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DESCRIPTION

Description

FIELD OF INVENTION

[0001] Use of a growth hormone protein and polynucleotides encoding same comprising an amino-terminal carboxy-terminal peptide (CTP) of chorionic gonadotrophin and two carboxy-terminal chorionic gonadotrophin CTPs attached to the growth hormone in methods of inducing weight loss or body fat reduction, method of increasing insulin-like growth factor (IGF-1) levels, and methods of reducing the dosing frequency of a growth hormone in a human subject are disclosed. Pharmaceutical compositions comprising the growth hormone and polynucleotides encoding the growth hormone of the invention and methods of using same are also disclosed.

BACKGROUND OF THE INVENTION

[0002] Polypeptides are susceptible to denaturation or enzymatic degradation in the blood, liver or kidney. Accordingly, polypeptides typically have short circulatory half-lives of several hours. Because of their low stability, peptide drugs are usually delivered in a sustained frequency so as to maintain an effective plasma concentration of the active peptide. Moreover, since peptide drugs are usually administered by infusion, frequent injection of peptide drugs cause considerable discomfort to a subject. Thus, there is a need for technologies that will prolong the half-lives of therapeutic polypeptides while maintaining a high pharmacological efficacy thereof. Such desired peptide drugs should also meet the requirements of enhanced serum stability, high activity and a low probability of inducing an undesired immune response when injected into a subject.

[0003] Unfavorable pharmacokinetics, such as a short serum half-life, can prevent the pharmaceutical development of many otherwise promising drug candidates. Serum half-life is an empirical characteristic of a molecule, and must be determined experimentally for each new potential drug. For example, with lower molecular weight polypeptide drugs, physiological clearance mechanisms such as renal filtration can make the maintenance of therapeutic levels of a drug unfeasible because of cost or frequency of the required dosing regimen. Conversely, a long serum half-life is undesirable where a drug or its metabolites has toxic side effects.

[0004] US 2010/081614 discloses a CTP-hGH-CTP-CTP polypeptide corresponding to SEQ ID NO: 11 of the present application, and its medical use in the treatment of hGH deficiency.

SUMMARY OF THE INVENTION

[0005] The present invention provides chorionic gonadotropin carboxy terminal peptide (CTP)-modified polypeptide for use in accordance with the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0006]

FIG. 1 is a Western blot illustrating the molecular weight & identity of hGH (SEQ ID NO: 5), hGH-CTP (SEQ ID NO: 9), hGH-CTP-CTP (SEQ ID NO: 10), CTP-hGH-CTP-CTP (SEQ ID NO: 11) and tCTP-hGH-CTP-CTP (SEQ ID NO: 12). PAGE SDS gel was blotted and stained using monoclonal anti-hGH antibodies. The photograph indicates that like commercial and wild type hGH, CTP-modified hGH variants are recognized by anti-hGH antibodies.

FIG. 2 is a bar graph illustrating the weight gain of hypophysectomized rats following administration of the GH-CTP polypeptides (the different MODs) of the present invention.

FIG. 3 includes two schemes (1) a map of CTP-hGH-CTP-CTP pCI-dhfr Plasmid and (2) structural protein formula of CTP-hGH-CTP-CTP.

FIG. 4 are graphs showing the mean plasma CTP-hGH-CTP-CTP or GH concentrations (pg/ml) following a single i.v. or s.c. dose of CTP-hGH-CTP-CTP or GH in rats (n=3-6 per dose/route).

FIG. 5 are graphs showing the mean incremental weight gain following a single s.c. doses of CTP-hGH-CTP-CTP (0.4, 0.8 and 4 mg/Kg) in hypophysectomized rats in comparison to daily GH injections (0.1 mg/Kg/Day) (n=10 per dose).

FIG. 6 is a graph showing the area Under the Curve following single injection of CTP-hGH-CTP-CTP correlates with Body Weight gain in Rats.

FIG. 7 is a graph showing the incremental weight gain following an s.c. doses of CTP-hGH-CTP-CTP (0.4, 0.8 and 4 mg/Kg) 4 days apart in hypophysectomized rats in comparison to daily GH injections (0.1 mg/Kg/Day) (n=10 per dose).

FIG. 8 is a graph showing hGH serum concentration in hypophysectomized rat following SC injection of CTP-hGH-CTP-CTP and commercial hGH. Single dose of CTP-hGH-CTP-CTP 0.6 or 1.8 mg/Kg and Biotropin 0.35 or 1.05 mg/Kg were injected subcutaneously to hypophysectomised rats for determination of PK/PD profile. Serum hGH post injection was measured using specific ELISA kits.

FIG. 9 is a graph showing IGF-1 serum levels in Hypophysectimized Rats Following SC injection of CTP-hGH-CTP-CTP and commercial hGH. Single dose of CTP-hGH-CTP-CTP 0.6 or 1.8 mg/Kg and Biotropin 0.35 or 1.05 mg/Kg were injected subcutaneously to

hypophysectomised rats for determination of PK/PD profile. Serum IGF-I post injection was measured using specific ELISA kits (Roche Diagnostics).

DETAILED DESCRIPTION OF THE INVENTION

[0007] In one embodiment, the present invention provides long-acting growth hormones and methods of producing and using same. In another embodiment, long-acting growth hormones comprise carboxy terminal peptide (CTP, also referred to as CTP unit). In another embodiment, long-acting polypeptides comprise carboxy terminal peptide (CTP) of human Chorionic Gonadotropin (hCG). In another embodiment, CTP acts as a protectant against degradation of growth hormone or polypeptides of interest. In another embodiment, CTP extends the Cmax of growth hormones or polypeptides of interest. In another embodiment, CTP extends the Tmax of growth hormones or polypeptides of interest. In another embodiment, CTP extends circulatory half-lives of growth hormones or polypeptides of interest. In some embodiments, CTP enhances the potency of growth hormones or polypeptides of interest.

[0008] In other embodiments, engineered growth hormones or polypeptides of interest of the invention comprising a single CTP attached to the amino terminus and two CTP peptides attached in tandem to the carboxy terminus are at least equivalent to the non CTP modified growth hormones or polypeptides of interest, in terms of biological activity. In other embodiments, engineered growth hormones or polypeptides of interest of the invention comprising a single CTP attached to the amino terminus and two CTP peptides attached in tandem to the carboxy terminus are at least equivalent to the non CTP modified growth hormones or polypeptides of interest, in terms of pharmacological measures such as pharmacokinetics and pharmacodynamics.

[0009] In another embodiment, the present invention provides a polypeptide comprising a growth hormone and at least one CTP peptide attached to an amino terminus of the growth hormone and at least two chorionic gonadotrophin carboxy terminal peptides attached to a carboxy terminus of the growth hormone. In another embodiment, the present invention provides a polypeptide comprising one chorionic gonadotrophin carboxy terminal peptide attached to an amino terminus of a growth hormone and two chorionic gonadotrophin carboxy terminal peptides attached to a carboxy terminus of a growth hormone.

[0010] In another embodiment, the terms "CTP peptide," "carboxy terminal peptide" and "CTP sequence" are used interchangeably herein. In another embodiment, the carboxy terminal peptide is a full-length CTP. In another embodiment, the carboxy terminal peptide is a truncated CTP. Each possibility represents a separate embodiment of the present invention.

[0011] In another embodiment, "signal sequence" and "signal peptide" are used

interchangeably herein. In another embodiment, "sequence" when in reference to a polynucleotide can refer to a coding portion. Each possibility represents a separate embodiment of the present invention.

[0012] In another embodiment, the invention provides a polypeptide consisting of a growth hormone, a single chorionic gonadotrophin carboxy terminal peptide attached to the amino terminus of the growth hormone, and two chorionic gonadotrophin carboxy terminal peptides attached to the carboxy terminus of the growth hormone. In another embodiment, the invention provides a polypeptide consisting of a growth hormone, a single chorionic gonadotrophin carboxy terminal peptide attached to the amino terminus of the growth hormone, two chorionic gonadotrophin carboxy terminal peptides attached to the carboxy terminus of the growth hormone, and a signal peptide attached to the amino terminus of one chorionic gonadotrophin carboxy terminal peptide.

[0013] In another embodiment, a growth hormone comprising CTPs as described herein has enhanced *in vivo* biological activity compared the same growth hormone without CTPs. In another embodiment, a growth hormone comprising at least one CTP attached to its amino terminus and at least two CTPs attached to its carboxy terminus has enhanced *in vivo* biological activity compared the same growth hormone without CTPs. In another embodiment, a growth hormone comprising one CTP attached to its amino terminus and two CTPs attached to its carboxy terminus has enhanced *in vivo* biological activity compared the same growth hormone without CTPs.

[0014] In another embodiment, a subject is a human subject. In one embodiment, the subject is male. In another embodiment, the subject is female.

[0015] In another embodiment, the configuration of CTP- growth hormone-CTP-CTP as described herein comprises a growth hormone or an active fragment thereof connected via a peptide bond to at least one CTP unit. In another embodiment, a CTP- growth hormone -CTP-CTP as described herein comprises a growth hormone or an active fragment thereof connected via a peptide bond to at least one CTP unit which is connected to an additional CTP unit via a peptide bond. In another embodiment, a polypeptide as described herein comprising a growth hormone fragments thereof and CTP units and/or fragments thereof are interconnected via a peptide bond. In another embodiment, one nucleic acid molecule encodes a polypeptide as described herein comprising a growth hormone and/or fragments thereof and CTP units and/or fragments thereof.

[0016] In another embodiment, the carboxy-terminal peptide (CTP) is attached to the growth hormone via a linker. In another embodiment, the linker which connects the CTP sequence to the growth hormone is a covalent bond. In another embodiment, the linker which connects the CTP sequence to the growth hormone is a peptide bond. In another embodiment, the linker which connects the CTP sequence to the growth hormone is a substituted peptide bond. In another embodiment, the carboxy-terminal peptide (CTP) sequence comprises an amino acid sequence selected from the sequences set forth in SEQ ID NO: 1 and SEQ ID NO: 2.

[0017] In another embodiment, SEQ ID NO: 1 comprise the following amino acid (AA) sequence: DPRFQDSSSSKAPPPSLPSPSRLPGPSDTPILQ (SEQ ID NO: 1). In another embodiment, SEQ ID NO: 2 comprise the following amino acid (AA) sequence: SSSSKAPPPSLPSPSRLPGPSDTPILPQ (SEQ ID NO: 2).

[0018] In another embodiment, the carboxy-terminal peptide (CTP) sequence is truncated. In another embodiment, a truncated CTP comprises the following amino acid sequence: SSSSKAPPPSLP (SEQ ID NO: 4).

[0019] In another embodiment, the carboxy terminal peptide (CTP) peptide of the present invention comprises the amino acid sequence from amino acid 112 to position 145 of a native human chorionic gonadotrophin peptide. In another embodiment, the CTP sequence of the present invention comprises the amino acid sequence from amino acid 118 to position 145 of a human chorionic gonadotropin peptide. In another embodiment, the CTP sequence also commences from any position between positions 112-118 and terminates at position 145 of human chorionic gonadotrophin peptide. In some embodiments, the CTP sequence peptide is 28, 29, 30, 31, 32, 33 or 34 amino acids long and commences at position 112, 113, 114, 115, 116, 117 or 118 of the gene bank deposited CTP amino acid sequence.

[0020] In another embodiment, the CTP peptide is a variant of chorionic gonadotrophin CTP which differs from the native CTP by 1-5 conservative amino acid substitutions as described in U.S. Pat. No. 5,712,122. In another embodiment, the CTP peptide is a variant of chorionic gonadotrophin CTP which differs from the native CTP by 1 conservative amino acid substitution. In another embodiment, the CTP peptide is a variant of chorionic gonadotrophin CTP which differs from the native CTP by 2 conservative amino acid substitutions. In another embodiment, the CTP peptide is a variant of chorionic gonadotrophin CTP which differs from the native CTP by 3 conservative amino acid substitutions. In another embodiment, the CTP peptide is a variant of chorionic gonadotrophin CTP which differs from the native CTP by 4 conservative amino acid substitutions. In another embodiment, the CTP peptide is a variant of chorionic gonadotrophin CTP which differs from the native CTP by 5 conservative amino acid substitutions. In another embodiment, the CTP peptide amino acid sequence of the present invention is at least 70% homologous to the native CTP amino acid sequence or a peptide thereof. In another embodiment, the CTP peptide amino acid sequence of the present invention is at least 80% homologous to the native CTP amino acid sequence or a peptide thereof. In another embodiment, the CTP peptide amino acid sequence of the present invention is at least 90% homologous to the native CTP amino acid sequence or a peptide thereof. In another embodiment, the CTP peptide amino acid sequence of the present invention is at least 95% homologous to the native CTP amino acid sequence or a peptide thereof.

[0021] In another embodiment, the CTP peptide DNA sequence of the present invention is at least 70% homologous to the native human CTP DNA sequence or a peptide thereof. In another embodiment, the CTP peptide DNA sequence of the present invention is at least 80%

homologous to the native human CTP DNA sequence or a peptide thereof. In another embodiment, the CTP peptide DNA sequence of the present invention is at least 90% homologous to the native CTP DNA sequence or a peptide thereof. In another embodiment, the CTP peptide DNA sequence of the present invention is at least 95% homologous to the native CTP DNA sequence or a peptide thereof.

[0022] In one embodiment, the truncated CTP comprises the first 11 amino acids of SEQ ID NO: 4. In one embodiment, the truncated CTP comprises the first 8 amino acids of SEQ ID NO: 4. In one embodiment, the truncated CTP comprises the first 13 amino acids of SEQ ID NO: 4. In one embodiment, the truncated CTP comprises the first 6 amino acids of SEQ ID NO: 4. In one embodiment, the truncated CTP comprises the first 5 amino acids of SEQ ID NO: 4.

[0023] In one embodiment, at least one of the chorionic gonadotrophin CTP amino acid sequences is glycosylated. In another embodiment, both of the chorionic gonadotrophin CTP amino acid sequences are glycosylated. In another embodiment, 2 of the chorionic gonadotrophin CTP amino acid sequences are glycosylated. In another embodiment, 2 or more of the chorionic gonadotrophin CTP amino acid sequences are glycosylated. In another embodiment, all of the chorionic gonadotrophin CTP amino acid sequences are glycosylated. In one embodiment, the CTP sequence of the present invention comprises at least one glycosylation site. In one embodiment, the CTP sequence of the present invention comprises 2 glycosylation sites. In one embodiment, the CTP sequence of the present invention comprises 3 glycosylation sites. In one embodiment, the CTP sequence of the present invention comprises 4 glycosylation sites.

[0024] In another embodiment, at least one carboxy-terminal peptide (CTP) sequence comprises an amino acid sequence selected from the sequences set forth in SEQ ID NO: 1 and SEQ ID NO: 2. In another embodiment, at least one carboxy-terminal peptide (CTP) is truncated.

[0025] In one embodiment, the terms "termini", "terminal", "terminal end" and "terminus" when in reference to a carboxy or amino end of a protein or peptide or fragment thereof provided herein, are used interchangeably herein. In another embodiment, the terms "C-terminal", "carboxy terminal" or "carboxyl terminal" are used interchangeably herein. In another embodiment, the terms "N-terminal" and "amino terminal" are used interchangeably herein. Each possibility represents a separate embodiment of the present invention.

[0026] In another embodiment, a CTP sequences at both the amino terminal end of a growth hormone and at the carboxy terminal end of the growth hormone provides enhanced protection against degradation of a growth hormone. In another embodiment, at least one CTP sequence at the amino terminal end of a growth hormone and two CTP units in the carboxy terminal end of a growth hormone provides enhanced protection against clearance. In another embodiment, at least one CTP sequence at the amino terminal end of a growth hormone and two CTP units in the carboxy terminal end of a growth hormone provides prolonged clearance time. In another embodiment, at least one CTP sequence at the amino terminal end of a

growth hormone and two CTP units in the carboxy terminal end of a growth hormone enhances the C_{\max} of a growth hormone. In another embodiment, at least one CTP sequence at the amino terminal end of a growth hormone and two CTP units in the carboxy terminal end of a growth hormone enhances the T_{\max} of a growth hormone. In another embodiment, at least one CTP sequence at the amino terminal end of a growth hormone and two CTP units in the carboxy terminal end of a growth hormone enhanced $T_{1/2}$ (half-life) of the growth hormone.

[0027] In another embodiment, CTP sequences at both the amino terminal end of a growth hormone and at the carboxy terminal end of the growth hormone extend the half-life of the modified growth hormone. In another embodiment, at least a single CTP sequence at the amino terminal end of a growth hormone and at least two CTP sequences at the carboxy terminal end of the growth hormone provide extended half-life to the modified growth hormone. In another embodiment, a single CTP sequence at the amino terminal end of a growth hormone and two CTP sequences at the carboxy terminal end of the growth hormone provide extended half-life to the attached growth hormone. In another embodiment, a single CTP sequence at the amino terminal end of a growth hormone and two CTP sequences in tandem at the carboxy terminal end of the growth hormone provide extended half-life to the modified growth hormone.

[0028] In another embodiment, a CTP sequence at the amino terminal end of a polypeptide, a CTP sequence at the carboxy terminal end of the growth hormone, and at least one additional CTP sequence attached in tandem to the CTP sequence at the carboxy terminus provide enhanced protection against degradation to a growth hormone. In some embodiments, a CTP sequence at the amino terminal end of a growth hormone, a CTP sequence at the carboxy terminal end of the growth hormone, and at least one additional CTP sequence attached in tandem to the CTP sequence at the carboxy terminus extend the half-life of the growth hormone. In some embodiments, a CTP sequence at the amino terminal end of a growth hormone, a CTP sequence at the carboxy terminal end of the growth hormone, and at least one additional CTP sequence attached in tandem to the CTP sequence at the carboxy terminus enhance the biological activity of the growth hormone.

[0029] In another embodiment, the growth hormone further comprises a signal peptide. In some embodiments, signal sequences include, but are not limited to the endogenous signal sequence. In some embodiments, signal sequences include, but are not limited to the endogenous signal sequence of any known growth hormone or growth hormones. In another embodiment, the polypeptides and methods of the present invention provide a growth hormone having additionally a signal peptide comprising the following amino acid sequence: MATGSRTSLLAFGLLCLPWLQEGSA (SEQ ID NO: 3).

[0030] In another embodiment, conjugated growth hormones of this invention are used in the same manner as unmodified growth hormones. In another embodiment, conjugated growth hormones of this invention have an increased circulating half-life and plasma residence time, decreased clearance, and increased clinical activity *in vivo*. In another embodiment, due to the

improved properties of the conjugated growth hormones as described herein, these conjugates are administered less frequently than unmodified growth hormones. In another embodiment, conjugated growth hormones as described herein are administered once a week to once every two weeks. In another embodiment, conjugated growth hormones as described herein are administered once every two weeks to once every three weeks. In another embodiment, conjugated growth hormones as described herein are administered once a day to three times a week. In another embodiment, decreased frequency of administration will result in improved patient compliance leading to improved treatment outcomes, as well as improved patient quality of life. In another embodiment, compared to conventional conjugates of growth hormones linked to poly(ethylene glycol) it has been found that growth hormone CTP conjugates having the molecular weight and linker structure of the conjugates of this invention have an improved potency, improved stability, elevated AUC levels, enhanced circulating half-life. In another embodiment, compared to conventional conjugates of growth hormones linked to polyethylene glycol) it has been found that growth hormones having the molecular weight and linker structure of the conjugates of this invention have an improved potency, improved stability, elevated AUC levels, enhanced circulating half-life. In another embodiment, a therapeutically effective amount of a conjugated growth hormone is the amount of conjugate necessary for the in vivo measurable expected biological activity. In another embodiment, a growth hormone utilized according to the teachings of the present invention exhibits increased potency. In another embodiment, the attachment of CTP sequence to both the amino and carboxy termini of a growth hormone results in prolonged in-vivo activity.

[0031] In another embodiment, a therapeutically effective amount of a conjugated growth hormone is determined according to factors as the exact type of condition being treated, the condition of the patient being treated, as well as the other ingredients in the composition. In another embodiment, a therapeutically effective amount of a conjugated growth hormone is 0.01 to 10 µg per kg body weight administered once a week. In another embodiment, a therapeutically effective amount of a conjugated growth hormone is 0.1 to 1 µg per kg body weight, administered once a week. In another embodiment, a pharmaceutical composition comprising a conjugated growth hormone is formulated at strength effective for administration by various means to a human patient.

[0032] In another embodiment, the growth hormone is any growth hormone known to one of skill in the art. In another embodiment, the growth hormone is a human growth hormone. In another embodiment, the nucleotide sequence and/or the amino acid sequence of a growth hormone is available in a gene bank database. In another embodiment, the growth hormone is a homologue. In another embodiment, a homologue also refers to a deletion, insertion, or substitution variant, including an amino acid substitution, thereof and biologically active polypeptide fragments thereof.

[0033] In another embodiment, the growth hormone is variant of hGH missing exons 2, 3, 4, or any combination thereof. In another embodiment, the growth hormone comprises a signal peptide. In another embodiment, the growth hormone comprises a signal cleavage site. In another embodiment, polypeptides comprising GH modified by CTPs of the present invention

comprise recombinant GH.

[0034] In another embodiment, a growth hormone as described herein is a member of the superfamily of growth hormone (GH)-like cytokines. In another embodiment, a growth hormone as described herein is human growth hormone (hGH). In another embodiment, a human growth hormone comprises the following amino acid sequence (Genbank Accession No. P01241):

MATGSRTSLLAFGLLCLPWLQEGSAFPTIPLSRLFDNAMLRAHRLHQLAFDTYQEFEEAYIP
KEQKYSFLQNPQTSCLFSESIPTPSNREETQQKSNLELLRISLLLIQSWLEPVQFLRSVFANSLVY
GASDSNVYDLLKDLEEGIQTLMGRLLEDGSPRTGQIFKQTYSKFDTNSHNDDALLKNYGLLYC
FRKDMDKVETFLRIVQCRSVEGSCGF (SEQ ID NO: 5). In another embodiment, a human growth hormone comprises the following amino acid sequence:
MFPTIPLSRLFDNAMLRAHRLHQLAFDTYQEFEEAYIPKEQKYSFLQNPQTSCLFSESIPTPSNR
EETQQKSNLELLRISLLLIQSWLEPVQFLRSVFANSLVYGASDSNVYDLLKDLEEGIQTLMGRL
EDGSPRTGQIFKQTYSKFDTNSHNDDALLKNYGLLYC FRKDMDKVETFLRIVQCRSVEGSCG F
(SEQ ID NO: 6). In another embodiment, a human growth hormone comprises the following amino acid sequence: MFPTIPLSRLFDNAMI,RAHRLHQLA (SEQ ID NO: 7). In another embodiment, an hGH comprises the following amino acid sequence:
MATGSRTSLLAFGLLCLPWLQEGSAFPTIPLSRLFDNAMLRAHRLHQLAFDTYQEFEEAYIP
KVQKYSFLQNPQTSCLFSESIPTPSNREETQQKSNLELLRISLLLIQSWLEPVQFLRSVFANSLV
YGASDSNVYDLLKDLEEGIQTLMGRLLEDGSPRTGQIFKQTYSKFDTNSHNDDALLKNYGLLY
CFRKDMDKVETFLRIVQCRSVEGSCGF (SEQ ID NO: 8). In another embodiment, an hGH is a substitution variant in which glutamine in position 65 of hGH is substituted by a valine.

[0035] In another embodiment, a growth hormone of the invention comprises the gene bank amino acid deposited sequence under accession no. AAA72260. In another embodiment, a growth hormone of the invention comprises the gene bank amino acid deposited sequence under accession no. AAK69708. In another embodiment, a growth hormone of the invention comprises the gene bank amino acid deposited sequence under accession no. CAA01435. In another embodiment, a growth hormone of the invention comprises the gene bank amino acid deposited sequence under accession no. CAA01329. In another embodiment, a growth hormone of the invention comprises the gene bank amino acid deposited sequence under accession no. CAA00380. In another embodiment, a growth hormone of the invention comprises the gene bank amino acid deposited sequence under accession no. AAA72555. In another embodiment, a growth hormone of the invention comprises the gene bank amino acid deposited sequence under accession no. NP_000506.2. In another embodiment, a growth hormone of the invention comprises the gene bank amino acid deposited sequence under accession no. NP_072053.1. In another embodiment, a growth hormone of the invention comprises the gene bank amino acid deposited sequence under accession no. NP_072054.1. In another embodiment, a growth hormone of the invention comprises the gene bank amino acid deposited sequence under accession no. NP_072055.1. In another embodiment, a growth hormone of the invention comprises the gene bank amino acid deposited sequence under accession no. NP_072056.1.

[0036] In another embodiment, the nucleic acid molecule encoding a growth hormone as described herein encodes any amino acid sequence of a growth hormone known to one of skill in the art. In another embodiment, the nucleic acid molecule encoding a growth hormone as described herein encodes an hGH. In another embodiment, the nucleic acid molecule encoding a growth hormone comprises the gene bank nucleic acid deposited sequence under accession no. NM_000515.3. In another embodiment, the nucleic acid molecule encoding a growth hormone comprises the gene bank nucleic acid deposited sequence under accession no. NM_022559.2. In another embodiment, the nucleic acid molecule encoding a growth hormone comprises the gene bank nucleic acid deposited sequence under accession no. NM_022560.2. In another embodiment, the nucleic acid molecule encoding a growth hormone comprises the gene bank nucleic acid deposited sequence under accession no. NM_022561.2. In another embodiment, the nucleic acid molecule encoding a growth hormone comprises the gene bank nucleic acid deposited sequence under accession no. NM_022562.2.

[0037] In another embodiment, a polypeptide comprising a growth hormone of the invention comprises one CTP attached to a carboxy terminus of a growth hormone (hGH-CTP) and having the following amino acid sequence:

MATGSRTSLLLAFGLLCLPWLQEGSAFPTIPLSRLFDNAMLRAHRLHQLAFDITYQEFEEAYIP
KEQKYSFLQNPQTSCLFSESIPTPSNREETQQKSNLELLRISLLLIQSWLEPVQFLRSVFANSLVY
GASDSNVYDLLKDLEEGIQTLMGRLLEDGSPRTGQIFKQTYSKFDTNSHNDDALLKNYGLLYC
FRKDMDKVETFLRIVQCRSVEGSCGFSSSSKAPPPSLPSPSRLPGPSDTPILPQ (SEQ ID NO: 9).

[0038] In another embodiment, a polypeptide comprising a growth hormone of the invention comprises two CTPs in tandem attached to a carboxy terminus of a growth hormone (hGH-CTP-CTP) and having the following amino acid sequence:

MATGSRTSLLLAFGLLCLPWLQEGSAFPTIPLSRLFDNAMLRAHRLHQLAFDITYQEFEEAYIP
KEQKYSFLQNPQTSCLFSESIPTPSNREETQQKSNLELLRISLLLIQSWLEPVQFLRSVFANSLVY
GASDSNVYDLLKDLEEGIQTLMGRLLEDGSPRTGQIFKQTYSKFDTNSHNDDALLKNYGLLYC
FRKDMDKVETFLRIVQCRSVEGSCGFSSSSKAPPPSLPSPSRLPGPSDTPILPQSSSSKAPPPSLP
PSRLPGPSDTPILPQ (SEQ ID NO: 10).

[0039] In another embodiment, a polypeptide comprising a growth hormone of the invention comprises two CTPs attached in tandem to a carboxy terminus of a growth hormone and one CTP attached to an amino terminus of a growth hormone (CTP-hGH-CTP-CTP) and having the following amino acid sequence:

MATGSRTSLLLAFGLLCLPWLQEGSASSSSKAPPPSLPSPSRLPGPSDTPILPQFPTIPLSRLFDN
AMLRAHRLHQLAFDITYQEFEEAYIPKEQKYSFLQNPQTSCLFSESIPTPSNREETQQKSNLELL
RISLLLIQSWLEPVQFLRSVFANSLVYGASDSNVYDLLKDLEEGIQTLMGRLLEDGSPRTGQIFK
QTYSKFDTNSHNDDALLKNYGLLYCFRKDMDKVETFLRIVQCRSVEGSCGFSSSSKAPPPSLP
PSRLPGPSDTPILPQSSSSKAPPPSLPSPSRLPGPSDTPILPQ (SEQ ID NO: 11).

[0040] In another embodiment, a polypeptide comprising a growth hormone of the invention comprises two CTPs in tandem attached to a carboxy terminus of a growth hormone, wherein one CTP of two CTPs is truncated, and one additional CTP attached to an amino terminus of a growth hormone (tCTP-hGH-CTP-CTP) and having the following amino acid sequence:

MATGSRTSLLLAFLGLCLPWLQEGSASSSSKAPPPSLPFPTIPLSRLFDNAMLRAHRLHQLAFD
TYQEFEEAYIPKEQKYSFLQNPQTSLCFSESIPTPSNREETQQKSNLELLRISLLLIQSWLEPVQF
LRSVFANSLVYGASDSNVYDLLKDLEEGIQTLMGRLEDGSPRTGQIFKQTYSKFDTNSHNDD
ALLKNYGLLYCFRKDMDKVETFLRIVQCRSVEGSCGFSSSSKAPPPSLPSPSRLPGPSDTPILPQ
SSSSKAPPPSLPSPSRLPGPSDTPILPQ (SEQ ID NO: 12).

[0041] In another embodiment, a polypeptide comprising a growth hormone of the invention comprises one CTP attached to a carboxy terminus of a growth hormone and one CTP attached to an amino terminus of a growth hormone (CTP-hGH-CTP) and having the following amino acid sequence:

MATGSRTSLLLAFLGLCLPWLQEGSASSSSKAPPPSLPSPSRLPGPSDTPILPQFPTIPLSRLFDN
AMLRAHRLHQLAFDQTYQEFEEAYIPKEQKYSFLQNPQTSLCFSESIPTPSNREETQQKSNLELL
RISLLLIQSWLEPVQFLRSVFANSLVYGASDSNVYDLLKDLEEGIQTLMGRLEDGSPRTGQIFK
QTYSKFDTNSHNDDALLKNYGLLYCFRKDMDKVETFLRIVQCRSVEGSCGFSSSSKAPPPSLP
SPSRLPGPSDTPILPQ (SEQ ID NO: 13).

[0042] In another embodiment, a polypeptide comprising a growth hormone and one CTP comprises the following amino acid sequence:

MATGSRTSLLLAFLGLCLPWLQEGSASSSSKAPPPSLPSPSRLPGPSDTPILPQFPTIPLSRLFDN
AMLRAHRLHQLAFDQTYQEFEEAYIPKEQKYSFLQNPQTSLCFSESIPTPSNREETQQKSNLELL
RISLLLIQSWLEPVQFLRSVFANSLVYGASDSNVYDLLKDLEEGIQTLMGRLEDGSPRTGQIFK
QTYSKFDTNSHNDDALLKNYGLLYCFRKDMDKVETFLRIVQCRSVEGSCGF (SEQ ID NO:
14).

[0043] In another embodiment, a polynucleotide molecule encoding a polypeptide having CTP-hGH-CTP comprises the following nucleic acid sequence:

tctagaggacatggccaccggcagcaggaccagcctgctgctggccttcggcctgctgctgctgcatggctgcaggagggcagcgccagctctt
cttctaaggctccacccccatctctgcccagcccagcagactgccgggccccagcgacacaccattctgccccagttccccaccatccccctga
gcaggctgttcgacaacgccatgctgagggtcacaggctgcaccagctggcctttgacacctaccaggagttcgaggaaacctacatccccaa
gagcagaagtacagcttcctgcagaacccccagacctcctgtgcttcagcgagagatccccacccccagcaacagagaggagaccagcaga
agagcaacctggagctgctgaggatctccctgctgctgatccagagctggctggagcccgctgcagttcctgagaagcgtgttcgccaacagcctgg
tgtacggcgccagcgacagcaacctgtacgacctgtgaaggacctggaggaggccatccagacctgatggccggctggaggacggcagc
cccaggaccggccagatcttaagcagacctacagcaagttcgacaccaacagccacaacgacgacgccctgctgaagaactacgggctgctgt
actgcttcagaaaggacatggacaagggtggagacctcctgaggatcgtgcagtgagaagcgtggaggggcagctgcggcttcagctccagcag
caaggccccctccccgagcctgccctcccccaagcagggtgctgggccccctccgacacaccaatcctgcctcagtgatgaaggcttgatgcggcc
gc (SEQ ID NO: 15).

[0044] In another embodiment, a polynucleotide molecule encoding a polypeptide having CTP-hGH-CTP-CTP comprises the following nucleic acid sequence:

tctaggacatggccaccggcagcaggaccagcctgctgctggccttcggcctgctgtgcctgcatggctgcaggagggcagcgcagctctt
cttctaaggctccacccccatctctgcccagccccagcagactgcccgggccccagcgacacaccattctgccccagttccccaccatccccctga
gcaggctgttcgacaacgccatgctgagggctcacaggctgcaccagctggcctttgacacctaccaggagttcgagggaagcctacatccccaag
gagcagaagtacagcttctgcagaacccccagacctccctgtgcttcagcgagagcatccccacccccagcaacagagaggagaccagcaga
agagcaacctggagctgctgaggatctccctgctgctgatccagagctggctggagcccgtgcagtctctgagaagcgtgttcgccaacagcctgg
tgtacggcgccagcgacagcaacgtgtacgacctgctgaaggacctggaggagggcatccagacctgatgggcccggctggaggacggcagc
cccaggaccggccagatcttaagcagacctacagcaagttcgacaccaacagccacaacgacgacgccctgctgaagaactacgggctgctgt
actgcttcagaaaggacatggacaagggtggagaccttctgaggatcgtgcagtgcagaagcgtggagggcagctgcggcttcagctccagcag
caaggccctccccgagcctgcccctcccaagcaggctgctgggcccctccgacacccaatctgccacagagcagctcctctaaggcccttc
ctccatccctgcatccccctccccggctgctggccccctctgacacccctatcctgcctcagtgatgaaggtctggatgcggccgc (SEQ ID
NO: 16).

[0045] In another embodiment, a polynucleotide molecule encoding a polypeptide having CTP-hGH-CTP-CTP comprises the following nucleic acid sequence:

tctaggacatggccaccggcagcaggaccagcctgctgctggccttcggcctgctgtgcctgcatggctgcaggagggcagcgcagctctt
cttctaaggctccacccccgagcctgcccctccccaccatccccctgagcaggctgttcgacaacgccatgctgagggctcacaggctgcaccagc
tggcctttgacacctaccaggagttcgagggaagcctacatcccaaggagcagaagtacagcttctgcagaacccccagacctccctgtgcttcag
cgagagcatccccacccccagcaacagagaggagaccagcagaagagcaacctggagctgctgaggatctccctgctgctgatccagagctg
gctggagcccgtgcagtctctgagaagcgtgttcgccaacagcctgggtacggcgccagcgacagcaacgtgtacacctgctgaaggacctgg
aggagggcatccagacctgatgggcccggctggaggacggcagccccaggaccggccagatcttcaagcagacctacagcaagttcgacacca
acagccacaacgacgacgcccctgctgaagaactacgggctgctgtactgcttcagaaaggacatggacaagggtggagaccttctgaggatcgtg
cagtgcagaagcgtggagggcagctgcggcttcagctccagcagaagggcccctccccgagcctgcccctcccaagcaggctgctgggccc
lccgacacaccaalccctgccacagagcagctccctcclaaggccccctccatccctgccatccccctccccggctgctggccccctcgacacccctat
cctgcctcagtgatgaaggtctggatgcggccgc (SEQ ID NO: 17).

[0046] In another embodiment, a growth hormone of the invention is homologous to a known sequence of a growth hormone. In another embodiment, a growth hormone of the invention is homologous to a growth hormone sequence as disclosed herein. In some embodiments, homology according to the present invention also encompasses deletions, insertions, or substitution variants, including an amino acid substitution, thereof and biologically active polypeptide fragments thereof. In one embodiment the substitution variant is one, in which the glutamine in position 65 of hGH is substituted by a valine [Gellerfors et al., J Pharm Biomed Anal 1989, 7: 173-83].

[0047] In one embodiment, the phrase "human growth hormone" (hGH) refers to a polypeptide, such as set forth in Genbank Accession No. P01241 exhibiting hGH activity (i.e. stimulation of growth).

[0048] In one embodiment, "human growth hormone" (hGH) refers to a polypeptide, such as set forth in Genbank Accession No. P01241, exhibiting hGH activity (i.e. stimulation of growth). In one embodiment, hGH of the present invention also refers to homologues. In one embodiment, hGH amino acid sequence of the present invention is at least 50% homologous to an hGH sequence set forth in GenBank Accession No. P01241 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). In one embodiment, hGH amino acid sequence of the present invention is at least 60% homologous to an hGH sequence set forth in GenBank Accession No. P01241 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). In one embodiment, hGH amino acid sequence of the present invention is at least 70% homologous to an hGH sequence set forth in GenBank Accession No. P01241 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). In one embodiment, hGH amino acid sequence of the present invention is at least 80% homologous to an hGH sequence set forth in GenBank Accession No. P01241 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). In one embodiment, hGH amino acid sequence of the present invention is at least 90% homologous to an hGH sequence set forth in GenBank Accession No. P01241 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). In one embodiment, hGH amino acid sequence of the present invention is at least 95% homologous to an hGH sequence set forth in GenBank Accession No. P01241 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters).

[0049] In another embodiment, polypeptides comprising hGH modified by CTPs bind adipocytes and stimulates them to break down triglyceride and suppresses their ability to take up and accumulate circulating lipids. In another embodiment, polypeptides comprising hGH modified by CTPs exert indirect effects mediated primarily by a insulin-like growth factor-I (IGF-I) (as shown in the examples section).

[0050] In another embodiment, polypeptides comprising hGH modified by CTPs stimulate body growth by stimulating the liver and other tissues to secrete IGF-I. In another embodiment, IGF-I stimulates proliferation of chondrocytes, resulting in bone growth. In another embodiment, IGF-I stimulates proliferation of skeletal muscle cells, resulting in muscle growth.

[0051] In another embodiment, polypeptides comprising hGH modified by CTPs induce a metabolic effect on protein, lipid, and carbohydrate metabolism. In another embodiment, polypeptides comprising hGH modified by CTPs have a direct effect. In another embodiment, polypeptides comprising hGH modified by CTPs have an indirect effect through induction of IGF-I. In another embodiment, polypeptides comprising hGH modified by CTPs further comprise a leader peptide. In another embodiment, polypeptides comprising hGH modified by CTPs include CTP truncated constructs.

[0052] In another embodiment, polypeptides comprising hGH modified by CTPs stimulate protein anabolism in a tissue. In another embodiment, polypeptides comprising hGH modified

by CTPs stimulate amino acid uptake, increased protein synthesis, and decreased oxidation of proteins .

[0053] In another embodiment, polypeptides comprising hGH modified by CTPs stimulate fat metabolism. In another embodiment, polypeptides comprising hGH modified by CTPs stimulate the utilization of fat by stimulating triglyceride breakdown and oxidation in adipocyte. In another embodiment, polypeptides comprising hGH modified CTPs reduce body fat.

[0054] In another embodiment, polypeptides comprising hGH modified by CTPs stimulate carbohydrate metabolism. In another embodiment, polypeptides comprising hGH modified by CTPs maintain blood glucose within a normal range. In another embodiment, polypeptides comprising hGH modified by CTPs comprise an anti-insulin activity. In another embodiment polypeptides comprising hGH modified by CTPs suppress the abilities of insulin to stimulate uptake of glucose in peripheral tissues and enhance glucose synthesis in the liver. In another embodiment, polypeptides comprising hGH modified by CTPs stimulate insulin secretion, leading to hyperinsulinemia.

[0055] In another embodiment, polypeptides comprising hGH modified by CTPs are used to compensate for limited or no production of growth hormone in a subject. In another embodiment, polypeptides comprising hGH modified by CTPs compensate for limited or no production of growth hormone-releasing hormone (GHRH). In another embodiment, polypeptides comprising hGH modified by CTPs compensate for the increased activity of somatostatin. In another embodiment, polypeptides comprising hGH modified by CTPs compensate for limited or no production of ghrelin.

[0056] In another embodiment, polypeptides comprising hGH modified by CTPs are used to treat diseases associated with lesions in either the hypothalamus, the pituitary, or in target cells. In another embodiment, polypeptides comprising hGH modified by CTPs are used to treat diseases associated with reduced target cell's response to the hormone.

[0057] In another embodiment, polypeptides comprising hGH modified by CTPs are used to treat children with severe growth retardation. In another embodiment, polypeptides comprising hGH modified by CTPs are used to treat children of pathologically short stature. In another embodiment, polypeptides comprising hGH modified by CTPs of the invention are used to enhance athletic performance. In another embodiment, polypeptides comprising hGH modified by CTPs of the invention are used to treat symptoms of aging. In another embodiment, polypeptides comprising hGH modified by CTPs of the invention are used to treat cosmetic symptoms of aging.

[0058] In another embodiment, treating GH deficient children with GH results in growth enhancement, whereas in GH-deficient adults, treatment with GH results in an increase in lean body mass and a decrease or reduction in body fat. In another embodiment, treatment with CTP modified forms of GH results in an enhancement of these effects in children and adults. In another embodiment, treatment with CTP-modified hGH in GH-deficient children results in an

enhancement of growth when compared to GH-deficient children receiving the same dose of daily, commercial, unmodified GH. In another embodiment, treatment with CTP-modified hGH in children results in an enhancement of growth when compared to children receiving the same dose of daily, commercial, unmodified GH.

[0059] In another embodiment, the methods of the present invention provide hGH having additionally at least one CTP amino acid peptide on the N-terminus and at least one CTP amino acid peptide on the C-terminus for the treatment of hGH deficiency. In another embodiment, the methods of the present invention provide polypeptides comprising hGH modified by CTPs for the treatment of hGH deficiency.

[0060] In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding a GH protein as described herein. In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding polypeptide comprising hGH modified by CTPs for stimulating muscle growth, increasing cardiac function, stimulating bone growth, maintaining muscle integrity, balancing muscle metabolism, inducing muscle buildup, inducing de-novo muscle build-up, enhancing bone load, treating symptoms associated with osteoporosis, treating a wasting disease, increasing lipolysis, improving fluid balance, treating osteoporosis, improving lung function, improving immunity, regrowing a vital organ, increasing sense of well-being, restoring REM sleep, or any combination thereof.

[0061] In some embodiments, human growth hormone (hGH) is utilized according to the teachings of the present invention. In some embodiments, the attachment of CTP sequence to both the amino and carboxy termini of the hGH protein results in increased potency (Figure 2). In some embodiments, the attachment of CTP sequence to both the amino and carboxy termini of the hGH protein results in prolonged in-vivo activity.

[0062] In some embodiments, "polypeptide" or "protein" as used herein encompasses native polypeptides (either degradation products, synthetically synthesized polypeptides or recombinant polypeptides) and peptidomimetics (typically, synthetically synthesized polypeptides), as well as peptoids and semipeptoids which are polypeptide analogs, which have, in some embodiments, modifications rendering the polypeptides even more stable while in a body or more capable of penetrating into cells.

[0063] In some embodiments, modifications include, but are not limited to N terminus modification, C terminus modification, polypeptide bond modification, including, but not limited to, CH₂-NH, CH₂-S, CH₂-S=O, O=C-NH, CH₂-O, CH₂-CH₂, S=C-NH, CH=CH or CF=CH, backbone modifications, and residue modification. Methods for preparing peptidomimetic compounds are well known in the art and are specified, for example, in Quantitative Drug Design, C.A. Ramsden Gd., Chapter 17.2, F. Choplin Pergamon Press (1992). Further details in this respect are provided hereinunder.

[0064] In some embodiments, polypeptide bonds (-CO-NH-) within the polypeptide are substituted. In some embodiments, the polypeptide bonds are substituted by N-methylated

bonds (-N(CH₃)-CO-). In some embodiments, the polypeptide bonds are substituted by ester bonds (-C(R)H-C-O-O-C(R)-N-). In some embodiments, the polypeptide bonds are substituted by ketomethylen bonds (-CO-CH₂-). In some embodiments, the polypeptide bonds are substituted by α -aza bonds (-NH-N(R)-CO-), wherein R is any alkyl, e.g., methyl, carba bonds (-CH₂-NH-). In some embodiments, the polypeptide bonds are substituted by hydroxyethylene bonds (-CH(OH)-CH₂-). In some embodiments, the polypeptide bonds are substituted by thioamide bonds (-CS-NH-). In some embodiments, the polypeptide bonds are substituted by olefinic double bonds (-CH=CH-). In some embodiments, the polypeptide bonds are substituted by retro amide bonds (-NH-CO-). In some embodiments, the polypeptide bonds are substituted by polypeptide derivatives (-N(R)-CH₂-CO-), wherein R is the "normal" side chain, naturally presented on the carbon atom. In some embodiments, these modifications occur at any of the bonds along the polypeptide chain and even at several (2-3 bonds) at the same time.

[0065] In some embodiments, natural aromatic amino acids of the polypeptide such as Trp, Tyr and Phe, are substituted for synthetic non-natural acid such as Phenylglycine, TIC, naphthylelanine (Nol), ring-methylated derivatives of Phe, halogenated derivatives of Phe or o-methyl-Tyr. In some embodiments, the polypeptides of the present invention include one or more modified amino acid or one or more non-amino acid monomers (e.g. fatty acid, complex carbohydrates etc).

[0066] In one embodiment, "amino acid" or "amino acid" is understood to include the 20 naturally occurring amino acid; those amino acid often modified post-translationally *in vivo*, including, for example, hydroxyproline, phosphoserine and phosphothreonine; and other unusual amino acid including, but not limited to, 2-aminoadipic acid, hydroxylysine, isodesmosine, nor-valine, nor-leucine and ornithine. In one embodiment, "amino acid" includes both D- and L-amino acid.

[0067] In some embodiments, the polypeptides of the present invention are utilized in therapeutics which requires the polypeptides to be in a soluble form. In some embodiments, the polypeptides of the present invention include one or more non-natural or natural polar amino acid, including but not limited to serine and threonine which are capable of increasing polypeptide solubility due to their hydroxyl-containing side chain.

[0068] In some embodiments, the polypeptides comprising hGH modified by CTPs of the present invention are utilized in a linear form, although it will be appreciated by one skilled in the art that in cases where cyclicization does not severely interfere with hGH modified by CTPs characteristics, cyclic forms of the growth hormones can also be utilized.

[0069] In some embodiments, the hGH modified by CTPs of present invention are biochemically synthesized such as by using standard solid phase techniques. In some embodiments, these biochemical methods include exclusive solid phase synthesis, partial solid phase synthesis, fragment condensation, or classical solution synthesis. In some embodiments, these methods are used when the growth hormones are relatively short (about 5-15kDa) and/or when it cannot be produced by recombinant techniques (i.e., not encoded by

a nucleic acid sequence) and therefore involves different chemistry.

[0070] In some embodiments, solid phase hGH modified by CTPs synthesis procedures are well known to one skilled in the art and further described by John Morrow Stewart and Janis Dillaha Young, Solid Phase Polypeptide Syntheses (2nd Ed., Pierce Chemical Company, 1984). In some embodiments, synthetic polypeptides are purified by preparative high performance liquid chromatography [Creighton T. (1983) Proteins, structures and molecular principles. WH Freeman and Co. N.Y.] and the composition of which can be confirmed via amino acid sequencing by methods known to one skilled in the art.

[0071] In some embodiments, recombinant protein techniques are used to generate the hGH modified by CTPs of the present invention. In some embodiments, recombinant protein techniques are used for generation of relatively long polypeptides (e.g., longer than 18-25 amino acid). In some embodiments, recombinant protein techniques are used for the generation of large amounts of the hGH modified by CTPs of the present invention. In some embodiments, recombinant techniques are described by Bitter et al., (1987) Methods in Enzymol. 153:516-544, Studier et al. (1990) Methods in Enzymol. 185:60-89, Brisson et al. (1984) Nature 310:511-514, Takamatsu et al. (1987) EMBO J. 6:307-311, Coruzzi et al. (1984) EMBO J. 3:1671-1680 and Brogli et al., (1984) Science 224:838-843, Gurley et al. (1986) Mol. Cell. Biol. 6:559-565 and Weissbach & Weissbach, 1988, Methods for Plant Molecular Biology, Academic Press, NY, Section VIII, pp 421-463.

[0072] In another embodiment, hGH modified by CTPs of the present invention are synthesized using a polynucleotide encoding a polypeptide of the present invention. In another embodiment, the polynucleotide encoding hGH modified by CTPs of the present invention is ligated into an expression vector, comprising a transcriptional control of a cis-regulatory sequence (e.g., promoter sequence). In another embodiment, the cis-regulatory sequence is suitable for directing constitutive expression of the growth hormones of the present invention. In another embodiment, the cis-regulatory sequence is suitable for directing tissue specific expression of the hGH modified by CTPs of the present invention. In another embodiment, the cis-regulatory sequence is suitable for directing inducible expression of the hGH modified by CTPs of the present invention.

[0073] In another embodiment, tissue-specific promoters suitable for use with the present invention include sequences which are functional in specific cell population, example include, but are not limited to promoters such as albumin that is liver specific [Pinkert et al., (1987) Genes Dev. 1:268-277], lymphoid specific promoters [Calame et al., (1988) Adv. Immunol. 43:235-275]; in particular promoters of T-cell receptors [Winoto et al., (1989) EMBO J. 8:729-733] and immunoglobulins; [Banerji et al. (1983) Cell 33:729-740], neuron-specific promoters such as the neurofilament promoter [Byrne et al. (1989) Proc. Natl. Acad. Sci. USA 86:5473-5477], pancreas-specific promoters [Edlun et al. (1985) Science 230:912-916] or mammary gland-specific promoters such as the milk whey promoter (U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166). Inducible promoters suitable for use with the present invention include for example the tetracycline-inducible promoter (Srour, M.A., et al.,

2003. Thromb. Haemost. 90: 398-405).

[0074] In one embodiment, the phrase "a polynucleotide" refers to a single or double stranded nucleic acid sequence which be isolated and provided in the form of an RNA sequence, a complementary polynucleotide sequence (cDNA), a genomic polynucleotide sequence and/or a composite polynucleotide sequences (e.g., a combination of the above).

[0075] In one embodiment, "complementary polynucleotide sequence" refers to a sequence, which results from reverse transcription of messenger RNA using a reverse transcriptase or any other RNA dependent DNA polymerase. In one embodiment, the sequence can be subsequently amplified *in vivo* or *in vitro* using a DNA polymerase.

[0076] In one embodiment, "genomic polynucleotide sequence" refers to a sequence derived (isolated) from a chromosome and thus it represents a contiguous portion of a chromosome.

[0077] In one embodiment, "composite polynucleotide sequence" refers to a sequence, which is at least partially complementary and at least partially genomic. In one embodiment, a composite sequence can include some exonal sequences required to encode the polypeptide of the present invention, as well as some intronic sequences interposing there between. In one embodiment, the intronic sequences can be of any source, including of other genes, and typically will include conserved splicing signal sequences. In one embodiment, intronic sequences include cis acting expression regulatory elements.

[0078] In another embodiment, polynucleotides of the present invention are prepared using PCR techniques as described in Example 1, or any other method or procedure known to one skilled in the art. In some embodiments, the procedure involves the ligation of two different DNA sequences (See, for example, "Current Protocols in Molecular Biology", eds. Ausubel et al., John Wiley & Sons, 1992).

[0079] In one embodiment, polynucleotides of the present invention are inserted into expression vectors (i.e., a nucleic acid construct) to enable expression of the recombinant polypeptide. In one embodiment, the expression vector of the present invention includes additional sequences which render this vector suitable for replication and integration in prokaryotes. In one embodiment, the expression vector of the present invention includes additional sequences which render this vector suitable for replication and integration in eukaryotes. In one embodiment, the expression vector of the present invention includes a shuttle vector which renders this vector suitable for replication and integration in both prokaryotes and eukaryotes. In another embodiment, cloning vectors comprise transcription and translation initiation sequences (e.g., promoters, enhancers) and transcription and translation terminators (e.g., polyadenylation signals).

[0080] In one embodiment, a variety of prokaryotic or eukaryotic cells can be used as host-expression systems to express the hGH modified by CTPs of the present invention. In some embodiments, these include, but are not limited to, microorganisms, such as bacteria

transformed with a recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vector containing the polypeptide coding sequence; yeast transformed with recombinant yeast expression vectors containing the polypeptide coding sequence; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors, such as Ti plasmid, containing the polypeptide coding sequence.

[0081] In another embodiment, non-bacterial expression systems are used (e.g. mammalian expression systems such as CHO cells) to express the growth hormones of the present invention. In one embodiment, the expression vector used to express polynucleotides of the present invention in mammalian cells is pCI-DHFR vector comprising a CMV promoter and a neomycin resistance gene. Construction of the pCI-dhfr vector is described, according to one embodiment, in Example 1.

[0082] In another embodiment, in bacterial systems of the present invention, a number of expression vectors can be advantageously selected depending upon the use intended for the polypeptide expressed. In one embodiment, large quantities of polypeptide are desired. In one embodiment, vectors that direct the expression of high levels of the protein product, possibly as a fusion with a hydrophobic signal sequence, which directs the expressed product into the periplasm of the bacteria or the culture medium where the protein product is readily purified are desired. In one embodiment, certain fusion protein engineered with a specific cleavage site to aid in recovery of the polypeptide. In one embodiment, vectors adaptable to such manipulation include, but are not limited to, the pET series of *E. coli* expression vectors [Studier et al., Methods in Enzymol. 185:60-89 (1990)].

[0083] In one embodiment, yeast expression systems are used. In one embodiment, a number of vectors containing constitutive or inducible promoters can be used in yeast as disclosed in U.S. Pat. Application. No: 5,932,447. In another embodiment, vectors which promote integration of foreign DNA sequences into the yeast chromosome are used.

[0084] In one embodiment, the expression vector of the present invention can further include additional polynucleotide sequences that allow, for example, the translation of several proteins from a single mRNA such as an internal ribosome entry site (IRES) and sequences for genomic integration of the promoter-chimeric polypeptide.

[0085] In another embodiment, mammalian expression vectors include, but are not limited to, pcDNA3, pcDNA3.1(+/-), pGL3, pZeoSV2(+/-), pSecTag2, pDisplay, pEF/myc/cyto, pCMV/myc/cyto, pCR3.1, pSinRep5, DH26S, DHBB, pNMT1, pNMT41, pNMT81, which are available from Invitrogen, pCI which is available from Promega, pMbac, pPbac, pBK-RSV and pBK-CMV which are available from Strategene, pTRES which is available from Clontech, and their derivatives.

[0086] In another embodiment, expression vectors containing regulatory elements from eukaryotic viruses such as retroviruses are used by the present invention. SV40 vectors

include pSVT7 and pMT2. In some embodiments, vectors derived from bovine papilloma virus include pBV-1MTHA, and vectors derived from Epstein Bar virus include pHEBO, and p2O5. Other exemplary vectors include pMSG, pAV009/A⁺, pMTO10/A⁺, pMAMneo-5, baculovirus pDSVE, and any other vector allowing expression of proteins under the direction of the SV-40 early promoter, SV-40 later promoter, metallothionein promoter, murine mammary tumor virus promoter, Rous sarcoma virus promoter, polyhedrin promoter, or other promoters shown effective for expression in eukaryotic cells.

[0087] In another embodiment, recombinant viral vectors are useful for *in vivo* expression of the GH modified by CTPs of the present invention since they offer advantages such as lateral infection and targeting specificity. In one embodiment, lateral infection is inherent in the life cycle of, for example, retrovirus and is the process by which a single infected cell produces many progeny virions that bud off and infect neighboring cells. In another embodiment, the result is that a large area becomes rapidly infected, most of which was not initially infected by the original viral particles. In one embodiment, viral vectors are produced that are unable to spread laterally. In another embodiment, this characteristic can be useful if the desired purpose is to introduce a specified gene into only a localized number of targeted cells.

[0088] In another embodiment, various methods can be used to introduce the expression vector of the present invention into cells. Such methods are generally described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Springs Harbor Laboratory, New York (1989, 1992), in Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley and Sons, Baltimore, Md. (1989), Chang et al., *Somatic Gene Therapy*, CRC Press, Ann Arbor, Mich. (1995), Vega et al., *Gene Targeting*, CRC Press, Ann Arbor Mich. (1995), *Vectors: A Survey of Molecular Cloning Vectors and Their Uses*, Butterworths, Boston Mass. (1988) and Gilboa et al. [*Biotechniques* 4 (6): 504-512, 1986] and include, for example, stable or transient transfection, lipofection, electroporation and infection with recombinant viral vectors. In addition, see U.S. Pat. Nos. 5,464,764 and 5,487,992 for positive-negative selection methods.

[0089] In another embodiment, introduction of nucleic acid by viral infection offers several advantages over other methods such as lipofection and electroporation, since higher transfection efficiency can be obtained due to the infectious nature of viruses.

[0090] In one embodiment, it will be appreciated that the GH modified by CTPs of the present invention can also be expressed from a nucleic acid construct administered to the individual employing any suitable mode of administration, described hereinabove (i.e., *in-vivo* gene therapy). In one embodiment, the nucleic acid construct is introduced into a suitable cell via an appropriate gene delivery vehicle/method (transfection, transduction, homologous recombination, etc.) and an expression system as needed and then the modified cells are expanded in culture and returned to the individual (i.e., *ex-vivo* gene therapy).

[0091] In one embodiment, *in vivo* gene therapy using a growth hormone has been conducted in animal models.

[0092] In one embodiment, plant expression vectors are used. In one embodiment, the expression of a polypeptide coding sequence is driven by a number of promoters. In some embodiments, viral promoters such as the 35S RNA and 19S RNA promoters of CaMV [Brisson et al., *Nature* 310:511-514 (1984)], or the coat protein promoter to TMV [Takamatsu et al., *EMBO J.* 6:307-311 (1987)] are used. In another embodiment, plant promoters are used such as, for example, the small subunit of RUBISCO [Coruzzi et al., *EMBO J.* 3:1671-1680 (1984); and Brogli et al., *Science* 224:838-843 (1984)] or heat shock promoters, e.g., soybean hsp17.5-E or hsp17.3-B [Gurley et al., *Mol. Cell. Biol.* 6:559-565 (1986)]. In one embodiment, constructs are introduced into plant cells using Ti plasmid, Ri plasmid, plant viral vectors, direct DNA transformation, microinjection, electroporation and other techniques well known to the skilled artisan. See, for example, Weissbach & Weissbach [Methods for Plant Molecular Biology, Academic Press, NY, Section VIII, pp 421-463 (1988)]. Other expression systems such as insects and mammalian host cell systems, which are well known in the art, can also be used by the present invention.

[0093] It will be appreciated that other than containing the necessary elements for the transcription and translation of the inserted coding sequence (encoding the polypeptide), the expression construct of the present invention can also include sequences engineered to optimize stability, production, purification, yield or activity of the expressed polypeptide.

[0094] Various methods, in some embodiments, can be used to introduce the expression vector of the present invention into the host cell system. In some embodiments, such methods are generally described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Springs Harbor Laboratory, New York (1989, 1992), in Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley and Sons, Baltimore, Md. (1989), Chang et al., *Somatic Gene Therapy*, CRC Press, Ann Arbor, Mich. (1995), Vega et al., *Gene Targeting*, CRC Press, Ann Arbor Mich. (1995), *Vectors: A Survey of Molecular Cloning Vectors and Their Uses*, Butterworths, Boston Mass. (1988) and Gilboa et al. [*Biotechniques* 4 (6): 504-512, 1986] and include, for example, stable or transient transfection, lipofection, electroporation and infection with recombinant viral vectors. In addition, see U.S. Pat. Nos. 5,464,764 and 5,487,992 for positive-negative selection methods.

[0095] In one embodiment, transformed cells are cultured under effective conditions, which allow for the expression of high amounts of recombinant polypeptide. In another embodiment, effective culture conditions include, but are not limited to, effective media, bioreactor, temperature, pH and oxygen conditions that permit protein production. In one embodiment, an effective medium refers to any medium in which a cell is cultured to produce the recombinant polypeptide of the present invention. In another embodiment, a medium typically includes an aqueous solution having assimilable carbon, nitrogen and phosphate sources, and appropriate salts, minerals, metals and other nutrients, such as vitamins. In another embodiment, cells of the present invention can be cultured in conventional fermentation bioreactors, shake flasks, test tubes, microtiter dishes and petri plates. In another embodiment, culturing is carried out at a temperature, pH and oxygen content appropriate for a recombinant cell. In another embodiment, culturing conditions are within the expertise of one of ordinary skill in the art.

[0096] In another embodiment, depending on the vector and host system used for production, resultant growth hormones of the present invention either remain within the recombinant cell, secreted into the fermentation medium, secreted into a space between two cellular membranes, such as the periplasmic space in *E. coli*; or retained on the outer surface of a cell or viral membrane.

[0097] In one embodiment, following a predetermined time in culture, recovery of the recombinant polypeptide is effected.

[0098] In one embodiment, the phrase "recovering the recombinant polypeptide" used herein refers to collecting the whole fermentation medium containing the polypeptide and need not imply additional steps of separation or purification.

[0099] In one embodiment, growth hormones of the present invention are purified using a variety of standard protein purification techniques, such as, but not limited to, affinity chromatography, ion exchange chromatography, filtration, electrophoresis, hydrophobic interaction chromatography, gel filtration chromatography, reverse phase chromatography, concanavalin A chromatography, chromatofocusing and differential solubilization.

[0100] In one embodiment, to facilitate recovery, the expressed coding sequence can be engineered to encode the polypeptide of the present invention and fused cleavable moiety. In one embodiment, a fusion protein can be designed so that the polypeptide can be readily isolated by affinity chromatography; e.g., by immobilization on a column specific for the cleavable moiety. In one embodiment, a cleavage site is engineered between the polypeptide and the cleavable moiety and the polypeptide can be released from the chromatographic column by treatment with an appropriate enzyme or agent that specifically cleaves the fusion protein at this site [e.g., see Booth et al., Immunol. Lett. 19:65-70 (1988); and Gardella et al., J. Biol. Chem. 265:15854-15859 (1990)].

[0101] In one embodiment, the polypeptide of the present invention is retrieved in "substantially pure" form.

[0102] In one embodiment, the phrase "substantially pure" refers to a purity that allows for the effective use of the protein in the applications described herein.

[0103] In one embodiment, the polypeptide of the present invention can also be synthesized using *in vitro* expression systems. In one embodiment, *in vitro* synthesis methods are well known in the art and the components of the system are commercially available.

[0104] In one embodiment, production of GH modified by CTPs using recombinant DNA technology is performed.

[0105] In another embodiment, the recombinant polypeptides are synthesized and purified;

their therapeutic efficacy can be assayed either *in vivo* or *in vitro*. In one embodiment, the binding activities of the recombinant GH modified by CTPs of the present invention can be ascertained using various assays.

[0106] In one embodiment, the present invention comprises CTP-hGH-CTP-CTP polypeptides. In one embodiment, recombinant DNA technology methods are used for the production of CTP-hGH-CTP-CTP polypeptides as illustrated in Example 1. In one embodiment, the therapeutic efficacy of the CTP-hGH-CTP-CTP polypeptides of the present invention is assayed either *in vivo*. In one embodiment, the therapeutic efficacy of the CTP-hGH-CTP-CTP polypeptides of the present invention is assayed either *in vitro*. In one embodiment, the binding activities of the recombinant hGH polypeptides of the present invention are measured using Nb2 (a prolactin-dependent rat lymphoma cell line (ECACC Cell Bank)) or a FCD-P1 murine cell line, previously transfected with human growth hormone receptor. In one embodiment, binding of hGH to these receptors induces cell proliferation which in one embodiment is measured by the levels of MTT cellular stain as a function of hGH activity. In one embodiment, *in vivo* activity is deduced by measuring weight gain over time in treated growth hormone deficient animals.

[0107] In one embodiment, the present invention provides a method of inducing growth or weight gain in a subject, comprising administering to the subject a therapeutically effective amount of a polypeptide comprising a growth hormone, one chorionic gonadotrophin carboxy terminal peptide (CTP) attached to an amino terminus of said growth hormone, and two chorionic gonadotrophin CTPs attached to a carboxy terminus of the growth hormone, thereby inducing growth or weight gain in a subject.

[0108] In another embodiment, the present invention provides a method of inducing growth in a human subject, comprising administering to said subject a therapeutically effective amount of a polypeptide comprising a growth hormone, one chorionic gonadotrophin carboxy terminal peptide (CTP) attached to the amino terminus of said growth hormone, and two chorionic gonadotrophin CTPs attached to the carboxy terminus of said growth hormone, thereby inducing growth in said subject. The human subject is a GH-deficient child.

[0109] In another embodiment, the present invention provides a method of inducing weight gain in a human subject, comprising administering to said subject a therapeutically effective amount of a polypeptide comprising a growth hormone, one chorionic gonadotrophin carboxy terminal peptide (CTP) attached to the amino terminus of said growth hormone, and two chorionic gonadotrophin CTPs attached to the carboxy terminus of said growth hormone, thereby inducing weight gain in said subject. The human subject is a GH-deficient child.

[0110] In another embodiment, provided herein a method of inducing growth in a subject comprising administering to a subject a growth hormone modified by CTPs as described herein. In one embodiment, the CTP-modified growth hormone is directly administered to the subject, while in another embodiment, a polynucleotide encoding said CTP-modified growth hormone is administered to the subject. In another embodiment, provided herein a method of

inducing growth in a subject comprising administering to a subject a composition consisting known excipients, known vehicles, and a polypeptide comprising a growth hormone, one chorionic gonadotrophin carboxy terminal peptide (CTP) attached to an amino terminus of the growth hormone, and two chorionic gonadotrophin carboxy terminal peptides attached to a carboxy terminus of the growth hormone. In another embodiment, provided herein a method of inducing growth in a subject comprising administering to a subject a composition consisting known excipients, known vehicles, and a polypeptide consisting a growth hormone, one chorionic gonadotrophin carboxy terminal peptide (CTP) attached to an amino terminus of the growth hormone, and two chorionic gonadotrophin carboxy terminal peptides attached to a carboxy terminus of the growth hormone.

[0111] In another embodiment, growth is measured by weight gain. In another embodiment, growth is measured by height gain. In another embodiment, growth is measured by weight gain. In another embodiment, growth is measured by muscle mass gain. In another embodiment, growth is measured by weight gain. In another embodiment, growth is measured by bone mass gain. In another embodiment, growth is measured by weight gain. In another embodiment, growth is measured by fat gain. In another embodiment, growth is measured by any known measure known to one of skill in the art. The subject wherein growth is measured is a GH-deficient child.

[0112] In another embodiment, polypeptides comprising GH modified by CTPs of the present invention are administered in a dose of 1-90 micrograms in 0.1-5 ml solution. In another embodiment, polypeptides comprising GH modified by CTPs are administered in a dose of 1-50 micrograms in 0.1-5 ml solution. In another embodiment, polypeptides comprising GH modified by CTPs are administered in a dose of 1-25 micrograms in 0.1-5 ml solution. In another embodiment, polypeptides comprising GH modified by CTPs are administered in a dose of 50-90 micrograms in 0.1-5 ml solution. In another embodiment, polypeptides comprising GH modified by CTPs are administered in a dose of 10-50 micrograms in 0.1-5 ml solution.

[0113] In another embodiment, polypeptides comprising GH modified by CTPs are administered in a dose of 1-90 micrograms in 0.1-5 ml solution by intramuscular (IM) injection, subcutaneous (SC) injection, or intravenous (IV) injection once a week. In another embodiment, polypeptides comprising GH modified by CTPs are administered in a dose of 1-90 micrograms in 0.1-5 ml solution by intramuscular (IM) injection, subcutaneous (SC) injection, or intravenous (IV) injection twice a week. In another embodiment, polypeptides comprising GH modified by CTPs are administered in a dose of 1-90 micrograms in 0.1-5 ml solution by intramuscular (IM) injection, subcutaneous (SC) injection, or intravenous (IV) injection three times a week. In another embodiment, polypeptides comprising GH modified by CTPs are administered in a dose of 1-90 micrograms in 0.1-5 ml solution by intramuscular (IM) injection, subcutaneous (SC) injection, or intravenous (IV) injection once every two weeks. In another embodiment, polypeptides comprising GH modified by CTPs are administered in a dose of 1-90 micrograms in 0.1-5 ml solution by intramuscular (IM) injection, subcutaneous (SC) injection, or intravenous (IV) injection once every 17 days. In another embodiment,

polypeptides comprising GH modified by CTPs are administered in a dose of 1-90 micrograms in 0.1-5 ml solution by intramuscular (IM) injection, subcutaneous (SC) injection, or intravenous (IV) injection once every 19 days weeks.

[0114] In another embodiment, protein drugs of molecular weight lower than 50,000 daltons, such as GH modified by CTPs of the present invention are in general short-lived species *in vivo*, having short circulatory half-lives of several hours. In another embodiment, the subcutaneous route of administration in general provides slower release into the circulation. In another embodiment, the CTP modified polypeptide of the invention prolongs the half-life of protein drugs of molecular weight lower than 50,000 daltons, such as GH. In another embodiment, the CTP modified polypeptide of the invention enable GH to exert its beneficial effects for a longer period of time.

[0115] In another embodiment, the immunogenicity of a CTP modified polypeptide comprising a GH modified by CTPs is equal to an isolated GH. In another embodiment, the immunogenicity of a CTP modified polypeptide comprising a GH modified by CTPs is comparable to an isolated GH. In another embodiment, modifying a GH as described herein with CTP peptides reduces the immunogenicity of the GH. In another embodiment, the CTP modified polypeptide comprising a GH is as active as an isolated GH protein. In another embodiment, the CTP modified polypeptide comprising a GH is more active than an isolated GH. In another embodiment, the CTP modified polypeptide comprising a GH maximizes the growth hormone's protective ability against degradation while minimizing reductions in bioactivity.

[0116] In another embodiment, the GH modified by CTPs of the present invention is provided to the individual *per se*. In one embodiment, the GH modified by CTPs of the present invention is provided to the individual as part of a pharmaceutical composition where it is mixed with a pharmaceutically acceptable carrier.

[0117] In another embodiment, a "pharmaceutical composition" refers to a preparation of one or more of the active ingredients described herein with other chemical components such as physiologically suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

[0118] In another embodiment, "active ingredient" refers to the polypeptide sequence of interest, which is accountable for the biological effect.

[0119] In one embodiment, the present invention provides combined preparations. In one embodiment, "a combined preparation" defines especially a "kit of parts" in the sense that the combination partners as defined above can be dosed independently or by use of different fixed combinations with distinguished amounts of the combination partners i.e., simultaneously, concurrently, separately or sequentially. In another embodiment, the parts of the kit of parts can then, e.g., be administered simultaneously or chronologically staggered, that is at different time points and with equal or different time intervals for any part of the kit of parts. The ratio of

the total amounts of the combination partners can be administered in the combined preparation. The combined preparation can be varied, e.g., in order to cope with the needs of a patient subpopulation to be treated or the needs of the single patient which different needs can be due to a particular disease, severity of a disease, age, sex, or body weight as can be readily made by a person skilled in the art.

[0120] In another embodiment, the phrases "physiologically acceptable carrier" and "pharmaceutically acceptable carrier" which be interchangeably used refer to a carrier or a diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound. An adjuvant is included under these phrases. In one embodiment, one of the ingredients included in the pharmaceutically acceptable carrier can be for example polyethylene glycol (PEG), a biocompatible polymer with a wide range of solubility in both organic and aqueous media (Mutter et al. (1979).

[0121] In another embodiment, "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient. In one embodiment, excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

[0122] Techniques for formulation and administration of drugs are found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition.

[0123] In another embodiment, suitable routes of administration, for example, include oral, rectal, transmucosal, transnasal, intestinal or parenteral delivery, including intramuscular, subcutaneous and intramedullary injections as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections.

[0124] In another embodiment, the preparation is administered in a local rather than systemic manner, for example, via injection of the preparation directly into a specific region of a patient's body.

The dosage is in a range of 1-60 mg/week

[0125] In another embodiment, the dosage is 1-5 mg/administration. In another embodiment, the dosage is 2 mg/administration. In another embodiment, the dosage is 4 mg/administration. In another embodiment, the dosage is 1.2 mg/administration. In another embodiment, the dosage is 1.8 mg/administration. In one embodiment, the composition is administered once a week. In another embodiment, the composition is administered once biweekly. In another embodiment, the composition is administered monthly. In another embodiment, the composition is administered daily.

[0126] In another embodiment, GH modified by CTPs is formulated in an intranasal dosage form. In another embodiment, GH modified by CTPs is formulated in an injectable dosage

form. In another embodiment, GH modified by CTPs is administered to a subject in a dose ranging from 0.0001 mg to 0.6 mg. In another embodiment, GH modified by CTPs is administered to a subject in a dose ranging from 0.001 mg to 0.005 mg. In another embodiment, GH modified by CTPs is administered to a subject in a dose ranging from 0.005 mg to 0.01 mg. In another embodiment, GH modified by CTPs is administered to a subject in a dose ranging from 0.01 mg to 0.3 mg. In another embodiment, a GH modified by CTPs is administered to a subject in a dose in a dose ranging from 0.2 mg to 0.6 mg.

[0127] In another embodiment, GH modified by CTPs is administered to a subject in a dose ranging from 1-100 micrograms. In another embodiment, a GH modified by CTPs is administered to a subject in a dose ranging from 10-80 micrograms. In another embodiment, a GH modified by CTPs is administered to a subject in a dose ranging from 20-60 micrograms. In another embodiment, a GH modified by CTPs is administered to a subject in a dose ranging from 10-50 micrograms. In another embodiment, a GH modified by CTPs is administered to a subject in a dose ranging from 40-80 micrograms. In another embodiment, a GH modified by CTPs is administered to a subject in a dose ranging from 10-30 micrograms. In another embodiment, a GH modified by CTPs is administered to a subject in a dose ranging from 30-60 micrograms.

[0128] In another embodiment, GH modified by CTPs is administered to a subject in a dose ranging from 0.2 mg to 2 mg. In another embodiment, a GH modified by CTPs is administered to a subject in a dose ranging from 2 mg to 6 mg. In another embodiment, a GH modified by CTPs is administered to a subject in a dose ranging from 4 mg to 10 mg. In another embodiment, a GH modified by CTPs is administered to a subject in a dose ranging from 5 mg and 15 mg.

[0129] In another embodiment, a GH modified by CTPs is injected into the muscle (intramuscular injection). In another embodiment, a GH modified by CTPs is injected below the skin (subcutaneous injection). In another embodiment, a GH modified by CTPs is injected into the muscle. In another embodiment, a GH modified by CTPs is injected below the skin.

[0130] In another embodiment, the methods of the invention result in increasing the compliance of patients afflicted with chronic illnesses that are in need of a GH therapy. In another embodiment, the methods of the invention enable reduction in the dosing frequency of a GH by modifying the GH with CTPs as described hereinabove. In another embodiment, the term compliance comprises adherence. In another embodiment, the methods of the invention include increasing the compliance of patients in need of a GH therapy by reducing the frequency of administration of the GH. In another embodiment, reduction in the frequency of administration of the GH is achieved due to the CTP modifications which render the CTP-modified GH more stable. In another embodiment, reduction in the frequency of administration of the GH is achieved as a result of increasing $T_{1/2}$ of the growth hormone. In another embodiment, reduction in the frequency of administration of the GH is achieved as a result of increasing the clearance time of the GH. In another embodiment, reduction in the frequency of administration of the growth hormone is achieved as a result of increasing the AUC measure of

the growth hormone.

[0131] In one embodiment, the present invention provides a method of maintaining insulin-like growth factor (IGF-1) levels within a normal therapeutic range in a subject, the method comprising administering to the subject a therapeutically effective amount of a polypeptide comprising a growth hormone, one chorionic gonadotrophin carboxy terminal peptide (CTP) attached to the amino terminus of the growth hormone, and two chorionic gonadotrophin CTPs attached to the carboxy terminus of the growth hormone, thereby maintaining IGF-I levels within a normal therapeutic range in the subject. In another embodiment, methods of maintaining IGF-1 levels within a normal therapeutic level are provided in Example 9, herein below.

[0132] In another embodiment, a GH modified by CTPs is administered to a subject once a day. In another embodiment, a polypeptide comprising a GH modified by CTPs is administered to a subject once every two days. In another embodiment, a GH modified by CTPs is administered to a subject once every three days. In another embodiment, a GH modified by CTPs is administered to a subject once every four days. In another embodiment, a GH modified by CTPs is administered to a subject once every five days. In another embodiment, a GH modified by CTPs is administered to a subject once every six days. In another embodiment, a GH modified by CTPs is administered to a subject once every week. In another embodiment, a GH modified by CTPs is administered to a subject once every 7-14 days. In another embodiment, a GH modified by CTPs is administered to a subject once every 10-20 days. In another embodiment, a GH modified by CTPs is administered to a subject once every 5-15 days. In another embodiment, a GH modified by CTPs is administered to a subject once every 15-30 days.

[0133] In another embodiment, the dosage is in a range of 50-500 mg/day. In another embodiment, the dosage is in a range of 50-150 mg/day. In another embodiment, the dosage is in a range of 100-200 mg/day. In another embodiment, the dosage is in a range of 150-250 mg/day. In another embodiment, the dosage is in a range of 200-300 mg/day. In another embodiment, the dosage is in a range of 250-400 mg/day. In another embodiment, the dosage is in a range of 300-500 mg/day. In another embodiment, the dosage is in a range of 350-500 mg/day.

[0134] In one embodiment, the dosage is 20 mg/day. In one embodiment, the dosage is 30 mg/day. In one embodiment, the dosage is 40 mg/day. In one embodiment, the dosage is 50 mg/day. In one embodiment, the dosage is 0.01 mg/day. In another embodiment, the dosage is 0.1 mg/day. In another embodiment, the dosage is 1 mg/day. In another embodiment, the dosage is 0.530 mg/day. In another embodiment, the dosage is 0.05 mg/day. In another embodiment, the dosage is 50 mg/day. In another embodiment, the dosage is 10 mg/day. In another embodiment, the dosage is 20-70 mg/day. In another embodiment, the dosage is 5 mg/day.

[0135] In another embodiment, the dosage is 1-90 mg/day. In another embodiment, the

dosage is 1-90 mg/2 days. In another embodiment, the dosage is 1-90 mg/3 days. In another embodiment, the dosage is 1-90 mg/4 days. In another embodiment, the dosage is 1-90 mg/5 days. In another embodiment, the dosage is 1-90 mg/6 days. In another embodiment, the dosage is 1-90 mg/week. In another embodiment, the dosage is 1-90 mg/9 days. In another embodiment, the dosage is 1-90 mg/11 days. In another embodiment, the dosage is 1-90 mg/14 days.

[0136] In another embodiment, the growth hormone dosage is 10-50 mg/day. In another embodiment, the dosage is 10-50 mg/2 days. In another embodiment, the dosage is 10-50 mg/3 days. In another embodiment, the dosage is 10-50 mg/4 days. In another embodiment, the dosage is 10-50 micrograms mg/5 days. In another embodiment, the dosage is 10-50 mg/6 days. In another embodiment, the dosage is 10-50 mg/week. In another embodiment, the dosage is 10-50 mg/9 days. In another embodiment, the dosage is 10-50 mg/11 days. In another embodiment, the dosage is 10-50 mg/14 days.

[0137] Oral administration, in one embodiment, comprises a unit dosage form comprising tablets, capsules, lozenges, chewable tablets, suspensions, emulsions and the like. Such unit dosage forms comprise a safe and effective amount of the desired growth hormone of the invention, each of which is in one embodiment, from about 0.7 or 3.5 mg to about 280 mg/70 kg, or in another embodiment, about 0.5 or 10 mg to about 210 mg/70 kg. The pharmaceutically-acceptable carriers suitable for the preparation of unit dosage forms for peroral administration are well-known in the art. In some embodiments, tablets typically comprise conventional pharmaceutically-compatible adjuvants as inert diluents, such as calcium carbonate, sodium carbonate, mannitol, lactose and cellulose; binders such as starch, gelatin and sucrose; disintegrants such as starch, alginic acid and croscarmellose; lubricants such as magnesium stearate, stearic acid and talc. In one embodiment, glidants such as silicon dioxide can be used to improve flow characteristics of the powder-mixture. In one embodiment, coloring agents, such as the FD&C dyes, can be added for appearance. Sweeteners and flavoring agents, such as aspartame, saccharin, menthol, peppermint, and fruit flavors, are useful adjuvants for chewable tablets. Capsules typically comprise one or more solid diluents disclosed above. In some embodiments, the selection of carrier components depends on secondary considerations like taste, cost, and shelf stability, which are not critical for the purposes of this invention, and can be readily made by a person skilled in the art.

[0138] In one embodiment, the oral dosage form comprises predefined release profile. In one embodiment, the oral dosage form of the present invention comprises an extended release tablets, capsules, lozenges or chewable tablets. In one embodiment, the oral dosage form of the present invention comprises a slow release tablets, capsules, lozenges or chewable tablets. In one embodiment, the oral dosage form of the present invention comprises an immediate release tablets, capsules, lozenges or chewable tablets. In one embodiment, the oral dosage form is formulated according to the desired release profile of the pharmaceutical active ingredient as known to one skilled in the art.

[0139] Peroral compositions, in some embodiments, comprise liquid solutions, emulsions,

suspensions, and the like. In some embodiments, pharmaceutically-acceptable carriers suitable for preparation of such compositions are well known in the art. In some embodiments, liquid oral compositions comprise from about 0.001% to about 0.933% of the desired compound or compounds, or in another embodiment, from about 0.01% to about 10 %.

[0140] In some embodiments, compositions for use in the methods of this invention comprise solutions or emulsions, which in some embodiments are aqueous solutions or emulsions comprising a safe and effective amount of the compounds of the present invention and optionally, other compounds, intended for topical intranasal administration. In some embodiments, h compositions comprise from about 0.001% to about 10.0% w/v of a GH modified by CTPs, more preferably from about 0.1% to about 2.0, which is used for systemic delivery of the compounds by the intranasal route.

[0141] In another embodiment, the pharmaceutical compositions are administered by intravenous, intra-arterial, or intramuscular injection of a liquid preparation. In some embodiments, liquid formulations include solutions, suspensions, dispersions, emulsions, oils and the like. In one embodiment, the pharmaceutical compositions are administered intravenously, and are thus formulated in a form suitable for intravenous administration. In another embodiment, the pharmaceutical compositions are administered intra-arterially, and are thus formulated in a form suitable for intra-arterial administration. In another embodiment, the pharmaceutical compositions are administered intramuscularly, and are thus formulated in a form suitable for intramuscular administration.

[0142] Further, in another embodiment, the pharmaceutical compositions are administered topically to body surfaces, and are thus formulated in a form suitable for topical administration. Suitable topical formulations include gels, ointments, creams, lotions, drops and the like. For topical administration, the compounds of the present invention are combined with an additional appropriate therapeutic agent or agents, prepared and applied as solutions, suspensions, or emulsions in a physiologically acceptable diluent with or without a pharmaceutical carrier.

[0143] In one embodiment, pharmaceutical compositions of the present invention are manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

[0144] In one embodiment, pharmaceutical compositions for use in accordance with the present invention is formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active ingredients into preparations which, can be used pharmaceutically. In one embodiment, formulation is dependent upon the route of administration chosen.

[0145] In one embodiment, injectables, of the invention are formulated in aqueous solutions. In one embodiment, injectables, of the invention are formulated in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological salt buffer. In some

embodiments, for transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

[0146] In one embodiment, the preparations described herein are formulated for parenteral administration, e.g., by bolus injection or continuous infusion. In some embodiments, formulations for injection are presented in unit dosage form, e.g., in ampoules or in multidose containers with optionally, an added preservative. In some embodiments, compositions are suspensions, solutions or emulsions in oily or aqueous vehicles, and contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

[0147] The compositions also comprise, in some embodiments, preservatives, such as benzalkonium chloride and thimerosal and the like; chelating agents, such as edetate sodium and others; buffers such as phosphate, citrate and acetate; tonicity agents such as sodium chloride, potassium chloride, glycerin, mannitol and others; antioxidants such as ascorbic acid, acetylcystine, sodium metabisulfite and others; aromatic agents; viscosity adjustors, such as polymers, including cellulose and derivatives thereof; and polyvinyl alcohol and acid and bases to adjust the pH of these aqueous compositions as needed. The compositions also comprise, in some embodiments, local anesthetics or other actives. The compositions can be used as sprays, mists, drops, and the like.

[0148] In some embodiments, pharmaceutical compositions for parenteral administration include aqueous solutions of the active preparation in water-soluble form. Additionally, suspensions of the active ingredients, in some embodiments, are prepared as appropriate oily or water based injection suspensions. Suitable lipophilic solvents or vehicles include, in some embodiments, fatty oils such as sesame oil, or synthetic fatty acid esters such as ethyl oleate, triglycerides or liposomes. Aqueous injection suspensions contain, in some embodiments, substances, which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol or dextran. In another embodiment, the suspension also contains suitable stabilizers or agents which increase the solubility of the active ingredients to allow for the preparation of highly concentrated solutions.

[0149] In another embodiment, the active compound can be delivered in a vesicle, in particular a liposome (see Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*).

[0150] In another embodiment, the pharmaceutical composition delivered in a controlled release system is formulated for intravenous infusion, implantable osmotic pump, transdermal patch, liposomes, or other modes of administration. In one embodiment, a pump is used (see Langer, *supra*; Sefton, *CRC Crit. Rev. Biomed. Eng.* 14:201 (1987); Buchwald et al., *Surgery* 88:507 (1980); Saudek et al., *N. Engl. J. Med.* 321:574 (1989). In another embodiment, polymeric materials can be used. In yet another embodiment, a controlled release system can be placed in proximity to the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in *Medical Applications of Controlled Release*, *supra*,

vol. 2, pp. 115-138 (1984). Other controlled release systems are discussed in the review by Langer (Science 249:1527-1533 (1990)).

[0151] In some embodiments, the active ingredient is in powder form for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water based solution, before use. Compositions are formulated, in some embodiments, for atomization and inhalation administration. In another embodiment, compositions are contained in a container with attached atomizing means.

[0152] In one embodiment, the preparation of the present invention is formulated in rectal compositions such as suppositories or retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other glycerides.

[0153] In another embodiment, pharmaceutical compositions suitable for use in context of the present invention include compositions wherein the active ingredients are contained in an amount effective to achieve the intended purpose. In another embodiment, a therapeutically effective amount means an amount of active ingredients effective to prevent, alleviate or ameliorate symptoms of disease or prolong the survival of the subject being treated.

[0154] In one embodiment, determination of a therapeutically effective amount is well within the capability of those skilled in the art.

[0155] Some examples of substances which can serve as pharmaceutically-acceptable carriers or components thereof are sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose, and methyl cellulose; powdered tragacanth; malt; gelatin; talc; solid lubricants, such as stearic acid and magnesium stearate; calcium sulfate; vegetable oils, such as peanut oil, cottonseed oil, sesame oil, olive oil, corn oil and oil of theobroma; polyols such as propylene glycol, glycerine, sorbitol, mannitol, and polyethylene glycol; alginic acid; emulsifiers, such as the TweenTM brand emulsifiers; wetting agents, such as sodium lauryl sulfate; coloring agents; flavoring agents; tableting agents, stabilizers; antioxidants; preservatives; pyrogen-free water; isotonic saline; and phosphate buffer solutions. The choice of a pharmaceutically-acceptable carrier to be used in conjunction with the compound is basically determined by the way the compound is to be administered. If the subject compound is to be injected, in one embodiment, the pharmaceutically-acceptable carrier is sterile, physiological saline, with a blood-compatible suspending agent, the pH of which has been adjusted to about 7.4.

[0156] In addition, the compositions further comprise binders (e.g. acacia, cornstarch, gelatin, carbomer, ethyl cellulose, guar gum, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, povidone), disintegrating agents (e.g. cornstarch, potato starch, alginic acid, silicon dioxide, croscarmellose sodium, crospovidone, guar gum, sodium starch glycolate), buffers (e.g., Tris-HCl, acetate, phosphate) of various pH and ionic strength, additives such as albumin or gelatin to prevent absorption to surfaces, detergents (e.g., Tween 20, Tween 80, Pluronic F68, bile acid salts), protease inhibitors, surfactants (e.g. sodium lauryl sulfate), permeation

enhancers, solubilizing agents (e.g., glycerol, polyethylene glycerol), antioxidants (e.g., ascorbic acid, sodium metabisulfite, butylated hydroxyanisole), stabilizers (e.g. hydroxypropyl cellulose, hydroxypropylmethyl cellulose), viscosity increasing agents (e.g. carbomer, colloidal silicon dioxide, ethyl cellulose, guar gum), sweeteners (e.g. aspartame, citric acid), preservatives (e.g., Thimerosal, benzyl alcohol, parabens), lubricants (e.g. stearic acid, magnesium stearate, polyethylene glycol, sodium lauryl sulfate), flow-aids (e.g. colloidal silicon dioxide), plasticizers (e.g. diethyl phthalate, triethyl citrate), emulsifiers (e.g. carbomer, hydroxypropyl cellulose, sodium lauryl sulfate), polymer coatings (e.g., poloxamers or poloxamines), coating and film forming agents (e.g. ethyl cellulose, acrylates, polymethacrylates) and/or adjuvants.

[0157] Typical components of carriers for syrups, elixirs, emulsions and suspensions include ethanol, glycerol, propylene glycol, polyethylene glycol, liquid sucrose, sorbitol and water. For a suspension, typical suspending agents include methyl cellulose, sodium carboxymethyl cellulose, cellulose (e.g. Avicel™, RC-591), tragacanth and sodium alginate; typical wetting agents include lecithin and polyethylene oxide sorbitan (e.g. polysorbate 80). Typical preservatives include methyl paraben and sodium benzoate. In another embodiment, peroral liquid compositions also contain one or more components such as sweeteners, flavoring agents and colorants disclosed above.

[0158] The compositions also include incorporation of the active material into or onto particulate preparations of polymeric compounds such as polylactic acid, polglycolic acid, hydrogels, etc, or onto liposomes, microemulsions, micelles, unilamellar or multilamellar vesicles, erythrocyte ghosts, or spheroplasts.) Such compositions will influence the physical state, solubility, stability, rate of *in vivo* release, and rate of *in vivo* clearance.

[0159] Also comprehended by the invention are particulate compositions coated with polymers (e.g. poloxamers or poloxamines) and the compound coupled to antibodies directed against tissue-specific receptors, ligands or antigens or coupled to ligands of tissue-specific receptors.

[0160] In another embodiment, compounds modified by the covalent attachment of water-soluble polymers such as polyethylene glycol, copolymers of polyethylene glycol and polypropylene glycol, carboxymethyl cellulose, dextran, polyvinyl alcohol, polyvinylpyrrolidone or polyproline. In another embodiment, the modified compounds exhibit substantially longer half-lives in blood following intravenous injection than do the corresponding unmodified compounds. In one embodiment, modifications also increase the compound's solubility in aqueous solution, eliminate aggregation, enhance the physical and chemical stability of the compound, and greatly reduce the immunogenicity and reactivity of the compound. In another embodiment, the desired *in vivo* biological activity is achieved by the administration of such polymer-compound adducts less frequently or in lower doses than with the unmodified compound.

[0161] In another embodiment, preparation of effective amount or dose can be estimated initially from *in vitro* assays. In one embodiment, a dose can be formulated in animal models

and such information can be used to more accurately determine useful doses in humans.

[0162] In one embodiment, toxicity and therapeutic efficacy of the active ingredients described herein can be determined by standard pharmaceutical procedures *in vitro*, in cell cultures or experimental animals. In one embodiment, the data obtained from these *in vitro* and cell culture assays and animal studies can be used in formulating a range of dosage for use in human. In one embodiment, the dosages vary depending upon the dosage form employed and the route of administration utilized. In one embodiment, the exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. [See e.g., Fingl, et al., (1975) "The Pharmacological Basis of Therapeutics", Ch. 1 p.1].

[0163] In one embodiment, depending on the severity and responsiveness of the condition to be treated, dosing can be of a single or a plurality of administrations, with course of treatment lasting from several days to several weeks or until cure is effected or diminution of the disease state is achieved.

[0164] In one embodiment, the amount of a composition to be administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

[0165] In one embodiment, compositions including the preparation of the present invention formulated in a compatible pharmaceutical carrier are also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition.

[0166] In another embodiment, a GH modified by CTPs is administered via systemic administration. In another embodiment, a growth hormone as described herein is administered by intravenous, intramuscular or subcutaneous injection. In another embodiment, a GH modified by CTPs is lyophilized (i.e., freeze-dried) preparation in combination with complex organic excipients and stabilizers such as nonionic surface active agents (i.e., surfactants), various sugars, organic polyols and/or human serum albumin. In another embodiment, a pharmaceutical composition comprises a lyophilized GH modified by CTPs as described in sterile water for injection. In another embodiment, a pharmaceutical composition comprises a lyophilized growth hormone as described in sterile PBS for injection. In another embodiment, a pharmaceutical composition comprises a lyophilized growth hormone as described in sterile 0.9% NaCl for injection.

[0167] In another embodiment, the pharmaceutical composition comprising a GH modified by CTPs as described herein and complex carriers such as human serum albumin, polyols, sugars, and anionic surface active stabilizing agents. See, for example, WO 89/10756 (Hara et al.- containing polyol and p-hydroxybenzoate). In another embodiment, the pharmaceutical composition comprises a growth hormone as described herein and lactobionic acid and an acetate/glycine buffer. In another embodiment, the pharmaceutical composition comprising a GH modified by CTPs as described herein and amino acids, such as arginine or glutamate that

increase the solubility of interferon compositions in water. In another embodiment, the pharmaceutical composition comprises a lyophilized GH modified by CTPs as described herein and glycine or human serum albumin (HSA), a buffer (e.g. acetate) and an isotonic agent (e.g. NaCl). In another embodiment, the pharmaceutical composition comprises a lyophilized GH modified by CTPs as described herein and phosphate buffer, glycine and HSA.

[0168] In another embodiment, the pharmaceutical composition comprising a GH modified by CTPs as described herein is stabilized when placed in buffered solutions having a pH between about 4 and 7.2. In another embodiment, the pharmaceutical composition comprising a GH modified by CTPs as described herein is stabilized with an amino acid as a stabilizing agent and in some cases a salt (if the amino acid does not contain a charged side chain).

[0169] In another embodiment, the pharmaceutical composition comprising a GH modified by CTPs as described herein is a liquid composition comprising a stabilizing agent at between about 0.3% and 5% by weight which is an amino acid.

[0170] In another embodiment, the pharmaceutical composition comprising a GH modified by CTPs as described herein provides dosing accuracy and product safety. In another embodiment, the pharmaceutical composition comprising a GH modified by CTPs as described herein provides a biologically active, stable liquid formulation for use in injectable applications. In another embodiment, the pharmaceutical composition comprises a non-lyophilized GH modified by CTPs as described herein.

[0171] In another embodiment, the pharmaceutical composition comprising a GH modified by CTPs as described herein provides a liquid formulation permitting storage for a long period of time in a liquid state facilitating storage and shipping prior to administration.

[0172] In another embodiment, the pharmaceutical composition comprising a GH modified by CTPs as described herein comprises solid lipids as matrix material. In another embodiment, the injectable pharmaceutical composition comprising a GH modified by CTPs as described herein comprises solid lipids as matrix material. In another embodiment, the production of lipid microparticles by spray congealing was described by Speiser (Speiser and al., Pharm. Res. 8 (1991) 47-54) followed by lipid nanopellets for peroral administration (Speiser EP 0167825 (1990)). In another embodiment, lipids, which are used, are well tolerated by the body (e.g. glycerides composed of fatty acids which are present in the emulsions for parenteral nutrition).

[0173] In another embodiment, the pharmaceutical composition comprising a GH modified by CTPs as described herein is in the form of liposomes (J. E. Diederichs and al., Pharm./nd. 56 (1994) 267-275).

[0174] In another embodiment, the pharmaceutical composition comprising a GH modified by CTPs as described herein comprises polymeric microparticles. In another embodiment, the injectable pharmaceutical composition comprising a GH modified by CTPs as described herein comprises polymeric microparticles. In another embodiment, the pharmaceutical composition

comprising a GH modified by CTPs as described herein comprises nanoparticles. In another embodiment, the pharmaceutical composition comprising a GH modified by CTPs as described herein comprises liposomes. In another embodiment, the pharmaceutical composition comprising a GH modified by CTPs as described herein comprises lipid emulsion. In another embodiment, the pharmaceutical composition comprising a GH modified by CTPs as described herein comprises microspheres. In another embodiment, the pharmaceutical composition comprising a GH modified by CTPs as described herein comprises lipid nanoparticles. In another embodiment, the pharmaceutical composition comprising a GH modified by CTPs as described herein comprises lipid nanoparticles comprising amphiphilic lipids. In another embodiment, the pharmaceutical composition comprising a GH modified by CTPs as described herein comprises lipid nanoparticles comprising a drug, a lipid matrix and a surfactant. In another embodiment, the lipid matrix has a monoglyceride content which is at least 50% w/w.

[0175] In one embodiment, compositions of the present invention are presented in a pack or dispenser device, such as an FDA approved kit, which contain one or more unit dosage forms containing the active ingredient. In one embodiment, the pack, for example, comprise metal or plastic foil, such as a blister pack. In one embodiment, the pack or dispenser device is accompanied by instructions for administration. In one embodiment, the pack or dispenser is accommodated by a notice associated with the container in a form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions or human or veterinary administration. Such notice, in one embodiment, is labeling approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert.

[0176] In one embodiment, it will be appreciated that the GH modified by CTPs of the present invention can be provided to the individual with additional active agents to achieve an improved therapeutic effect as compared to treatment with each agent by itself. In another embodiment, measures (e.g., dosing and selection of the complementary agent) are taken to adverse side effects which are associated with combination therapies.

[0177] Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

EXAMPLES

[0178] Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols

in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Culture of Animal Cells - A Manual of Basic Technique" by Freshney, Wiley-Liss, N. Y. (1994), Third Edition; "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak et al., "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996). Other general references are provided throughout this document.

EXAMPLE 1

Generation of hGH constructs

MATERIALS AND METHODS

[0179] Four hGH clones (variants of 20kD protein) were synthesized. Xba I -Not I fragments containing hGH sequences from the four variants were ligated into the eukaryotic expression vector pCI-dhfr previously digested with XbaI -NotI. DNA from the 4 clones (401-0, 1, 2, 3 and 4) was prepared. Another partial hGH clone (1-242 bp) from 22kD protein was also synthesized (0606114). Primers were ordered from Sigma-Genosys. The primer sequences used to generate the hGH modified by CTPs polypeptides of the present invention are summarized in Table 1, hereinbelow.

Table 1

Primer number	SEQ ID NO	sequence	Restriction site (underlined in sequence)
25	18	5' <u>CTCTAGAGG</u> ACATGGCCAC 3'	XbaI
32 ^R	19	5' ACAGGGAGGTCTGGGGGTTCTGCA 3'	
33	20	5' TGCAGAACCCCCAGACCTCCCTGTGC 3'	
4 ^R	21	5' CCAAACATCATCAATGTATCTTA 3'	
25	22	5' <u>CTCTAGAGG</u> ACATGGCCAC 3'	XbaI
35 ^R	23	5' CGAACTCCTGGTAGGTGTCAAAGGC 3'	
34	24	5' GCCTTTGACACCTACCAGGAGTTTCG 3'	
37 ^R	25	5'ACGCGGCCCGCATCCAGACCTTCATCACTGAGGC 3'	NotI
39 ^R	26	5' <u>GCGGCCCGCGG</u> ACTCATCAGAAGCCGCAGCTGCCC 3'	

[0180] Construction of 402-0-p69-1 (hGH) (SEQ ID NO: 5): hGH is the wild type recombinant human growth hormone (without CTP) which was prepared for use as control in the below described experiments.

[0181] Three PCR reactions were performed. The first reaction was conducted with primer 25 and primer 32^R and plasmid DNA of 0606114 (partial clone of hGH 1-242 bp) as a template; as a result of the PCR amplification, a 245 bp product was formed.

[0182] The second reaction was conducted with primer 33 and primer 4^R and plasmid DNA of 401-0-p57-2 as a template; as a result of the PCR amplification, a 542 bp product was formed.

[0183] The last reaction was conducted with primers 25 and 4^R and a mixture of the products of the previous two reactions as a template; as a result of the PCR amplification, a 705 bp product was formed and ligated into the TA cloning vector (Invitrogen, catalog K2000-01). The XbaI -NotI fragment containing hGH-0 sequence was ligated into the eukaryotic expression vector pCI-dhfr. The vector was transfected into DG-44 CHO cells. Cells were grown in protein-free medium.

[0184] Construction of 402-1-p83-5 (hGH-CTP) - SEQ ID NO: 9 and 402-2-p72-3(hGH-CTPx2) - SEQ ID NO: 10: hGH-CTP is a recombinant human growth hormone which was fused to 1 copy of the C-terminal peptide of the beta chain of human Chorionic Gonadotropin

(CTP). The CTP cassette of hGH-CTP was attached to the C-terminus (one cassette). hGH-CTP-CTP is a recombinant human growth hormone which was fused to 2 copies of the C-terminal peptide of the beta chain of human Chorionic Gonadotropin (CTP). The two CTP cassettes of hGH-CTP-CTP were attached to the C-terminus (two cassettes).

[0185] Construction of hGH-CTP and hGH-CTP-CTP was performed in the same way as the construction of hGH-0. pCI-dhfr-401-1-p20-1 (hGH*-ctp) and pCI-dhfr-401-2-p21-2 (hGH*-ctp x2) were used as templates in the second PCR reaction.

[0186] hGH-CTP and hGH-CTP-CTP were expressed in DG-44 CHO cells. Cells were grown in protein-free medium. The molecular weight of hGH-CTP is ~30.5 kD since hGH has a MW of 22 kD while each "CTP cassette" contributes 8.5 kD to the overall molecular weight (see Figure 1). The molecular weight of hGH-CTP-CTP is ~39 kD (see Figure 1).

[0187] Construction of 402-3-p81-4 (CTP-hGH-CTP-CTP) - SEQ ID NO: 11 and 402-4-p82-9(CTP*hGH-CTP-CTP) - SEQ ID NO: 12: CTP-hGH-CTP-CTP is a recombinant human growth hormone which was fused to 3 copies of the C-terminal peptide of the beta chain of human Chorionic Gonadotropin (CTP). The three CTP cassettes of CTP-hGH-CTP-CTP were attached to both N-terminus (one cassette) and the C-terminus (two cassettes). tCTP-hGH-CTP-CTP is a recombinant human growth hormone which is fused to 1 truncated and 2 complete copies of the C-terminal peptide of the beta chain of human Chorionic Gonadotropin (CTP). The truncated CTP cassette of tCTP-hGH-CTP-CTP was attached to the N-terminus and two CTP cassettes were attached to the C-terminus (two cassettes).

[0188] Three PCR reactions were performed. The first reaction was conducted with primer 25 and primer 35^R and plasmid DNA of p401-3-p12-5 or 401-4-p22-1 as a template; as a result of the PCR amplification, a 265 or 220 bp product was formed. The second reaction was conducted with primer 34 and primer 37^R and plasmid DNA of TA-hGH-2-q65-1 as a template; as a result of the PCR amplification, a 695 bp product was formed. The last reaction was conducted with primers 25 and 37^R and a mixture of the products of the previous two reactions as a template; as a result of the PCR amplification, a 938 or 891bp product was formed and ligated into TA cloning vector (Invitrogen, catalog K2000-01). Xba I -Not I fragment containing hGH sequence was ligated into our eukaryotic expression vector pCI-dhfr.

[0189] CTP-hGH-CTP-CTP and tCTP-hGH-CTP-CTP were expressed in DG-44 CHO cells. Cells were grown in protein-free medium. The molecular weight of CTP-hGH-CTP-CTP is ~47.5 kD (see Figure 1) and the molecular weight of tCTP-hGH-CTP-CTP is ~43.25 kD (see Figure 1).

[0190] Construction of 402-6-p95a-8 (CTP-hGH-CTP) - SEQ ID NO: 13: Construction of hGH-6 was performed in the same way as the construction of hGH-3. pCI-dhfr-402-1-p83-5 (hGH-ctp) was used as a template in the second PCR reaction.

[0191] Construction of 402-5-p96-4 (CTP-hGH) - SEQ ID NO: 14: PCR reaction was performed using primer 25 and primer 39^R and plasmid DNA of pCI-dhfr- ctp-EPO-ctp (402-6-p95a-8) as a template; as a result of the PCR amplification , a 763 bp product was formed and ligated into TA cloning vector (Invitrogen, catalog K2000-01). Xba I -Not I fragment containing ctp-hGH sequence was ligated into our eukaryotic expression vector pCI-dhfr to yield 402-5-p96-4 clone.

EXAMPLE 2

In vivo bioactivity tests of hGH-CTPpolypeptides of the present invention

[0192] The following experiment was performed in order to test the potential long acting biological activity of hGH-CTP polypeptides in comparison with commercial recombinant human GH and hGH.

MATERIALS AND METHODS

[0193] Female hypophysectomized rats (60-100 g) received a weekly S.C. injection of 21.7 µg hGH-CTP polypeptides or a once daily 5 µg S.C. injection of control commercial rhGH.

[0194] Weight was measured in all animals before treatment, 24 hours following first injection and then every other day until the end of the study on day 21. Each point represents the group's average weight gain percentage ((Weight day 0-weight last day)/Weight day 0). Average weight gain was normalized against once-daily injection of commercial hGH. The treatment schedule is summarized in Table 2.

Table 2

No.	Drug	N	Route	Treatment Schedule	Equimolar Dose (µg/rat)	Accumulate Dosage (µg/rat)	Dose Vol.(ml)
1	Vehicle	7	s.c.	days 1, 7 and 13; 1/W	NA	NA	0.25
2	Mock	7	s.c	days 1, 7 and 13;1/W	NA	NA	0.25
3	hGH	7	s.c	days 1, 7 and 13; 1/W	21.7	65	0.25
	SEQ ID NO: 5						
4	hGH-CTP	7	s.c.	days 1, 7	21.7	65	0.25

No.	Drug	N	Route	Treatment Schedule	Equimolar Dose ($\mu\text{g/rat}$)	Accumulate Dosage ($\mu\text{g/rat}$)	Dose Vol.(ml)
	SEQ ID NO: 9			and 13; 1/W			
5	hGH-CTP-CTP	7	s.c.	days 1, 7 and 13; 1/W	21.7	65	0.25
	SEQ ID NO: 10						
6	CTP-hGH-CTP-CTP	7	s.c.	days 1, 7 and 13; 1/W	21.7	65	0.25
	SEQ ID NO: 11						
7	tCTP-hGH-CTP-CTP	7	s.c.	days 1, 7 and 13; 1/W	21.7	65	0.25
	SEQ ID NO: 12						
8	Commercial	7	s.c.	days 1, 7 and 13; 1/W	21.7	65	0.25
	hGH v.1						
9	Commercial	7	s.c.	days 1-13; d/W	5	65	0.25
	hGH v.1						

RESULTS

[0195] Results are summarized in Figure 2. These results show that CTP-hGH-CTP-CTP (SEQ ID NO: 11) and tCTP-hGH-CTP-CTP (SEQ ID NO: 12) induced over 120% weight gain compared to commercial rhGH which induced 100% weight gain.

CONCLUSION

[0196] 3 weekly doses (Days of injections: 1, 7, and 13) of 21.7 μg of CTP-hGH-CTP-CTP (SEQ ID NO: 11) and tCTP-hGH-CTP-CTP (SEQ ID NO: 12) induced a 30% greater weight increase in hypophysectomised rats compared to commercial rhGH injected at the same accumulated dose which was administered once per day at a dose of 5 μg for 13 days.

EXAMPLE 3

Pharmacokinetic studies of CTP-modified GH

[0197] Single-dose pharmacokinetic studies were conducted in Sprague-Dawley rats. All animal experimentation was conducted in accordance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and under the supervision and approval of the Institutional Animal Care and Use Committees of Modigene, Biotechnology General Ltd. Rats were housed either individually or two per cage in rooms with a 12-h light/dark cycle. Access to water (municipal supply) and noncertified rodent chow was provided ad libitum.

[0198] To compare the pharmacokinetics of CTP-hGH-CTP-CTP and GH in rats, four groups of Sprague-Dawley rats (270-290 g), three to six male rats per group. The rats were randomly assigned to four treatment groups (see Table 3). Rats were administered a single s.c. or i.v. injection of GH (50 µg/kg i.v. or s.c.) or CTP-hGH-CTP-CTP (108 µg/kg i.v. or s.c.). With the exception of the predose sample, which was collected under isoflurane anesthesia, blood collection was performed in unanesthetized animals. Blood samples (approximately 0.25 ml) were collected in EDTA-coated microtainers for ELISA analyses of CTP-hGH-CTP-CTP plasma concentration at the times outlined in Table 3. After each sampling, the blood volume was replaced with an equal volume of sterile 0.9% saline. Samples were stored on ice for up to 1 h prior to centrifugation and plasma harvest. Plasma samples were stored at approximately -20 °C prior to analysis.

Table 3. Experimental design of rat pharmacokinetic study

[illegible]

Trt Gr p.	Test Article	No. of animals / group/ timepoint	Dose Route	Gender	Dose Level ($\mu\text{g/kg}$)	Injected Vol. (μl)	Concentration ($\mu\text{g/ml}$) /Total vol. (ml)	Time-Points * (hours post-dose) samples
# 3 rats per time point.								

[0199] A commercial sandwich ELISA kit specific for detection of human growth hormone (Roche Diagnostics) was used for evaluation of the rat plasma samples. This kit detects human growth hormone in plasma by means of an antibody sandwich ELISA format. This kit was initially used to measure the concentration of CTP-hGH-CTP-CTP in rat plasma. For these plasma samples, a CTP-hGH-CTP-CTP standard curve (1.2-100 ng/ml) was used and the concentrations of CTP-hGH-CTP-CTP in rat plasma were interpolated from this curve.

[0200] Standard pharmacokinetic parameters, including clearance (CL or CLIP), volume of distribution (V_d or V_d/F), half-life ($t_{1/2}$), area under the plasma concentration versus time curve (AUC), maximal observed plasma concentration (C_{\max}) and time to maximal observed plasma concentration (T_{\max}), were obtained from plasma albutropin or GH concentration/time curves by noncompartmental analysis using the modeling program WinNonlin (Pharsight, version 3.1). Plasma CTP-hGH-CTP-CTP or GH concentration data were uniformly weighted for this analysis. The AUC was calculated using the log-linear trapezoidal analysis for the i.v. data and the linear-up/log-down trapezoidal method for the s.c. data. Plasma concentration profiles for each rat (with the exception of the s.c. albutropin data) or monkey were analyzed individually, and mean \pm standard error of the mean (S.E.M.) values for the pharmacokinetic parameters are reported in Table 4 and Figure 4.

[0201] CTP-hGH-CTP-CTP is a single chain protein of 275 amino acids and up to twelve O-linked carbohydrates. The structure consists of modified human Growth Hormone (Somatotropin) attached to three copies of the C-terminal peptide (CTP) of the beta chain of human Chorionic Gonadotropin (hCG); one copy at the N-terminus and two copies (in tandem) at the C terminus. Human Growth Hormone is comprised of 191 amino acids. CTP is comprised of 28 amino acids and four O-linked sugar chains.

EXAMPLE 4

Pharmacokinetics of CTP-modified GH in SD rats

[0202] The pharmacokinetics of CTP-hGH-CTP-CTP was evaluated and compared to that of commercial hGH (Biotropin).

Table 4. Mean pharmacokinetic parameters following single-dose i.v. and s.c. administration of

CTP-hGH-CTP-CTP and GH (Biotropin) in Sprague-Dawley rats.

PK Statistics	SC		IV		
	Units	Biotropin	CTP-hGH-CTP-CTP	Biotropin	CTP-hGH-CTP-CTP
Dose	mg/Kg	50	50	50	50
AUClast	hr*ng/mL	41	680	162.7	1568.3
Cmax	ng/ml	13	36.8	275.8	926
Tmax	hr	0.5	8	0	0
MRT	hr	2.5	12.9	0.5	9.9
T1/2 alpha	hr		1.58		0.74
T1/2 beta	hr	1.73	9	0.5	6.9

Data statistical analysis

[0203] Analysis of serum samples was performed in order to determine specific concentration levels for each sample. Concentration and time-point data were processed using WinNonLin noncompartmental analysis.

[0204] Parameters that were determined included: AUC, MRT, t1/2, Cmax, and Tmax. Figure 4 demonstrates the superior pharmacokinetic profile of CTP-hGH-CTP-CTP plasma concentration compared to GH concentrations (pg/ml) following a single i.v. or s.c. dose of CTP-hGH-CTP-CTP or GH in rats ($n=3-6$ per dose/route).

[0205] Following a single S.C. injection of 50 µg/kg, clearance of CTP-hGH-CTP-CTP from SD rat's blood was significantly slower than that of CTP-hGH-CTP and of Biotropin. The corresponding calculated half-life times and AUCs were:

Biotropin	T1/2 1.7h,	AUC	41 hr*ng/mL
CTP-hGH-CTP	T1/2 8.5h,	AUC	424 hr*ng/mL
CTP-hGH-CTP-CTP	T1/2 9.0h,	AUC	680 hr*ng/mL

Conclusion:

[0206] CTP-hGH-CTP-CTP was chosen as the final candidate out of 6 other variants. CTP-hGH-CTP-CTP demonstrated superior performance in terms of biological activity and pharmacokinetics.

EXAMPLE 5***Weight Gain Assay (WGA) for Single dose/Repeated dose of CTP-modified GH***

[0207] Hypophysectomized (interaural method) male rats, 3-4 weeks of age, were obtained from CRL Laboratories. During a post-surgical acclimation period of 3 weeks, rats were examined and weighed twice weekly to eliminate animals deemed to have incomplete hypophysectomy evidenced by weight gain similar to that of sham-operated rats. Those rats with incomplete hypophysectomized were eliminated from the study. The average body weights of the hypophysectomized were 70-90 grams, at the time of the experiment. This is the standard USP and EP bioassay for hGH. Hypophysectomized rats (rats from which the pituitary gland was removed) lose their ability to gain weight. Injections of hGH (and of CTP-hGH-CTP-CTP) to these rats results in weight gain. Based on the measured weight gain along a defined period of time and the amount of hGH injected, the specific activity of hGH (and CTP-hGH-CTP-CTP) is determined. Rats were administered either a single s.c. doses of 0.4, 0.8 and 4 mg/Kg or repeated s.c. doses of 0.6 and 1.8 mg/Kg 4 days apart for 3 weeks. Individual body weights of all animals are determined at randomization, prior to the first dosing, thereafter every two days or in case of decedents at the time of death, and prior to sacrifice.

Single dose and repeated dose weight gain assay

[0208] The results comparing whole body growth response following different dosing patterns of CTP-hGH-CTP-CTP in hypophysectomized rats are demonstrated in Figure 5. The results demonstrate that a single injection of 0.4 & 0.8 mg/Kg/day doses of hGH-CTP were equivalent to 4 daily injections of 0.1 mg/Kg/day of Biotropin. The peak of the hGH-CTP effect was after 2 days.

[0209] Figure 6 further demonstrates that the area under the curve following single injection of CTP-hGH-CTP-CTP correlates with Body Weight gain in Rats. Thus, the collective data demonstrates that body weight gain is closely correlated with cumulative AUC.

[0210] The hGH-CTP construct administered 4 days apart promotes the same weight gain as daily injections of Biotropin as demonstrated in Figure 7. Half-life of hGH in humans is expected to be 5x better than in rats - indicating potential peak effect in humans after 10 days for one single injection. These results support administration of hGH-CTP construct, CTP-hGH-CTP-CTP, once weekly or biweekly in humans.

EXAMPLE 6

Pharmacodynamics/Pharmacokinetics studies of CTP-modified GH

[0211] Hypophysectomized (interaural method) male rats, 3-4 weeks of age, were obtained from CRL Laboratories. During a post-surgical acclimation period of 3 weeks, rats were examined and weighed twice weekly to eliminate animals deemed to have incomplete hypophysectomy evidenced by weight gain similar to that of sham-operated rats. Those rats with incomplete hypophysectomized were eliminated from the study. The average body weights of the hypophysectomized and sham rats were 70 and 150 g, respectively, at the time of the experiment.

[0212] Rats were administered a single s.c. with CTP-hGH-CTP-CTP, vehicle, human growth hormone CTP-hGH-CTP-CTP or human growth hormone (20 µg/rat) was administered s.c. in an injection volume of 0.2 ml/rat. The dose of GH was 0.35 and 1.05 µg/Kg, a dose of growth hormone that was equimolar with the amount of GH in a corresponding 0.6 and 1.8 µg/Kg dose of CTP-hGH-CTP-CTP. The treatment groups are summarized in Table 4. Following injection, plasma samples for IGF-1 analyses were obtained at the times described in Table 5. Samples were analyzed for IGF-1 concentration using a commercial ELISA (R&D systems).

[0213] Weekly administration of CTP-modified hGH was successful in maintaining IGF-I within the normal range as standard GH and in addition it also maintains body weight as standard GH.

Table 5. Treatment schedule for hypophysectomized rat study

Trt. Grp.	Test Article	No. of animals/ group/ timepoint	Dose Route	Eq. Dose (mg/rat)	Eq. Dosage (mg/Kg)	CTP-hGH-CTP-CTP Conc. mg/ml	Dose Vol. (ml)	Time-Points * (hours post-dose)
M7	Biotropin	9	SC	0.032	0.35	0.16	0.2	0 (Pre-dose) 0.5, 2, 4, 8, 24, 48, 72, 96
M8	Biotropin	9	SC	0.095	1.05	0.475	0.2	0 (Pre-dose) 0.5, 2, 4, 8, 24, 48, 72, 96
M9	EN648-01-08-005	12	SC	0.032 (0.055)	0.35 (0.6)	0.275	0.2	1, 2, 4, 8, 24, 48, 72, 96
M10	EN648-01-08-005	12	SC	0.095 (0.165)	1.05 (1.8)	0.825	0.2	1, 2, 4, 8, 24, 48, 72, 96

Trt. Grp.	Test Article	No. of animals/ group/ timepoint	Dose Route	Eq. Dose (mg/rat)	Eq. Dosage (mg/Kg)	CTP-hGH-CTP-CTP Conc. mg/ml	Dose Vol. (ml)	Time-Points * (hours post-dose)
Volume of blood sample/time point - 500 µl								Terminal blood samples

[0214] Non-compartmental pharmacokinetic analysis was performed on the mean serum concentration versus time curves for each group. CTP-hGH-CTP-CTP Cmax was significantly higher than Biotropin Cmax. The terminal half-life of CTP-hGH-CTP-CTP was 6 times higher than Biotropin.

Table 6: Pharmacokinetic Parameter Estimates of CTP-hGH-CTP-CTP and Biotropin Following a Single Subcutaneous Injection in hypophysectomized Rats

Group	Dose mg/kg	Gender	Cmax ng/mL	Tmax hr	AUC _{0-∞} ng-hr/mL	AUC _{0-t} ng-hr/mL	CL/F mL/hr/kg	T _{1/2} hr
CTP-								
hGH-								
CTP-								
CTP	1.8	M	2,150	8	37,713	37,695	0.928	6.86
	0.6	M	681	8	11,505	11,489	3.042	6.8
hGH	1.05	M	1,078	0.5	3,541	3,540	9.884	1
	0.35	M	439	0.5	1,279	1,279	27.36	1

[0215] The AUC_{0-t} and the AUC_{0-∞} were very similar suggesting the duration of sampling was adequate to characterize the pharmacokinetic profiles. AUC of CTP-hGH-CTP-CTP was 10 times higher than of Biotropin. Moreover, Cmax was generally proportional to dose and for CTP-hGH-CTP-CTP and it was twice higher than Cmax of Biotropin. However, as shown in Figure 8, Tmax of CTP-hGH-CTP-CTP was 8 hr as compare to 0.5 hr of Biotropin, and the terminal half-lives did not appear to vary with dose level. T_{1/2} of CTP-hGH-CTP-CTP was 6.8 times longer than of Biotropin.

[0216] Indirect effects of GH are mediated primarily by an insulin-like growth factor-I (IGF-I), a hormone that is secreted from the liver and other tissues in response to growth hormone. A majority of the growth promoting effects of growth hormone is actually due to IGF-I acting on its target cells. Accordingly, the effect of the CTP-hGH construct, CTP-hGH-CTP-CTP, on IGF-1 serum levels in Hypophysectomized Rats was measured. Figure 9 presents results of IGF-1

serum levels in Hypophysectimized Rats Following SC injection of CTP-hGH-CTP-CTP and commercial hGH.

[0217] Single dose of CTP-hGH-CTP-CTP 0.6 or 1.8 mg/Kg and Biotropin 0.35 or 1.05 mg/Kg were injected subcutaneously to hypophysectomised rats for determination of PK/PD profile. Serum IGF-I post injection was measured using specific ELISA kits (Roche Diagnostics).

[0218] The cumulative serum levels of IGF-I following injection of CTP-hGH-CTP-CTP was significantly higher than following injection of Biotropin. Cmax was generally proportional to dose and for CTP-hGH-CTP-CTP it was 3-4 times higher than Cmax of Biotropin. Tmax of CTP-hGH-CTP-CTP was 36-48 hr as compare to 20-24 hr of Biotropin. In conclusion, higher hGH levels and longer presence in serum result in significant increase in IGF-1 levels.

EXAMPLE 7

Carbohydrate Content and Sialic Acid Content of CTP-modified GH

[0219] Analysis of O-glycans is based on a Prozyme kit. O-glycans are chemically and enzymatically cleaved from the protein and separated from peptides using paper chromatography. Sequencing of the O-glycan pool is performed by sequential enzymatic digestions (exo-glycosidases) followed by HPLC analysis compared to standards.

Glycoprofiling with Sequence Analysis

[0220] Glycoprofiling was performed by Ludger Ltd. Two samples (EN648 and RS0708) were taken through triplicate releases and each release was also analyzed by HPLC in triplicate. Triplicate 300µg samples of EN648 and RS0708 and a single 100µl sample of citrate/sodium chloride buffer, plus a positive control fetuin (250µg) and a 100µl water negative control, were ultra-filtrated by centrifugation using a molecular weight cut off membrane of 10,000Da to replace the buffer with water, then taken through hydrazinolysis under O-mode conditions (6 h at 60°C). Released glycans were re-N-acetylated and cleaned up by LudgerClean CEX cartridges. An aliquot of the released glycans was then labeled with 2-aminobenzamide (2AB), cleaned up with Ludger Clean S cartridges and analyzed by LudgerSep-N2 HILIC-HPLC.

Monosaccharide Content

[0221] Analysis of neutral monosaccharides requires hydrolysis of glycans to their constituent monosaccharide components. The hydrolysis was performed by Ludger Ltd, on intact glycoprotein samples. Specifically, 50µg of intact glycoprotein was acid hydrolyzed, 2-AB (2-

aminobenzamide) labeled and run on a reverse phase HPLC column. This method hydrolyzes all glycans present on the glycoprotein inclusive of N and O linked types.








Sialic Acid Profiling

[0222] Two samples (EN648 and RS0708) and a buffer control were analyzed. Sialic acid analysis requires mild acid release of the monosaccharides followed by DMB fluorophore labeling and HPLC analysis on a LudgerSep-R1 column. 50µg of intact glycoprotein was acid hydrolyzed for each analysis.

Glyco analysis of CTP-hGH-CTP-CTP

[0223]

Table 7. Glycan analysis. Structural assignments and percentage areas of peaks are based upon HPLC and enzyme array digests.

Peak ID ^a	GU ^b	Structure ^c	name	Percent from total glycans ^e			
				Und ^d	NAN1	ABS	ABS BTG
1 ^f	0.92	 +bgd	GalNAc	0.4	0.4	0.6	53.0
2 ^f	1.02	 +bgd	galactose	1.9	9.7	23.8	26.5
*	1.72			4.3	4.6	2.3	
3	1.79		Galβ1-3GalNAc	2.3	67.7	69.4	17.1 ^h
49	2.25		NeuNAcα2-3Gal	19.8	13.0 ^h		
*	2.57			1.5	1.9	1.1	1.1
5	2.90		NeuNAcα2-3Galβ1-3 GalNAc	70.6			
*	3.58			0.6	0.7	0.6	
6	3.22		Galβ1-3[NeuNAcα2-6]GalNAc	0.9	2.3		
7	4.42		NeuNAcα2-3Galβ1-3	1.8			
			[NeuNAcα2-6]GalNAc				

[0224] The monosaccharide profiles indicate that the CTP-hGH-CTP-CTP glycoprotein samples contain predominantly O-link type glycans. The major O-glycan peak is sialylated core 1 (Neu5Ac α 2-3Gal β 1-3GalNAc). The major sialic acid is Neu5Ac and there are some minor peaks suggesting the presence of 3-4% of di-acetylated sialic acid N-acetyl-9-O-acetylneuraminic acid (Neu5, 9Ac2) and less than 1% N-glycolylneuraminic acid. There are also small amounts of Neu5Ac α 2-6(Gal β 1-3)GalNAc.

EXAMPLE 8

Pharmacokinetic/Toxicokinetic Analysis of CTP-modified GH in Rhesus monkeys

[0225] Serum concentrations versus time curves were generated for each animal. Non-compartmental analysis was performed with WinNonlin professional version 5.2.1 (Pharsight Corporation, Mt View CA.). The estimated pharmacokinetic parameters are shown in Table 8 below:

Table 8: Estimates of CTP-hGH-CTP-CTP Pharmacokinetic Parameters (Mean \pm SD) from Non-compartmental Analysis Following A Single Subcutaneous Injection in Rhesus Monkeys

Parameter	1.8 mg/kg	90 mg/kg
C _{max} (μ g/mL)	2.073 \pm 0.417	108.7 \pm 46.0
T _{max} (hr)	4 \pm 0	11 \pm 7
AUC _{0-t} (μ g-hr/mL)	38.7 \pm 7.4	2,444 \pm 394
AUC _{0-∞} (μ g-hr/mL)	39.0 \pm 7.3	2,472 \pm 388
CLIP (mL/hr/kg)	47.5 \pm 9.0	37.04 \pm 4.78
T _{1/2} (hr)	10.00 \pm 1.47	9.85 \pm 1.07
Vz/F (mL/kg)	701 \pm 236	529 \pm 104

[0226] The AUC_{0-t} and the AUC_{0- ∞} were very similar suggesting the duration of sampling was adequate to characterize the pharmacokinetic profiles. C_{max} was proportional to dose. T_{max} was later at the higher dose. T_{max} was at 4 hours for all animals in the low dose group and was at 8 or 24 hours in the high dose group. Terminal half-lives are similar for the two dose groups.

[0227] AUC was approximately proportional to dose with a slightly larger than proportional AUC at the higher dose producing a slightly lower estimate for CL/F and Vz/F compared to the lower dose. It is not possible to say if CL and Vz are lower at the higher dose or if F is lower at the lower dose. There was overlap between the groups so it is questionable that this represents a meaningful difference in CL/F and Vz/F.

[0228] Pharmacokinetic parameters estimated by the model were very similar to those from non-compartment analysis. Absorption and elimination half-lives are shown in Table 9 below:

Table 9: Estimates of CTP-hGH-CTP-CTP Absorption and Elimination Half-lives (Mean \pm SD) Following Subcutaneous Injection Derived From Pharmacokinetic Modeling in Rhesus Monkeys

Dose	T _{1/2} abs (hr)	T _{1/2} el (hr)
1.8 mg/kg	1.17 \pm 0.40	10.41 \pm 2.36
90 mg/kg	6.49 \pm 1.87	7.26 \pm 1.85

[0229] The data indicate that the elimination rates are fairly similar between the groups with a slightly longer T_{1/2} el in the lower dose group. The absorption, however, is more than 5-fold slower following subcutaneous administration of 90 mg/kg compared to that following 1.8 mg/kg. As in the case of the non-compartmental analysis, modeling indicated a later T_{max} at the high dose.

[0230] Although GH supplementation is effective in the treatment of GH deficiency in children and adults, the disadvantages of daily injections over extended periods of time limit its use by physicians in certain patient populations as well as increase the risk of dosing error, the number of care givers, the cost of treatment and/noncompliance. Especially important in certain populations, such as children of short stature who may not understand the implications of not following the prescribed GH dosing regimen, is the necessity of compliance to achieve the optimal benefit from GH therapy. The issue of finding a more suitable alternative to daily GH injections and subsequent compliance gains further importance as GH-deficient children transition into adults with a continuing need for GH treatment. The requirement of daily therapy is largely due to recombinant GH's short plasma half-life and has led to the development of a sustained release form of GH (Reiter EO. Attire KM., Mashing TJ. Silverman BL. Kemp SF. Neolith RB. Ford KM. and Sanger P. A multimember study of the efficacy and safety of sustained release GH in the treatment of naive pediatric patients with GH deficiency. J. Clin. Endocrinol. Metab. 86 (2001), pp. 4700-4706.).

[0231] GH-CTP, a recombinant human growth hormone-CTP fusion protein, as provided herein, has a pharmacokinetic profile in the rat that is longer in duration than that of GH. This unique pharmacokinetic profile allows for intermittent administration of GH-CTP to achieve pharmacodynamic effects in growth-hormone-deficient rat as evidenced by growth and elevations in plasma IGF-1 levels, respectively.

[0232] GH-CTP offers a superior pharmacokinetic profile compared with that of GH when administered s.c. in the rat. There are substantial differences in plasma clearance of GH-CTP compared to GH. Specifically, plasma is cleared of GH-CTP at more than 6 times more slowly than GH following s.c. dosing. The terminal half-life and mean residence time of GH-CTP were approximately six times longer than that of GH in rats following s.c. administration. In addition,

the CIIP following s.c. dosing is 10 times lower for GH-CTP than for GH.

[0233] In an effort to examine whether the pharmacokinetic advantages in the rat translated to a pharmacodynamic benefit, the possibility that GH-CTP might stimulate growth in GH-deficient hypophysectomized rats with dosing regimens less frequent than daily was tested at equimolar CTP-hGH-CTP-CTP and GH dose levels. Single SC injection of GH-CTP promoted incremental weight gain which was equal to 4 daily consecutive injections of GH. In addition, the every fourth day administration schedule for GH-CTP shows enhanced body weight gain over GH.

[0234] Pharmacodynamically, the long circulation time of GH-CTP relative to GH in the hypophysectomized rats resulted in a prolonged IGF-1 response measured in blood plasma following a single s.c. injection. Subcutaneous administration of a single dose of GH-CTP increased circulating IGF-1 concentrations in a dose-dependent manner in the hypophysectomized rats. At the highest albutropin dose, IGF-1 concentrations were elevated above baseline for as long as 75 hours after a single administration. Thus, the enhanced circulation time of a single dose of GH-CTP resulted in substantial pharmacodynamic improvement over a single dose of GH, raising the possibility that GH-CTP could offer similar growth enhancement and decrease in body fat with reduced dosing frequency compared with standard GH treatment regimens.

[0235] Single CTPs modified hGH- dose of 90mg/kg in Rhesus and 180 mg/kg in rats were well tolerated in both species. The allometric factor between rats and primates is approximately X2 which is based on the anticipated clearance of proteins in these animals. In-line with industry-accepted extrapolation models for therapeutic proteins' half-life increase between species (FDA Guidance). 90 mg/kg in Primates has a PK profile slightly better than 180 mg/kg of CTPs modified hGH in Rat. Thus, allometric extrapolation to humans supports weekly or once/2w injection.

[0236] The present concept utilizing a CTP-GH construct, reduced dosing frequency compared to the commercial GH recombinant product. Nutropin Depot[®] is a sustained release formulation of GH approved for use in pediatric populations; however, comparisons to historical controls have revealed that 1- and 2-year growth rates are significantly ($p < 0.001$) lower in children given Nutropin Depot[®] (1-year growth rate 8.2 ± 1.8 cm/year) than in children treated with GH (one-year growth rate 10.1 ± 2.8 cm/year) (Silverman BL. Blethen SL. Reiter EO. Attie KM. Neuwirth RB. and Ford KM. A long-acting human growth hormone (Nutropin Depot[®]): efficacy and safety following two years of treatment in children with growth hormone deficiency. J. Pediatr. Endocrinol. Metab. 15 (2002), pp. 715-722.). The local effects of subcutaneously administered Nutropin Depot[®] include nodules, erythema, pain at the injection site, headache and vomiting. Preclinical toxicology studies in both rat and monkey have shown that s.c. administration of CTP-hGH-CTP-CTP produces no local reactions compared to vehicle. Given the medical need for a less frequently administered form of GH, the pharmacologic properties of CTP-hGH-CTP-CTP in this study in rats suggest that this product is favorable also in terms of toxicology and patient compliance. The sustained activity of CTP-hGH-CTP-CTP in the rat

support its potential utility as an agent that requires only intermittent administration to attain a therapeutic benefit that is currently achieved with daily dosing.

COMPARATIVE EXAMPLE 9

Long-acting CTP-modified version of human growth hormone (hGH-CTP) was highly effective in growth hormone deficient adults - Phase II Clinical Trial

[0237] A randomized, open-label, Phase II Clinical Trial was conducted to evaluate the safety, tolerability, pharmacokinetics and pharmacodynamic properties of hGH-CTP injected either weekly or twice-monthly in patients who currently receive daily injections of growth hormone. The trial was conducted at multiple sites in six countries. The three main cohorts in the trial received a single weekly dose of hGH-CTP, containing 30%, 45% or 100% of the equivalent cumulative commercial hGH dose that growth hormone-deficient adult patients receive over the course of seven days in the form of daily injections (referred to as the "30%", "45%" and "100%" cohorts, respectively). The data reflect results from 39 patients, 13 in each cohort. 2 females were included in each cohort.

[0238] In addition to the three main cohorts, growth hormone deficient adults were enrolled in an experimental fourth cohort, which is conducted outside of the formal Phase II trial. The patients in the experimental fourth cohort receive a single injection of hGH-CTP once every two weeks that contains 50% of the cumulative commercial dose of that growth hormone-deficient adult patients receive over a two-week period in the form of daily injections.

[0239] Efficacy for the three main cohorts receiving a single weekly injection of hGH-CTP is defined by measuring daily insulin-like growth factor 1 (IGF-1) levels within the desired therapeutic range over a period of seven days (during the last week of treatment in the study). The desired therapeutic range is defined as between +2 standard deviations through -2 standard deviations from the average IGF-1 levels expected in a normal population, stratified by age group and gender. In addition, the trial measured IGF-1 levels within a narrower range of +/-1.5 standard deviations for the purpose of observing the variance of the patients within the normal range.

Results:

[0240] Table 10 contains the average percent of days within the normal therapeutic range (+/- 2 SD), average percent of days within a narrower normal therapeutic range (+/- 1.5 SD), and average C_{max} (highest concentration level) of IGF-1 for males, measured during the last treatment week, expressed in standard deviations from the normal population mean IGF-1 levels.

Table 10: Human Phase II Clinical Trial Results.

Cohort	% Days Within Narrow Normal Range of IGF-1 (+/- 1.5 SD)	% Days Within Normal Range of IGF-1 (+/- 2 SD)	Avg. Cmax of IGF-1 (preferred below +2 SD)
30%	57%	100%	-0.9
45%	100%	100%	0.1
100%	86%	100%	0.4

[0241] Two mg per week of hGH-CTP, containing 50% of the cumulative weekly hGH dose that an adult patient would usually be prescribed as the initial treatment dose, has a high likelihood of being defined as the starting dose for males and females in the adult Phase III.

[0242] There was no evidence of safety and/or tolerability issues, and no indication that hGH-CTP, when used in high doses, induced excessive levels of IGF-1 in patients or even levels above the normal range.

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Patentkrav

- 1.** Choriogonadotropin-carboxyterminalpeptid (CTP)-modificeret polypeptid omfattende et væksthormon til anvendelse i en fremgangsmåde til behandling af en væksthormonmangel hos et menneske-barn målt ved bevarelse af niveauer af insulinlignende vækstfaktor (IGF-1) inden for et normalt terapeutisk interval, hvor det CTP-modificerede polypeptid omfatter ét choriogonadotropin-carboxyterminalpeptid (CTP), der er forbundet med væksthormonets aminoterminal, og to choriogonadotropin-CTP'er, der efter hinanden er forbundet med væksthormonets carboxyterminal, hvor fremgangsmåden omfatter administration til menneske-barnet af en terapeutisk effektiv mængde af det CTP-modificerede polypeptid én gang om ugen i en dosis på 1 til 60 milligram pr. uge (mg/uge).
- 2.** CTP-modificeret polypeptid til anvendelse ifølge krav 1, hvor mindst ét CTP er glycosyleret.
- 3.** CTP-modificeret polypeptid til anvendelse ifølge et hvilket som helst af krav 1 eller krav 2, hvor mindst ét CTP er forbundet med væksthormonet *via* en linker.
- 4.** CTP-modificeret polypeptid til anvendelse ifølge krav 3, hvor linkeren er en peptidbinding.
- 5.** CTP-modificeret polypeptid til anvendelse ifølge et hvilket som helst af kravene 1 til 4, hvor væksthormonet er humant væksthormon.
- 6.** CTP-modificeret polypeptid til anvendelse ifølge et hvilket som helst af kravene 1 til 5, hvor mindst ét CTP har aminosyresekvensen af SEQ ID NO: 1 eller SEQ ID NO: 2.
- 7.** CTP-modificeret polypeptid til anvendelse ifølge et hvilket som helst af kravene 1 til 6, hvor aminosyresekvensen af nævnte polypeptid omfatter sekvensen af aminosyre 27 til 301 af SEQ ID NO: 11.

- 8.** CTP-modificeret polypeptid til anvendelse ifølge et hvilket som helst af kravene 1 til 7, hvor den terapeutisk effektive mængde omfatter en dosis på 5 til 10 mg/uge én gang om ugen.
- 5 **9.** CTP-modificeret polypeptid til anvendelse ifølge et hvilket som helst af kravene 1 til 8, hvor menneske-barnet er en dreng.
- 10.** CTP-modificeret polypeptid til anvendelse ifølge et hvilket som helst af kravene 1 til 9, hvor det CTP-modificerede polypeptid administreres subkutant til
- 10 menneske-barnet.

DRAWINGS

Drawing

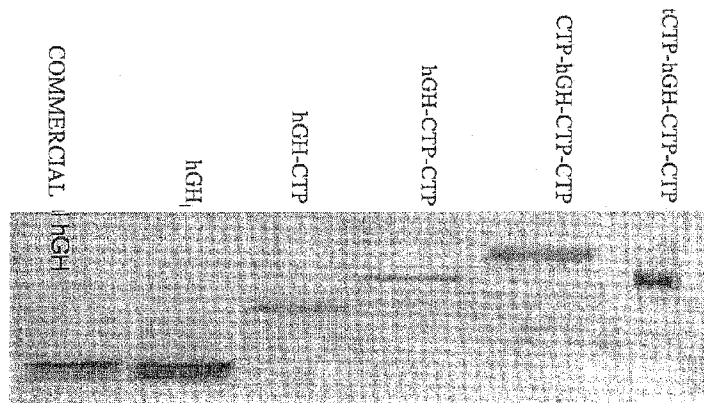


FIGURE 1

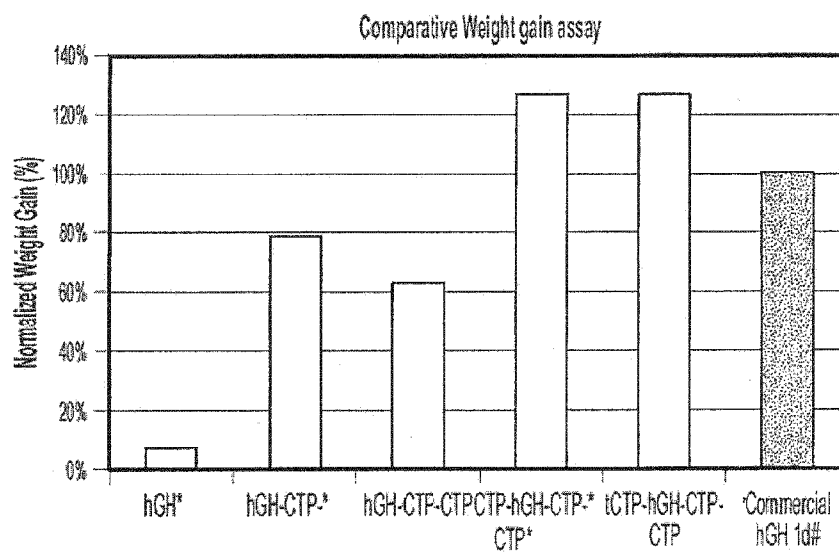


FIGURE 2

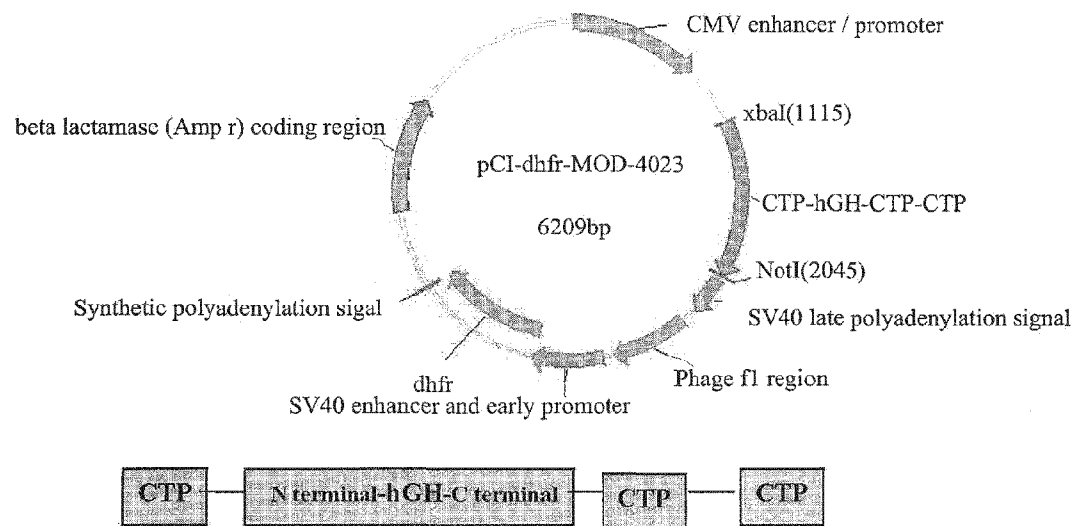


FIGURE 3

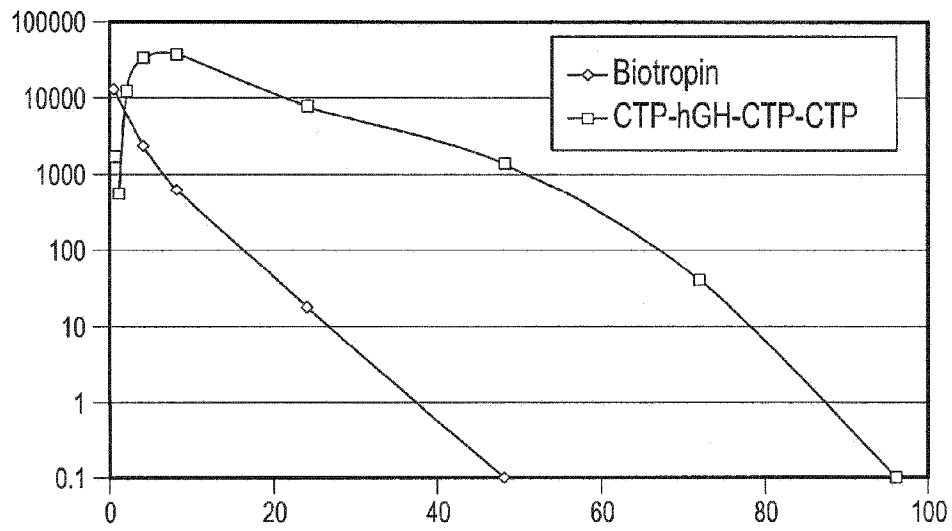


FIGURE 4A

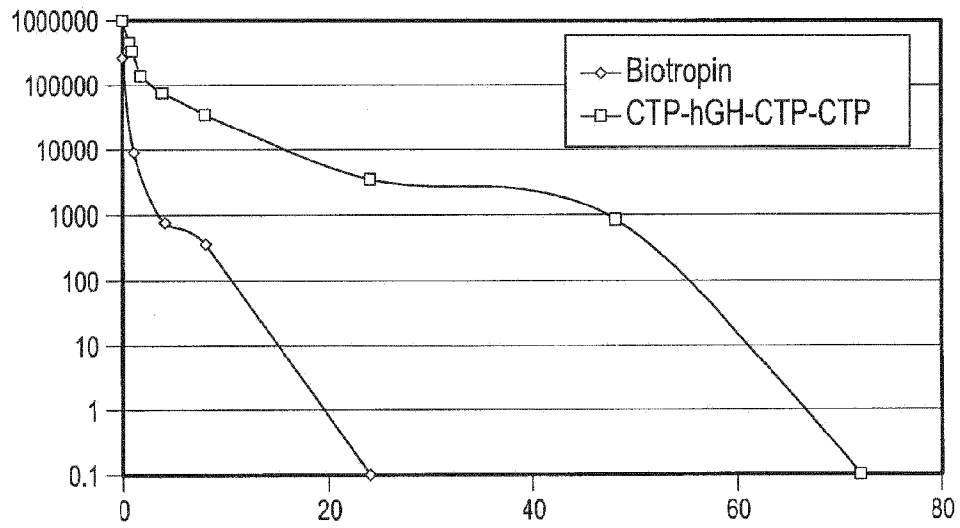


FIGURE 4B

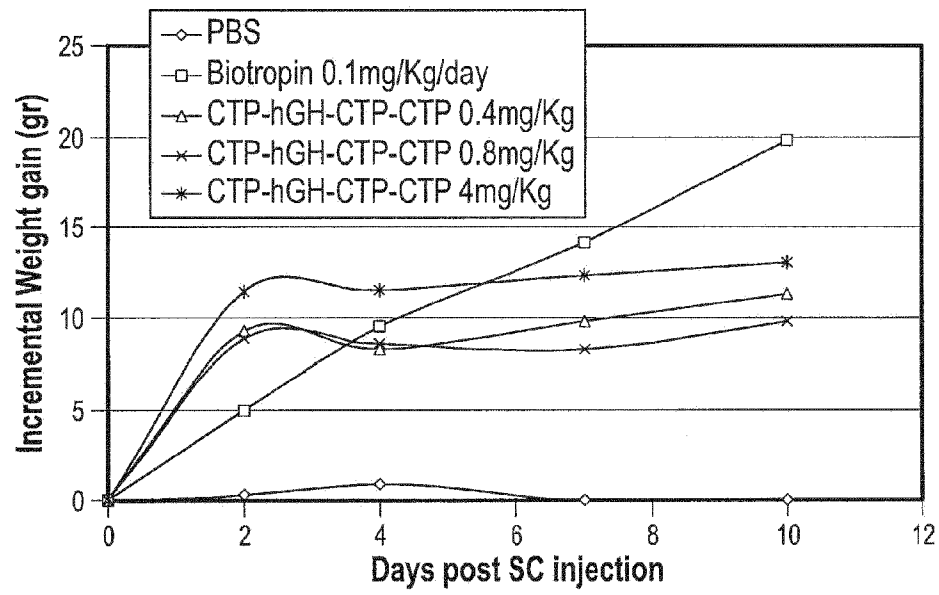
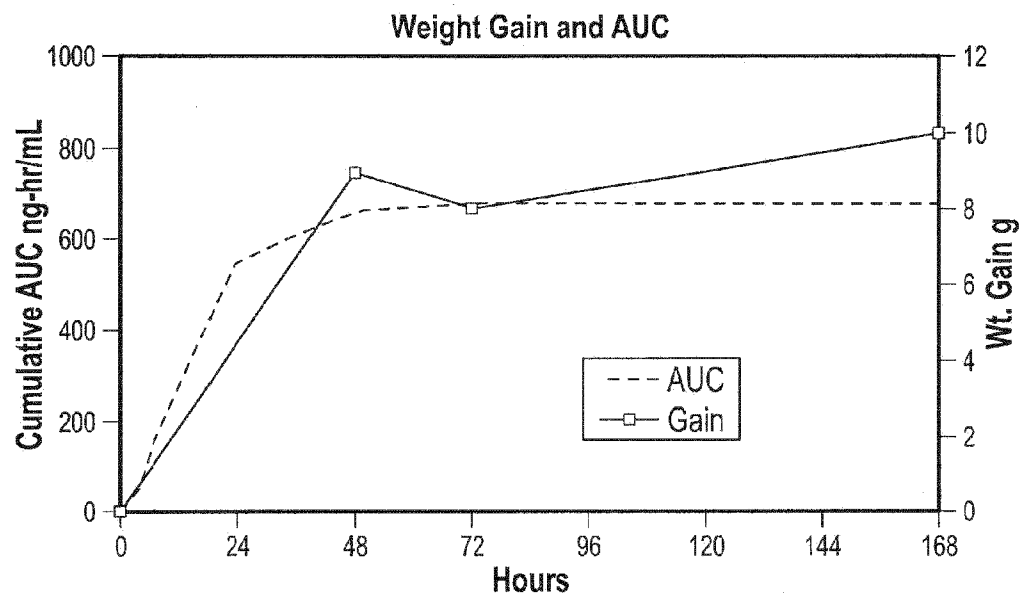
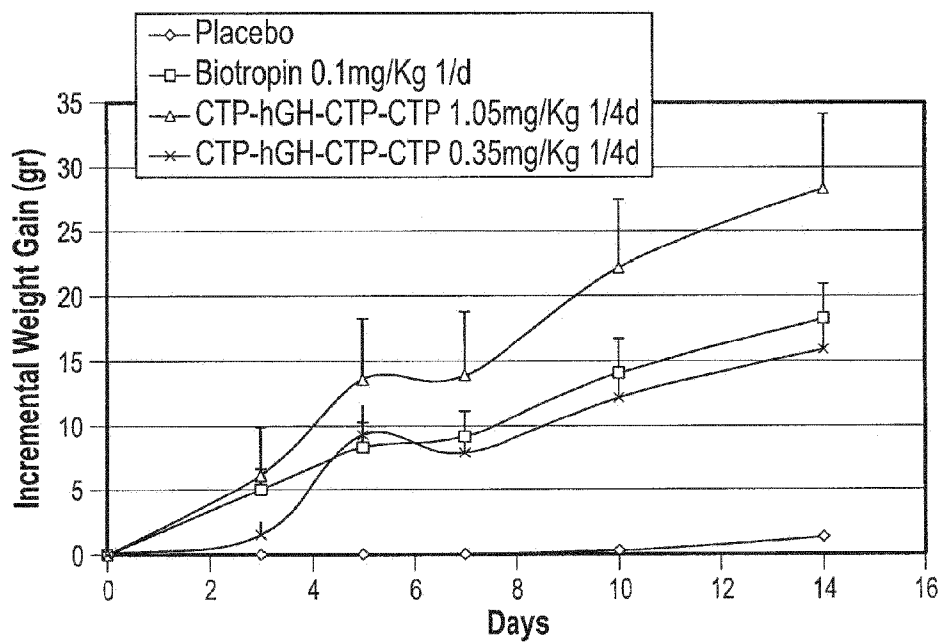


FIGURE 5

**FIGURE 6****FIGURE 7**

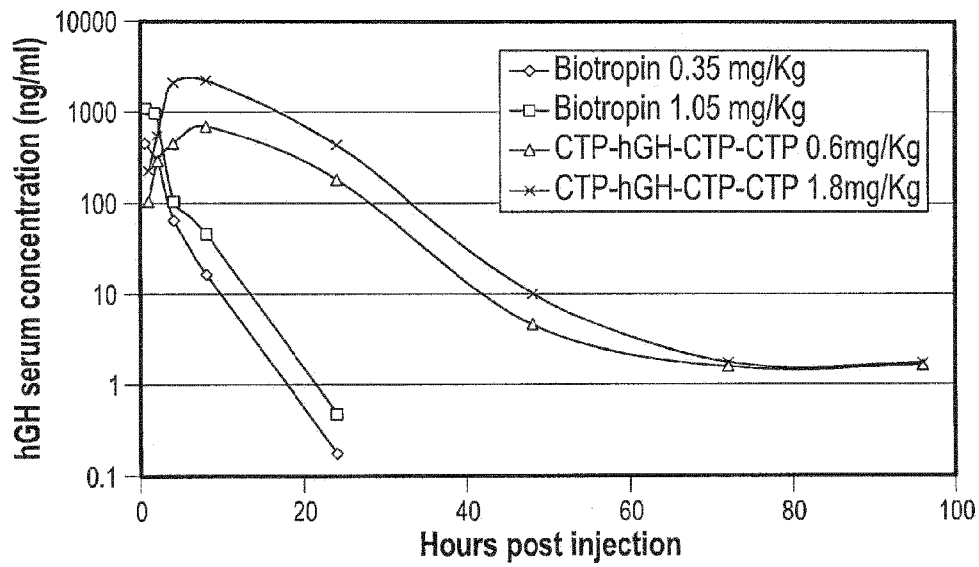


FIGURE 8

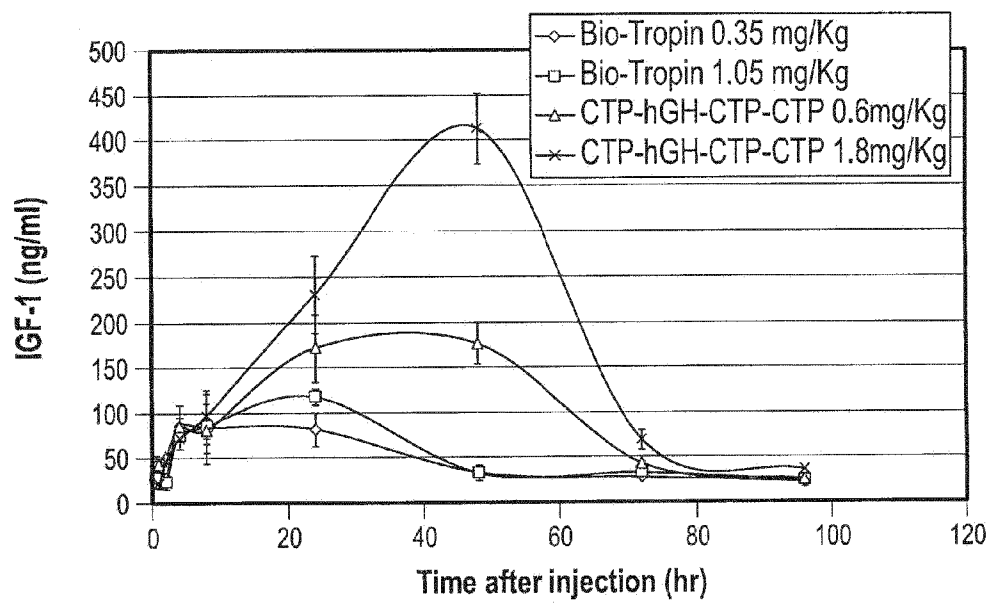


FIGURE 9

SEKVENSLISTE

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