/mL. In further embodiments, the GHRH molecule, preferably *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or a pharmaceutically acceptable salt thereof, is at a concentration of about 7.5, 7.6, 7.7, 7.8 or 7.9 to about 8.1, 8.2,

8.3, 8.4 or 8.5 mg/ml in the pharmaceutical composition. In further embodiments, the GHRH molecule, preferably *trans*-3-hexenoyl-$GHRH_{(1-44)}$-NH_2 or a pharmaceutically acceptable salt thereof, is at a concentration of about 7.80, 7.82, 7.84, 7.86, 7.88, 7.9, 7.92, 7.94, 7.95, 7.96, 7.97, 7.98 or 7.99 to about 8.01, 8.02, 8.03, 8.04, 8.05, 8.06, 8.08, 8.1, 8.12, 8.14, 8.16, 8.18 or 8.2 mg/mL in the pharmaceutical composition. In a further embodiment, the GHRH molecule, preferably *trans*-3-hexenoyl-$GHRH_{(1-44)}$-NH_2 or a pharmaceutically acceptable salt thereof, is at a concentration of about 8 mg/mL.

[0027] The term "GHRH molecule" as used in the context of the present disclosure includes, without limitation, human native $GHRH_{(1-44)}$ and fragments thereof (*e.g.*, $GHRH_{(1-40)}$, $GHRH_{(1-29)}$, fragments ranging between 1-29 and the 1-44 sequence), and any other fragments; GHRH from other species and fragments thereof; GHRH variants containing amino acid(s) substitution(s), addition(s) and/or deletion(s); derivatives or analogs of GHRH or fragments or variants thereof having for example an organic group or a moiety coupled to the GHRH amino acid sequence at the N-terminus, the C-terminus or on the side-chain; and pharmaceutically acceptable salts of GHRH (human or from other species), as well as pharmaceutically acceptable salts of GHRH fragments, variants, analogs and derivatives. The GHRH molecules of the present disclosure also encompass the GHRH molecules currently known in the art, including, without limitation, albumin-conjugated GHRH (U.S. Patent No. 7,268,113); pegylated GHRH peptide (U.S. Patent Nos. 7,256,258 and 6,528,485); porcine GHRH (1-40) (U.S. Patent No. 6,551,996); canine GHRH (U.S. patent application no. 2005/0064554); GHRH 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^0-GHRHpeptide and variants thereof (U.S. Patent No. 5,137,872).

[0028] The GHRH analogs 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-GHRH Peptide (A)

wherein the GHRH peptide is a peptide of the following formula B (SEQ ID NO:2):

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-A31-A32-A33-A34-A35-A36-A37-A38-A39-A40-A41-A42- A43-A44-R0 (B)

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;

A30 is absent or is any amino acid, preferably Gln;

A31 is absent or is any amino acid, preferably Gln;

A32 is absent or is any amino acid, preferably Gly;

A33 is absent or is any amino acid, preferably Glu;

A34 is absent or is any amino acid, preferably Ser;

A35 is absent or is any amino acid, preferably Asn;

A36 is absent or is any amino acid, preferably Gln;

A37 is absent or is any amino acid, preferably Glu;

A38 is absent or is any amino acid, preferably Arg;

A39 is absent or is any amino acid, preferably Gly;

A40 is absent or is any amino acid, preferably Ala;

A41 is absent or is any amino acid, preferably Arg;

A42 is absent or is any amino acid, preferably Ala;

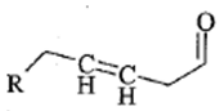
A43 is absent or is any amino acid, preferably Arg;

A44 is absent or is any amino acid, preferably Leu; and

R0 is NH_2 or NH-(CH_2)n-$CONH_2$, with n = 1 to 12.

[0029] The group X is a hydrophobic tail anchored via an amide bond to the N-terminus of the peptide and the hydrophobic tail defining a backbone of 5 to 7 atoms. The backbone can be substituted by C_{1-6} alkyl, C_{3-6} cycloalkyl, or C_{6-12} aryl and the backbone comprises at least one rigidifying moiety connected to at least two atoms of the backbone. The rigidifying moiety is a double bond, triple bond, saturated or unsaturated C_{3-9} cycloalkyl, or C_{6-12} aryl.

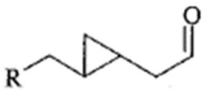
[0030] In an embodiment, group X is:



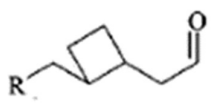
1 (R=H or CH_3 or CH_2CH_3),



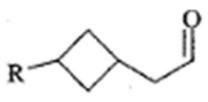
2 (R=H or CH_3 or CH_2CH_3),



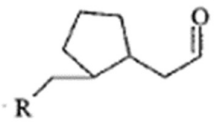
3 (R=H or CH_3 or CH_2CH_3),



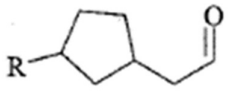
4 (R=H or CH_3 or CH_2CH_3),



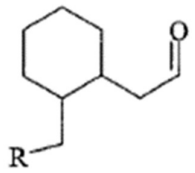
5 (R=H or CH_3 or CH_2CH_3),



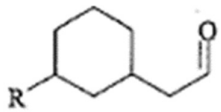
6 (R=H or CH_3 or CH_2CH_3),



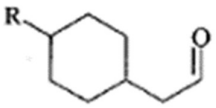
7 (R=H or CH_3 or CH_2CH_3),



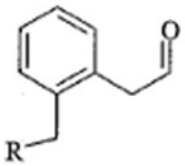
8 (R=H or CH_3 or CH_2CH_3),



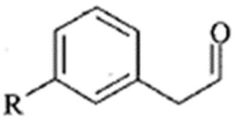
9 (R=H or CH_3 or CH_2CH_3),



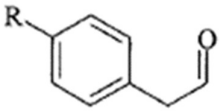
10 (R=H or CH_3 or CH_2CH_3),



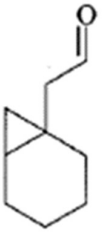
11 (R=H or CH_3 or CH_2CH_3),



12 (R=H or CH_3 or CH_2CH_3),



13 (R=H or CH_3 or CH_2CH_3), or



.

[0031] In an embodiment, in formula B, A30-A44 are: (a) absent; (b) an amino acid sequence corresponding to positions 30-44 of a native GHRH peptide (SEQ ID NO: 3), or (c) the amino acid sequence of (b) having a 1-14 amino acid deletion from its C-terminus.

[0032] In an embodiment, the GHRH peptide is a polypeptide comprising the amino acid sequence of SEQ ID NO: 4.

[0033] In an embodiment, the GHRH molecule is (hexenoyl trans-3)hGHRH$_{(1-44)}$NH$_2$ (SEQ ID NO: 1) or a pharmaceutically acceptable salt thereof. [trans-3-hexenoyl]hGHRH$_{(1-44)}$ amide (also referred to as (hexenoyl trans-3)hGHRH(1-44)NH$_2$) is a synthetic human GHRH (hGHRH) analog that comprises the 44-amino acid sequence of hGHRH on which a hexenoyl moiety, a C_6 side chain, has been anchored on the amino-terminal tyrosine residue. The structure of [trans-3-hexenoyl]hGHRH$_{(1-44)}$ amide is depicted at **FIG. 1**.

[0034] The term "pharmaceutically acceptable salt" refers to salts of GHRH molecules that are pharmacologically acceptable and substantially non-toxic to the subject to which they are administered. More specifically, these salts retain the biological effectiveness and properties of the GHRH molecule and are formed from suitable non-toxic organic or inorganic acids or bases.

[0035] For example, these salts include acid addition salts of GHRH molecules which are sufficiently basic to form such salts. Such acid addition salts include acetates, adipates, alginates, lower alkanesulfonates such as a methanesulfonates, trifluoromethanesulfonates or ethanesulfonates, arylsulfonates such as a benzenesulfonates, 2-naphthalenesulfonates, or toluenesulfonates (also known as tosylates), ascorbates, aspartates, benzoates,

benzenesulfonates, bisulfates, borates, butyrates, citrates, camphorates, camphorsulfonates, cinnamates, cyclopentanepropionates, digluconates, dodecylsulfates, ethanesulfonates, fumarates, glucoheptanoates, glycerophosphates, hemisulfates, heptanoates, hexanoates, hydrochlorides, hydrobromides, hydroiodides, hydrogen sulphates, 2-hydroxyethanesulfonates, itaconates, lactates, maleates, mandelates, methanesulfonates, nicotinates, nitrates, oxalates, pamoates, pectinates, perchlorates, persulfates, 3-phenylpropionates, phosphates, picrates, pivalates, propionates, salicylates, succinates, sulfates, sulfonates, tartrates, thiocyanates, undecanoates and the like.

[0036] Additionally, acids which are generally considered suitable for the formation of pharmaceutically useful salts from basic pharmaceutical compounds are discussed, for example, by P. Stahl *et al*., Camille G. (eds.) *Handbook of Pharmaceutical Salts. Properties, Selection and Use*. (2002) Zurich: Wiley-VCH; S. Berge et al, *Journal of Pharmaceutical Sciences* (1977) 66(1) 1–19; P. Gould, *International J. of Pharmaceutics* (1986) 33 201–217; Anderson et al, *The Practice of Medicinal Chemistry* (1996), Academic Press, New York; and in *The Orange Book* (Food & Drug Administration, Washington, D.C. on their website).

[0037] Such salts can be formed quite readily by those skilled in the art using standard techniques. Indeed, the chemical modification of a pharmaceutical compound (i.e. drug) into a salt is a technique well known to pharmaceutical chemists, (See, *e.g*., H. Ansel *et. al*., Pharmaceutical Dosage Forms and Drug Delivery Systems (6^{th} Ed. 1995) at pp. 196 and 1456–1457). Salts of the GHRH molecules may be formed, for example, by reacting the GHRH molecule with an amount of acid or base, such as an equivalent amount, in a medium such as one in which the salt precipitates or in an aqueous medium followed by lyophilization.

[0038] In an embodiment, the pharmaceutically acceptable salt of the GHRH molecule, preferably [trans-3-hexenoyl]hGHRH$_{(1-44)}$ amide, is an acetate salt.

[0039] The term “pharmaceutically acceptable excipient” as used herein has its normal meaning in the art and is any ingredient that is not an active ingredient (drug) itself. Excipients include for example binders, lubricants, diluents, bulking agents (fillers), thickening agents, disintegrants, plasticizers, coatings, barrier layer formulations, lubricants, stabilizing agent, release-delaying agents and other components. "Pharmaceutically acceptable excipient" as used herein refers to any excipient that does not interfere with effectiveness of the biological activity of the active ingredients and that is not toxic to the subject, *i.e*., is a type of excipient and/or is for use in an amount which is not toxic to the subject. Excipients are well known in the art, and the present composition is not limited in these respects. In certain embodiments, the pharmaceutical composition comprises one or more excipients, including for example and without limitation, one or more binders (binding agents), thickening agents, surfactants, diluents, release-delaying agents, colorants, flavoring agents, fillers, disintegrants/dissolution promoting agents, lubricants, plasticizers, silica flow conditioners, glidants, anti-caking agents, anti-tacking agents, stabilizing

agents, anti-static agents, swelling agents and any combinations thereof. As those of skill would recognize, a single excipient can fulfill more than two functions at once, *e.g.*, can act as both a binding agent and a thickening agent. As those of skill will also recognize, these terms are not necessarily mutually exclusive. Therapeutic formulations are prepared using standard methods known in the art by mixing the active ingredient having the desired degree of purity with one or more optional pharmaceutically acceptable carriers, excipients and/or stabilizers. The excipient(s) may be suitable, for example, for intravenous, parenteral, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intrathecal, epidural, intracisternal, intraperitoneal, intranasal or pulmonary (*e.g.*, aerosol) administration (see Remington: *The Science and Practice of Pharmacy*, by Loyd V Allen, Jr, 2012, 22nd edition, Pharmaceutical Press; *Handbook of Pharmaceutical Excipients*, by Rowe *et al.*, 2012, 7th edition, Pharmaceutical Press). In an embodiment, the pharmaceutical composition is an injectable composition, such as an injectable solution or suspension. In an embodiment, the pharmaceutical composition comprises one or more excipients for subcutaneous administration/injection.

[0040] In an embodiment, the pharmaceutical composition comprises a bulking agent. The term "bulking agent" as used herein refers to a compound used to provide an adequate or desired tonicity of the solution resulting from the reconstitution of the lyophilized formulation. Preferably, the adequate or desired tonicity of the solution is equal to or approximates isotonicity with physiological fluid of the subject to which the solution is administered. For example, one or more sugars may be used as the bulking agent. Sugars, as used herein, include, but are not limited to, monosaccharides, oligosaccharides and polysaccharides. Examples of suitable sugars include, but are not limited to, mannose, sorbose, xylose, maltose, lactose, sucrose, and dextran. Sugar also includes sugar alcohols, such as mannitol, inositol, dulcitol, xylitol and arabitol. Mixtures of sugars may also be used in accordance with the present disclosure. In an embodiment, the bulking agent is mannitol. For example, one or more amino acids, such as glycine, may be used as the bulking agent. The bulking agent is in concentration of about 1% to about 10% (w/w) or about 2% to about 8% (w/w) in the pharmaceutical composition. In an embodiment, the bulking agent is in concentration of about 3 to about 5% (w/w) in the pharmaceutical composition. In a further embodiment, the bulking agent is in concentration of about 4% (w/w) in the pharmaceutical composition.

[0041] In an embodiment, the pharmaceutical composition of the present disclosure may further comprise a surfactant. Typical examples of surfactants include sorbitan fatty acid esters such as sorbitan monocaprylate, sorbitan monolaurate, sorbitan monopalmitate; glycerin fatty acid esters such as glycerin monocaprylate, glycerin monomyristate, glycerin monostearate; polyglycerin fatty acid esters such as decaglyceryl monostearate, decaglyceryl distearate, decaglyceryl monolinoleate; polyoxyethylene sorbitan fatty acid esters such as polyoxyethylene sorbitan monolaurate, polyoxyethylene sorbitan monooleate, polyoxyethylene sorbitan monostearate,

polyoxyethylene sorbitan monopalmitate, polyoxyethylene sorbitan trioleate, polyoxyethylene sorbitan tristearate; polyoxyethylene sorbitol fatty acid esters such as polyoxyethylene sorbitol tetrastearate, polyoxyethylene sorbitol tetraoleate; polyoxyethylene glycerin fatty acid esters such as polyoxyethylene glyceryl monostearate; polyethylene glycol fatty acid esters such as polyethylene glycol distearate; polyoxyethylene alkyl ethers such as polyoxyethylene lauryl ether; polyoxyethylene polyoxypropylene alkyl ethers such as polyoxyethylene polyoxypropylene glycol ether, polyoxyethylene polyoxypropylene propyl ether, polyoxyethylene polyoxypropylene cetyl ether; polyoxyethylene alkyl phenyl ethers such as polyoxyethylene nonyl phenyl ether; polyoxyethylene hardened castor oils such as polyoxyethylene castor oil, polyoxyethylene hardened castor oil (polyoxyethylene hydrogenated castor oil); polyoxyethylene beeswax derivatives such as polyoxyethylene sorbitol beeswax; polyoxyethylene lanolin derivatives such as polyoxyethylene lanolin; polyoxyethylene fatty acid amides such as polyoxyethylene stearic acid amide; alkyl sulfates having a C_{10-18} alkyl group such as sodium cetyl sulfate, sodium lauryl sulfate, sodium oleyl sulfate; polyoxyethylene alkyl ether sulfates having an average EO mole number of 2-4 and a C_{10-18} alkyl group such as sodium polyoxyethylene lauryl sulfate; alkyl sulfosuccinic acid ester salts having a C_{8-18} alkyl group such as sodium laurylsulfosuccinate; lecithin; glycerophospholipids; sphingophospholipids such as sphingomyelin; sucrose fatty acid esters of C_{12-18} fatty acids.

[0042] In an embodiment, the surfactant the pharmaceutical composition of the present disclosure is a non-ionic surfactant. In a further embodiment, the surfactant the pharmaceutical composition of the present disclosure is a polyoxyethylene sorbitan alkyl ester, *e.g.* polysorbate. In yet a further embodiment, the surfactant the pharmaceutical composition of the present disclosure is polysorbate-20 (T20 or Tween-20™).

[0043] In another embodiment, the amount of surfactant in the pharmaceutical composition of the present disclosure is about 0.0001% to about 10% (w/w). In a further embodiment, the amount of surfactant in the pharmaceutical composition of the present disclosure is about 0.001% to about 5%, 1% or 0.1% (w/w) or about 0.005% to about 0.05%. In yet a further embodiment, the amount of surfactant in the pharmaceutical composition of the present disclosure is about 0.01% (w/w).

[0044] In an embodiment, the pharmaceutical composition of the present disclosure may further comprise one or more stabilizing agents or stabilizers. As used herein, the term "stabilizer" is intended to mean a compound used to stabilize the therapeutic agent against physical, chemical, or biochemical process that would reduce the therapeutic activity of the agent. Suitable stabilizers are non-reducing sugars including, by way of example and without limitation, sucrose (or saccharose) and trehalose; and non-reducing polyols or sugar alcohols including, by way of example and without limitation, sorbitol, mannitol, maltitol, xylitol, glycol, glycerol and ethylene glycol. In an embodiment, the amount of stabilizing agent in the pharmaceutical composition of the present disclosure is about 0.05% to about 10% (w/w). In a further embodiment, the amount

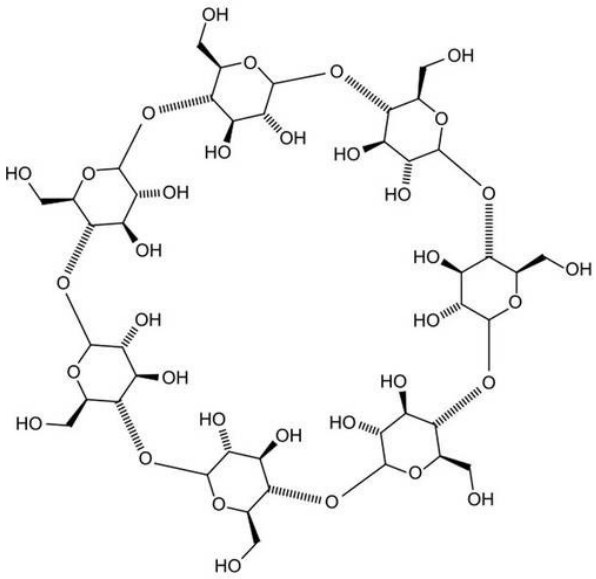
of stabilizing agent in the pharmaceutical composition of the present disclosure is about 1% to about 5%, about 2% to about 4% or about 2.5% to about 3.5% (w/w). In yet a further embodiment, the amount of surfactant in the pharmaceutical composition of the present disclosure is about 3% (w/w).

[0045] In an embodiment, the pharmaceutical composition of the present disclosure comprises a non-reducing sugar. "Non-reducing sugar" as used herein refers to a sugar that does not contain a hemi-acetal, for example a carbohydrate or sugar characterized by having a glycosidic bond formed between the reducing ends of the sugar units, and not between a reducing end of one sugar unit and a non-reducing end of the other sugar unit. In a further embodiment, the above-mentioned non-reducing sugar is trehalose or sucrose. In a further embodiment, the above-mentioned non-reducing sugar is sucrose. In an embodiment, the non-reducing sugar is in a concentration of about 0.1% to about 5% (w/w) in the pharmaceutical composition of the disclosure. In an embodiment, the non-reducing sugar is in a concentration of about 1% to about 3% (w/w). In a further embodiment, the non-reducing sugar is in a concentration of about 2% (w/w).

[0046] In an embodiment, the pharmaceutical composition of the present disclosure comprises a buffering agent, i.e. an agent that maintains the pH of the pharmaceutical composition near a chosen value. Examples of buffering agents include acetate buffers, succinate buffers, citrate buffers, phosphate buffers and histidine buffers. In an embodiment, the buffering agent is a histidine buffer. In an embodiment, the concentration of histidine in the pharmaceutical composition is about 0.01% to about 1%, for example about 0.05% to about 0.5% or about 0.1% to about 0.3%. In a further embodiment, the histidine sugar is in a concentration of about 0.15%.

[0047] In an embodiment, the pharmaceutical composition of the present disclosure comprises an oligosaccharide, for example a cyclic oligosaccharide such as a cyclodextrin. The term "cyclodextrin" as used herein refers to a family of cyclic oligosaccharides, comprising a macrocyclic ring of glucopyranoside subunits (5 or more) joined by α-1,4 glycosidic bonds. Examples of cyclodextrins include α-cyclodextrin, β-cyclodextrin and γ-cyclodextrin, which comprise 6, 7 and 8 glucopyranoside subunits, respectively, as well as analogs thereof (*e.g.*, modified cyclodextrins). In an embodiment, the cyclodextrin is a β-cyclodextrin or a modified β-cyclodextrin.

[0048] β–cyclodextrin has the following structure:



[0049] One or more of the hydroxyl groups of one or more of the sugar units may be modified, for example with an alkyl, alkenyl or alkynyl group, or with a substituted alkyl, alkenyl or alkynyl group. Therefore, in embodiments, the β-cyclodextrin may be unmodified or unsubstituted, or may be modified or substituted. As such, in a further embodiment, the β-cyclodextrin is a modified β-cyclodextrin. "Modified β-cyclodextrin" as used herein refers to a β-cyclodextrin that contains a modification at one or more hydroxyl groups of one or more sugar units of the β-cyclodextrin, i.e., a group or moiety that is attached to one or more hydroxyl groups of one or more sugar units of the β-cyclodextrin. As such, in embodiments, the modified β-cyclodextrin is an alkyl-, alkenyl-, alkynyl, substituted alkyl-, substituted alkenyl or substituted alkynyl-β-cyclodextrin (*e.g.*, with a hydroxyl substitution). In embodiments, the alkyl, alkenyl or alkynyl groups are (C_1-C_6)alkyl, (C_1-C_6)alkenyl or (C_1-C_6)alkynyl groups. In a further embodiment, the modified β-cyclodextrin is a (C_1-C_6)alkyl β-cyclodextrin, in a further embodiment methyl-β-cyclodextrin (M-β-CD). In a further embodiment, the modified β-cyclodextrin is a hydroxy(C_1-C_6)alkyl β–cyclodextrin, in a further embodiment hydroxypropyl-β-cyclodextrin (HP-β-CD).

[0050] In an embodiment, the cyclodextrin is present in the pharmaceutical composition at a concentration of about 2 to about 15 % (w/v), in a further embodiment about 2 to about 12.5 % (w/v), for example about 2 to about 10 % (w/v), about 2.5 to about 15 % (w/v), about 2.5 to about 12.5 % (w/v), about 2.5 to about 10 % (w/v), about 5 to about 15 % (w/v), about 5 to about 12.5 % (w/v), about 5 to about 10 % (w/v), about 7.5 to about 12.5 % (w/v), about 7.5 to about 10 % (w/v), about 5, 7.5, 10, 12.5 or 15 % (w/v), or about 10 % (w/v).

[0051] In an embodiment, the pharmaceutical composition of the present disclosure has a pH of about 4.5 to about 6.5, for example about 5.0 to about 6.0. According to another embodiment, the pharmaceutical composition has a pH of about 5.0. According to a further embodiment, the pharmaceutical composition has a pH of about 5.5. According to another further embodiment, the

pharmaceutical composition has a pH of about 5.9-6.0.

[0052] In an embodiment, the pharmaceutical composition of the present disclosure comprises a diluent, for example an aqueous solution. In a further embodiment, the pharmaceutical composition comprises (typically sterile) water.

[0053] The pharmaceutical composition of the present disclosure may further contain other diluents, solubilizing agents, excipients, pH-modifiers, soothing agents, buffers, sulfur-containing reducing agents, antioxidants or the like, if desired. For example, sulfur-containing reducing agents include N-acetylcysteine, N-acetylhomocysteine, thioctic acid, thiodiglycol, thioethanolamine, thioglycerol, thiosorbitol, thioglycolic acid and salts thereof, sodium thiosulfate, glutathione, methionine and sulfhydryl-containing compounds such as thioalkanoic acid having 1 to 7 carbon atoms. Antioxidants include methionine, erythorbic acid, dibutylhydroxytoluene, butylhydroxyanisole, α-tocopherol, tocopherol acetate, L-ascorbic acid and salts thereof, L-ascorbyl palmitate, L-ascorbyl stearate, sodium bisulfite, sodium sulfite, triamyl gallate, propyl gallate or chelating agents such as disodium ethylenediamine tetraacetate (EDTA), sodium pyrophosphate, sodium metaphosphate. Other components commonly added may also be contained, *e.g.*, inorganic salts such as sodium chloride, potassium chloride, calcium chloride, sodium phosphate, potassium phosphate, sodium bicarbonate; and organic salts such as sodium citrate, potassium citrate, sodium acetate.

[0054] In an embodiment, the pharmaceutical composition is stable at room temperature. A stable composition is a composition in which the active principal ingredient, i.e. the GHRH molecule (*e.g.*, [trans-3-hexenoyl]hGHRH (1-44) amide) therein essentially retains its physical and chemical stability and integrity upon storage. Various analytical techniques for measuring protein or peptide stability are available in the art and are reviewed in Peptide and Protein Drug Delivery, 247-301, Vincent Lee Ed., Marcel Dekker, Inc., New York, N.Y., Pubs. (1991) and Jones, A. Adv. Drug Delivery Rev. 10: 29-90 (1993). Stability can be measured at a selected temperature for a selected time period. For rapid screening, the composition may be kept, for example, at 40°C for 2 weeks to 1 month (and for up to 6 months), at which time stability is measured. The composition may also be kept, for example, at in ambient room temperature conditions (about 15-30°C, preferably about 20-25°C) for at least 6 months, at which time stability is measured. The composition of the present disclosure preserves the stability of the GHRH molecule (*e.g.*, [trans-3-hexenoyl]hGHRH (1-44) amide) in lyophilized form for a period of storage at room temperature (i.e. 20-25°C) for at least 1 week, at least 2 weeks, at least 4 weeks, at least 6 weeks, at least 8 weeks, at least 3 months, at least 4 months, at least 6 months, or at least 12 months. For example, a "stable" composition may be one wherein more than about 80%, more than about 90%, more than about 95%, more than about 96%, more than about 97%, more than about 98%, or more than about 99% of the non-degraded active agent is present in the composition upon the storage period. The stability of the composition of the present disclosure may for example be measured

using reverse phase high pressure liquid chromatography (RP-HPLC).

[0055] In an embodiment, the pharmaceutical composition has a pH of about 5.8 to about 6.2 and comprises:

- about 7.8 to about 8.2 mg/ml of the GHRH molecule (*e.g.*, [trans-3-hexenoyl]hGHRH (1-44) amide)
- about 8% to about 12% of a cyclodextrin, for example a β-cyclodextrin such as hydroxypropyl-β-cyclodextrin (HP-β-CD)
- about 2% to about 4% of a sugar alcohol, such as mannitol.

[0056] In an embodiment, the pharmaceutical composition has a pH of about 5.9 to about 6.1 and comprises:

- about 7.9 to about 8.1, or about 8 mg/ml, of the GHRH molecule (*e.g.*, [trans-3-hexenoyl]hGHRH (1-44) amide)
- about 9 to about 11%, or about 10%, of a cyclodextrin, for example a β-cyclodextrin such as hydroxypropyl-β-cyclodextrin (HP-β-CD)
- about 2.5% to about 3.5%, or about 3%, of a sugar alcohol, such as mannitol.

[0057] The pharmaceutical composition of the present disclosure may be useful for inducing or increasing GH secretion in a subject.

[0058] Accordingly, in another aspect, the present disclosure provides a method for inducing or increasing GH secretion in a subject in need thereof, said method comprising administering to said subject an effective amount of the above-mentioned formulation or composition.

[0059] In another aspect, the present disclosure provides a use of the above-mentioned formulation or composition, for inducing or increasing growth hormone secretion in a subject.

[0060] In another aspect, the present disclosure provides a use of the above-mentioned formulation or composition, for the preparation of a medicament for inducing or increasing GH secretion in a subject.

[0061] The terms "stimulating," "increasing," or "inducing" or any variations of these terms as used herein, refer to a measurable increase of a biological activity. In embodiments, the increase is at least a 10%, 20%, 40%, 60%, 80%, 90%, 95%, 100% (2-fold), 200% (3-fold) increase in the biological activity relative to a control. For example, a GRF analog is found to stimulate GHRHr activity when an increase in GH levels is measured following administration of the GHRH molecule to a subject (*e.g.*, animal, human) in comparison to a subject not administered with the GHRH molecule.

[0062] In view of their GHRHr agonist activity and GH-releasing properties, the compositions of the disclosure may be useful as a medicament, for prophylactic and/or therapeutic applications in which stimulation of GH secretion is desirable, for example for the treatment or prevention of conditions/disorders/diseases associated with GHRH and/or GH function (*e.g.*, in which reduced GH and/or GHRH function is involved in the etiology of the disease/disorder). Diseases and

conditions in which administration of GH, GHRH or GHRH analogs/derivatives may be beneficial have been extensively described in the art (see, *e.g.*, WO 2009/009727, WO 2006/042408, WO 2005/037307, WO 2004/105789). Such conditions/disorders/diseases include, for example, syndromes associated with fat accumulation, hypercholesterolemia, obesity, syndrome X, lipohypertrophy, lipoatrophy, lipodystrophy (*e.g.*, HIV-associated lipodystrophy syndrome), impaired cognitive function, impaired daytime vigilance, declined function of the immune system (*e.g.*, immunodeficiencies such as T-cell deficiencies), muscle protein catabolism, diseases/conditions associated with muscle wasting such as sarcopenia, frailty, radiotherapy- and/or chemotherapy-related side effects (*e.g.*, in HIV-infected and cancer patients), cachexia (*e.g.*, in cancer patients), hypothalamic pituitary dwarfism, burns, osteoporosis, renal failure, non-union bone fracture, acute/chronic debilitating illness or infection, wound healing, post-surgical problems, lactation failure, infertility in women, neurodegenerative conditions, GRF receptor-dependent tumors, conditions related to aging, sleep disorders/impairment, liver disease or conditions such as Non-Alcoholic Fatty Liver Disease (NAFLD) or Nonalcoholic steatohepatitis (NASH), with or without fibrosis, or cirrhosis. Thus, in an embodiment, the subject to whom the composition or formulation is administered suffers from one or more of the diseases or conditions described herein. In an embodiment, the subject suffers from lipodystrophy (*e.g.*, HIV-associated lipodystrophy syndrome). In an embodiment, the subject suffers from NAFLD or NASH.

[0063] Therefore, in other aspects, the present disclosure provides a method for (1) stimulating daytime vigilance and/or cognitive function, *e.g.* in conditions related to aging, mild cognitive impairment (MCI), pre-Alzheimer's symptoms (Pre-Onset Alzheimer's), dementia and/or sleep impairment (*e.g.*, age-related sleep impairment), (2) improving/preventing/treating metabolic conditions associated with fat accumulation and/or hypercholesterolemia (obesity, abdominal obesity/adiposity, abdominal obesity with metabolic disorders, abdominal obesity with relative GH deficiency, metabolic syndrome or syndrome X, lipohypertrophy, lipoatrophy, lipodystrophy (*e.g.*, HIV-associated lipodystrophy syndrome), dyslipidemia, hypertriglyceridemia), NAFLD/NASH (3) improving anabolism in catabolic/wasting conditions, such as those observed in acute or chronic renal failure (*e.g.*, acute or chronic renal failure wasting), chronic heart failure (*e.g.*, chronic heart failure wasting), chronic obstructive pulmonary disease (COPD), cystic fibrosis (*e.g.*, cystic fibrosis wasting in adults), frailty, burns, infections (sepsis), muscular dystrophy, congestive heart failure, neurodegenerative conditions (Alzheimer's, pre-Alzheimer's syndromes, amyotrophic lateral sclerosis (ALS), Acquired Immune Deficiency Syndrome (AIDS), protein malnutrition following long-term corticosteroid therapy, following non-union bone fracture, hip fracture, trauma, or major surgery (post-surgical problems), osteoporosis, long-term immobilization, cancer-related cachexia, sarcopenia (*e.g.*, age-related sarcopenia), gastro-intestinal (GI) malabsorption (Short Bowel Syndrome (SBS), Crohn's disease) particularly in elderly subjects, for example to increase muscle mass and/or function, (4) improving immune function or reconstitution of immunodeficient

states (*e.g.*, T-cell immunodeficiencies) such as that associated aging, HIV infection/AIDS or following high-dose chemotherapy and/or radiotherapy (in HIV-infected and cancer patients), (5) altering a lipid parameter ((a) decreasing cholesterol; (b) decreasing non-HDL cholesterol; (c) decreasing triglycerides; and/or (d) decreasing the ratio of total cholesterol/HDL cholesterol); (6) altering a body composition parameter ((a) increasing lean body mass; (b) decreasing trunk fat; (c) decreasing visceral fat; (d) decreasing abdominal girth; (e) decreasing visceral adipose tissue (VAT); and/or (f) decreasing the VAT/subcutaneous adipose tissue (SAT) ratio), (7) enhancing fertility or treating infertility (in women), treating lactation failure, (8) treating GH deficiency (*e.g.*, GH deficiency with abdominal obesity), providing GH replacement therapy, *e.g.*, in adults, treating idiopathic short stature (ISS) (9) treating GHRH receptor-related tumors, (10) treating hypothalamic pituitary dwarfism, (11) improving wound healing, (12) treating burns, (13) treating acute/chronic debilitating illness or infection, and/or (14) preventing/treating a condition characterized by deficient or decreased bone formation *(e.g.*, osteoporosis); the method comprising administering an effective amount of the above-mentioned composition, to a subject in need thereof.

[0064] In other aspects, the present disclosure provides a use of the above-mentioned composition for achieving one or more of the biological/therapeutic effects (1) to (14) noted above, e.g. for improving, preventing and/or treating the conditions, diseases or disorders noted above, or for the preparation/manufacture of a medicament for improving, preventing and/or treating the conditions, diseases or disorders noted above. In other aspects, the present disclosure provides the above-mentioned composition for use in improving, preventing and/or treating the conditions, diseases or disorders noted above, or for the preparation/manufacture of a medicament for improving, preventing and/or treating the conditions, diseases or disorders noted above.

[0065] The term "treatment" as used herein, is defined as the application or administration of a therapeutic agent to a subject, or application or administration of a therapeutic agent to an isolated tissue or cell line from a subject, who has a disorder, a disease, a symptom of disorder or disease, or a predisposition toward a disorder or disease, with the purpose to cure, heal, alleviate, delay, relieve, alter, remedy, ameliorate, improve or affect the disorder/disease, the symptoms of disorder/disease or the predisposition toward disorder/disease.

[0066] In another aspect, the present disclosure provides a method of administering a GHRH molecule to a subject, preferably *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$, to a subject to obtain plasmatic levels of the GHRH molecule that are bioequivalent to administration of 2 mg of the GHRH molecule at a concentration of 1 mg/mL (*e.g.*, the EGRIFTA™ formulation comprising 5% mannitol), the method comprising administering to the subject more than 1.2 mg and less than 1.36 mg, for example about 1.21 to about 1.35, about 1.22 to about 1.33 or 1.34, or about 1.23 to about 1.32 mg of the GHRH molecule at a concentration of about 7.5 mg/mL or more. In an embodiment, the GHRH molecule, preferably *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$, is formulated in

the pharmaceutical composition described herein. In an embodiment, the subject suffers from one or more of the conditions, diseases or disorders noted above. In a further embodiment, the subject suffers from HIV-associated lipodystrophy. In another embodiment, the subject suffers from NAFLD or NASH.

[0067] The term "bioequivalent" as used herein means that one or more pharmacokinetic (PK) parameters following administration of the GHRH molecule to subjects do not significantly differ between the two treatment regimens, as determined using a suitable statistical standard. In an embodiment, at least two PK parameters do not significantly differ between the two treatment regimens. In an embodiment, at least three PK parameters do not significantly differ between the two treatment regimens. In an embodiment, the one or more PK parameters comprise the maximum plasmatic concentration (C_{max}). In an embodiment, the one or more PK parameters comprise the area under the plasma concentration time curve extrapolated to infinity ($AUC_{0-\infty}$). In an embodiment, the one or more PK parameters comprise the cumulative area under the plasma concentration time curve calculated from 0 to T_{LQC} (time of last observed quantifiable plasma concentration) using the linear trapezoidal method (AUC_{0-T}). In an embodiment, bioequivalent means that the 90% CI of the relative mean C_{max}, $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ is within 80% to 125% of the reference (EGRIFTA®) in the fasting state.

[0068] In an embodiment, the natural logarithmic (ln) transformation of the one or more PK parameters is used for the statistical analysis. In an embodiment, the statistical standard used is the ratio of geometric LSmeans with corresponding 90% confidence interval (CI) for the exponential of the difference between the two treatment regimens for the Least-squares means (LSmeans) of the ln-transformed PK parameter(s) that is within the 80.00% to 125.00% range, as described in the Examples below.

[0069] In an embodiment, the method permits to achieve a maximum plasmatic concentration (C_{max}) of the GHRH molecule of about 1500 to about 4500 pg/mL in a human subject. In another embodiment, the method permits to achieve an average C_{max} of the GHRH molecule of about 2500 to about 3500 pg/mL in a population of human subjects. In further embodiments, the method permits to achieve an average maximum plasmatic concentration C_{max} of the GHRH molecule of about 2600 or 2700 to about 3000, 3100 or 3200 pg/mL in a population of human subjects.

[0070] In an embodiment, the method permits to achieve an area under the plasma concentration time curve extrapolated to infinity ($AUC_{0-\infty}$) of the GHRH molecule of about 300 to about 1400 pg·h/mL in a subject. In an embodiment, the method permits to achieve an average $AUC_{0-\infty}$ of the GHRH molecule of about 500 to about 1000 pg·h/mL in a population of human subjects. In further embodiments, the method permits to achieve an average $AUC_{0-\infty}$ of the GHRH molecule of about 600, 650 or 700 to about 750, 800, 850 or 900 pg/mL in a population of human subjects.

[0071] In an embodiment, the method comprises: (a) resuspending a lyophilized GHRH molecule, preferably *trans*-3-hexenoyl-$GHRH_{(1-44)}$-NH_2 or a pharmaceutically acceptable salt

thereof, in a suitable volume of a pharmaceutically acceptable diluent to obtain a GHRH molecule solution at about 7.5 mg/mL or more; and (b) administering a suitable volume of the GHRH solution so that more than 1.2 mg and less than 1.36 mg, for example about 1.21 to about 1.35, about 1.22 to about 1.33 or 1.34, or about 1.23 to about 1.32 mg of the GHRH molecule, preferably *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or a pharmaceutically acceptable salt thereof, is administered to the subject.

[0072] In an embodiment, the method comprises: (a) resuspending a lyophilized GHRH molecule, preferably *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or a pharmaceutically acceptable salt thereof, in a suitable volume of a pharmaceutically acceptable diluent to obtain a GHRH molecule solution at about 7.5 to about 8.5 mg/mL; and (b) administering about 0.144 to about 0.176 mL of the GHRH molecule solution of (a) to the subject, thereby administering about 1.23 to about 1.32 mg of the GHRH molecule.

[0073] In an embodiment, the method comprises: (a) resuspending a lyophilized GHRH molecule, preferably *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or a pharmaceutically acceptable salt thereof, in a suitable volume of a pharmaceutically acceptable diluent to obtain a GHRH molecule solution at about 7.8 to about 8.2 mg/mL; and (b) administering about 0.150 to about 0.170 mL of the GHRH molecule solution of (a) to the subject, thereby administering about 1.23 to about 1.32 mg of the GHRH molecule.

[0074] In a further embodiment, the method comprises: (a) resuspending lyophilized GHRH molecule, preferably *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or a pharmaceutically acceptable salt thereof, in a suitable volume of a pharmaceutically acceptable diluent to obtain a GHRH molecule solution at about 8 mg/mL; and (b) administering about 0.16 mL of the GHRH molecule solution of (a) to the subject, thereby administering about 1.28 mg of the GHRH molecule.

[0075] In a further embodiment, the method comprises: (a) resuspending about 12.5 mg of lyophilized GHRH molecule, preferably *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or a pharmaceutically acceptable salt thereof, in about 1.4 mL of a pharmaceutically acceptable diluent to obtain a GHRH molecule solution at about 8 mg/mL; and (b) administering about 0.16 mL of the GHRH molecule solution of (a) to the subject, thereby administering about 1.28 mg of the GHRH molecule.

[0076] In an embodiment, the lyophilized GHRH molecule, preferably *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or a pharmaceutically acceptable salt thereof, is in a container, preferably a sealed container, such as a vial. In an embodiment, the lyophilized GHRH molecule is resuspended using a syringe. In an embodiment, the GHRH molecule solution is administered by injection, *e.g.*, subcutaneous injection.

[0077] As used herein, the term "subject" or "patient" are taken to mean a warm-blooded animal such as a mammal, for example, a cat, a dog, a mouse, a guinea pig, a horse, a bovine cow, a sheep or a human. In an embodiment, the subject is a mammal. In a further embodiment, the

above-mentioned subject is a human.

[0078] In another aspect, the present disclosure also provides a kit comprising: (a) a first container comprising at least about 1.21 mg, for example at least about 1.23 to about 1.32 mg of lyophilized GHRH molecule, preferably *trans*-3-hexenoyl-$GHRH_{(1-44)}$-NH_2 or a pharmaceutically acceptable salt thereof; (b) a second container comprising a pharmaceutically acceptable diluent; and (c) instructions for resuspending the lyophilized GHRH molecule, preferably *trans*-3-hexenoyl-$GHRH_{(1-44)}$-NH_2 or a pharmaceutically acceptable salt thereof, in the pharmaceutically acceptable diluent to obtain a GHRH molecule solution at about 7.5 mg/mL or more.

[0079] In an embodiment, the kit comprises: (a) a first container comprising at least about 1.21 mg, for example at least about 1.23 to about 1.32 mg of lyophilized GHRH molecule, preferably *trans*-3-hexenoyl-$GHRH_{(1-44)}$-NH_2 or a pharmaceutically acceptable salt thereof; (b) a second container comprising at least 0.16 mL of a pharmaceutically acceptable diluent; and (c) instructions for resuspending the lyophilized GHRH molecule, preferably *trans*-3-hexenoyl-$GHRH_{(1-44)}$-NH_2 or a pharmaceutically acceptable salt thereof, in the pharmaceutically acceptable diluent to obtain a GHRH molecule solution at about 7.8 to about 8.2 mg/mL.

[0080] In an embodiment, the first container comprises about 12.5 mg of lyophilized *trans*-3-hexenoyl-$GHRH_{(1-44)}$-NH_2 or a pharmaceutically acceptable salt thereof. In an embodiment, the second container comprises about 1.4 mL of the pharmaceutically acceptable diluent.

[0081] In an embodiment, the pharmaceutically acceptable diluent is an aqueous solution, for example sterile water.

[0082] In an embodiment, the lyophilized GHRH molecule, preferably *trans*-3-hexenoyl-$GHRH_{(1-44)}$-NH_2 or a pharmaceutically acceptable salt thereof, is in a sealed container, such as a vial. In an embodiment, the kit further comprises at least one syringe.

[0083] In an embodiment, the kit further comprises instructions for administering more than 1.2 mg and less than 1.36 mg, for example about 1.21 to about 1.35, about 1.22 to about 1.33 or 1.34, or about 1.23 to about 1.32 mg of the GHRH molecule to the subject, *e.g.*, by subcutaneous injection.

EXAMPLES

[0084] The present disclosure is illustrated in further detail by the following non-limiting examples.

Example 1: Bioequivalence study in humans with 1.2 mg, 1.36 mg and 1.6 mg of an 8 mg/ml tesamorelin formulation

[0085] The study was a single center, randomized, single dose, blinded, 4-treatment, 4-period, 4-sequence, crossover design in 16 healthy male and female subjects. The following investigational products (IPs) were administered under fasting conditions:

[0086] Test product: Tesamorelin 12.5 mg/vial sterile lyophilized powder for SC injection, resuspended at 8 mg/ml in a solution comprising 10% hydroxypropyl-β-cyclodextrin (HP-β-CD)

and 3% Mannitol (pH 5.9-6).

[0087] Reference product: *EGRIFTA*® (tesamorelin) 1 mg/vial sterile lyophilized powder for SC injection resuspended at 1 mg/ml in a solution comprising 5% Mannitol.

[0088] A single SC dose of one of the following 4 treatments was administered in each study period according to the randomization scheme:

[0089] Treatment-A: A single 1.2 mg (0.15 mL) SC dose of the Test Product

[0090] Treatment-B: A single 1.36 mg (0.17 mL) SC dose of the Test Product

[0091] Treatment-C: A single 1.6 mg (0.20 mL) SC dose of the Test Product

[0092] Treatment-D: A single 2 mg (2.00 mL) SC dose of the Reference Product

[0093] The treatments were administered according to Table 1:

Table 1: Study sequences

	Period 1	Period 2	Period 3	Period 4
Sequence ABDC (n = 4)	Treatment-A	Treatment-B	Treatment-D	Treatment-C
Sequence BCAD (n = 4)	Treatment-B	Treatment-C	Treatment-A	Treatment-D
Sequence CDBA (n = 4)	Treatment-C	Treatment-D	Treatment-B	Treatment-A
Sequence DACB (n = 4)	Treatment-D	Treatment-A	Treatment-C	Treatment-B

[0094] Inclusion Criteria

1. Provision of signed and dated ICF
2. Stated willingness to comply with all study procedures and availability for the duration of the study
3. Healthy adult male or female
4. If female, met one of the following criteria:
    a) Was of childbearing potential and agreed to use one of the accepted contraceptive regimens from at least 28 days prior to the first study drug administration through to at least 30 days after the last dose of the study drug. An acceptable method of contraception included one of the following:
        - Abstinence from heterosexual intercourse

- Systemic contraceptives (combined birth control pills, injectable/implant/insertable hormonal birth control products, transdermal patch)
- Intrauterine device (with or without hormones)
- Male condom with spermicide or male condom with a vaginal spermicide (gel, foam, or suppository)
- Male partner vasectomized at least 6 months prior to the first study drug administration

or

b) Male partner vasectomized less than 6 months prior to dosing, and agreed to use an additional acceptable contraceptive method from the first study drug administration through to at least 30 days after the last dose of the study drug

or

c) Was of non-childbearing potential, defined as surgically sterile (i.e. had undergone complete hysterectomy, bilateral oophorectomy, or tubal ligation) or was in a postmenopausal state (i.e. at least 1 year without menses without an alternative medical condition prior to the first study drug administration)

5. Aged at least 18 years but not older than 55 years
6. Body mass index within 18.5 kg/m^2 to 30.0 kg/m^2, inclusively
7. Light-, non- or ex-smoker *(A light smoker was defined as someone using 10.0 nicotine units or less per day for at least 90 days prior to the first study drug administration. An ex-smoker was defined as someone who completely stopped using nicotine products for at least 180 days prior to the first study drug administration)*
8. Clinical laboratory values within the laboratory's stated normal range; if not within this range, they must have been without clinical significance, as determined by an investigator
9. Had no clinically significant (CS) diseases captured in the medical history or evidence of CS findings in the physical examination (including vital signs), glycemia measurements and/or ECG, as determined by an investigator

[0095] Exclusion Criteria

1. Female who was lactating at screening
2. Female who was pregnant according to the pregnancy test at screening or prior to the

first study drug administration

3. History of significant hypersensitivity to tesamorelin, mannitol, betadex or any related products (including excipients of the formulations) as well as severe hypersensitivity reactions (like angioedema) to any drugs

4. Presence or history of significant gastrointestinal, liver or kidney disease, or any other condition that is known to interfere with drug absorption, distribution, metabolism or excretion, or known to potentiate or predispose to undesired effects

5. History of significant cardiovascular, pulmonary, hematologic, neurological, psychiatric, endocrine, immunologic or dermatologic disease

6. Presence of CS ECG abnormalities at the screening visit, as defined by medical judgment

7. Presence of scars, bruises, reddening, infection or irritation at the injection site (abdomen)

8. Presence of any tattoo, skin discoloration or abnormal skin texture at the injection site (abdomen) which may have affected visual skin evaluation

9. Maintenance therapy with any drug or significant history of drug dependency or alcohol abuse (> 3 units of alcohol per day, intake of excessive alcohol, acute or chronic)

10. Any CS illness in the 28 days prior to the first study drug administration

11. Use of any prescription drugs (with the exception of hormonal contraceptives or hormone replacement therapy) in the 28 days prior to the first study drug administration, that in the opinion of an investigator would have put into question the status of the participant as healthy

12. Any history of tuberculosis

13. Positive test result for alcohol and/or drugs of abuse at screening or prior to the first study drug administration

14. Positive screening results to HIV Ag/Ab Combo, Hepatitis B surface Antigen or Hepatitis C Virus tests

15. Inclusion in a previous group for this clinical study

16. History of tesamorelin intake

17. Intake of an IP in the 28 days prior to the first study drug administration

18. Donation of 50 mL or more of blood in the 28 days prior to the first study drug administration

19. Donation of 500 mL or more of blood (Canadian Blood Services, Hema-Quebec, clinical studies, etc.) in the 56 days prior to the first study drug administration

[0096] A total of 16 subjects were included in this study and, after randomization, all 16 subjects received Treatment-A, Treatment-B, Treatment-C and Treatment-D. All subjects completed the study.

[0097] Blood samples were collected prior to and up to 4.00 hours after drug administration in K_2EDTA Vacutainers. Samples were processed and stored under conditions that have been shown not to cause significant degradation of the analyte. Briefly, samples were centrifuged at 4°C and at approximately 1000*g* for 10 minutes. The plasma obtained was transferred in a polypropylene transfer tube. Thereafter, 1800 µL of generated plasma were transferred into a polypropylene tube containing 200 µL of stabilization solution (10% of final volume). The stabilized plasma samples were put immediately on dry ice and stored frozen at -80°C until assayed.

[0098] Tesamorelin plasma levels were assessed using a validated ELISA assay. The lower limit of quantitation (LOQ) and upper limit of quantitation were 150 pg/mL and 6000 pg/mL, respectively.

[0099] The main PK parameters of interest for this study were:

- C_{max} (Maximum observed plasma concentration),
- AUC_{0-T} (cumulative area under the plasma concentration time curve calculated from 0 to Time of last observed quantifiable plasma concentration (T_{LQC}) using the linear trapezoidal method); and
- AUC_{0-INF} (Area under the plasma concentration time curve extrapolated to infinity, calculated as $AUC_{0-T} + C_{LQC}/\lambda_z$, where C_{LQC} is the measured concentration at time T_{LQC}, and λ_z is the apparent elimination rate constant, estimated by linear regression of the terminal linear portion of the log concentration *versus* time curve)

[00100] Other parameters such as T_{max} (Time of maximum observed plasma concentration; if it occurs at more than one time point, T_{max} is defined as the first time point with this value), $AUC_{0-T/INF}$ (Relative percentage of AUC_{0-T} with respect to AUC_{0-INF}), λ_Z and T_{half} (Terminal elimination half-life, calculated as $\ln(2)/\lambda_z$) were also determined.

[00101] The main absorption and disposition parameters were estimated using a non-compartmental approach with a log-linear terminal phase assumption. The trapezoidal rule was used to estimate the AUC (linear trapezoidal linear interpolation) and the terminal phase was estimated by maximizing the coefficient of determination estimated from the log-linear regression model. However, disposition parameters were not estimated for individual concentration-time profiles where the terminal log-linear phase could be reliably characterized using the following criteria:

- Phoenix® WinNonlin® Best fit range selection: R^2 of at least 80%
- The corresponding terminal half-life value was lower than or equal to 2 times the time interval over which λ_Z was estimated (i.e. $T_{half} \leq$ twice the time interval difference between T_{LQC} and T_{LIN}).

[00102] Descriptive statistics were calculated for plasma concentrations at each individual time point and for all PK parameters. The individual plasma concentration/time profiles were presented using the actual sampling times whereas the mean plasma concentration/time profiles were presented using the theoretical sampling times.

[00103] The natural logarithmic transformation of C_{max}, AUC_{0-T} and AUC_{0-INF} was used for all statistical inference. The parameter T_{max} was analyzed using a non-parametric approach. Test of fixed period, sequence and treatment effects was based on the Wilcoxon's rank sum test (Mann-Whitney U-test). All other PK parameters were statistically analyzed using an Analysis of Variance (ANOVA) model.

[00104] Bioequivalence demonstration was based on the 8 mg/mL to 1 mg/mL ratio of geometric Least-Square means (LSmeans) with corresponding 90% CI for C_{max}, AUC_{0-T} and AUC_{0-INF} being within the 80% to 125% acceptance range. The 90% confidence interval (CI) for the exponential of the difference in LSmeans between the Test and Reference products was calculated for the ln-transformed parameters (Treatment-A, -B or -C to Treatment-D ratio of geometric LSmeans).

[00105] The formula to estimate the intra-subject CV was: $\sqrt{e^{MSE}-1}$, where MSE is the Mean Square Error obtained from the ANOVA model of the ln-transformed parameters.

[00106] Safety was assessed by qualified study staff by evaluating the following: reported adverse events (AEs), clinical laboratory test results, vital signs measurements, ECG findings, physical examination findings, visual skin evaluation and glycemia.

[00107] Results

[00108] A summary of the statistical analysis of C_{max} and AUC for tesamorelin is given in Table 2 ((Treatment-A *vs* Treatment-D), Table 3 (Treatment-B *vs* Treatment-D) and Table 4 (Treatment-C *vs* Treatment-D).

Table 2: Summary of the Statistical Analysis of Tesamorelin (Treatment-A *vs* Treatment-D)

Parameter	Intra-Subject C.V. (%)	Geometric LSmeans[a]		Ratio (%)	90% Confidence Limits (%)	
		Treatment-A (1.2 mg) (n=16)[b]	Treatment-D (2 mg) (n=16)[b]		Lower	Upper
C_{max}	21.9	2633.0	2840.9	92.68	81.48	105.43
AUC_{0-T}	21.6	702.0	831.3	84.44	74.35	95.91
$AUC_{0-\infty}$	20.6	767.1	914.1	83.93	73.90	95.32

[a] units are pg/mL for C_{max} and pg·h/mL for AUC_{0-T} and $AUC_{0-\infty}$.
[b] n=15 for $AUC_{0-\infty}$

[00109] Following a 1.2 mg SC dose of the Test Product (Treatment-A), tesamorelin was rapidly absorbed, with a median T_{max} value of 0.15 hour (range = 0.10 to 0.20 hour), and was generally similar to the T_{max} observed for the 2 mg SC dose of the Reference Product (Treatment-

D, median [range] = 0.20 [0.15 to 0.25] hour). The C_{max} , AUC_{0-T} , and $AUC_{0-\infty}$ values observed for the 1.2 mg Test Product were 2889.6 pg/mL, 807.6 pg·h/mL, and 879.2 pg·h/mL respectively, relative to 3097.7 pg/mL, 949.3 pg·h/mL, and 1057.2 pg·h/mL for the 2 mg Reference Product, respectively. The geometric least squares mean ratio point estimate were 92.68%, 84.44% and 83.93% for C_{max}, AUC_{0-T} and $AUC_{0-\infty}$, respectively. The lower bound of the 90% confidence intervals for the Treatment-A to Treatment-D ratios of geometric LSmeans for AUC were below the 80.00% bioequivalence criteria. Thus, administration of 1.2 mg of the 8 mg/ml formulation is not bioequivalent to administration of 2 mg of EGRIFTA®.

Table 3: Summary of the Statistical Analysis of Tesamorelin (Treatment-B *vs* Treatment-D)

Parameter	Intra-Subject C.V. (%)	Geometric LSmeans[a]		Ratio (%)	90% Confidence Limits (%)	
		Treatment-B (1.36 mg) (n=15)[b]	Treatment-D (2 mg) (n=16)[c]		Lower	Upper
C_{max}	21.9	3203.8	2840.9	112.77	98.85	128.66
AUC_{0-T}	21.6	917.1	831.3	110.32	96.54	126.06
$AUC_{0-\infty}$	20.6	990.0	914.1	108.31	95.06	123.41

[a] units are pg/mL for C_{max} and pg·h/mL for AUC_{0-T} and $AUC_{0-\infty}$.
[b] n=14 for AUC_{0-T} and $AUC_{0-\infty}$
[c] n=15 for $AUC_{0-\infty}$

[00110] Following a 1.36 mg SC dose of the Test Product (Treatment-B), tesamorelin was rapidly absorbed, with a median T_{max} value of 0.15 hour (range = 0.10 to 0.25 hour), and was generally similar to the T_{max} observed for the 2 mg SC dose of the Reference Product (Treatment-D, median [range] = 0.20 [0.15 to 0.25] hour). The C_{max}, AUC_{0-T}, and $AUC_{0-\infty}$ values observed for the 1.36 mg Test Product were 3462.6 pg/mL, 957.1 pg·h/mL and 1029.8 pg·h/mL, respectively, relative to 3097.7 pg/mL, 949.3 pg·h/mL and 1057.2 pg·h/mL for the 2 mg Reference Product, respectively. The geometric least squares mean ratio point estimates were 112.77%, 110.32% and 108.31% for C_{max}, AUC_{0-T} and $AUC_{0-\infty}$, respectively. The upper bound of the 90% confidence intervals for the Treatment-B to Treatment-D ratios of geometric LSmeans for C_{max} and AUC_{0-T} were just above the 125.00% bioequivalence criteria. Thus, administration of 1.36 mg of the 8 mg/ml formulation is not bioequivalent to administration of 2 mg of EGRIFTA®.

Table 4: Summary of the Statistical Analysis of Tesamorelin (Treatment-C *vs* Treatment-D)

Parameter	Intra-Subject C.V. (%)	Geometric LSmeans[a]		Ratio (%)	90% Confidence Limits (%)	
		Treatment-C (1.6 mg) (n=16)	Treatment-D (2 mg) (n=16)[b]		Lower	Upper
C_{max}	21.9	3740.0	2840.9	131.65	115.74	149.75
AUC_{0-T}	21.6	1039.6	831.3	125.05	110.11	142.03
$AUC_{0-\infty}$	20.6	1115.5	914.1	122.04	107.78	138.18

[a] units are pg/mL for C_{max} and pg·h/mL for AUC_{0-T} and $AUC_{0-\infty}$.
[b] n=15 for $AUC_{0-\infty}$

[00111] Following a 1.6 mg SC dose of the Test Product (Treatment-C), tesamorelin was rapidly absorbed, with a median T_{max} value of 0.15 hour (range = 0.10 to 0.25 hour), and was generally similar to the T_{max} observed for the 2 mg SC dose of the Reference Product (Treatment-D, median [range] = 0.20 [0.15 to 0.25] hour). The C_{max}, AUC_{0-T}, and $AUC_{0-\infty}$ values observed for the 1.6 mg Test Product were 3918.1 pg/mL, 1126.6 pg·h/mL and 1260.1 pg·h/mL, respectively, relative to 3097.7 pg/mL, 949.3 pg·h/mL and 1057.2 pg·h/mL for the 2 mg Reference Product, respectively. The geometric least squares mean ratio point estimates were 131.65%, 125.05% and 122.04% for C_{max}, AUC_{0-T} and $AUC_{0-\infty}$, respectively. The upper bound of the 90% confidence intervals for the Treatment-C to Treatment-D ratios of geometric LSmeans for C_{max} and AUC were above the 125.00% bioequivalence criteria. Thus, administration of 1.36 mg of the 8 mg/ml formulation is not bioequivalent to administration of 2 mg of EGRIFTA®.

[00112] C_{max} and AUC_{0-T} for the Test Products A, B and C seemed to increase dose proportionally with an 1.36-fold increase in C_{max} and 1.39-fold increase in AUC_{0-T} for the 1.33-fold increase in dose from 1.2 mg to 1.6 mg.

[00113] Overall, these results suggest that administration of a dose above 1.2 mg and below 1.36 mg of the 8 mg/ml formulation would be suitable to obtain bioequivalence to administration of 2 mg of EGRIFTA®.

Example 2: Bioequivalence study in humans with 1.28 mg of an 8 mg/ml tesamorelin formulation

[00114] The primary objective of this study was to evaluate the pharmacokinetic (PK) of 2 tesamorelin formulations (1 mg/vial and 12.5 mg/vial) after a single subcutaneous (SC) dose administration in healthy subjects.

[00115] The investigational products under study were as follows:

- Test**:** Tesamorelin for injection, sterile lyophilized powder, 12.5 mg/vial (8 mg/mL after resuspension in a solution comprising 10% hydroxypropyl-β-cyclodextrin (HP-β-CD) and 3% Mannitol (pH 5.9-6)).

- Reference**:** EGRIFTA®, tesamorelin for injection, sterile lyophilized powder, 1 mg/vial (1 mg/mL solution after resuspension in a solution comprising 5% mannitol).

[00116] Subjects received 1.28 mg of the Test formulation and 2 mg of the Reference subcutaneously, as an injection in the abdomen in a single dose, cross-over, and open-label study. Thus, the following products were administered:

Table 5: Description of formulations studied

Parameter	Study Drug - Test	Study Drug - Reference
Product	tesamorelin	tesamorelin
Strength	8 mg/mL	1 mg/mL
Dosage Form	Lyophilised powder to be reconstituted for injection solution	Lyophilised powder to be reconstituted for injection solution
Dose Administered	1.28 mg (0.16 mL)	2 mg (2.0 mL)
Route of Administration	Subcutaneous injection (abdomen)	Subcutaneous injection (abdomen)
Inactive Ingredients	10% hydroxypropyl-β-cyclodextrin (HP-β-CD) 3% Mannitol	5% Mannitol

[00117] Per Food and Drug Administration (FDA) and Therapeutic Products Directorate (TPD) regulations, relative bioavailability was assessed using different standards:

[00118] FDA:

- The ratio of geometric LSmeans with corresponding 90% confidence interval calculated from the exponential of the difference between the Test and Reference product for the ln-transformed parameters C_{max}, AUC_{0-t} and AUC_{0-INF} were all fall within the 80.00 to 125.00% bioequivalence range.

[00119] TPD:

- The ratio of geometric LSmeans calculated from the exponential of the difference between the Test and Reference product for the ln-transformed parameter C_{max} was to fall within the 80.0 to 125.0% bioequivalence range.
- The ratio of geometric LSmeans with corresponding 90% confidence interval calculated from the exponential of the difference between the Test and Reference product for the ln-transformed parameter AUC_{0-t} was to fall within the 80.0 to 125.0% bioequivalence range.

[00120] Thirty-three (33) of the 36 dosed subjects were included in the pharmacokinetic and statistical analysis. The pharmacokinetic and statistical analyses presented herein were based on QC'ed unaudited concentration data. Actual time was used to perform pharmacokinetic

analysis.

[00121] The inclusion/exclusion criteria, study protocol and data analysis were similar to those of the study reported in Example 1.

[00122] **Results**

[00123] A summary of the statistical analysis of C_{max} and AUC for tesamorelin is given in Table 6.

Table 6: Statistical Results

Parameter	Intra-Subject C.V. (%)	Geometric LSmeans[a]		Ratio[C] (%)	90% Confidence Limits (%)[c]		Ratio[C] (%)	90% Confidence Limits (%)[c]	
		Test (n=33)	Reference (n=33)		Lower	Lower		Lower	Lower
					FDA			TPD	
C_{max}	31.0	3447.6	3193.4	107.96	95.06	122.61	108.0	95.1	122.6
AUC_{0-t}	28.8	930.5	960.5	96.87	86.04	109.07	96.9	86.0	109.1
AUC_{0-INF}[b]	22.6	1047.8	1038.2	100.93	91.78	110.99	N/AP	N/AP	N/AP

[a] units are pg/mL for C_{max} and pg·h/mL for AUC_{0-t} and AUC_{0-INF}

[b] n=32 for AUC_{0-INF}

[C] Decimals are different per regulation requirements. (2 decimals for FDA, and 1 decimal for TPD)

[00124] Statistical results confirmed similar rate and extent of absorption between the 1.28 mg dose (Test) and 2 mg dose (Reference) as all PK endpoints (C_{max}, AUC_{0-t} and AUC_{0-INF}) were within the pre-defined acceptable range of 80-125%.

[00125] TPD additional requirements: No outliers were found. Moreover, the measured drug content of the lots of the reference and test products did not differ by more than 5% from each other (percent of the label claim), thus the potency-corrected content was not used for the ratios and confidence intervals.

[00126] A dose of tesamorelin 1.28 mg (0.16 mL) of a 8 mg/mL formulation is judged to be bioequivalent to a dose of 2 mg (2 mL) of the 1 mg/mL formulation and was found to be safe and well tolerated in the subjects.

[00127] Although the present disclosure has been described hereinabove by way of specific embodiments thereof, it can be modified, without departing from the spirit and nature of the subject disclosure 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 context clearly dictates otherwise.

CLAIMS

WHAT IS CLAIMED IS:

1. A pharmaceutical composition comprising (i) 1.23 to 1.32 mg of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or a pharmaceutically acceptable salt thereof at a concentration of 7.5 to 8.5 mg/mL; and (ii) at least one pharmaceutically acceptable excipient.

2. The pharmaceutical composition of claim 1, comprising 1.25 to 1.30 mg of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or pharmaceutically acceptable salt thereof.

3. The pharmaceutical composition of claim 1 or 2, comprising 1.27 to 1.29 mg of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or pharmaceutically acceptable salt thereof.

4. The pharmaceutical composition of any one of claims 1 to 3, comprising 1.28 *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or pharmaceutically acceptable salt thereof.

5. The pharmaceutical composition of any one of claims 1 to 4, wherein the *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or pharmaceutically acceptable salt thereof is at a concentration of about 8 mg/mL.

6. The pharmaceutical composition of any one of claims 1 to 5, wherein the at least one pharmaceutically acceptable excipient comprises a bulking agent.

7. The pharmaceutical composition of claim 6, wherein the bulking agent is mannitol.

8. The pharmaceutical composition of any one of claims 1 to 7, wherein the at least one pharmaceutically acceptable excipient comprises a cyclodextrin.

9. The pharmaceutical composition of claim 8, wherein the cyclodextrin is a β-cyclodextrin.

10. The pharmaceutical composition of any one of claims 1 to 9, wherein the pharmaceutically acceptable salt of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ is an acetate salt.

11. Use of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or a pharmaceutically acceptable salt thereof for the preparation of a pharmaceutical composition for producing plasmatic levels of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or a pharmaceutically acceptable salt thereof in a subject that are bioequivalent to administration of 2 mg of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ at a concentration of 1 mg/mL, wherein the pharmaceutical composition is for administration of 1.23 to 1.32 mg of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or a pharmaceutically acceptable salt thereof at a concentration of 7.5 to 8.5 mg/mL to the subject.

12. The use of claim 11, wherein the pharmaceutical composition is for administration of 1.28 mg of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or a pharmaceutically acceptable salt thereof.

13. The use of claim 11 or 12, wherein the pharmaceutical composition is for administration of the *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or pharmaceutically acceptable salt thereof at a concentration of 8 mg/mL.

14. The use of any one of claims 11 to 13, wherein the pharmaceutically acceptable salt of *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ is an acetate salt.

15. The use of any one of claims 11 to 14, wherein the subject suffers from HIV-associated lipodystrophy.

16. The use of any one of claims 11 to 15, wherein the *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or pharmaceutically acceptable salt thereof is for administration by subcutaneous injection.

17. The use of any one of claims 11 to 16, further comprising resuspending lyophilized *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or pharmaceutically acceptable salt thereof in a suitable amount of a pharmaceutically acceptable diluent to obtain a *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ or *trans*-3-hexenoyl-GHRH$_{(1-44)}$-NH$_2$ salt solution at a concentration of 7.5 to 8.5 mg/mL; thereby to provide the pharmaceutical composition for administration.