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Canadian Intellectual Property Office

CA 3149645 A1 2021/02/18

(21) 3 149 645

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(86) Date de dépôt PCT/PCT Filing Date: 2020/08/14
(87) Date publication PCT/PCT Publication Date: 2021/02/18
(85) Entrée phase nationale/National Entry: 2022/02/02
(86) N° demande PCT/PCT Application No.: US 2020/046375
(87) N° publication PCT/PCT Publication No.: 2021/030687
(30) Priorités/Priorities: 2019/08/15 (US62/887,049); 2020/06/05 (US63/035,521)

(51) Cl.Int./Int.Cl. *A61K 38/08* (2019.01), *A61P 3/04* (2006.01)

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(54) Titre : PEPTIDES THERAPEUTIQUES
(54) Title: THERAPEUTIC PEPTIDES

(57) Abrégé/Abstract:
The disclosures herein relate to the fields of cell biology and the modulation of cellular mechanisms controlling cell viability, cell proliferation, and metabolic processes. More specifically disclosed herein are peptides effective to modulate cellular mechanisms controlling cell viability, cell proliferation, and metabolic processes, including cell signaling associated with aberrant cellular proliferation and malignancy. Also disclosed herein are peptides effective in modulating cellular mechanisms controlling cell viability, treating metabolic diseases, and as cytoprotective agents. Also disclosed herein are peptides effective as apelin receptor agonists.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 February 2021 (18.02.2021)

WIPO | PCT

(10) International Publication Number
WO 2021/030687 A8

(51) International Patent Classification:
A61K 38/08 (2019.01) *A61P 3/04* (2006.01)
A61K 38/10 (2006.01)

(21) International Application Number:
PCT/US2020/046375

(22) International Filing Date:
14 August 2020 (14.08.2020)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/887,049 15 August 2019 (15.08.2019) US
63/035,521 05 June 2020 (05.06.2020) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *with sequence listing part of description (Rule 5.2(a))*

(48) Date of publication of this corrected version:
08 April 2021 (08.04.2021)

(15) Information about Correction:
see Notice of 08 April 2021 (08.04.2021)

(54) Title: THERAPEUTIC PEPTIDES

(57) Abstract: The disclosures herein relate to the fields of cell biology and the modulation of cellular mechanisms controlling cell viability, cell proliferation, and metabolic processes. More specifically disclosed herein are peptides effective to modulate cellular mechanisms controlling cell viability, cell proliferation, and metabolic processes, including cell signaling associated with aberrant cellular proliferation and malignancy. Also disclosed herein are peptides effective in modulating cellular mechanisms controlling cell viability, treating metabolic diseases, and as cytoprotective agents. Also disclosed herein are peptides effective as apelin receptor agonists.

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THERAPEUTIC PEPTIDES

[001] This application claims priority benefit of U.S. Provisional Application Nos. 63/035,521, filed June 5, 2020, and 62/887,049, filed August 15, 2019, both incorporated herein by reference in their entirety.

TECHNICAL FIELD

[002] This disclosure relates to the fields of cell biology and the modulation of cell viability and metabolic processes. More specifically disclosed are peptides effective to modulate cell signaling associated with aberrant cellular proliferation and malignancy. Also disclosed are peptides effective in modulating cell viability, treating metabolic diseases and as cytoprotective agents. Also disclosed herein are peptides effective as apelin receptor agonists.

INCORPORATION BY REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[003] The Sequence Listing, which is a part of the present disclosure, is submitted concurrently with the specification as a text file, and incorporated herein by reference. The file containing the Sequence Listing is “54008A_Seqlisting.txt", created on August 14, 2020, and is 21,928 bytes in size.

BACKGROUND

[004] The control of cellular behavior is not clearly understood. Dysregulation of cellular metabolic pathways can lead to imbalance in energy homeostasis and may result in a wide range of metabolic disorders, including but not limited to obesity, diabetes, hypertension, arteriosclerosis, high cholesterol, hyperlipidemia, fatty liver disease, non-alcoholic steatohepatitis (NASH), and other diseases. The precise cellular mechanisms regulating cellular apoptosis are not completely known. Dysregulation of apoptosis has been implicated in a number of human diseases. An inappropriate suppression of apoptosis in a cell may lead to the uncontrolled propagation of that cell, potentially favoring the development of cancer. In contrast, a failure to control the extent of apoptotic cell death may lead to degeneration of specific tissues and cell-types, such as occurs in neurodegeneration, autoimmune disorders, and other diseases.

[005] There is a need for more effective therapies modulating cellular mechanisms that control the activity of cells, including for example cell metabolism, cell proliferation, and cell viability. More specifically, there remains a great need for more effective treatments that can address a wide range of metabolic disorders by safely regulating metabolic pathways. There is a need for more effective therapies modulating cellular mechanisms including those that induce or suppress apoptosis in cells and/or tissues of individuals suffering from disorders characterized by inappropriate cell proliferation or inappropriate cell death.

[006] Mitochondria, central to metabolic processes in eukaryotic cells, are involved in numerous cellular processes, including among others energy production, ATP synthesis, reactive oxygen species (ROS) generation, programmed cell death, signaling, cellular differentiation, and control of the cell cycle and cell growth. A small number of mitochondrial DNA-derived signaling peptides have been identified to date with diverse structures and widely differing biological properties. Despite this effort, the natural occurrence and function of the vast majority of theoretical mitochondrial DNA-derived peptide sequences remains undefined, while their potential biological activity as exogenous peptides is completely unknown and cannot be predicted from their structure. The inventors have identified therapeutically useful isolated peptides with unexpected properties based on mitochondrial DNA and conceived novel analogs and derivatives with improved properties.

SUMMARY

[007] Disclosed are peptides comprising amino acid sequences of Formulas I-II that exhibit activity in modulating cellular mechanisms. Also disclosed are peptides comprising amino acid sequences SEQ ID NOs: 1-64, analogs and derivatives thereof.

[008] The present disclosure moreover includes pharmaceutical compositions comprising peptides described herein, including but not limited to peptides comprising amino acid sequences of SEQ ID NOs: 1-64, analogs and derivatives thereof described herein and a pharmaceutically acceptable excipient, as well as a method of treating or preventing a disease or medical condition (e.g., cancer, metabolic diseases) in a patient using peptides and compositions described herein. The method comprises administering to the patient a presently disclosed peptide, derivative or analog, optionally formulated into a pharmaceutical composition, in an amount effective to treat an appropriate disease or medical condition. Similarly disclosed are uses of the peptides, derivatives, analogs, and compositions described herein to treat or prevent the aforementioned diseases or medical conditions.

[009] The present disclosure further includes nucleic acids, e.g., DNA or RNA, that comprise nucleotide sequences that encode peptides described herein; vectors that comprise or contain such nucleic acids; and host cells transformed or transfected with such nucleic acids or vectors; as well as therapeutic methods and uses thereof. Other aspects of the invention will be apparent from the detailed description and claims that follow.

DETAILED DESCRIPTION

[010] In one aspect, peptides that therapeutically modulate cellular mechanisms are disclosed.

[011] In one embodiment, a peptide of any one or more of the amino acid sequences set forth in any one of SEQ ID NOs: 1-64 are disclosed.

[012] An embodiment comprises a peptide of the amino acid sequence of **Formula I**

$$X^1\text{-}R\text{-}X^2\text{-}X^3\text{-}X^4\text{-}X^5\text{-}X^6\text{-}Q\text{-}X^7\text{-}L\text{-}X^8\text{-}X^9 \quad \text{(I) (SEQ ID NO: 1)}$$

[013] wherein X^1 is absent or if present is an amino acid having a polar side chain or a non-polar side chain; X^2 is an amino acid having a polar side chain or a non-polar side chain; X^3 is absent or if present is one to three amino acids, each amino acid independently having a polar side chain or a non-polar side chain; X^4 is an amino acid having a polar side chain or a non-polar side chain; X^5 is an amino acid having a non-polar side chain; X^6 is an amino acid having a polar side chain or a non-polar side chain; X^7 is an amino acid having a polar side chain; X^8 is an amino acid having a polar side chain; and X^9 is absent or if present is one to three amino acids, each amino acid independently having a polar side chain or a non-polar side chain; or an analog of said peptide having a deletion, insertion or substitution of one, two, three, or four amino acids; or C-terminal acids or amides, or N-acetyl derivatives thereof; or pharmaceutically acceptable salts thereof.

[014] An embodiment comprises a peptide of the amino acid sequence of Formula I wherein X^3 is absent, or if present is $-X^{12}X^{11}X^{10}-$; wherein X^{10} is absent, or if present is an amino acid having a non-polar side chain; X^{11} is absent, or if present is an amino acid having a non-polar side chain; and X^{12} is an amino acid having a polar side chain or a non-polar side chain; or C-terminal acids or amides, or N-acetyl derivatives thereof; or pharmaceutically acceptable salts thereof.

[015] An embodiment comprises a peptide of the amino acid sequence of Formula I wherein X^9 is absent, or if present is $-X^{13}X^{14}X^{15}$; wherein X^{13} is an amino acid having a non-polar side chain; X^{14} is absent, or if present is an amino acid having a non-polar side chain; and X^{15} is absent, or if present is an amino acid having a polar side chain; or C-terminal acids or amides, or N-acetyl derivatives thereof; or pharmaceutically acceptable salts thereof.

[016] An embodiment comprises a peptide of the amino acid sequence of Formula I wherein X^1 is absent, or if present is selected from D, (dD), E, (dE), K, (dK), R, (dR), H, (dH), N, (dN), Q, (dQ), S, (dS), T, (dT), Y, (dY), C, (dC), G, A, (dA), V, (dV), L, (dL), I, (dI), F, (dF), W, (dW), P (dP), M and (dM); X^2 is selected from D, (dD), E, (dE), K, (dK), R, (dR), H, (dH), N, (dN), Q, (dQ), S, (dS), T, (dT), Y, (dY), C, (dC), G, A, (dA), V, (dV), L, (dL), I, (dI), F, (dF), W, (dW), P (dP), M and (dM); X^3 is absent or if present is D, (dD), E, (dE), K, (dK), R, (dR), H, (dH), N, (dN), Q, (dQ), S, (dS), T, (dT), Y, (dY), C, (dC), G, A, (dA), V, (dV), L, (dL), I, (dI), F, (dF), W, (dW), P (dP), M, (dM) or-$X^{12}X^{11}X^{10}$-; X^4 is an amino acid selected from D, (dD), E, (dE), K, (dK), R, (dR), H, (dH), N, (dN), Q, (dQ), S, (dS), T, (dT), Y, (dY), C, (dC), G, A, (dA), V, (dV), L, (dL), I, (dI), F, (dF), W, (dW), P (dP), M and (dM); X^5 is an amino acid selected from G, A, (dA), V, (dV), L, (dL), I, (dI), F, (dF), W, (dW), P (dP), M and (dM); X^6 is an amino acid selected from D, (dD), E, (dE), K, (dK), R, (dR), H, (dH), N, (dN), Q, (dQ), S, (dS), T, (dT), Y, (dY), C, (dC), G, A, (dA), V, (dV), L, (dL), I, (dI), F, (dF), W, (dW), P (dP), M and (dM); X^7 is an amino acid selected from D, (dD), E, (dE), K, (dK), R, (dR), H, (dH), N, (dN), Q, (dQ), S, (dS), T, (dT), Y, (dY), C, and

(dC); X^8 is an amino acid selected from D, (dD), E, (dE), K, (dK), R, (dR), H, (dH), N, (dN), Q, (dQ), S, (dS), T, (dT), Y, (dY), C, and (dC); X^9 is absent or if present is an amino acid independently selected from G, A, (dA), V, (dV), L, (dL), I, (dI), F, (dF), W, (dW), P (dP), M and (dM) or -$X^{12}X^{13}X^{14}$; X^{10} is absent, or if present is an amino acid selected from G, A, (dA), V, (dV), L, (dL), I, (dI), F, (dF), W, (dW), P (dP), M and (dM); X^{11} is absent, or if present is an amino acid selected from G, A, (dA), V, (dV), L, (dL), I, (dI), F, (dF), W, (dW), P (dP), M and (dM); X^{12} is an amino acid selected from G, A, (dA), V, (dV), L, (dL), I, (dI), F, (dF), W, (dW), P (dP), M and (dM); X^{13} is an amino acid selected from G, A, (dA), V, (dV), L, (dL), I, (dI), F, (dF), W, (dW), P (dP), M and (dM); X^{14} is absent, or if present is an amino acid selected from G, A, (dA), V, (dV), L, (dL), I, (dI), F, (dF), W, (dW), P (dP), M and (dM); and X^{15} is absent, or if present is an amino acid selected from D, (dD), E, (dE), K, (dK), R, (dR), H, (dH), N, (dN), Q, (dQ), S, (dS), T, (dT), Y, (dY), C, and (dC); or C-terminal acids or amides, or N-acetyl derivatives thereof; or pharmaceutically acceptable salts thereof.

[017] An embodiment comprises a peptide of the amino acid sequence of Formula I wherein X^1 is M, K, or absent; X^2 is R or Aib; X^3 is absent or if present is M, E, -MMG-, -II(dA)-, -Nle-Nle-G- or -IIG-; X^4 is M, E, I or Nle; X^5 is V, A or G; X^6 is F, Y, A or E; X^7 is C, S or E; X^8 is C, S or E; and X^9 is -GL, -G(dA), -G(dA)K, -(dA)L, G or absent; or C-terminal acids or amides, or N-acetyl derivatives thereof; or pharmaceutically acceptable salts thereof.

[018] An embodiment comprises a peptide of the amino acid sequence of Formula I wherein X^1 is (PEG12)-K, and/or wherein X^9 is -G(dA)-K(PEG12).

[019] An embodiment comprising a peptide of the amino acid sequence of **Formula II**:

X^{16}-M-M-G-M-X^{17} (II) (SEQ ID NO: 64)

wherein X^{16} is absent or if present is R- or R-R-; and X^{17} is absent or if present is selected from -V, -VF, -VFQ, -VFQS, -VFQSL, and -VFQSLCG(dA); C-terminal acids or amides, or N-acetyl derivatives thereof; or pharmaceutically acceptable salt thereof.

[020] An embodiment comprises a peptide of the amino acid sequence of Formula II wherein X^{16} is R- or RR-; and X^{17} is selected from VF, -VFQ, -VFQS, -VFQSL, and -VFQSLCG(dA; C-terminal acids or amides, or N-acetyl derivatives thereof; or pharmaceutically acceptable salt thereof.

[021] An embodiment comprising an amino acid sequence selected from MMGMVF (SEQ ID NO: 45); RMMGMVFQ (SEQ ID NO: 51); RMMGMVFQS (SEQ ID NO: 52); RMMGMVFQSL (SEQ ID NO: 53); RMMGMVFQSLCG(dA) (SEQ ID NO: 54); RRMMGMVF (SEQ ID NO: 57); Acetyl-RRMMGMVFQSLCG(dA) (SEQ ID NO: 61); RRMMGMVFQSLCG(dA)-Amide (SEQ ID NO: 62); and Acetyl-RRMMGMVFQSLCG(dA)-Amide (SEQ ID NO: 63); or pharmaceutically acceptable salt thereof.

[022] An embodiment comprises a peptide of the amino acid sequence of Formulas I-II further comprising solvates and/or co-crystals thereof.

[023] An embodiment comprises a peptide of the amino acid sequence MRRIIGIVFQCLCGL (SEQ ID NO: 2). In some embodiments a peptide is in a modified form of SEQ ID NO: 2 comprising up to 5 amino acid modifications relative to SEQ ID NO: 2. In some embodiments a peptide is in a modified form of SEQ ID NO: 2 comprising up to 5 amino acid modifications relative to SEQ ID NO: 2, the modification(s) being in one or more of the positions 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 wherein the amino acid numbering corresponds to SEQ ID NO: 2. In some embodiments a peptide comprises up to 5 amino acid modifications relative to SEQ ID NO: 2, the modification(s) being in one or more of the positions 1, 4, 5, 6, 7, 9, 11, 13, 14 or 15, wherein the amino acid numbering corresponds to SEQ ID NO: 2.

[024] An embodiment comprises a peptide selected from MRRIIGIVFQCLCGL (SEQ ID NO: 2); MRRMMGMVFQCLCGL (SEQ ID NO: 7); RRMMGMVFQCLCG(dA) (SEQ ID NO: 8); RRII(dA)IVFQCLC(dA)L (SEQ ID NO: 9); RRMMGMVYQCLCG(dA) (SEQ ID NO: 10); RRMMGMVEQCLCG(dA) (SEQ ID NO: 12); RRMMGMVFQSLCG(dA) (SEQ ID NO: 15); (PEG12)KRRMMGMVFQSLCG(dA) (SEQ ID NO: 36); RRMMGMVEQSLCG(dA) (SEQ ID NO: 38); RRIIGIVFQSLCG(dA) (SEQ ID NO: 43); or pharmaceutically acceptable salts thereof.

[025] In some embodiments, a peptide is represented by the peptides listed in Table 1.

TABLE 1

Sequence	SEQ ID NO:
MRRIIGIVFQCLCGL	2
RRIIGIVFQCLCGL	3
RRIIGIVFQCLCG	4
RRIIGIVFQCLC	5
RRIIGIVFQCLC(dA)L	6
MRRMMGMVFQCLCGL	7
RRMMGMVFQCLCG(dA)	8
RRII(dA)IVFQCLC(dA)L	9
RRMMGMVYQCLCG(dA)	10
RRMMGMVAQCLCG(dA)	11
RRMMGMVEQCLCG(dA)	12
RRMMGMVFQELCG(dA)	13
RRMMGMVFQCLEG(dA)	14
RRMMGMVFQSLCG(dA)	15
RRMMGMVFQCLSG(dA)	16
RRMMGMVFQSLSG(dA)	17
RR(Nle)(Nle)G(Nle)VFQCLCG(dA)	18
RRMVFQCLCG(dA)	19

(PEG12)KRRMMGMVFQCLCG(dA)	20
RRMMGMVFQCLCG(dA)K(PEG12)	21
RRMVYQCLCG(dA)	22
RRMVFQCLEG(dA)	23
RRMVYQCLEG(dA)	24
RREMVYQCLCG(dA)	25
RREMVYQCLEG(dA)	26
RRMAYQCLEG(dA)	27
RRMGYQCLEG(dA)	28
RRMMGMVYQCLEG(dA)	29
RRMMGMVAQCLEG(dA)	30
RRMEVYQCLCG(dA)	31
RRMEVYQCLEG(dA)	32
RRLLGLVFQSLCG(dA)	33
R(AIB)MMGMVFQSLCG(dA)	34
R(AIB)LLGLVFQSLCG(dA)	35
(PEG12)KRRMMGMVFQSLCG(dA)	36
(PEG12)KRRLLGLVFQSLCG(dA)	37
RRMMGMVEQSLCG(dA)	38
RRMMGMVFQSLEG(dA)	39
RRLLGLVEQSLCG(dA)	40
RRLLGLVFQSLEG(dA)	41
(PEG12)KRRIIGIVFQCLCG(dA)	42
RRIIGIVFQSLCG(dA)	43
MMGMV	44
MMGMVF	45
MMGMVFQ	46
MMGMVFQS	47
MMGMVFQSL	48
MMGMVFQSLCG(dA)	49
RMMGMVF	50
RMMGMVFQ	51
RMMGMVFQS	52
RMMGMVFQSL	53
RMMGMVFQSLCG(dA)	54
RRMMGM	55
RRMMGMV	56

RRMMGMVF	57
RRMMGMVFQ	58
RRMMGMVFQS	59
RRMMGMVFQSL	60
Acetyl-RRMMGMVFQSLCG(dA)	61
RRMMGMVFQSLCG(dA)-Amide	62
Acetyl-RRMMGMVFQSLCG(dA)-Amide	63.

[026] In exemplary embodiments, the peptide or peptide derivative is a PEG, acetyl, biotin or fatty acid derivative thereof. In exemplary embodiments, the peptide derivative includes PEG12.

[027] In exemplary aspects, the peptide or peptide analog of the present disclosure decreases free fatty acid levels in adipocytes, e.g., human primary adipocytes. In exemplary aspects, the free fatty acids level is decreased by at least or about 5%, relative to a control. In exemplary aspects, the free fatty acids level is decreased by at least or about 10%, at least or about 20%, at least or about 30%, at least or about 40%, at least or about 50%, at least or about 60%, at least or about 70%, at least or about 80%, at least or about 90%, relative to a control. In exemplary aspects, the free fatty acids level is decreased by greater than 90%, relative to a control. In exemplary aspects, the peptide or peptide analog of the present disclosure decreases free fatty acid levels in adipocytes, e.g., human primary adipocytes, to a better extent relative to that achieved by or associated with a MOTS-c peptide (e.g., the peptide consisting of SEQ ID NO: 2). In exemplary aspects, the peptide or peptide analog of the present disclosure decreases free fatty acid levels in adipocytes, e.g., human primary adipocytes, to an extent which is at least or about 10%, at least or about 20%, at least or about 30%, at least or about 40%, at least or about 50%, at least or about 60%, at least or about 70%, at least or about 80%, at least or about 90%, lower than the decrease caused by or associated with a MOTS-c peptide (e.g., the peptide consisting of SEQ ID NO: 2). Suitable methods of assaying free fatty acid levels in adipocytes are known, a few exemplary methods of which are described here in Examples 2-5 and 17. In exemplary aspects, the peptide or peptide analog of the present disclosure decreases free fatty acid levels in adipocytes, e.g., human primary adipocytes, as assayed by a method described in one of Examples 2-5 and 17. In exemplary aspects, the peptide or peptide analog of the present disclosure decreases free fatty acid levels in adipocytes, e.g., human primary adipocytes, as assayed by a single dose assay described in one of Examples 2-5 and 17.

[028] In exemplary aspects, the peptide or peptide analog of the present disclosure decreases body weight, blood glucose levels, and/or fat mass in mammals, e.g., DIO mice, humans. In exemplary aspects, body weight, blood glucose levels, and/or fat mass is decreased by at least or about 5%, relative to a control, in a mammal. In exemplary aspects, body weight, blood glucose levels, and/or fat mass is decreased by at least or about 10%, at least or about 20%, at least or about 30%,

at least or about 40%, at least or about 50%, at least or about 60%, at least or about 70%, at least or about 80%, relative to a control, in a mammal. In exemplary aspects, the peptide or peptide analog of the present disclosure decreases body weight, blood glucose levels, and/or fat mass in mammals, e.g., DIO mice, humans, to a better extent relative to that achieved by or associated with a MOTS-c peptide (e.g., the peptide consisting of SEQ ID NO: 2). In exemplary aspects, the peptide or peptide analog of the present disclosure decreases body weight, blood glucose levels, and/or fat mass in mammals, e.g., DIO mice, humans, to an extent which is at least or about 10%, at least or about 20%, at least or about 30%, at least or about 40%, at least or about 50%, at least or about 60%, at least or about 70%, at least or about 80%, at least or about 90%, lower than the decrease caused by or associated with a MOTS-c peptide (e.g., the peptide consisting of SEQ ID NO: 2). In exemplary aspects, the peptide or peptide analog of the present disclosure decreases serum triglyceride levels and/or serum levels of enzyme markers of liver damage (e.g., AST, ALT). In exemplary aspects, serum triglyceride levels and/or serum levels of enzyme markers of liver damage (e.g., AST, ALT) are decreased by at least or about 5%, relative to a control, in a mammal. In exemplary aspects, serum triglyceride levels and/or serum levels of enzyme markers of liver damage (e.g., AST, ALT) are decreased by at least or about 10%, at least or about 20%, at least or about 30%, at least or about 40%, at least or about 50%, at least or about 60%, at least or about 70%, at least or about 80%, at least or about 90%, relative to a control, in a mammal. In exemplary aspects, serum triglyceride levels and/or serum levels of enzyme markers of liver damage (e.g., AST, ALT) are decreased by greater than 90%, relative to a control, in a mammal. In exemplary aspects, the peptide or peptide analog of the present disclosure decreases serum triglyceride levels and/or serum levels of enzyme markers of liver damage (e.g., AST, ALT) to a better extent relative to that achieved by or associated with a MOTS-c peptide (e.g., the peptide consisting of SEQ ID NO: 2). In exemplary aspects, the peptide or peptide analog of the present disclosure decreases serum triglyceride levels and/or serum levels of enzyme markers of liver damage (e.g., AST, ALT), to an extent which is at least or about 10%, at least or about 20%, at least or about 30%, at least or about 40%, at least or about 50%, at least or about 60%, at least or about 70%, at least or about 80%, at least or about 90%, lower than the decrease caused by or associated with a MOTS-c peptide (e.g., the peptide consisting of SEQ ID NO: 2). Suitable methods of assaying body weight, blood glucose levels, fat mass, serum triglyceride levels, and serum levels of enzyme markers of liver damage in a mammal are known in the art, a few exemplary methods of which are described here in Examples 6-9 and 18-20. In exemplary aspects, the peptide or peptide analog of the present disclosure decreases body weight, blood glucose levels, and/or fat mass in mammals, e.g., DIO mice, humans, as assayed by a method described in one of Examples 6-9 and 18-20, e.g., once or twice daily by subcutaneous or intraperitoneal injection at a dose of 15 mg/kg/dose for 10 days (Example 6), twice daily by appropriate routes at a dose of 15 mg/kg/dose for 21 days (Example 7), once daily by appropriate routes at a dose of 5

mg/kg/dose for 21 days (Example 8), twice daily by appropriate routes at a dose of 15 mg/kg/dose for 21 days (Example 9).

[029] In exemplary aspects, the peptide or peptide analog of the present disclosure exhibits at least a 10% stability in mouse plasma for 60 minutes at 37 degrees Celsius. In other words, at least 10% of the starting assay amount of the peptide or peptide analog is present in an intact state (e.g., not degraded, cleaved, etc.) after being incubated in mouse plasma for 60 minutes at 37 degrees Celsius. In exemplary aspects, the peptide or peptide analog exhibits at least a 20% stability, at least or about a 30% stability, at least or about a 40% stability, at least or about a 50% stability, at least or about a 60% stability, at least or about a 70% stability, at least or about a 80% stability, or at least or about a 90% stability, in plasma for 60 minutes at 37 degrees Celsius. Suitable methods of assaying the stability of peptides in plasma (included mouse plasma) are known in the art,. In exemplary aspects, the peptide or peptide analog of the present disclosure exhibits at least a 10% stability in mouse plasma for 60 minutes at 37 degrees Celsius. In exemplary aspects, the peptide or peptide analog of the present disclosure exhibits at least a 10% stability in mouse plasma for 60 minutes at 37 degrees Celsius, as assayed by a single peptide dose/concentration assay.

Peptide Length

[030] In exemplary embodiments, the peptide or peptide analog of the present disclosure is a peptide or peptide analog comprising at least four amino acids connected via peptide bonds or other covalent linkages, as described herein. In exemplary aspects, the peptide or peptide analog is about 4 to about 50 amino acids in length. All integer subranges of 4 to 50 amino acids are specifically contemplated for peptides herein. In exemplary aspects, the peptide or peptide analog is about 5 to about 35 amino acids in length, about 5 to about 30 amino acids in length, about 5 to about 25 amino acids in length, or about 5 to about 20 amino acids in length. In exemplary aspects, the peptide or peptide analog is about 6 to about 35 amino acids in length, about 7 to about 30 amino acids in length, about 6 to about 25 amino acids in length, or about 6 to about 20 amino acids in length. In exemplary aspects, the peptide or peptide analog is about 7 to about 35 amino acids in length, about 7 to about 30 amino acids in length, about 7 to about 25 amino acids in length, or about 7 to about 20 amino acids in length. In exemplary aspects, the peptide or peptide analog is about 8 to about 35 amino acids in length, about 8 to about 30 amino acids in length, about 8 to about 25 amino acids in length, or about 8 to about 20 amino acids in length. In exemplary aspects, the peptide is about 8 to about 17 or 18 or about 9 to about 16 or 17 amino acids in length. In exemplary aspects, the peptide is about 10 to about 17 or about 12 to about 16 or 17 or about 14 to about 16 amino acids in length. In some embodiments, the peptide is a 5-mer, 6-mer, 7-mer, 8-mer, 9-mer-10-mer, 11-mer, 12-mer, 13-mer, 14-mer, 15-mer, 16-mer, 17-mer, 18-mer, 19-mer, or 20-mer.

Peptide Modifications

[031] Peptides of the disclosure include peptides that have been modified in any way and for any reason, for example, to: (1) reduce susceptibility to proteolysis, (2) alter binding affinities, and (3) confer or modify other physicochemical or functional properties. For example, single or multiple amino acid substitutions (e.g., equivalent, conservative or non-conservative substitutions, deletions or additions) may be made in a sequence. In exemplary aspects, the peptide or peptide analog of the present disclosure comprises a sequence listed in Table 1, or a modified sequence thereof. In exemplary embodiments of the present disclosure, the peptide or peptide analog is lipidated (e.g., myritoylated, palmitoylated, linked to a C_7-C_{20} lipid moiety), glycosylated, amidated, carboxylated, phosphorylated, esterified, acylated, acetylated, cyclized, pegylated (e.g., linked to a 5-20 kDa PEG, linked to a 5 kDa PEG, 12 kDa PEG, 20 kDa PEG) to or converted into an acid addition salt and/or optionally dimerized or polymerized, or conjugated, as further described herein. PEG in sizes of 200-4600 mol wt also would be of use for modifying the peptides of the current invention. PEG that are linear, branched and star geometries also would be of use for modifying the peptides of the current invention. PEG600 is also known as PEG12. In exemplary embodiments of the present disclosure, the peptide or peptide analog is acetylated at the N-terminus, amidated at the C-terminus, and/or phosphorylated on a Tyr residue. In exemplary aspects, the peptide or peptide analog is linked to a lipid moiety at the N-terminus or side chain of an internal residue. In exemplary aspects, the peptide or peptide analog is directly linked to a lipid moiety. In exemplary aspects, the peptide or peptide analog is indirectly linked to a lipid moiety. For example, the lipid moiety may be attached to the peptide via a linker. The linker may be an amino acid. In exemplary aspects, the lipid moiety is attached to a Lys residue of the peptide or peptide analog via a Glu residue optionally attached via the epsilon amine. Examples of modified peptides of the invention are found in Table 1.

[032] In some embodiments, peptides disclosed herein comprise a sequence having at least 66% sequence identity to any one of amino acid sequences SEQ ID NOs: 1-64. In certain embodiments, the % identity is selected from, *e.g.,* at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95%, or more sequence identity to a given sequence. In certain embodiments, the % identity is in the range of, *e.g.,* about 65% to about 70%, about 70% to about 80%, about 80% to about 85%, about 85% to about 90%, or about 90% to about 95%; %; between about 70% and about 80%, between about 80% and about 90% and between about 90% and about 99% sequence identity.

[033] In certain embodiments, the peptide comprises a sequence having at least 66% sequence identity to any one of amino acid sequences SEQ ID NOs: 1-64. In certain embodiments, the % identity is selected from, *e.g.,* at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95%, or more sequence identity to a given sequence. In certain embodiments, the % identity is in

the range of, *e.g.,* about 65% to about 70%, about 70% to about 80%, about 80% to about 85%, about 85% to about 90%, or about 90% to about 95%; %; between about 70% and about 80%, between about 80% and about 90% and between about 90% and about 99% sequence identity, but does not comprise the sequence set forth in SEQ ID NO: 2.

[034] Peptides of the disclosure include peptides that have been modified in any way and for any reason, for example, to: (1) reduce susceptibility to proteolysis, (2) alter binding affinities, and (3) confer or modify other physicochemical or functional properties. For example, single or multiple amino acid substitutions (e.g., equivalent, conservative or non-conservative substitutions, deletions or additions) may be made in a sequence.

[035] A conservative amino acid substitution refers to the substitution in a peptide of an amino acid with a functionally similar amino acid having similar properties, e.g., size, charge, hydrophobicity, hydrophilicity, and/or aromaticity. The following six groups each contain amino acids that are conservative substitutions for one another are found in Table 2.

TABLE 2

i. Alanine (A), Serine (S), and Threonine (T)
ii. Aspartic acid (D) and Glutamic acid (E)
iii. Asparagine (N) and Glutamine (Q)
iv. Arginine (R) and Lysine (K)
v. Isoleucine (I), Leucine (L), Methionine (M), and Valine (V)
vi. Phenylalanine (F), Tyrosine (Y), and Tryptophan (W)

[036] Additionally, within the meaning of the term "equivalent amino acid substitution" as applied herein, one amino acid may be substituted for another, in one embodiment, within the groups of amino acids indicated herein below:

1. Amino acids with polar side chains (Asp, Glu, Lys, Arg, His, Asn, Gln, Ser, Thr, Tyr, and Cys,)
2. Amino acids with small nonpolar or slightly polar residues (Ala, Ser, Thr, Pro, Gly);
3. Amino acids with non-polar side chains (Gly, Ala, Val, Leu, Ile, Phe, Trp, Pro, and Met)
4. Amino acids with large, aliphatic, nonpolar residues (Met, Leu, Ile, Val, Cys, Norleucine (Nle), homocysteine)
5. Amino acids with aliphatic side chains (Gly, Ala Val, Leu, Ile)
6. Amino acids with cyclic side chains (Phe, Tyr, Trp, His, Pro)
7. Amino acids with aromatic side chains (Phe, Tyr, Trp)
8. Amino acids with acidic side chains (Asp, Glu)
9. Amino acids with basic side chains (Lys, Arg, His)
10. Amino acids with amide side chains (Asn, Gln)
11. Amino acids with hydroxy side chains (Ser, Thr)

12. Amino acids with sulphur-containing side chains (Cys, Met),
13. Neutral, weakly hydrophobic amino acids (Pro, Ala, Gly, Ser, Thr)
14. Hydrophilic, acidic amino acids (Gln, Asn, Glu, Asp), and
15. Hydrophobic amino acids (Leu, Ile, Val).

[037] In some embodiments, the amino acid substitution is not a conservative amino acid substitution, e.g., is a non-conservative amino acid substitution. This class generally includes corresponding D-amino acids, homo-amino acids, N-alkyl amino acids, beta amino acids and other unnatural amino acids. The non-conservative amino acid substitutions still fall within the descriptions identified for the equivalent amino acid substitutions above [e.g. polar, nonpolar, etc.]. Examples of non-conservative amino acids are provided below.

[038] Non limiting examples for alanine non-conservative amino acids are: D-alanine [Dala, (dA), a], *N*-Acetyl-3-(3,4-dimethoxyphenyl)-D-alanine, *N*-Me-D-Ala-OH, *N*-Me-Ala-OH, H-β-Ala-β-naphthalene, L-(−)-2-Amino-3-ureidopropionic acid, (*R*)-(+)-α-Allylalanine, (*S*)-(−)-α-Allylalanine, D-2-Aminobutyric acid, L-2-Aminobutyric acid, DL-2-Aminobutyric acid, 2-Aminoisobutyric acid, α-Aminoisobutyric acid, (*S*)-(+)-2-Amino-4-phenylbutyric acid ethyl ester, Benzyl α-aminoisobutyrate, Abu-OH, Aib-OH, β-(9-anthryl)-Ala-OH, β-(3-benzothienyl)-Ala-OH, β-(3-benzothienyl)-D-Ala-OH, Cha-OH, Cha-OMe, β-(2-furyl)-Ala-OH, β-(2-furyl)-D-Ala-OH, β-iodo-Ala-OBzl, β-iodo-D-Ala-OBzl, 3-iodo-D-Ala-OMe, β-iodo-Ala-OMe, 1-Nal-OH, D-1-Nal-OH, 2-Nal-OH, D-2-Nal-OH, (*R*)-3-(2-naphthyl)-β-Ala-OH, (*S*)-3-(2-naphthyl)-β-Ala-OH, β-phenyl-Phe-OH, 3-(2-pyridyl)-Ala-OH, 3-(3-pyridyl)-Ala-OH, 3-(3-pyridyl)-D-Ala-OH, (*S*)-3-(3-pyridyl)-β-Ala-OH, 3-(4-pyridyl)-Ala-OH, 3-(4-pyridyl)-D-Ala-OH, β-(2-quinolyl)-Ala-OH, 3-(2-quinolyl)-DL-Ala-OH, 3-(3-quinolyl)-DL-Ala-OH, 3-(2-quinoxalyl)-DL-Ala-OH, β-(4-thiazolyl)-Ala-OH, β-(2-thienyl)-Ala-OH, β-(2-thienyl)-D-Ala-OH, β-(3-thienyl)-Ala-OH, β-(3-thienyl)-D-Ala-OH, 3-Chloro-D-alanine methyl ester, *N*-[(4-Chlorophenyl)sulfonyl]-β-alanine, 3-Cyclohexyl-D-alanine, 3-Cyclopentyl-DL-alanine, (−)-3-(3,4-Dihydroxyphenyl)-2-methyl-L-alanine, 3,3-Diphenyl-D-alanine, 3,3-Diphenyl-L-alanine, *N*-[(*S*)-(+)-1-(Ethoxycarbonyl)-3-phenylpropyl]-L-alanine, *N*-[1-(*S*)-(+)-Ethoxycarbonyl-3-phenylpropyl]-L-alanyl carboxyanhydride, N-(3-fluorobenzyl)alanine, *N*-(3-Indolylacetyl)-L-alanine, Methyl (*RS*)-2-(aminomethyl)-3-phenylpropionate, 3-(2-Oxo-1,2-dihydro-4-quinolinyl)alanine, 3-(1-Pyrazolyl)-L-alanine, 3-(2-Pyridyl)-D-alanine, 3-(2-Pyridyl)-L-alanine, 3-(3-Pyridyl)-L-alanine, 3-(4-Pyridyl)-D-alanine, 3-(4-Pyridyl)-L-alanine, 3-(2-Quinolyl)-DL-alanine, 3-(4-Quinolyl)-DL-alanine, D-styrylalanine, L-styrylalanine, 3-(2-Thienyl)-L-alanine, 3-(2-Thienyl)-DL-alanine, 3-(2-Thienyl)-DL-alanine, 3,3,3-Trifluoro-DL-alanine, *N*-Methyl-L-alanine, 3-Ureidopropionic acid, Aib-OH, Cha-OH, Dehydro-Ala-*O*Me, dehydro-Ala-OH, D-2-Nal-OH, β-Ala-ONp, β-Homoala-OH, β-D-Homoala-OH, β-Alanine, β-Alanine ethyl ester, β-Alanine methyl ester, (*S*)- diphenyl-β-Homoala-OH, (*R*)-4-(4-pyridyl)-β-Homoala-OH, (*S*)-4-(4-pyridyl)-β-Homoala-OH, β-Ala-OH, (*S*)-diphenyl-β-

Homoala-OH, L-β-Homoalanine, (*R*)-4-(3-pyridyl)-β-Homoala-OH, α-methyl-α-naphthylalanine [Manap], N-methyl-cyclohexylalanine [Nmchexa], cyclohexylalanine [Chexa], N-methyl-cyclopentylalanine [Nmcpen], cyclopentylalanine [Cpen], N-methyl-α-naphthylalanine [Nmanap], α-naphthylalanine [Anap], L-N-methylalanine [Nmala], D-N-methylalanine [Dnmala], α-methyl-cyclohexylalanine [Mchexa], α-methyl-cyclopentylalanine [Mcpen]. Each possibility represents a separate embodiment.

[039] Non limiting examples for arginine non-conservative amino acids are: homoarginine (hArg), N-methyl arginine (NMeArg), citruline, 2-amino-3-guanidinopropionic acid, N-iminoethyl-L-ornithine, Nω-monomethyl-L-arginine, Nω-nitro-L-arginine, D-arginine, 2-amino-3-ureidopropionic acid, Nω,ω-dimethyl-L-arginine, Nω-Nitro-D-arginine, L-α-methylarginine [Marg], D-α-methylarginine [Dmarg], L-N-methylarginine [Nmarg], D-N-methylarginine [Dnmarg], β-Homoarg-OH, L-Homoarginine, N-(3-guanidinopropyl)glycine [Narg], and D-arginine [Darg, (dR), r]. Each possibility represents a separate embodiment.

[040] Non limiting examples for asparagine non-conservative amino acids are: L-α-methylasparagine [Masn], D-α-methylasparagine [Dmasn], L-N-methylasparagine [Nmasn], D-N-methylasparagine [Dnmasn], N-(carbamylmethyl)glycine [Nasn] and D-asparagine [Dasn, (dN), n]. Each possibility represents a separate embodiment.

[041] Non limiting examples for aspartic acid non-conservative amino acids are: L-α-methylaspartate [Masp], D-α-methylaspartate [Dmasp], L-N-methylaspartic acid [Nmasp], D-N-methylasparatate [Dnmasp], N-(carboxymethyl)glycine [Nasp] and D-aspartic acid [Dasp, (dD), d]. Each possibility represents a separate embodiment.

[042] Non limiting examples for cysteine non-conservative amino acids are: L-Cysteic acid, L-Cysteinesulfinic acid, D-Ethionine, S-(2-Thiazolyl)-L-cysteine, DL-Homocysteine, L-Homocysteine, L-Homocystine, L-α-methylcysteine [Mcys], D-α-methylcysteine [Dmcys], L-N-methylcysteine [Nmcys], D-N-methylcysteine [Dnmcys], N-(thiomethyl)glycine [Ncys] and D-cysteine [Dcys, (dC), c]. Each possibility represents a separate embodiment.

[043] Non limiting examples for glutamic acid non-conservative amino acids are: γ-Carboxy-DL-glutamic acid, 4-Fluoro-DL-glutamic acid, β-Glutamic acid, L-β-Homoglutamic acid, L-α-methylglutamate [Mglu], D-α-methyl glutamic acid [Dmglu], L-N-methylglutamic acid [Nmglu], D-N-methylglutamate [Dnmglu], N-(2-carboxyethyl)glycine [Nglu], and D-glutamic acid [Dglu, (dE), e]. Each possibility represents a separate embodiment.

[044] Non limiting examples for glutamine non-conservative amino acids are: Cit-OH, D-Citrulline, Thio-L-citrulline, β-Gln-OH, L-β-Homoglutamine, L-α-methylglutamine [Mgln], D-α-methylglutamine [Dmgln], L-N-methylglutamine [Nmgln], D-N-methylglutamine [Dnmgln], N-(2-carbamylethyl)glycine [Ngln], and D-glutamine [Dgln, (dQ), q]. Each possibility represents a separate embodiment.

[045] Non limiting examples for glycine non-conservative amino acids are: tBu-Gly-OH ,D-Allylglycine, *N*-[Bis(methylthio)methylene]glycine methyl ester, Chg-OH, D-Chg-OH, D-cyclopropylglycine, L-cyclopropylglycine, (*R*)-4-fluorophenylglycine, (*S*)-4-fluorophenylglycine, iminodiacetic acid, (2-indanyl)-Gly-OH, (±)-α-phosphonoglycine trimethyl ester, D-propargylglycine, propargyl-Gly-OH, (*R*)-2-thienylglycine, (*S*)-2-thienylglycine, (*R*)-3-thienylglycine, (*S*)-3-thienylglycine, 2-(4-trifluoromethyl-phenyl)-DL-glycine, (2S,3R,4S)-α-(Carboxycyclopropyl)glycine, *N*-(Chloroacetyl)glycine ethyl ester, (S)-(+)-2-chlorophenylglycine methyl ester, N-(2-chlorophenyl)-N-(methylsulfonyl)glycine, D-α-Cyclohexylglycine, L-α-Cyclopropylglycine, Di-*tert*-butyl-iminodicarboxylate, Ethyl acetamidocyanoacetate, N-(2-fluorophenyl)-N-(methylsulfonyl) glycine, N-(4-fluorophenyl)-N-(methylsulfonyl)glycine, *N*-(2-Furfurylideneacetyl)glycine methyl ester, *N*-(2-Furoyl)glycine, *N*-(2-Hydroxyethyl)iminodiacetic acid, *N*-(4-Hydroxyphenyl)glycine, Iminodiacetic acid, *N*-Lauroylsarcosine sodium salt, L-α-Neopentylglycine, *N*-(Phosphonomethyl)glycine, D-Propargylglycine, L-C-Propargylglycine, Sarcosine, *N*,*N*-Dimethylglycine, *N*,*N*-Dimethylglycine ethyl ester, D-Chg-OH, α-Phosphonoglycine trimethyl ester, N-cyclobutylglycine [Ncbut], L-α-methylethylglycine [Metg], N-cycloheptylglycine [Nchep], L-α-methyl-i-butylglycine [Mtbug], N-methylglycine [Nmgly], L-N-methyl-ethylglycine [Nmetg], L-ethylglycine [Etg], L-N-methyl-t-butylglycine [Nmtbug], L-t-butylglycine [Tbug], N-cyclohexylglycine [Nchex], N-cyclodecylglycine [Ncdec], N-cyclododecylglycine [Ncdod], N-cyclooctylglycine [Ncoct], N-cyclopropylglycine [Ncpro], N-cycloundecylglycine [Ncund], N-(2-aminoethyl)glycine [Naeg], N-(N-(2,2-diphenylethyl) diphenylethyl)glycine [Nnbhm], N-(2,2- carbamylmethyl-glycine [Nbhm], N-(N-(3,3-diphenylpropyl) diphenylpropyl)glycine [Nnbhe] and N-(3,3- carbamylmethyl-glycine [Nbhe]. Each possibility represents a separate embodiment.

[046] Non limiting examples for histidine non-conservative amino acids are: L-α-methylhistidine [Mhis], D-α-methylhistidine [Dmhis], L-N-methylhistidine [Nmhis], D-N-methylhistidine [Dnmhis], N-(imidazolylethyl)glycine [Nhis], and D-histidine [Dhis, (dH), h]. Each possibility represents a separate embodiment.

[047] Non limiting examples for isoleucine non-conservative amino acids are: *N*-Methyl-L-isoleucine [Nmile], *N*-(3-Indolylacetyl)-L-isoleucine, allo-Ile-OH, D-allo-Isoleucine, L-β-Homoisoleucine, L-α-methylisoleucine [Mile], D-α-methylisoleucine [Dmile], D-N-methylisoleucine [Dnmile], N-(1 -methylpropyl)glycine [Nile], and D-isoleucine [Dile, (dD), i]. Each possibility represents a separate embodiment.

[048] Non limiting examples for leucine non-conservative amino acids are: D-leuine [Dleu, (dL), l]. Cycloleucine, DL-leucine, *N*-Formyl-Leu-OH, D-*tert*-Leucine, L-*tert*-Leucine, DL-*tert*-Leucine, L-*tert*-Leucine methyl ester, 5,5,5-Trifluoro-DL-leucine, D-β-Leu-OH, L-β-Leucine, DL-β-Leucine, L-β-Homoleucine, DL-β-Homoleucine, L-N-methyl-leucine [Nmleu], D-N-methyl-leucine [Dnmleu], L-α-methyl-leucine [Mleu], D-α-methyl-leucine [Dmleu], N-(2-

methylpropyl)glycine [Nleu], D-leucine [Dleu, l], D-Norleucine, L-Norleucine, DL-Norleucine, L-N-methylnorleucine [Nmnle] and L-norleucine [Nle]. Each possibility represents a separate embodiment.

[049] Non limiting examples for lysine non-conservative amino acids are: DL-5-Hydroxylysine, (5*R*)-5-Hydroxy-L-lysine, β-Lys-OH, L-β-Homolysine, L-α-methyl-lysine [Mlys], D-α-methyl-lysine [Dmlys], L-N-methyl-lysine [Nmlys], D-N-methyl-lysine [Dnmlys], N-(4-aminobutyl)glycine [Nlys], and D-lysine [Dlys, (dK), k]. Each possibility represents a separate embodiment.

[050] Non limiting examples for methionine non-conservative amino acids are: L-β-Homomethionine, DL-β-Homomethionine, L-α-methylmethionine [Mmet], D-α-methylmethionine [Dmmet], L-N-methylmethionine [Nmmet], D-N-methylmethionine [Dnmmet], N-(2-methylthioethyl)glycine [Nmet], and D-methionine [Dmet, (dM), m]. Each possibility represents a separate embodiment.

[051] Non limiting examples for phenylalanine non-conservative amino acids are: *N*-Acetyl-2-fluoro-DL-phenylalanine, *N*-Acetyl-4-fluoro-DL-phenylalanine, 4-Amino-L-phenylalanine, 3-[3,4-bis(trifluoromethyl)phenyl]-L-alanine, Bpa-OH, D-Bpa-OH, 4-*tert*-butyl-Phe-OH, 4-*tert*-butyl-D-Phe-OH, 4-(amino)-L-phenylalanine, *rac*-β^2-homophenylalanine, 2-methoxy-L-phenylalanine, (*S*)-4-methoxy-β-Phe-OH, 2-nitro-L-phenylalanine, pentafluoro-D-phenylalanine, pentafluoro-L-phenylalanine, Phe(4-Br)-OH, D-Phe(4-Br)-OH, Phe(2-CF_3)-OH, D-Phe(2-CF_3)-OH, Phe(3-CF_3)-OH, D-Phe(3-CF_3)-OH, Phe(4-CF_3)-OH, D-Phe(4-CF_3)-OH, Phe(2-Cl)-OH, D-Phe(2-Cl)-OH, Phe(2,4-Cl_2)-OH, D-Phe(2,4-Cl_2)-OH, D-Phe(3-Cl)-OH, Phe(3,4-Cl_2)-OH, Phe(4-Cl)-OH, D-Phe(4-Cl)-OH, Phe(2-CN)-OH, D-Phe(2-CN)-OH, D-Phe(3-CN)-OH, Phe(4-CN)-OH, D-Phe(4-CN)-OH, Phe(2-Me)-OH, D-Phe(2-Me)-OH, Phe(3-Me)-OH, D-Phe(3-Me)-OH, Phe(4-Me)-OH, Phe(4-NH_2)-OH, Phe(4-NO_2)-OH, Phe(2-F)-OH, D-Phe(2-F)-OH, Phe(3-F)-OH, D-Phe(3-F)-OH, Phe(3,4-F_2)-OH, D-Phe(3,4-F_2)-OH, Phe(3,5-F_2)-OH, Phe(4-F)-OH, D-Phe(4-F)-OH, Phe(4-I)-OH, D-3,4,5-trifluorophenylalanine, *p*-Bromo-DL-phenylalanine, 4-Bromo-L-phenylalanine, β-phenyl-D-phenylalanine, 4-Chloro-L-phenylalanine, DL-2,3-Difluorophenylalanine, DL-3,5-Difluorophenylalanine, 3,4-Dihydroxy-L-phenylalanine, 3-(3,4-Dimethoxyphenyl)-L-alanine, *N*-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-2-methoxy-L-phenylalanine, *o*-Fluoro-DL-phenylalanine, *m*-Fluoro-L-phenylalanine, *m*-Fluoro-DL-phenylalanine, *p*-Fluoro-L-phenylalanine, *p*-Fluoro-DL-phenylalanine, 4-Fluoro-D-phenylalanine, 2-fluoro-L-phenylalanine methyl ester, *p*-fluoro-DL-Phe-OMe, D-3-bromophenylalanine, D-4-bromophenylalanine, L-β-(6-chloro-4-pyridinyl)alanine, D-3,5-difluorophenylalanine, L-3-fluorophenylalanine, L-4-fluorophenylalanine, L-β-(1*H*-5-indolyl)alanine, 2-nitro-L-phenylalanine, pentafluoro-L-phenylalanine, phe(3-br)-oh, Phe(4-Br)-OH, Phe(2-CF_3)-OH, D-Phe(2-CF_3)-OH, Phe(3-CF_3)-OH, D-Phe(3-CF_3)-OH, Phe(4-CF_3)-OH, D-Phe(4-CF_3)-OH, Phe(2-Cl)-OH, D-Phe(2-Cl)-OH, Phe(2,4-Cl_2)-OH, D-Phe(2,4-Cl_2)-OH, Phe(3,4-Cl_2)-OH, D-Phe(3,4-Cl_2)-OH, Phe(4-Cl)-OH, D-Phe(4-Cl)-OH, Phe(2-CN)-OH, D-Phe(2-CN)-OH, D-Phe(3-CN)-OH, Phe(4-CN)-OH, Phe(2-Me)-OH, Phe(3-Me)-OH, D-Phe(3-Me)-OH, Phe(4-NO_2)-OH, D-Phe(4-NO_2)-OH, D-Phe(2-F)-OH, Phe(3-F)-OH, D-Phe(3-F)-OH, Phe(3,4-F_2)-

OH, Phe(3,5-F_2)-OH, D-Phe(4-F)-OH, Phe(4-I)-OH, D-Phe(4-I)-OH, 4-(phosphonomethyl)-Phe-OH, L-4-trifluoromethylphenylalanine, 3,4,5-trifluoro-D-phenylalanine, L-3,4,5-trifluorophenylalanine, 6-Hydroxy-DL-DOPA, 4-(Hydroxymethyl)-D-phenylalanine, *N*-(3-Indolylacetyl)-L-phenylalanine, *p*-Iodo-D-phenylalanine, 4-Iodo-L-phenylalanine, α-Methyl-D-phenylalanine, α-Methyl-L-phenylalanine, α-Methyl-DL-phenylalanine, α-Methyl-DL-phenylalanine methyl ester, 4-Nitro-D-phenylalanine, 4-Nitro-L-phenylalanine, 4-Nitro-DL-phenylalanine, (*S*)-(+)-4-Nitrophenylalanine methyl ester, 2-(Trifluoromethyl)-D-phenylalanine, 2-(Trifluoromethyl)-L-phenylalanine, 3-(Trifluoromethyl)-D-phenylalanine, 3-(Trifluoromethyl)-L-phenylalanine, 4-(Trifluoromethyl)-D-phenylalanine, 3,3′,5-Triiodo-L-thyronine, , (*R*)-4-bromo-β-Phe-OH, N-Acetyl-DL-β-phenylalanine, (*S*)-4-bromo-β-Phe-OH, (*R*)-4-chloro-β-Homophe-OH, (*S*)-4-chloro-β-Homophe-OH, (*R*)-4-chloro-β-Phe-OH, (*S*)-4-chloro-β-Phe-OH, (*S*)-2-cyano-β-Homophe-OH, (*R*)- 4-cyano-β-Homophe-OH, (*S*)-4-cyano-β-Homophe-OH, (*R*)-3-cyano-β-Phe-OH, (*R*)-4-cyano-β-Phe-OH, (*S*)-4-cyano-β-Phe-OH, (*R*)-3,4-dimethoxy-β-Phe-OH, (*S*)-3,4-dimethoxy-β-Phe-OH, (*R*)-4-fluoro-β-Phe-OH, (*S*)-4-fluoro-β-Phe-OH, (*S*)-4-iodo-β-Homophe-OH, (*S*)-3-cyano-β-Homophe-OH, (*S*)-3,4-difluoro-β-Homophe-OH, (*R*)-4-fluoro-β-Homophe-OH, (*S*)-β2-homophenylalanine, (*R*)-3-methoxy-β-Phe-OH, (*S*)-3-methoxy-β-Phe-OH, (*R*)-4-methoxy-β-Phe-OH, (*S*)-4-methyl-β-Homophe-OH, (*R*)-2-methyl-β-Phe-OH, (*S*)-2-methyl-β-Phe-OH, (*R*)-3-methyl-β-Phe-OH, (*S*)-3-methyl-β-Phe-OH, (*R*)-4-methyl-β-Phe-OH, (*S*)-4-methyl-β-Phe-OH, β-Phe-OH, D-β-Phe-OH, (*S*)-2-(trifluoromethyl)-β-Homophe-OH, (*S*)-2-(trifluoromethyl)-β-Homophe-OH, (*S*)-3-(trifluoromethyl)-β-Homophe-OH, (*R*)-4-(trifluoromethyl)-β-Homophe-OH, (*S*)-2-(trifluoromethyl)-β-Phe-OH, (*R*)-3-(trifluoromethyl)-β-Phe-OH, (*S*)-3-(trifluoromethyl)-β-Phe-OH, (*R*)-4-(trifluoromethyl)-β-Phe-OH, (*S*)-4-(trifluoromethyl)-β-Phe-OH, β-Homophe-OH, D-β-Homophe-OH, (*S*)-2-methyl-β-Homophe-OH, (*S*)-3-methyl-β-Homophe-OH, β-Phe-OH, β-D-Phe-OH, (*S*)-3-(trifluoromethyl)-β-Homophe-OH, L-β-Homophenylalanine, DL-β-Homophenylalanine, DL-β-Phenylalanine, DL-homophenylalanine methyl ester, D-Homophenylalanine, L-Homophenylalanine, DL-Homophenylalanine, D-Homophenylalanine ethyl ester, (*R*)-β^2-homophenylalanine, L-α-methyl-homophenylalanine [Mhphe], L-α-methylphenylalanine [Mphe], D-a-methylphenylalanine [Dmphe], L-N-methyl- homophenylalanine [Nm phe], L-homophenylalanine [Hphe], L-N-methylphenylalanine [Nmphe], D-N-methylphenylalanine [Dnmphe], N-benzylglycine [Nphe] and D-phenylalanine [Dphe, (dF), f]. Each possibility represents a separate embodiment.

[052] Non limiting examples for proline non-conservative amino acids are: homoproline (hPro), (4-hydroxy)Pro (4HyP), (3-hydroxy)Pro (3HyP), gamma-benzyl-proline, gamma-(2-fluoro-benzyl)-proline, gamma-(3-fluoro-benzyl)-proline, gamma-(4-fluoro-benzyl)-proline, gamma-(2-chloro-benzyl)- proline, gamma-(3-chloro-benzyl)-proline, gamma-(4-chloro-benzyl)-proline, gamma-(2-bromo-benzyl)-proline, gamma-(3-bromo-benzyl)-proline, gamma-(4-bromo-benzyl)-proline, gamma-(2-methyl-benzyl)-proline, gamma-(3-methyl-benzyl)-proline, gamma-(4-methyl-benzyl)-

proline, gamma-(2-nitro-benzyl)-proline, gamma-(3-nitro-benzyl)-proline, gamma-(4-nitro-benzyl)-proline, gamma-(l-naphthalenylmethyl)- proline, gamma-(2-naphthalenylmethyl)-proline, gamma-(2,4-dichloro-benzyl)-proline, gamma-(3,4-dichloro-benzyl)-proline, gamma-(3,4-difluoro-benzyl)-proline, gamma-(2-trifluoro-methyl-benzyl)-proline, gamma-(3-trifluoro-methyl-benzyl)-proline, gamma-(4-trifluoro-methyl-benzyl)-proline, gamma-(2-cyano-benzyl)-proline, gamma-(3-cyano-benzyl)-proline, gamma-(4-cyano-benzyl)-proline, gamma-(2-iodo-benzyl)-proline, gamma-(3-iodo-benzyl)-proline, gamma-(4-iodo-benzyl)-proline, gamma-(3-phenyl-allyl-benzyl)-proline, gamma-(3-phenyl-propyl-benzyl)-proline, gamma-(4-tert-butyl-benzyl)-proline, gamma-benzhydryl-proline, gamma-(4-biphenyl-methyl)-proline, gamma-(4-thiazolyl-methyl)-proline, gamma-(3-benzothienyl-methyl)-proline, gamma-(2-thienyl-methyl)-proline, gamma-(3-thienyl-methyl)- proline, gamma-(2-furanyl-methyl)-proline, gamma-(2-pyridinyl-methyl)-proline, gamma-(3-pyridinyl-methyl)-proline, gamma-(4-pyridinyl-methyl)- proline, gamma-allyl-proline, gamma-propynyl-proline, alpha-modified-proline residues , pipecolic acid, azetidine-3-carboxylicacid, L-β-Homoproline, L-β^3-homoproline, L-β-Homohydroxyproline**,** hydroxyproline [Hyp], L-□-methylproline [Mpro], D-□-methylproline [Dmpro], L-N-methylproline [Nmpro], D-N-methylproline [Dnmpro], and D-proline [Dpro, (dP), p].. Each possibility represents a separate embodiment.

[053] Non limiting examples for serine non-conservative amino acids are: (2*R*,3*S*)-3-phenylisoserine, D-cycloserine, L-Isoserine, DL-Isoserine, DL-3-Phenylserine, L-β-Homoserine, D-Homoserine, D-Homoserine, L-3-Homoserine, L-homoserine, L-α-methylserine [Mser], D-α-methylserine [Dmser], L-N-methylserine [Nmser], D-N-methylserine [Dnmser], D-serine [Dser, (dS), s], N-(hydroxymethyl)glycine [Nser] and phosphoserine [pSer]. Each possibility represents a separate embodiment.

[054] Non limiting examples for threonine non-conservative amino acids are: L-*allo*-Threonine, D-Thyroxine, L-β-Homothreonine, L-α-methylthreonine [Mthr], D-α-methylthreonine [Dmthr], L-N-methylthreonine [Nmthr], D-N-methylthreonine [Dnmthr], D-threonine [Dthr, (dT), t], N-(1-hydroxyethyl)glycine [Nthr] and phosphothreonine [pThr]. Each possibility represents a separate embodiment.

[055] Non limiting examples for tryptophan non-conservative amino acids are: 5-Fluoro-L-tryptophan, 5-Fluoro-DL-tryptophan, 5-Hydroxy-L-tryptophan, 5-Methoxy-DL-tryptophan, L-abrine, 5-Methyl-DL-tryptophan, H-Tpi-OMe. β-Homotrp-OMe, L-β-Homotryptophan, L-α-methyltryptophan [Mtrp], D-α-methyltryptophan [Dmtrp], L-N-methyltryptophan [Nmtrp], D-N-methyltryptophan [Dnmtrp], N-(3-indolylethyl)glycine [Nhtrp], D-tryptophan [Dtrp, (dW), w]. Each possibility represents a separate embodiment.

[056] Non limiting examples for tyrosine non-conservative amino acids are: 3,5 diiodotyrosine (3,5-dITyr), 3,5 diBromotyrosine (3,5-dBTyr), homotyrosine, D-tyrosine, 3-amino-L-tyrosine, 3-amino-D-tyrosine, 3- iodo- L- tyrosine, 3- iodo- D- tyrosine, 3-methoxy-L-tyrosine, 3-methoxy-D-

tyrosine, L-thyroxine, D-thyroxine, L-thyronine, D-thyronine, O-methyl-L-tyrosine, O-methyl-D-tyrosine, D-thyronine, O-ethyl-L-tyrosine, O-ethyl-D-tyrosine, 3,5,3'-triiodo-L-thyronine, 3,5,3'-triiodo-D-thyronine, 3,5-diiodo-L-thyronine, 3,5-diiodo-D-thyronine, D-meta-tyrosine, L-meta-tyrosine, D-ortho- tyrosine, L-ortho-tyrosine, phenylalanine, substituted phaenylalanine, N-nitro phenylalanine, p-nitro phenylalanine, 3-chloro-Dtyr-oh, Tyr(3,5-diI), 3-Chloro-L-tyrosine, Tyr(3-NO_2)-OH , Tyr(3,5-diI)-OH, *N*-Me-Tyr-OH, α-Methyl-DL-tyrosine, 3-Nitro-L-tyrosine, DL-*o*-Tyrosine, β-Homotyr-OH, (*R*)-β-Tyr-OH, (*S*)-β-Tyr-OH, L-α-methyltyrosine [Mtyr], D-α-methyltyrosine [Dmtyr], L-N-methyltyrosine [Nmtyr], D-N-methyltyrosine [Dnmtyr], D-tyrosine [Dtyr, (dY), y], O-methyl-tyrosine, and phosphotyrosine [pTyr]. Each possibility represents a separate embodiment.

[057] Non limiting examples for valine non-conservative amino acids are: 3-Fluoro-DL-valine, 4,4,4,4′,4′,4′-Hexafluoro-DL-valine, D-valine [Dval, (dV), v], *N*-Me-Val-OH [Nmval], *N*-Me-Val-OH, L-α-methylvaline [Mval], D-α-methylvaline [Dmval], (*R*)-(+)-α-Methylvaline, (*S*)-(−)-α-Methylvaline and D-N-methylvaline [Dnmval]. Each possibility represents a separate embodiment.

[058] Other non-natural amino acids that may be substituted as non-conservative replacements include: Ornithine and its modifications : D-Ornithine [Dorn], L-Ornithine [Orn], DL-Ornithine, L-α-methylornithine [Morn], D-α-methylornithine [Dmorn], L-N-methylornithine [Nmorn], D-N-methylornithine [Dnmorn] and N-(3-aminopropyl)glycine [Norn]. Each possibility represents a separate embodiment.

[059] Alicyclic amino acids : L-2,4-Diaminobutyric acid, L-2,3-Diaminopropionic Acid, N-Me-Aib-OH, (*R*)-2-(amino)-5-hexynoic acid, piperidine-2-carboxylic acid, aminonorbornyl- carboxylate [Norb], alpha-aminobutyric acid [Abu], aminocyclopropane-carboxylate [Cpro], (*cis*)-3-Aminobicyclo[2.2.1]heptane-2-carboxylic acid, exo-cis-3-Aminobicyclo[2.2.1]hept-5-ene-2-carboxylic acid, 1-Amino-1-cyclobutanecarboxylic acid, *cis*-2-Aminocycloheptanecarboxylic acid, 1-Aminocyclohexanecarboxylic acid, *cis*-2-Aminocyclohexanecarboxylic acid, *trans*-2-Aminocyclohexanecarboxylic acid, *cis*-6-Amino-3-cyclohexene-1-carboxylic acid, 2-(1-Aminocyclohexyl)acetic acid, *cis*-2-Amino-1-cyclooctanecarboxylic acid, *cis*-2-Amino-3-cyclooctene-1-carboxylic acid, (1*R*,2*S*)-(−)-2-Amino-1-cyclopentanecarboxylic acid, (1*S*,2*R*)-(+)-2-Amino-1-cyclopentanecarboxylic acid, *cis*-2-Amino-1-cyclopentanecarboxylic acid, 2-(1-Aminocyclopentyl)acetic acid, *cis*-2-Amino-2-methylcyclohexanecarboxylic acid, *cis*-2-Amino-2-methylcyclopentanecarboxylic acid, 3-Amino-3-(4-nitrophenyl)propionic acid, 3-Azetidinecarboxylic acid, amchc-oh, 1-aminocyclobutane carboxylic acid, 1-(amino)cyclohexanecarboxylic acid, *cis*-2-(amino)-cyclohexanecarboxylic acid, *trans*-2-(amino)-cyclohexanecarboxylic acid, *cis*-4-(amino)cyclohexanecarboxylic acid, *trans*-4-(amino)cyclohexanecarboxylic acid, (±)-*cis*-2-(amino)-3-cyclohexene-1-carboxylic acid, (±)-*cis*-6-(amino)-3-cyclohexene-1-carboxylic acid, 2-(1-aminocyclohexyl)acetic acid, *cis*-[4-

(amino)cyclohexyl]acetic acid, 1-(amino)cyclopentanecarboxylic acid, (±)-*cis*-2-(amino)cyclopentanecarboxylic acid, (1R,4S)-(+)-4-(amino)-2-cyclopentene-1-carboxylic acid, (±)-*cis*-2-(amino)-3-cyclopentene-1-carboxylic acid, 2-(1-aminocyclopentyl)acetic acid, 1-(amino)cyclopropanecarboxylic acid, Ethyl 1-aminocyclopropanecarboxylate, 1,2-trans-achec-oh, 1-(amino)cyclobutanecarboxylic acid, 1-(amino)cyclohexanecarboxylic acid, *cis*-2-(amino)-cyclohexanecarboxylic acid, *trans*-2-(amino)cyclohexanecarboxylic acid, *cis*-4-(amino)cyclohexanecarboxylic acid, *trans*-4-(amino)cyclohexanecarboxylic acid, *cis*-[4-(amino)cyclohexyl]acetic acid, 1-(amino)cyclopentanecarboxylic acid, (1*R*,4*S*)-(+)-4-(amino)-2-cyclopentene-1-carboxylic acid, (1*S*,4*R*)-(−)-4-(amino)-2-cyclopentene-1-carboxylic acid, 1-(amino)cyclopropanecarboxylic acid, *trans*-4-(aminomethyl)cyclohexanecarboxylic acid, β-Dab-OH, 3-Amino-3-(3-bromophenyl)propionic acid, 3-Aminobutanoic acid, *cis*-2- Amino-3-cyclopentene-1-carboxylic acid, DL-3-Aminoisobutyric acid, (*R*)-3-Amino-2-phenylpropionic acid, (±)-3-(amino)-4-(4-biphenylyl)butyric acid, *cis*-3-(amino)cyclohexanecarboxylic acid, (1*S*,3*R*)-(+)-3-(amino)cyclopentanecarboxylic acid, (2*R*,3*R*)-3-(amino)-2-hydroxy-4-phenylbutyric acid, (2*S*,3*R*)-3-(amino)-2-hydroxy-4-phenylbutyric acid, 2-(aminomethyl)phenylacetic acid, (*R*)-3-(amino)-2-methylpropionic acid, (*S*)-3-(amino)-2-methylpropionic acid, (*R*)-3-(amino)-4-(2-naphthyl)butyric acid, (*S*)-3-(amino)-4-(2-naphthyl)butyric acid, (*R*)-3-(amino)-5-phenylpentanoic acid, (*R*)-3-(amino)-2-phenylpropionic acid, Ethyl 3-(benzylamino)propionate, *cis*-3-(amino)cyclohexanecarboxylic acid, (*S*)-3-(amino)-5-hexenoic acid, (*R*)-3-(amino)-2-methylpropionic acid, (*S*)-3-(amino)-2-methylpropionic acid, (*R*)-3-(amino)-4-(2-naphthyl)butyric acid, (*S*)-3-(amino)-4-(2-naphthyl)butyric acid, (*R*)-(−)-Pyrrolidine-3-carboxylic acid, (S)-(+)-Pyrrolidine-3-carboxylic acid, N-methyl- γ -aminobutyrate [Nmgabu], γ-aminobutyric acid [Gabu], N-methyl- α-amino- α- methylbutyrate [Nmaabu], α-amino- α-methylbutyrate [Aabu], N-methyl- α- aminoisobutyrate [Nmaib], α-aminoisobutyric acid [Aib], α-methyl-y-aminobutyrate [Mgabu]. Each possibility represents a separate embodiment.

[060] Phenyl glycine and its modifications: Phg-OH, D-Phg-OH, 2-(piperazino)-2-(3,4-dimethoxyphenyl)acetic acid, 2-(piperazino)-2-(2-fluorophenyl)acetic acid, 2-(4-piperazino)-2-(3-fluorophenyl)acetic acid, 2-(4-piperazino)-2-(4-methoxyphenyl)acetic acid, 2-(4-piperazino)-2-(3-pyridyl)acetic acid, 2-(4-piperazino)-2-[4-(trifluoromethyl)phenyl]acetic acid, L-(+)-2-Chlorophenylglycine, (±)-2-Chlorophenylglycine, (±)-4-Chlorophenylglycine, (*R*)-(−)-2-(2,5-Dihydrophenyl)glycine, (*R*)-(−)-*N*-(3,5-Dinitrobenzoyl)-α-phenylglycine, (*S*)-(+)-*N*-(3,5-Dinitrobenzoyl)-α-phenylglycine, 2,2-Diphenylglycine, 2-Fluoro-DL-α-phenylglycine, 4-Fluoro-D-α-phenylglycine, 4-Hydroxy-D-phenylglycine, 4-Hydroxy-L-phenylglycine, 2-Phenylglycine, D-(−)-α-Phenylglycine, D–(−)-α-Phenylglycine, DL-α-Phenylglycine, L–(+)-α-Phenylglycine, *N*-Phenylglycine, (*R*)-(−)-2-Phenylglycine methyl ester, (*S*)-(+)-2-Phenylglycine methyl ester, 2-Phenylglycinonitrile hydrochloride, α-Phenylglycinonitrile, 3-(Trifluoromethyl)-DL-

phenylglycine, and 4-(Trifluoromethyl)-L-phenylglycine. Each possibility represents a separate embodiment.

[061] Penicillamine and its modifications: N-Acetyl-D-penicillamine, D-Penicillamine, L-Penicillamine [Pen], DL-Penicillamine. α -methylpenicillamine [Mpen], N-methylpenicillamine [Nmpen]. Each possibility represents a separate embodiment.

[062] β-Homopyrrolidine. Each possibility represents a separate embodiment.

[063] Aromatic amino acids: 3-Acetamidobenzoic acid, 4-Acetamidobenzoic acid, 4-Acetamido-2-methylbenzoic acid, *N*-Acetylanthranilic acid, 3-Aminobenzoic acid, 3-Aminobenzoic acid hydrochloride, 4-Aminobenzoic acid, 4-Aminobenzoic acid, 4-Aminobenzoic acid, 4-Aminobenzoic acid, 4-Aminobenzoic acid, 4-Aminobenzoic acid, 2-Aminobenzophenone-2′-carboxylic acid, 2-Amino-4-bromobenzoic acid, 2-Amino-5-bromobenzoic acid, 3-Amino-2-bromobenzoic acid, 3-Amino-4-bromobenzoic acid, 3-Amino-5-bromobenzoic acid, 4-Amino-3-bromobenzoic acid, 5-Amino-2-bromobenzoic acid, 2-Amino-3-bromo-5-methylbenzoic acid, 2-Amino-3-chlorobenzoic acid, 2-Amino-4-chlorobenzoic acid, 2-Amino-5-chlorobenzoic acid, 2-Amino-5-chlorobenzoic acid, 2-Amino-6-chlorobenzoic acid, 3-Amino-2-chlorobenzoic acid, 3-Amino-4-chlorobenzoic acid, 4-Amino-2-chlorobenzoic acid, 4-Amino-3-chlorobenzoic acid, 5-Amino-2-chlorobenzoic acid, 5-Amino-2-chlorobenzoic acid, 4-Amino-5-chloro-2-methoxybenzoic acid, 2-Amino-5-chloro-3-methylbenzoic acid, 3-Amino-2,5-dichlorobenzoic acid, 4-Amino-3,5-dichlorobenzoic acid, 2-Amino-4,5-dimethoxybenzoic acid, 4-(2-Aminoethyl)benzoic acid hydrochloride, 2-Amino-4-fluorobenzoic acid, 2-Amino-5-fluorobenzoic acid, 2-Amino-6-fluorobenzoic acid, 4-Amino-2-fluorobenzoic acid, 2-Amino-5-hydroxybenzoic acid, 3-Amino-4-hydroxybenzoic acid, 4-Amino-3-hydroxybenzoic acid, 2-Amino-5-iodobenzoic acid, 5-Aminoisophthalic acid, 2-Amino-3-methoxybenzoic acid, 2-Amino-4-methoxybenzoic acid, 2-Amino-5-methoxybenzoic acid, 3-Amino-2-methoxybenzoic acid, 3-Amino-4-methoxybenzoic acid, 3-Amino-5-methoxybenzoic acid, 4-Amino-2-methoxybenzoic acid, 4-Amino-3-methoxybenzoic acid, 5-Amino-2-methoxybenzoic acid, 2-Amino-3-methylbenzoic acid, 2-Amino-5-methylbenzoic acid, 2-Amino-6-methylbenzoic acid, 3-(Aminomethyl)benzoic acid, 3-Amino-2-methylbenzoic acid, 3-Amino-4-methylbenzoic acid, 4-(Aminomethyl)benzoic acid, 4-Amino-2-methylbenzoic acid, 4-Amino-3-methylbenzoic acid, 5-Amino-2-methylbenzoic acid, 3-Amino-2-naphthoic acid, 6-Amino-2-naphthoic acid, 2-Amino-3-nitrobenzoic acid, 2-Amino-5-nitrobenzoic acid, 2-Amino-5-nitrobenzoic acid, 4-Amino-3-nitrobenzoic acid, 5-Amino-2-nitrobenzoic acid, 3-(4-Aminophenyl)propionic acid, 3-Aminophthalic acid, 4-Aminophthalic acid, 3-Aminosalicylic acid, 4-Aminosalicylic acid, 5-Aminosalicylic acid, 5-Aminosalicylic acid, 2-Aminoterephthalic acid, 2-Amino-3,4,5,6-tetrafluorobenzoic acid, 4-Amino-2,3,5,6-tetrafluorobenzoic acid, (*R*)-2-Amino-1,2,3,4-tetrahydronaphthalene-2-carboxylic acid, (*S*)-2-Amino-1,2,3,4-tetrahydro-2-naphthalenecarboxylic acid, 2-Amino-3-(trifluoromethyl)benzoic acid, 2-Amino-3-(trifluoromethyl)benzoic acid, 3-Amino-5-(trifluoromethyl)benzoic acid, 5-

Amino-2,4,6-triiodoisophthalic acid, 2-Amino-3,4,5-trimethoxybenzoic acid, 2-Anilinophenylacetic acid, 2-Abz-OH, 3-Abz-OH, 4-Abz-OH, 2-(aminomethyl)benzoic acid, 3-(aminomethyl)benzoic acid, 4-(aminomethyl)benzoic acid, *tert*-Butyl 2-aminobenzoate, *tert*-Butyl 3-aminobenzoate, *tert*-Butyl 4-aminobenzoate, 4-(Butylamino)benzoic acid, 2,3-Diaminobenzoic acid, 3,4-Diaminobenzoic acid, 3,5-Diaminobenzoic acid, 3,5-Diaminobenzoic acid, 3,5-Dichloroanthranilic acid, 4-(Diethylamino)benzoic acid, 4,5-Difluoroanthranilic acid, 4-(Dimethylamino)benzoic acid, 4-(Dimethylamino)benzoic acid, 3,5-Dimethylanthranilic acid, 5-Fluoro-2-methoxybenzoic acid, 2-Abz-OH, 3-Abz-OH, 4-Abz-OH, 3-(aminomethyl)benzoic acid, 4-(aminomethyl)benzoic acid, 4-(2-hydrazino)benzoic acid, 3-Hydroxyanthranilic acid, 3-Hydroxyanthranilic acid, Methyl 3-aminobenzoate, 3-(Methylamino)benzoic acid, 4-(Methylamino)benzoic acid, Methyl 2-amino-4-chlorobenzoate, Methyl 2-amino-4,5-dimethoxybenzoate, 4-Nitroanthranilic acid, *N*-Phenylanthranilic acid, *N*-Phenylanthranilic acid, and Sodium 4-aminosalicylate. Each possibility represents a separate embodiment.

[064] Other amino acids: (*S*)-α-Amino-γ-butyrolactone, DL-2-Aminocaprylic acid, 7-Aminocephalosporanic acid , 4-Aminocinnamic acid, (*S*)-(+)-α-Aminocyclohexanepropionic acid, (*R*)-Amino-(4-hydroxyphenyl)acetic acid methyl ester, 5-Aminolevulinic acid, 4-Amino-nicotinic acid, 3-Aminophenylacetic acid, 4-Aminophenylacetic acid, 2-Amino-2-phenylbutyric acid, 4-(4-Aminophenyl)butyric acid, 2-(4-Aminophenylthio)acetic acid, DL-α-Amino-2-thiopheneacetic acid, 5-Aminovaleric acid, 8-Benzyl (S)-2-aminooctanedioate, 4-(amino)-1-methylpyrrole-2-carboxylic acid, 4-(amino)tetrahydrothiopyran-4-carboxylic acid , (1*R*,3*S*,4*S*)-2-azabicyclo[2.2.1]heptane-3-carboxylic acid , L-azetidine-2-carboxylic acid, azetidine-3-carboxylic acid, 4-(amino)piperidine-4-carboxylic acid, diaminoacetic acid, Inp-OH, (*R*)-Nip-OH, (*S*)-4-oxopiperidine-2-carboxylic acid, 2-(4-piperazino)-2-(4-fluorophenyl)acetic acid, 2-(4-piperazino)-2-phenylacetic acid, 4-piperidineacetaldehyde, 4-piperidylacetic acid, (−)-L-thioproline, Tle-OH, 3-piperidinecarboxylic acid, L-(+)-Canavanine, (±)-Carnitine, Chlorambucil, 2,6-Diaminopimelic acid, *meso*-2,3-Diaminosuccinic acid, 4-(Dimethylamino)cinnamic acid, 4-(Dimethylamino)phenylacetic acid , Ethyl (*S*)-*N*-Boc-piperidine-3-carboxylate, Ethyl piperazinoacetate , 4-[2-(amino)ethyl]piperazin-1-ylacetic acid, (*R*)-4-(amino)-5-phenylpentanoic acid, (*S*)-azetidine-2-carboxylic acid, azetidine-3-carboxylic acid, guvacine, Inp-OH, (*R*)-Nip-OH, DL-Nip-OH, 4-phenyl-piperidine-4-carboxylic acid, 1-piperazineacetic acid, 4-piperidineacetic acid, (*R*)-piperidine-2-carboxylic acid, (*S*)-piperidine-2-carboxylic acid, (*S*)-1,2,3,4-tetrahydronorharmane-3-carboxylic acid, Tic-OH, D-Tic-OH, Iminodiacetic acid, Indoline-2-carboxylic acid, DL-Kynurenine, L-aziridine-2-carboxylate, Methyl 4-aminobutyrate, (*S*)-2-Piperazinecarboxylic acid, 2-(1-Piperazinyl)acetic acid, (*R*)-(−)-3-Piperidinecarboxylic acid, 2-Pyrrolidone-5-carboxylic acid, (*R*)-(+)-2-Pyrrolidone-5-carboxylic acid, (*R*)-1,2,3,4-Tetrahydro-3-isoquinolinecarboxylic acid, (*S*)-1,2,3,4-Tetrahydro-3-isoquinolinecarboxylic acid, L-4-

Thiazolidinecarboxylic acid, (4*R*)-(−)-2-Thioxo-4-thiazolidinecarboxylic acid, hydrazinoacetic acid, and 3,3′,5-Triiodo-L-thyronine. Each possibility represents a separate embodiment.

[065] The present disclosure provides peptides comprising peptidomimetic compounds having further improved stability and cell permeability properties. Some embodiments comprise a peptide according to any of SEQ ID NOs: 1-64, wherein one of more peptide bonds (-CO-NH-) within the peptide may be substituted, for example, by N-methylated amide bonds (-N(CH_3)-CO-), ester bonds (-C(=O)-O-), ketomethylene bonds (-CO-CH_2-), sulfinylmethylene bonds (-S(=O)-CH_2-), α-aza bonds (-NH-N(R)-CO-), wherein R is any alkyl (e.g., methyl), amine bonds (-CH_2-NH-), sulfide bonds (-CH_2-S-), ethylene bonds (-CH_2CH_2-), hydroxyethylene bonds (-CH(OH)-CH_2-), thioamide bonds (-CS-NH-), olefinic double bonds (-CH=CH-), fluorinated olefinic double bonds (-CF=CH-), or retro amide bonds (-NH-CO-), peptide derivatives (-N(R^x)-CH_2-CO-), wherein R^x is the "normal" side chain, naturally present on the carbon atom. These modifications can occur at any of the bonds along the peptide chain and even at several (2-3) bonds at the same time.

[066] Size variants of the peptides described herein are specifically contemplated. Exemplary peptides are composed of 6 to 50 amino acids. All integer subranges of 6-50 amino acids (e.g., 7 – 50 aa, 8-50 aa, 9-50 aa, 6-49 aa, 6-48 aa, 7-49 aa, and so on) are specifically contemplated as genera of the invention; and all interger values are contemplated as species of the invention. In exemplary embodiments, the peptide comprises at least seven or eight amino acids connected via peptide bonds. In exemplary aspects, the peptide is at least about 9 amino acids in length, at least about 10 amino acids in length, at least about 11 amino acids in length, at least about 12 amino acids in length, or at least about 13 amino acids in length. In exemplary aspects, the peptide is at least about 14 amino acids in length, at least about 15 amino acids in length, at least about 16 amino acids in length, or at least about 17 amino acids in length. In exemplary aspects, the peptide is at least about 18 amino acids in length, at least about 19 amino acids in length, or at least about 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 amino acids in length. In exemplary aspects, the peptide is less than about 50 amino acids in length, less than about 40 amino acids, or less than about 30 amino acids, or less than about 25 amino acids in length. In exemplary aspects, the peptide is about 8 to about 30 amino acids in length or about 8 to about 20 amino acids in length. In exemplary aspects, the peptide is about 10 to about 10 amino acids in length, about 14 to about 20 amino acids in length. In exemplary aspects, the peptide is 8-9, 10-11, 12-13, 14-15, or 16-17 amino acids in length. In some embodiments, the peptide is a 8 mer, 9-mer, 10-mer, 11-mer, 12-mer, 13-mer, 14-mer, 15-mer, 16-mer, 17-mer, 18-mer, 19-mer, or 20-mer.

[067] The peptides of some embodiments are preferably utilized in a linear form, although it will be appreciated that in cases where cyclization does not severely interfere with peptide characteristics, cyclic forms of the peptide can also be utilized and are contemplated as embodiments.

[068] According to some embodiments conjugates comprising any of the peptides and analogs described herein conjugated to a moiety for extending half-life or increasing cell penetration. For example,

the half-life extending moiety may be a peptide or protein and the conjugate is a fusion protein or chimeric polypeptide. Alternatively, the half-life extending moiety may be a polymer, e.g., a polyethylene glycol. The present disclosures furthermore provide dimers and multimers comprising any of the peptides and analogs described herein.

[069] Any moiety known in the art to facilitate actively or passively or enhance permeability of the peptides into cells may be used for conjugation with the peptide core. Non-limitative examples include: hydrophobic moieties such as fatty acids, steroids and bulky aromatic or aliphatic compounds; moieties which may have cell-membrane receptors or carriers, such as steroids, vitamins and sugars, natural and non-natural amino acids and transporter peptides. According to a preferred embodiment, the hydrophobic moiety is a lipid moiety or an amino acid moiety. The permeability-enhancing moiety may be connected to any position in the peptide moiety, directly or through a spacer or linker, preferably to the amino terminus of the peptide moiety. The hydrophobic moiety may preferably comprise a lipid moiety or an amino acid moiety. According to a specific embodiment the hydrophobic moiety is selected from the group consisting of: phospholipids, steroids, sphingosines, ceramides, octyl-glycine, 2-cyclohexylalanine, benzolylphenylalanine, propionoyl (C_3); butanoyl (C_4); pentanoyl (C_5); caproyl (C_6); heptanoyl (C_7); capryloyl (C_8); nonanoyl (C_9); capryl (C_{10}); undecanoyl (C_{11}); lauroyl (C_{12}); tridecanoyl (C_{13}); myristoyl (C_{14}); pentadecanoyl (C_{15}); palmitoyl (C_{16}); phtanoyl ($(CH_3)_4$); heptadecanoyl (C_{16}); stearoyl (C_{18}); nonadecanoyl (C_{19}); arachidoyl (C_{20}); heniecosanoyl (C_{21}); behenoyl (C_{22}); trucisanoyl (C_{23}); and lignoceroyl (C_{24}); wherein said hydrophobic moiety is attached to said chimeric polypeptide with amide bonds, sulfhydryls, amines, alcohols, phenolic groups, or carbon-carbon bonds. Other examples of lipidic moieties which may be used include: Lipofectamine, Transfectace, Transfectam, Cytofectin, DMRIE, DLRIE, GAP-DLRIE, DOTAP, DOPE, DMEAP, DODMP, DOPC, DDAB, DOSPA, EDLPC, EDMPC, DPH, TMADPH, CTAB, lysyl-PE, DC-Cho, -alanyl cholesterol; DCGS, DPPES, DCPE, DMAP, DMPE, DOGS, DOHME, DPEPC, Pluronic, Tween, BRIJ, plasmalogen, phosphatidylethanolamine, phosphatidylcholine, glycerol-3-ethylphosphatidylcholine, dimethyl ammonium propane, trimethyl ammonium propane, diethylammonium propane, triethylammonium propane, dimethyldioctadecylammonium bromide, a sphingolipid, sphingomyelin, a lysolipid, a glycolipid, a sulfatide, a glycosphingolipid, cholesterol, cholesterol ester, cholesterol salt, oil, N-succinyldioleoylphosphatidylethanolamine, 1,2-dioleoyl-glycerol, 1,3-dipalmitoyl-2-succinylglycerol, 1,2-dipalmitoyl-3-succinylglycerol, l-hexadecyl-2-palmitoylglycerophosphatidylethanolamine, palmitoylhomocystiene, N,N'-Bis (dodecyaminocarbonylmethylene)-N,N'-bis((-N,N,N-trimethylammoniumethyl-ami nocarbonylmethylene)ethylenediamine tetraiodide; N,N"-Bis(hexadecylaminocarbonylmethylene)-N,N', N"-tris((-N,N,N-trimethylammonium-ethylaminocarbonylmethylenediethylenetri amine hexaiodide; N,N'- Bis(dodecylaminocarbonylmethylene)-N,N"-bis((-N,N,N-trimethylammonium ethylaminocarbonylmethylene)cyclohexylene-l,4-diamine tetraiodide; l,7,7-tetra-((N,N,N,N-

tetramethylammoniumethylamino-carbonylmethylene)-3-hexadecylarninocarbonyl-methylene-l,3,7-triaazaheptane heptaiodide; N,N,N',N'-tetra((N,N,N-trimethylammonium-ethylaminocarbonylmethylene)-N'-(1,2-dioleoylglycero-3-phosphoethanolamino-carbonylmethylene)diethylenetriamine tetraiodide; dioleoylphosphatidylethanolamine, a fatty acid, a lysolipid, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylglycerol, phosphatidylinositol, a sphingolipid, a glycolipid, a glucolipid, a sulfatide, a glycosphingolipid, phosphatidic acid, palmitic acid, stearic acid, arachidonic acid, oleic acid, a lipid bearing a polymer, a lipid bearing a sulfonated saccharide, cholesterol, tocopherol hemisuccinate, a lipid with an ether-linked fatty acid, a lipid with an ester-linked fatty acid, a polymerized lipid, diacetyl phosphate, stearylamine, cardiolipin, a phospholipid with a fatty acid of 6-8 carbons in length, a phospholipid with asymmetric acyl chains, 6-(5-cholesten-3b-yloxy)-l-thio-b-D-galactopyranoside, digalactosyldiglyceride, 6-(5-cholesten-3b-yloxy)hexyl-6-amino-6-deoxy-l-thio-b-D-galactopyranoside , 6-(5-cholesten-3b-yloxy)hexyl-6-amino-6-deoxyl-l-thio-a-D-mannopyranoside, 12-(((7'-diethylamino-coumarin-3-yl)carbonyl)methylamino)-octadecanoic acid; N-[12-(((7'-diethylaminocoumarin-3-yl)carbonyl)methyl-amino) octadecanoyl]-2-aminopalmitic acid; cholesteryl)4'-trimethyl-ammonio)butanoate; N-succinyldioleoyl-phosphatidylethanolamine; 1,2-dioleoyl-glycerol; 1,2-dipalmitoyl-3-succinyl-glycerol; 1,3-dipalmitoyl-2-succinylglycerol, l-hexadecyl-2-palmitoylglycero-phosphoethanolamine, and palmitoylhomocysteine.

[070] The peptides disclosed herein may be conjugated to one or more moieties that cause the conjugate to function as a prodrug. For example, the N-amino acid related moieties described in US Pat. No. 8969288 and US Pub. 20160058881 can be conjugated to the peptides disclosed herein and such conjugates are included in this disclosure.

[071] According to some embodiments the peptides may be attached (either covalently or non-covalently) to a penetrating agent. As used herein the phrase "penetrating agent" refers to an agent which enhances translocation of any of the attached peptide across a cell membrane. Typically, peptide based penetrating agents have an amino acid composition containing either a high relative abundance of positively charged amino acids such as lysine or arginine, or have sequences that contain an alternating pattern of polar/charged amino acids and non-polar, hydrophobic amino acids. By way of a non-limiting example, cell penetrating peptide (CPP) sequences may be used in order to enhance intracellular penetration. CPPs may include short and long versions of the protein transduction domain (PTD) of HIV TAT protein, such as for example, YARAAARQARA (SEQ ID NO: 65), YGRKKRR (SEQ ID NO: 66), YGRKKRRQRRR (SEQ ID NO: 67), or RRQRR (SEQ ID NO: 68). However, the disclosure is not so limited, and any suitable penetrating agent may be used, as known by those of skill in the art. Another method of enhancing cell penetration is via N-terminal myristoilation. In this protein modification, a myristoyl group (derived from

myristic acid) is covalently attached via an amide bond to the alpha-amino group of an N-terminal amino acid of the peptide.

[072] According to some embodiments the peptide is modified to include a duration enhancing moiety. The duration enhancing moiety can be a water soluble polymer, or a long chain aliphatic group. In some embodiments, a plurality of duration enhancing moieties may be attached to the peptide, in which case each linker to each duration enhancing moiety is independently selected from the linkers described herein.

[073] According to some embodiments the amino terminus of the peptide is modified, e.g. acylated. According to additional embodiments the carboxy terminus is modified, e.g., it may be acylated, amidated, reduced or esterified. In accordance with some embodiments, the peptide comprises an acylated amino acid (e.g., a non-coded acylated amino acid (e.g., an amino acid comprising an acyl group which is non-native to a naturally-occurring amino acid)). In accordance with one embodiment, the peptide comprises an acyl group which is attached to the peptide via an ester, thioester, or amide linkage for purposes of prolonging half-life in circulation and/or delaying the onset of and/or extending the duration of action and/or improving resistance to proteases. Acylation can be carried out at any position within the peptide, (e.g., the amino acid at the C-terminus), provided that activity is retained, if not enhanced. The peptide in some embodiments can be acylated at the same amino acid position where a hydrophilic moiety is linked, or at a different amino acid position. The acyl group can be covalently linked directly to an amino acid of the peptide, or indirectly to an amino acid of the peptide via a spacer, wherein the spacer is positioned between the amino acid of the peptide and the acyl group.

[074] In specific aspects, the peptide is modified to comprise an acyl group by direct acylation of an amine, hydroxyl, or thiol of a side chain of an amino acid of the peptide. In this regard, the acylated peptide can comprise the amino acid sequence of any of SEQ ID NOs: 1-64, or a modified amino acid sequence thereof comprising one or more of the amino acid modifications described herein.

[075] In some embodiments, the peptide comprises a spacer between the analog and the acyl group. In some embodiments, the peptide is covalently bound to the spacer, which is covalently bound to the acyl group. In some embodiments, the spacer is an amino acid comprising a side chain amine, hydroxyl, or thiol, or a dipeptide or tripeptide comprising an amino acid comprising a side chain amine, hydroxyl, or thiol. The amino acid to which the spacer is attached can be any amino acid (e.g., a singly or doubly α-substituted amino acid) comprising a moiety which permits linkage to the spacer. For example, an amino acid comprising a side chain NH_2, -OH, or -COOH (e.g., Lys, Orn, Ser, Asp, or Glu) is suitable. In some embodiments, the spacer is an amino acid comprising a side chain amine, hydroxyl, or thiol, or a dipeptide or tripeptide comprising an amino acid comprising a side chain amine, hydroxyl, or thiol. When acylation occurs through an amine group of a spacer, the acylation can occur through the alpha amine of the amino acid or a side chain

amine. In the instance in which the alpha amine is acylated, the amino acid of the spacer can be any amino acid. For example, the amino acid of the spacer can be a hydrophobic amino acid, e.g., Gly, Ala, Val, Leu, Ile, Trp, Met, Phe, Tyr, 6-amino hexanoic acid, 5-aminovaleric acid, 7-aminoheptanoic acid, and 8-aminooctanoic acid. Alternatively, the amino acid of the spacer can be an acidic residue, e.g., Asp, Glu, homoglutamic acid, homocysteic acid, cysteic acid, gamma-glutamic acid. In the instance in which the side chain amine of the amino acid of the spacer is acylated, the amino acid of the spacer is an amino acid comprising a side chain amine. In this instance, it is possible for both the alpha amine and the side chain amine of the amino acid of the spacer to be acylated, such that the peptide is diacylated. Embodiments include such diacylated molecules. When acylation occurs through a hydroxyl group of a spacer, the amino acid or one of the amino acids of the dipeptide or tripeptide can be Ser. When acylation occurs through a thiol group of a spacer, the amino acid or one of the amino acids of the dipeptide or tripeptide can be Cys. In some embodiments, the spacer is a hydrophilic bifunctional spacer. In certain embodiments, the hydrophilic bifunctional spacer comprises two or more reactive groups, e.g., an amine, a hydroxyl, a thiol, and a carboxyl group or any combinations thereof. In certain embodiments, the hydrophilic bifunctional spacer comprises a hydroxyl group and a carboxylate. In other embodiments, the hydrophilic bifunctional spacer comprises an amine group and a carboxylate. In other embodiments, the hydrophilic bifunctional spacer comprises a thiol group and a carboxylate.

[076] In a specific embodiment, the spacer comprises an amino poly(alkyloxy)carboxylate. In this regard, the spacer can comprise, for example, $NH_2(CH_2CH_2O)_n(CH_2)_mCOOH$, wherein m is any integer from 1 to 6 and n is any integer from 2 to 12, such as, e.g., 8-amino-3,6-dioxaoctanoic acid, which is commercially available from Peptides International, Inc. (Louisville, Ky.). In some embodiments, the spacer is a hydrophobic bifunctional spacer. Hydrophobic bifunctional spacers are known in the art. See, e.g., *Bioconjugate Techniques*, G. T. Hermanson (Academic Press, San Diego, Calif., 1996), which is incorporated by reference in its entirety. In certain embodiments, the hydrophobic bifunctional spacer comprises two or more reactive groups, e.g., an amine, a hydroxyl, a thiol, and a carboxyl group or any combinations thereof. In certain embodiments, the hydrophobic bifunctional spacer comprises a hydroxyl group and a carboxylate. In other embodiments, the hydrophobic bifunctional spacer comprises an amine group and a carboxylate. In other embodiments, the hydrophobic bifunctional spacer comprises a thiol group and a carboxylate. Suitable hydrophobic bifunctional spacers comprising a carboxylate and a hydroxyl group or a thiol group are known in the art and include, for example, 8-hydroxyoctanoic acid and 8-mercaptooctanoic acid. In some embodiments, the bifunctional spacer is not a dicarboxylic acid comprising an unbranched, methylene of 1-7 carbon atoms between the carboxylate groups. In some embodiments, the bifunctional spacer is a dicarboxylic acid comprising an unbranched, methylene of 1-7 carbon atoms between the carboxylate groups. The spacer (e.g., amino acid,

dipeptide, tripeptide, hydrophilic bifunctional spacer, or hydrophobic bifunctional spacer) in specific embodiments is 3 to 10 atoms (e.g., 6 to 10 atoms, (e.g., 6, 7, 8, 9, or 10 atoms) in length. In more specific embodiments, the spacer is about 3 to 10 atoms (e.g., 6 to 10 atoms) in length and the acyl group is a C_{12} to C_{18} fatty acyl group, e.g., C_{14} fatty acyl group, C_{16} fatty acyl group, such that the total length of the spacer and acyl group is 14 to 28 atoms, e.g., about 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28 atoms. In some embodiments, the length of the spacer and acyl group is 17 to 28 (e.g., 19 to 26, 19 to 21) atoms. In accordance with certain foregoing embodiments, the bifunctional spacer can be a synthetic or naturally occurring amino acid (including, but not limited to, any of those described herein) comprising an amino acid backbone that is 3 to 10 atoms in length (e.g., 6-amino hexanoic acid, 5-aminovaleric acid, 7-aminoheptanoic acid, and 8-aminooctanoic acid). Alternatively, the spacer can be a dipeptide or tripeptide spacer having a peptide backbone that is 3 to 10 atoms (e.g., 6 to 10 atoms) in length. Each amino acid of the dipeptide or tripeptide spacer can be the same as or different from the other amino acid(s) of the dipeptide or tripeptide and can be independently selected from the group consisting of: naturally-occurring or coded and/or non-coded or non-naturally occurring amino acids, including, for example, any of the D or L isomers of the naturally-occurring amino acids (Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Arg, Ser, Thr, Val, Trp, Tyr), or any D or L isomers of the non-naturally occurring or non-coded amino acids selected from the group consisting of: β-alanine (β-Ala), N-α-methyl-alanine (Me-Ala), aminobutyric acid (Abu), γ-aminobutyric acid (7-Abu), aminohexanoic acid (ε-Ahx), aminoisobutyric acid (Aib), aminomethylpyrrole carboxylic acid, aminopiperidinecarboxylic acid, aminoserine (Ams), aminotetrahydropyran-4-carboxylic acid, arginine N-methoxy-N-methyl amide, β-aspartic acid (β-Asp), azetidine carboxylic acid, 3-(2-benzothiazolyl)alanine, α-tert-butylglycine, 2-amino-5-ureido-n-valeric acid (citrulline, Cit), β-Cyclohexylalanine (Cha), acetamidomethyl-cysteine, diaminobutanoic acid (Dab), diaminopropionic acid (Dpr), dihydroxyphenylalanine (DOPA), dimethylthiazolidine (DMTA), γ-Glutamic acid (γ-Glu), homoserine (Hse), hydroxyproline (Hyp), isoleucine N-methoxy-N-methyl amide, methyl-isoleucine (MeIle), isonipecotic acid (Isn), methyl-leucine (MeLeu), methyl-lysine, dimethyl-lysine, trimethyl-lysine, methanoproline, methionine-sulfoxide (Met(O)), methionine-sulfone (Met(O_2)), norleucine (Nle), methyl-norleucine (Me-Nle), norvaline (Nva), ornithine (Orn), para-aminobenzoic acid (PABA), penicillamine (Pen), methylphenylalanine (MePhe), 4-Chlorophenylalanine (Phe(4-Cl)), 4-fluorophenylalanine (Phe(4-F)), 4-nitrophenylalanine (Phe(4-NO_2)), 4-cyanophenylalanine ((Phe(4-CN)), phenylglycine (Phg), piperidinylalanine, piperidinylglycine, 3,4-dehydroproline, pyrrolidinylalanine, sarcosine (Sar), selenocysteine (Sec), O-Benzyl-phosphoserine, 4-amino-3-hydroxy-6-methylheptanoic acid (Sta), 4-amino-5-cyclohexyl-3-hydroxypentanoic acid (ACHPA), 4-amino-3-hydroxy-5-phenylpentanoic acid (AHPPA), 1,2,3,4,-tetrahydro-isoquinoline-3-carboxylic acid (Tic), tetrahydropyranglycine, thienylalanine (Thi), O-benzyl-phosphotyrosine, O-Phosphotyrosine, methoxytyrosine,

ethoxytyrosine, O-(bis-dimethylamino-phosphono)-tyrosine, tyrosine sulfate tetrabutylamine, methyl-valine (MeVal), and alkylated 3-mercaptopropionic acid. In some embodiments, the spacer comprises an overall negative charge, e.g., comprises one or two negative-charged amino acids. In some embodiments, the dipeptide is not any of the dipeptides of general structure A-B, wherein A is selected from the group consisting of Gly, Gln, Ala, Arg, Asp, Asn, Ile, Leu, Val, Phe, and Pro, wherein B is selected from the group consisting of Lys, His, Trp. In some embodiments, the dipeptide spacer is selected from the group consisting of: Ala-Ala, β-Ala-β-Ala, Leu-Leu, Pro-Pro, γ-aminobutyric acid-γ-aminobutyric acid, Glu-Glu, and γ-Glu-γ-Glu.

[077] Suitable methods of peptide acylation via amines, hydroxyls, and thiols are known in the art. See, for example, Miller, *Biochem Biophys Res Commun* 218: 377-382 (1996); Shimohigashi and Stammer, *Int J Pept Protein Res* 19: 54-62 (1982); and Previero et al., *Biochim Biophys Acta* 263: 7-13 (1972) (for methods of acylating through a hydroxyl); and San and Silvius, *J Pept Res* 66: 169-180 (2005) (for methods of acylating through a thiol); Bioconjugate Chem. "Chemical Modifications of Proteins: History and Applications" pages 1, 2-12 (1990); Hashimoto et al., Pharmaceutical Res. "Synthesis of Palmitoyl Derivatives of Insulin and their Biological Activity" Vol. 6, No: 2 pp. 171-176 (1989). The acyl group of the acylated amino acid can be of any size, e.g., any length carbon chain, and can be linear or branched. In some specific embodiments, the acyl group is a C_4 to C_{30} fatty acid. For example, the acyl group can be any of a C_4 fatty acid, C_6 fatty acid, C_8 fatty acid, C_{10} fatty acid, C_{12} fatty acid, C_{14} fatty acid, C_{16} fatty acid, C_{18} fatty acid, C_{20} fatty acid, C_{22} fatty acid, C_{24} fatty acid, C_{26} fatty acid, C_{28} fatty acid, or a C_{30} fatty acid. In some embodiments, the acyl group is a C_8 to C_{20} fatty acid, e.g., a C_{14} fatty acid or a C_{16} fatty acid. In an alternative embodiment, the acyl group is a bile acid. The bile acid can be any suitable bile acid, including, but not limited to, cholic acid, chenodeoxycholic acid, deoxycholic acid, lithocholic acid, taurocholic acid, glycocholic acid, and cholesterol acid. In some embodiments, the peptide comprises an acylated amino acid by acylation of a long chain alkane on the peptide. In specific aspects, the long chain alkane comprises an amine, hydroxyl, or thiol group (e.g., octadecylamine, tetradecanol, and hexadecanethiol) which reacts with a carboxyl group, or activated form thereof, of the peptide. The carboxyl group, or activated form thereof, of the peptide can be part of a side chain of an amino acid (e.g., glutamic acid, aspartic acid) of the peptide or can be part of the analog backbone. In certain embodiments, the peptide is modified to comprise an acyl group by acylation of the long chain alkane by a spacer which is attached to the peptide. In specific aspects, the long chain alkane comprises an amine, hydroxyl, or thiol group which reacts with a carboxyl group, or activated form thereof, of the spacer. Suitable spacers comprising a carboxyl group, or activated form thereof, are described herein and include, for example, bifunctional spacers, e.g., amino acids, dipeptides, tripeptides, hydrophilic bifunctional spacers and hydrophobic bifunctional spacers.

[078] As used herein, the term "activated form" of a carboxyl group refers to a carboxyl group with the general formula R(C=O)X, wherein X is a leaving group and R is the peptide or the spacer. For example, activated forms of a carboxyl groups may include, but are not limited to, acyl chlorides, anhydrides, and esters. In some embodiments, the activated carboxyl group is an ester with a N-hydroxysuccinimide ester (NHS) leaving group.

[079] With regard to these aspects, in which a long chain alkane is acylated by the peptide or the spacer, the long chain alkane may be of any size and can comprise any length of carbon chain. The long chain alkane can be linear or branched. In certain aspects, the long chain alkane is a C4 to C30 alkane. For example, the long chain alkane can be any of a C_4 alkane, C_6 alkane, C_8 alkane, C_{10} alkane, C_{12} alkane, C_{14} alkane, C_{16} alkane, C_{18} alkane, C_{20} alkane, C_{22} alkane, C_{24} alkane, C_{26} alkane, C_{28} alkane, or a C_{30} alkane. In some embodiments, the long chain alkane comprises a C_8 to C_{20} alkane, e.g., a C_{14} alkane, C_{16} alkane, or a C_{18} alkane.

[080] Also, in some embodiments, an amine, hydroxyl, or thiol group of the peptide is acylated with a cholesterol acid. In a specific embodiment, the peptide is linked to the cholesterol acid through an alkylated des-amino Cys spacer, i.e., an alkylated 3-mercaptopropionic acid spacer. The alkylated des-amino Cys spacer can be, for example, a des-amino-Cys spacer comprising a dodecaethylene glycol moiety.

[081] The peptides described herein can be further modified to comprise a hydrophilic moiety. In some specific embodiments the hydrophilic moiety can comprise a polyethylene glycol (PEG) chain. The incorporation of a hydrophilic moiety can be accomplished through any suitable means, such as any of the methods described herein. In this regard, the acylated peptide can of any of SEQ ID NOs: 1-64, including any of the modifications described herein, in which at least one of the amino acids comprises an acyl group and at least one of the amino acids is covalently bonded to a hydrophilic moiety (e.g., PEG). In some embodiments, the acyl group is attached via a spacer comprising Cys, Lys, Orn, homo-Cys, or Ac-Phe, and the hydrophilic moiety is incorporated at a Cys residue.

[082] Alternatively, the peptides can comprise a spacer, wherein the spacer is both acylated and modified to comprise the hydrophilic moiety. Nonlimiting examples of suitable spacers include a spacer comprising one or more amino acids selected from the group consisting of Cys, Lys, Orn, homo-Cys, and Ac-Phe.

[083] In accordance with some embodiments, the peptide comprises an alkylated amino acid (e.g., a non-coded alkylated amino acid (e.g., an amino acid comprising an alkyl group which is non-native to a naturally-occurring amino acid)). Alkylation can be carried out at any positions within the peptides, including any of the positions described herein as a site for acylation, including but not limited to, any of amino acid positions, at a position within a C-terminal extension, or at the C-terminus, provided that the biological activity is retained. The alkyl group can be covalently linked directly to an amino acid of the peptides, or indirectly to an amino acid of the peptides via a

spacer, wherein the spacer is positioned between the amino acid of the peptides and the alkyl group. The peptides may be alkylated at the same amino acid position where a hydrophilic moiety is linked, or at a different amino acid position. In specific aspects, the peptides may be modified to comprise an alkyl group by direct alkylation of an amine, hydroxyl, or thiol of a side chain of an amino acid of the peptides. In this regard, the alkylated peptides can comprise an amino acid sequence with at least one of the amino acids modified to any amino acid comprising a side chain amine, hydroxyl, or thiol. In yet other embodiments, the amino acid comprising a side chain amine, hydroxyl, or thiol is a disubstituted amino acid. In some embodiments, the alkylated peptide comprises a spacer between the peptide and the alkyl group. In some embodiments, the peptide is covalently bound to the spacer, which is covalently bound to the alkyl group. In some exemplary embodiments, the peptide is modified to comprise an alkyl group by alkylation of an amine, hydroxyl, or thiol of a spacer, which spacer is attached to a side chain of an amino acid. The amino acid to which the spacer is attached can be any amino acid comprising a moiety which permits linkage to the spacer. For example, an amino acid comprising a side chain NH_2, -OH, or -COOH (e.g., Lys, Orn, Ser, Asp, or Glu) is suitable. In some embodiments, the spacer is an amino acid comprising a side chain amine, hydroxyl, or thiol or a dipeptide or tripeptide comprising an amino acid comprising a side chain amine, hydroxyl, or thiol. When alkylation occurs through an amine group of a spacer, the alkylation can occur through the alpha amine of an amino acid or a side chain amine. In the instance in which the alpha amine is alkylated, the amino acid of the spacer can be any amino acid. For example, the amino acid of the spacer can be a hydrophobic amino acid, e.g., Gly, Ala, Val, Leu, Ile, Trp, Met, Phe, Tyr, 6-amino hexanoic acid, 5-aminovaleric acid, 7-aminoheptanoic acid, and 8-aminooctanoic acid. Alternatively, the amino acid of the spacer can be an acidic residue, e.g., Asp and Glu, provided that the alkylation occurs on the alpha amine of the acidic residue. In the instance in which the side chain amine of the amino acid of the spacer is alkylated, the amino acid of the spacer is an amino acid comprising a side chain amine, e.g., an amino acid of Formulas I-II (e.g., Lys or Orn). In this instance, it is possible for both the alpha amine and the side chain amine of the amino acid of the spacer to be alkylated, such that the peptide is dialkylated. Embodiments include such dialkylated molecules. When alkylation occurs through a hydroxyl group of a spacer, the amino acid can be Ser. When alkylation occurs through a thiol group of spacer, the amino acid can be Cys. In some embodiments, the spacer is a hydrophilic bifunctional spacer. In certain embodiments, the hydrophilic bifunctional spacer comprises two or more reactive groups, e.g., an amine, a hydroxyl, a thiol, and a carboxyl group or any combinations thereof. In certain embodiments, the hydrophilic bifunctional spacer comprises a hydroxyl group and a carboxylate. In other embodiments, the hydrophilic bifunctional spacer comprises an amine group and a carboxylate. In other embodiments, the hydrophilic bifunctional spacer comprises a thiol group and a carboxylate. In a specific embodiment, the spacer comprises an amino poly(alkyloxy)carboxylate. In this regard, the

spacer can comprise, for example, $NH_2(CH_2CH_2O)_n(CH_2)_mCOOH$, wherein m is any integer from 1 to 6 and n is any integer from 2 to 12, such as, e.g., 8-amino-3,6-dioxaoctanoic acid, which is commercially available from Peptides International, Inc. (Louisville, Ky.). Suitable hydrophobic bifunctional spacers comprising a carboxylate and a hydroxyl group or a thiol group are known in the art and include, for example, 8-hydroxyoctanoic acid and 8-mercaptooctanoic acid. The spacer (e.g., amino acid, dipeptide, tripeptide, hydrophilic bifunctional spacer, or hydrophobic bifunctional spacer) in specific embodiments is 3 to 10 atoms (e.g., 6 to 10 atoms, (e.g., 6, 7, 8, 9, or 10 atoms)) in length. In more specific embodiments, the spacer is about 3 to 10 atoms (e.g., 6 to 10 atoms) in length and the alkyl is a C_{12} to C_{18} alkyl group, e.g., C_{14} alkyl group, C_{16} alkyl group, such that the total length of the spacer and alkyl group is 14 to 28 atoms, e.g., about 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28 atoms. In some embodiments, the length of the spacer and alkyl is 17 to 28 (e.g., 19 to 26, 19 to 21) atoms. In accordance with certain foregoing embodiments, the bifunctional spacer can be a synthetic or non-naturally occurring or non-coded amino acid comprising an amino acid backbone that is 3 to 10 atoms in length (e.g., 6-amino hexanoic acid, 5-aminovaleric acid, 7-aminoheptanoic acid, and 8-aminooctanoic acid). Alternatively, the spacer can be a dipeptide or tripeptide spacer having a peptide backbone that is 3 to 10 atoms (e.g., 6 to 10 atoms) in length. The dipeptide or tripeptide spacer can be composed of naturally-occurring or coded and/or non-coded or non-naturally occurring amino acids, including, for example, any of the amino acids taught herein. In some embodiments, the spacer comprises an overall negative charge, e.g., comprises one or two negative-charged amino acids. In some embodiments, the dipeptide spacer is selected from the group consisting of: Ala-Ala, β-Ala-β-Ala, Leu-Leu, Pro-Pro, γ-aminobutyric acid-γ-aminobutyric acid, and γ-Glu-γ-Glu. Suitable methods of peptide alkylation via amines, hydroxyls, and thiols are known in the art. For example, a Williamson ether synthesis can be used to form an ether linkage between a hydroxyl group of the peptides and the alkyl group. Also, a nucleophilic substitution reaction of the peptide with an alkyl halide can result in any of an ether, thioether, or amino linkage. The alkyl group of the alkylated peptides can be of any size, e.g., any length carbon chain, and can be linear or branched. In some embodiments, the alkyl group is a C_4 to C_{30} alkyl. For example, the alkyl group can be any of a C_4 alkyl, C_6 alkyl, C_8 alkyl, C_{10} alkyl, C_{12} alkyl, C_{14} alkyl, C_{16} alkyl, C_{18} alkyl, C_{20} alkyl, C_{22} alkyl, C_{24} alkyl, C_{26} alkyl, C_{28} alkyl, or a C_{30} alkyl. In some embodiments, the alkyl group is a C_8 to C_{20} alkyl, e.g., a C_{14} alkyl or a C_{16} alkyl. In some embodiments of the disclosure, the peptide comprises an alkylated amino acid by reacting a nucleophilic, long chain alkane with the peptide, wherein the peptide comprises a leaving group suitable for nucleophilic substitution. In specific aspects, the nucleophilic group of the long chain alkane comprises an amine, hydroxyl, or thiol group (e.g., octadecylamine, tetradecanol, and hexadecanethiol). The leaving group of the peptide can be part of a side chain of an amino acid or can be part of the peptide backbone. Suitable leaving groups include, for example, N-hydroxysuccinimide, halogens, and sulfonate esters. In

certain embodiments, the peptide is modified to comprise an alkyl group by reacting the nucleophilic, long chain alkane with a spacer which is attached to the peptide, wherein the spacer comprises the leaving group. In specific aspects, the long chain alkane comprises an amine, hydroxyl, or thiol group. In certain embodiments, the spacer comprising the leaving group can be any spacer discussed herein, e.g., amino acids, dipeptides, tripeptides, hydrophilic bifunctional spacers and hydrophobic bifunctional spacers further comprising a suitable leaving group. With regard to these aspects of the disclosure, in which a long chain alkane is alkylated by the peptides or the spacer, the long chain alkane may be of any size and can comprise any length of carbon chain. The long chain alkane can be linear or branched. In certain aspects, the long chain alkane is a C_4 to C_{30} alkane. For example, the long chain alkane can be any of a C_4 alkane, C_6 alkane, C_8 alkane, C_{10} alkane, C_{12} alkane, C_{14} alkane, C_{16} alkane, C_{18} alkane, C_{20} alkane, C_{22} alkane, C_{24} alkane, C_{26} alkane, C_{28} alkane, or a C_{30} alkane. In some embodiments, the long chain alkane comprises a C_8 to C_{20} alkane, e.g., a C_{14} alkane, C_{16} alkane, or a C_{18} alkane. Also, in some embodiments, alkylation can occur between the peptides and a cholesterol moiety. For example, the hydroxyl group of cholesterol can displace a leaving group on the long chain alkane to form a cholesterol- peptides product. The alkylated peptides described herein can be further modified to comprise a hydrophilic moiety. In some specific embodiments, the hydrophilic moiety can comprise a polyethylene glycol (PEG) chain. The incorporation of a hydrophilic moiety can be accomplished through any suitable means, such as any of the methods described herein. Alternatively, the alkylated peptides can comprise a spacer, wherein the spacer is both alkylated and modified to comprise the hydrophilic moiety. Nonlimiting examples of suitable spacers include a spacer comprising one or more amino acids selected from the group consisting of Cys, Lys, Orn, homo-Cys, and Ac-Phe.

[084] In some embodiments, the peptide comprises at position 1 or 2, or at both positions 1 and 2, an amino acid which achieves resistance of the peptides to peptidase cleavage. In some embodiments, the peptide comprises at position 1 an amino acid selected from the group consisting of: D-histidine, desaminohistidine, hydroxyl-histidine, acetyl-histidine, homo-histidine, N-methyl histidine, alpha-methyl histidine, imidazole acetic acid, or alpha, alpha-dimethyl imidazole acetic acid (DMIA). In some embodiments, the peptide comprises at position 2 an amino acid selected from the group consisting of: D-serine, D-alanine, valine, glycine, N-methyl serine, N-methyl alanine, or alpha, aminoisobutyric acid. In some embodiments, the peptide comprises at position 2 an amino acid which achieves resistance of the peptide to peptidases and the amino acid which achieves resistance of the peptide to peptidases is not D-serine. In some embodiments, this covalent bond is an intramolecular bridge other than a lactam bridge. For example, suitable covalent bonding methods include any one or more of olefin metathesis, lanthionine-based cyclization, disulfide bridge or modified sulfur-containing bridge formation, the use of α,ω-

diaminoalkane tethers, the formation of metal-atom bridges, and other means of peptide cyclization.

[085] In some embodiments, the peptide is modified by amino acid substitutions and/or additions that introduce a charged amino acid into the C-terminal portion of the analog. In some embodiments, such modifications enhance stability and solubility. As used herein the term "charged amino acid" or "charged residue" refers to an amino acid that comprises a side chain that is negative-charged (i.e., de-protonated) or positive-charged (i.e., protonated) in aqueous solution at physiological pH. In some aspects, these amino acid substitutions and/or additions that introduce a charged amino acid modifications may be at a C-terminal position. In some embodiments, one, two or three (and in some instances, more than three) charged amino acids may be introduced at the C-terminal position. In exemplary embodiments, one, two or all of the charged amino acids may be negative-charged. The negative-charged amino acid in some embodiments is aspartic acid, glutamic acid, cysteic acid, homocysteic acid, or homoglutamic acid. In some aspects, these modifications increase solubility.

[086] In accordance with some embodiments, the peptides disclosed herein may be modified by truncation of the C-terminus by one or two amino acid residues. In this regard, the peptides can comprise the sequences (SEQ ID NOs: 1-64), optionally with any of the additional modifications described herein.

[087] In some embodiments, the peptide comprises a modified SEQ ID NOs: 1-64 in which the carboxylic acid of the C-terminal amino acid is replaced with a charge-neutral group, such as an amide or ester. Accordingly, in some embodiments, the peptide is an amidated peptide, such that the C-terminal residue comprises an amide in place of the alpha carboxylate of an amino acid. As used herein a general reference to a peptide or analog is intended to encompass peptides that have a modified amino terminus, a modified carboxy terminus, or modifications of both amino and carboxy termini. For example, an amino acid chain composing an amide group in place of the terminal carboxylic acid is intended to be encompassed by an amino acid sequence designating the standard amino acids.

[088] In accordance with some embodiments, the peptides disclosed herein may be modified by conjugation on at least one amino acid residue. In this regard, the peptides can comprise the sequences (SEQ ID NOs: 1-64), optionally with any of the additional conjugations described herein.

[089] The disclosure further provides conjugates comprising one or more of the peptides described herein conjugated to a heterologous moiety. As used herein, the term "heterologous moiety" is synonymous with the term "conjugate moiety" and refers to any molecule (chemical or biochemical, naturally-occurring or non-coded) which is different from the peptides described herein. Exemplary conjugate moieties that can be linked to any of the analogs described herein include but are not limited to a heterologous peptide or polypeptide (including for example, a

plasma protein), a targeting agent, an immunoglobulin or portion thereof (e.g., variable region, CDR, or Fc region), a diagnostic label such as a radioisotope, fluorophore or enzymatic label, a polymer including water soluble polymers, or other therapeutic or diagnostic agents. In some embodiments a conjugate is provided comprising a peptide and a plasma protein, wherein the plasma protein is selected from the group consisting of albumin, transferin, fibrinogen and globulins. In some embodiments the plasma protein moiety of the conjugate is albumin or transferin.

[090] The conjugate in some embodiments comprises one or more of the peptides described herein and one or more of: a different peptide (which is distinct from the peptides described herein), a polypeptide, a nucleic acid molecule, an antibody or fragment thereof, a polymer, a quantum dot, a small molecule, a toxin, a diagnostic agent, a carbohydrate, an amino acid. In some embodiments, the heterologous moiety is a polymer. In some embodiments, the polymer is selected from the group consisting of: polyamides, polycarbonates, polyalkylenes and derivatives thereof including, polyalkylene glycols, polyalkylene oxides, polyalkylene terepthalates, polymers of acrylic and methacrylic esters, including poly(methyl methacrylate), poly(ethyl methacrylate), poly(butylmethacrylate), poly(isobutyl methacrylate), poly(hexylmethacrylate), poly(isodecyl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), and poly(octadecyl acrylate), polyvinyl polymers including polyvinyl alcohols, polyvinyl ethers, polyvinyl esters, polyvinyl halides, poly(vinyl acetate), and polyvinylpyrrolidone, polyglycolides, polysiloxanes, polyurethanes and co-polymers thereof, celluloses including alkyl cellulose, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitro celluloses, methyl cellulose, ethyl cellulose, hydroxypropyl cellulose, hydroxy-propyl methyl cellulose, hydroxybutyl methyl cellulose, cellulose acetate, cellulose propionate, cellulose acetate butyrate, cellulose acetate phthalate, carboxylethyl cellulose, cellulose triacetate, and cellulose sulphate sodium salt, polypropylene, polyethylenes including poly(ethylene glycol), poly(ethylene oxide), and poly(ethylene terephthalate), and polystyrene. In some aspects, the polymer is a biodegradable polymer, including a synthetic biodegradable polymer (e.g., polymers of lactic acid and glycolic acid, polyanhydrides, poly(ortho)esters, polyurethanes, poly(butic acid), poly(valeric acid), and poly(lactide-cocaprolactone)), and a natural biodegradable polymer (e.g., alginate and other polysaccharides including dextran and cellulose, collagen, chemical derivatives thereof (substitutions, additions of chemical groups, for example, alkyl, alkylene, hydroxylations, oxidations, and other modifications routinely made by those skilled in the art), albumin and other hydrophilic proteins (e.g., zein and other prolamines and hydrophobic proteins)), as well as any copolymer or mixture thereof. In general, these materials degrade either by enzymatic hydrolysis or exposure to water in vivo, by surface or bulk erosion. In some aspects, the polymer is a bioadhesive polymer, such as a bioerodible hydrogel described by H. Sawhney, et al., [Macromolecules, 1993, 26, 581-587] the teachings of which are incorporated herein,

polyhyaluronic acids, casein, gelatin, glutin, polyanhydrides, polyacrylic acid, alginate, chitosan, poly(methyl methacrylates), poly(ethyl methacrylates), poly(butylmethacrylate), poly(isobutyl methacrylate), poly(hexylmethacrylate), poly(isodecyl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), and poly(octadecyl acrylate).

[091] In some embodiments, the polymer is a water-soluble polymer or a hydrophilic polymer. Hydrophilic polymers are further described herein under "Hydrophilic Moieties." Suitable water-soluble polymers are known in the art and include, for example, polyvinylpyrrolidone, hydroxypropyl cellulose (HPC; Klucel), hydroxypropyl methylcellulose (HPMC; Methocel), nitrocellulose, hydroxypropyl ethylcellulose, hydroxypropyl butylcellulose, hydroxypropyl pentylcellulose, methyl cellulose, ethylcellulose (Ethocel), hydroxyethyl cellulose, various alkyl celluloses and hydroxyalkyl celluloses, various cellulose ethers, cellulose acetate, carboxymethyl cellulose, sodium carboxymethyl cellulose, calcium carboxymethyl cellulose, vinyl acetate/crotonic acid copolymers, poly-hydroxyalkyl methacrylate, hydroxymethyl methacrylate, methacrylic acid copolymers, polymethacrylic acid, polymethylmethacrylate, maleic anhydride/methyl vinyl ether copolymers, poly vinyl alcohol, sodium and calcium polyacrylic acid, polyacrylic acid, acidic carboxy polymers, carboxypolymethylene, carboxyvinyl polymers, polyoxyethylene polyoxypropylene copolymer, polymethylvinylether co-maleic anhydride, carboxymethylamide, potassium methacrylate divinylbenzene co-polymer, polyoxyethyleneglycols, polyethylene oxide, and derivatives, salts, and combinations thereof. In specific embodiments, the polymer is a polyalkylene glycol, including, for example, polyethylene glycol (PEG).

[092] In some embodiments, the heterologous moiety is a carbohydrate. In some embodiments, the carbohydrate is a monosaccharide (e.g., glucose, galactose, fructose), a disaccharide (e.g., sucrose, lactose, maltose), an oligosaccharide (e.g., raffinose, stachyose), a polysaccharide (a starch, amylase, amylopectin, cellulose, chitin, callose, laminarin, xylan, mannan, fucoidan, galactomannan.

[093] In some embodiments, the heterologous moiety is a lipid. The lipid, in some embodiments, is a fatty acid, eicosanoid, prostaglandin, leukotriene, thromboxane, N-acyl ethanolamine), glycerolipid (e.g., mono-, di-, tri-substituted glycerols), glycerophospholipid (e.g., phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine, phosphatidylserine), sphingolipid (e.g., sphingosine, ceramide), sterol lipid (e.g., steroid, cholesterol), prenol lipid, saccharolipid, or a polyketide, oil, wax, cholesterol, sterol, fat-soluble vitamin, monoglyceride, diglyceride, triglyceride, a phospholipid.

[094] In some embodiments, the heterologous moiety is attached via non-covalent or covalent bonding to the peptide of the present disclosure. In certain aspects, the heterologous moiety is attached to the peptide of the present disclosure via a linker. Linkage can be accomplished by covalent chemical

bonds, physical forces such electrostatic, hydrogen, ionic, van der Waals, or hydrophobic or hydrophilic interactions. A variety of non-covalent coupling systems may be used, including biotin-avidin, ligand/receptor, enzyme/substrate, nucleic acid/nucleic acid binding protein, lipid/lipid binding protein, cellular adhesion molecule partners; or any binding partners or fragments thereof which have affinity for each other. The peptide in some embodiments is linked to conjugate moieties via direct covalent linkage by reacting targeted amino acid residues of the analog with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues of these targeted amino acids. Reactive groups on the analog or conjugate moiety include, e.g., an aldehyde, amino, ester, thiol, α-haloacetyl, maleimido or hydrazino group. Derivatizing agents include, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride or other agents known in the art. Alternatively, the conjugate moieties can be linked to the analog indirectly through intermediate carriers, such as polysaccharide or polypeptide carriers. Examples of polysaccharide carriers include aminodextran. Examples of suitable polypeptide carriers include polylysine, polyglutamic acid, polyaspartic acid, co-polymers thereof, and mixed polymers of these amino acids and others, e.g., serines, to confer desirable solubility properties on the resultant loaded carrier. Cysteinyl residues are most commonly reacted with α-haloacetates (and corresponding amines), such as chloroacetic acid, chloroacetamide to give carboxymethyl or carboxyamidomethyl derivatives. Cysteinyl residues also may be derivatized by reaction with bromotrifluoroacetone, alpha-bromo-β-(5-imidozoyl)propionic acid, chloroacetyl phosphate, N-alkylmaleimides, 3-nitro-2-pyridyl disulfide, methyl 2-pyridyl disulfide, p-chloromercuribenzoate, 2-chloromercuri-4-nitrophenol, or chloro-7-nitrobenzo-2-oxa-1,3-diazole. Histidyl residues may be derivatized by reaction with diethylpyrocarbonate at pH 5.5-7.0 because this agent is relatively specific for the histidyl side chain. Para-bromophenacyl bromide also is useful; the reaction is preferably performed in 0.1 M sodium cacodylate at pH 6.0. Lysinyl and amino-terminal residues may be reacted with succinic or other carboxylic acid anhydrides. Derivatization with these agents has the effect of reversing the charge of the lysinyl residues. Other suitable reagents for derivatizing alpha-amino-containing residues include imidoesters such as methyl picolinimidate, pyridoxal phosphate, pyridoxal, chloroborohydride, trinitrobenzenesulfonic acid, O-methylisourea, 2,4-pentanedione, and transaminase-catalyzed reaction with glyoxylate. Arginyl residues may be modified by reaction with one or several conventional reagents, among them phenylglyoxal, 2,3-butanedione, 1,2-cyclohexanedione, and ninhydrin. Derivatization of arginine residues requires that the reaction be performed in alkaline conditions because of the high pKa of the guanidine functional group. Furthermore, these reagents may react with the groups of lysine as well as the arginine epsilon-amino group. The specific modification of tyrosyl residues may be made, with particular interest in introducing spectral labels into tyrosyl residues by reaction with aromatic diazonium

compounds or tetranitromethane. Most commonly, N-acetylimidizole and tetranitromethane are used to form O-acetyl tyrosyl species and 3-nitro derivatives, respectively. Carboxyl side groups (aspartyl or glutamyl) may be selectively modified by reaction with carbodiimides (R-N=C=N-R′), where R and R′ are different alkyl groups, such as 1-cyclohexyl-3-(2-morpholinyl-4-ethyl) carbodiimide or 1-ethyl-3-(4-azonia-4,4-dimethylpentyl) carbodiimide. Furthermore, aspartyl and glutamyl residues may be converted to asparaginyl and glutaminyl residues by reaction with ammonium ions. Other modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the alpha-amino groups of lysine, arginine, and histidine side chains (T. E. Creighton, Proteins: Structure and Molecular Properties, W.H. Freeman & Co., San Francisco, pp. 79-86 (1983)), deamidation of asparagine or glutamine, acetylation of the N-terminal amine, and/or amidation or esterification of the C-terminal carboxylic acid group. Another type of covalent modification involves chemically or enzymatically coupling glycosides to the peptide. Sugar(s) may be attached to (a) arginine and histidine, (b) free carboxyl groups, (c) free sulfhydryl groups such as those of cysteine, (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline, (e) aromatic residues such as those of tyrosine, or tryptophan, or (f) the amide group of glutamine. These methods are described in WO87/05330 published 11 Sep. 1987, and in Aplin and Wriston, CRC Crit. Rev. Biochem., pp. 259-306 (1981). In some embodiments, the peptide is conjugated to a heterologous moiety via covalent linkage between a side chain of an amino acid of the peptides and the heterologous moiety. In some aspects, the amino acid covalently linked to a heterologous moiety (e.g., the amino acid comprising a heterologous moiety) is a Cys, Lys, Orn, homo-Cys, or Ac-Phe, and the side chain of the amino acid is covalently bonded to a heterologous moiety. In some embodiments, the conjugate comprises a linker that joins the peptide to the heterologous moiety. In some aspects, the linker comprises a chain of atoms from 1 to about 60, or 1 to 30 atoms or longer, 2 to 5 atoms, 2 to 10 atoms, 5 to 10 atoms, or 10 to 20 atoms long. In some embodiments, the chain atoms may be all carbon atoms. In some embodiments, the chain atoms in the backbone of the linker may be selected from the group consisting of C, O, N, and S. Chain atoms and linkers may be selected according to their expected solubility (hydrophilicity) so as to provide a more soluble conjugate. In some embodiments, the linker provides a functional group that is subject to cleavage by an enzyme or other catalyst or hydrolytic conditions found in the target tissue or organ or cell. In some embodiments, the length of the linker is long enough to reduce the potential for steric hindrance. If the linker is a covalent bond or a peptidyl bond and the conjugate is a polypeptide, the entire conjugate can be a fusion protein. Such peptidyl linkers may be any length. Exemplary linkers may be from about 1 to 50 amino acids in length, 5 to 50, 3 to 5, 5 to 10, 5 to 15, or 10 to 30 amino acids in length. Such fusion proteins may alternatively be produced by recombinant genetic engineering methods known to one of ordinary skill in the art.

[095] As noted above, in some embodiments, the peptides may be conjugated, e.g., fused to an immunoglobulin or portion thereof (e.g., variable region, CDR, or Fc region). Known types of immunoglobulins (Ig) include IgG, IgA, IgE, IgD or IgM. The Fc region is a C-terminal region of an Ig heavy chain, which is responsible for binding to Fc receptors that carry out activities such as recycling (which results in prolonged half-life), antibody dependent cell-mediated cytotoxicity (ADCC), and complement dependent cytotoxicity (CDC). For example, according to some definitions the human IgG heavy chain Fc region stretches from Cys226 to the C-terminus of the heavy chain. The "hinge region" generally extends from Glu216 to Pro230 of human IgG1 (hinge regions of other IgG isotypes may be aligned with the IgG1 sequence by aligning the cysteines involved in cysteine bonding). The Fc region of an IgG includes two constant domains, CH2 and CH3. The CH2 domain of a human IgG Fc region usually extends from amino acids 231 to amino acid 341. The CH3 domain of a human IgG Fc region usually extends from amino acids 342 to 447. References made to amino acid numbering of immunoglobulins or immunoglobulin fragments, or regions, are all based on Kabat et al. 1991, Sequences of Proteins of Immunological Interest, U.S. Department of Public Health, Bethesda, Md. In related embodiments, the Fc region may comprise one or more native or modified constant regions from an immunoglobulin heavy chain, other than CH1, for example, the CH2 and CH3 regions of IgG and IgA, or the CH3 and CH4 regions of IgE. Suitable conjugate moieties include portions of immunoglobulin sequence that include the FcRn binding site. FcRn, a salvage receptor, is responsible for recycling immunoglobulins and returning them to circulation in blood. The region of the Fc portion of IgG that binds to the FcRn receptor has been described based on X-ray crystallography (Burmeister et al. 1994, Nature 372:379). The major contact area of the Fc with the FcRn is near the junction of the CH2 and CH3 domains. Fc-FcRn contacts are all within a single Ig heavy chain. The major contact sites include amino acid residues 248, 250-257, 272, 285, 288, 290-291, 308-311, and 314 of the CH2 domain and amino acid residues 385-387, 428, and 433-436 of the CH3 domain. Some conjugate moieties may or may not include FcγR binding site(s). FcγR are responsible for ADCC and CDC. Examples of positions within the Fc region that make a direct contact with FcγR are amino acids 234-239 (lower hinge region), amino acids 265-269 (B/C loop), amino acids 297-299 (C'/E loop), and amino acids 327-332 (F/G) loop (Sondermann et al., Nature 406: 267-273, 2000). The lower hinge region of IgE has also been implicated in the FcRI binding (Henry, et al., Biochemistry 36, 15568-15578, 1997). Residues involved in IgA receptor binding are described in Lewis et al., (J Immunol. 175:6694-701, 2005). Amino acid residues involved in IgE receptor binding are described in Sayers et al. (J Biol Chem. 279(34):35320-5, 2004). Amino acid modifications may be made to the Fc region of an immunoglobulin. Such variant Fc regions comprise at least one amino acid modification in the CH3 domain of the Fc region (residues 342-447) and/or at least one amino acid modification in the CH2 domain of the Fc region (residues 231-341). Mutations believed to impart an increased affinity for FcRn include T256A, T307A,

E380A, and N434A (Shields et al. 2001, J. Biol. Chem. 276:6591). Other mutations may reduce binding of the Fc region to FcγRI, FcγRIIA, FcγRIIB, and/or FcγRIIIA without significantly reducing affinity for FcRn. For example, substitution of the Asn at position 297 of the Fc region with Ala or another amino acid removes a highly conserved N-glycosylation site and may result in reduced immunogenicity with concomitant prolonged half-life of the Fc region, as well as reduced binding to FcγRs (Routledge et al. 1995, Transplantation 60:847; Friend et al. 1999, Transplantation 68:1632; Shields et al. 1995, J. Biol. Chem. 276:6591). Amino acid modifications at positions 233-236 of IgG1 have been made that reduce binding to FcγRs (Ward and Ghetie 1995, Therapeutic Immunology 2:77 and Armour et al. 1999, Eur. J. Immunol. 29:2613). Some exemplary amino acid substitutions are described in U.S. Pat. Nos. 7,355,008 and 7,381,408, each incorporated by reference herein in its entirety. In certain embodiments, a peptide described herein is inserted into a loop region within the immunoglobulin molecule. In other embodiments, a peptide described herein replaces one or more amino acids of a loop region within the immunoglobulin molecule.

[096] The peptides described herein can be further modified to improve its solubility and stability in aqueous solutions at physiological pH, while retaining the biological activity. Hydrophilic moieties such as PEG groups can be attached to the analogs under any suitable conditions used to react a protein with an activated polymer molecule. Any means known in the art can be used, including via acylation, reductive alkylation, Michael addition, thiol alkylation or other chemoselective conjugation/ligation methods through a reactive group on the PEG moiety (e.g., an aldehyde, amino, ester, thiol, α-haloacetyl, maleimido or hydrazino group) to a reactive group on the target compound (e.g., an aldehyde, amino, ester, thiol, α-haloacetyl, maleimido or hydrazino group). Activating groups which can be used to link the water soluble polymer to one or more proteins include without limitation sulfone, maleimide, sulfhydryl, thiol, triflate, tresylate, azidirine, oxirane, 5-pyridyl, and alpha-halogenated acyl group (e.g., alpha-iodo acetic acid, alpha-bromoacetic acid, alpha-chloroacetic acid). If attached to the analog by reductive alkylation, the polymer selected should have a single reactive aldehyde so that the degree of polymerization is controlled. See, for example, Kinstler et al., *Adv. Drug. Delivery Rev.* 54: 477-485 (2002); Roberts et al., *Adv. Drug Delivery Rev.* 54: 459-476 (2002); and Zalipsky et al., *Adv. Drug Delivery Rev.*16: 157-182 (1995). In specific aspects, an amino acid residue of the peptides having a thiol is modified with a hydrophilic moiety such as PEG. In some embodiments, the thiol is modified with maleimide-activated PEG in a Michael addition reaction to result in a PEGylated analog comprising a thioether linkage. In some embodiments, the thiol is modified with a haloacetyl-activated PEG in a nucleophilic substitution reaction to result in a PEGylated analog comprising a thioether linkage. Suitable hydrophilic moieties include polyethylene glycol (PEG), polypropylene glycol, polyoxyethylated polyols (e.g., POG), polyoxyethylated sorbitol, polyoxyethylated glucose, polyoxyethylated glycerol (POG), polyoxyalkylenes, polyethylene glycol

propionaldehyde, copolymers of ethylene glycol/propylene glycol, monomethoxy-polyethylene glycol, mono-(C_1-C_{10}) alkoxy- or aryloxy-polyethylene glycol, carboxymethylcellulose, polyacetals, polyvinyl alcohol (PVA), polyvinyl pyrrolidone, poly-1,3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, poly (.beta.-amino acids) (either homopolymers or random copolymers), poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers (PPG) and other polyakylene oxides, polypropylene oxide/ethylene oxide copolymers, colonic acids or other polysaccharide polymers, Ficoll or dextran and mixtures thereof. Dextrans are polysaccharide polymers of glucose subunits, predominantly linked by α1-6 linkages. Dextran is available in many molecular weight ranges, e.g., about 1 kD to about 100 kD, or from about 5, 10, 15 or 20 kD to about 20, 30, 40, 50, 60, 70, 80 or 90 kD. Linear or branched polymers are contemplated. Resulting preparations of conjugates may be essentially monodisperse or polydisperse, and may have about 0.5, 0.7, 1, 1.2, 1.5 or 2 polymer moieties per analog.

[097] In some embodiments, the peptide is conjugated to a hydrophilic moiety via covalent linkage between a side chain of an amino acid of the peptide and the hydrophilic moiety. In some embodiments, the peptide is conjugated to a hydrophilic moiety via the side chain of an amino acid, a position within a C-terminal extension, or the C-terminal amino acid, or a combination of these positions. In some aspects, the amino acid covalently linked to a hydrophilic moiety (e.g., the amino acid comprising a hydrophilic moiety) is a Cys, Lys, Orn, homo-Cys, or Ac-Phe, and the side chain of the amino acid is covalently bonded to a hydrophilic moiety (e.g., PEG). In some embodiments, the conjugate of the present disclosure comprises the peptide fused to an accessory analog which is capable of forming an extended conformation similar to chemical PEG (e.g., a recombinant PEG (rPEG) molecule), such as those described in International Patent Application Publication No. WO2009/023270 and U.S. Patent Application Publication No. US20080286808. The rPEG molecule in some aspects is a polypeptide comprising one or more of glycine, serine, glutamic acid, aspartic acid, alanine, or proline. In some aspects, the rPEG is a homopolymer, e.g., poly-glycine, poly-serine, poly-glutamic acid, poly-aspartic acid, poly-alanine, or poly-proline. In other embodiments, the rPEG comprises two types of amino acids repeated, e.g., poly(Gly-Ser), poly(Gly-Glu), poly(Gly-Ala), poly(Gly-Asp), poly(Gly-Pro), poly(Ser-Glu), etc. In some aspects, the rPEG comprises three different types of amino acids, e.g., poly(Gly-Ser-Glu). In specific aspects, the rPEG increases the half-life of the peptide. In some aspects, the rPEG comprises a net positive or net negative charge. The rPEG in some aspects lacks secondary structure. In some embodiments, the rPEG is greater than or equal to 10 amino acids in length and in some embodiments is about 40 to about 50 amino acids in length. The accessory peptide in some aspects is fused to the N- or C-terminus of the peptide of the present disclosure through a peptide bond or a proteinase cleavage site, or is inserted into the loops of the peptide of the present disclosure. The rPEG in some aspects comprises an affinity tag or is linked to a PEG that is greater than 5 kDa. In some embodiments, the rPEG confers the peptide of the present disclosure with an increased

hydrodynamic radius, serum half-life, protease resistance, or solubility and in some aspects confers the analog with decreased immunogenicity.

[098] The peptides comprising the sequences (SEQ ID NOs: 1-64), optionally with any of the conjugations described herein are contemplated as an embodiment.

[099] The disclosure further provides multimers or dimers of the peptides disclosed herein, including homo- or hetero-multimers or homo- or hetero-dimers. Two or more of the analogs can be linked together using standard linking agents and procedures known to those skilled in the art. For example, dimers can be formed between two peptides through the use of bifunctional thiol crosslinkers and bi-functional amine crosslinkers, particularly for the analogs that have been substituted with cysteine, lysine ornithine, homocysteine or acetyl phenylalanine residues. The dimer can be a homodimer or alternatively can be a heterodimer. In certain embodiments, the linker connecting the two (or more) analogs is PEG, e.g., a 5 kDa PEG, 20 kDa PEG. In some embodiments, the linker is a disulfide bond. For example, each monomer of the dimer may comprise a Cys residue (e.g., a terminal or internally positioned Cys) and the sulfur atom of each Cys residue participates in the formation of the disulfide bond. In some aspects, the monomers may be connected via terminal amino acids (e.g., N-terminal or C-terminal), via internal amino acids, or via a terminal amino acid of at least one monomer and an internal amino acid of at least one other monomer. In specific aspects, the monomers are not connected via an N-terminal amino acid. In some aspects, the monomers of the multimer may be attached together in a "tail-to-tail" orientation in which the C-terminal amino acids of each monomer may be attached together.

[0100] Peptides disclosed herein may be made in a variety of ways. Suitable methods of de novo synthesizing peptides are described in, for example, Merrifield, J. Am. Chem. Soc, 85, 2149 (1963); Davis et al., Biochem. Intl., 10, 394-414 (1985); Larsen et al., J. Am. Chem. Soc, 115, 6247 (1993); Smith et al., J. Peptide Protein Res., 44, 183 (1994); O'Donnell et al., J. Am. Chem. Soc, 118, 6070 (1996); Stewart and Young, Solid Phase Peptide Synthesis, Freeman (1969); Finn et al., The Proteins, 3 ed., vol. 2, pp. 105-253 (1976); Erickson et al., The Proteins, 3rd ed., vol. 2, pp. 257-527 (1976); and Chan et al., Fmoc Solid Phase Peptide Synthesis, Oxford University Press, Oxford, United Kingdom, 2005. The disclosure contemplates synthetic peptides. Methods of making the peptides are themselves embodiments of the invention.

[0101] Alternatively, the peptide can be expressed recombinantly by introducing a nucleic acid that comprises or consists of a nucleotide sequence encoding a peptide into host cells, which may be cultured to express the encoded peptide using standard recombinant methods. See, for instance, Sambrook et al., Molecular Cloning: A Laboratory Manual. 3rd ed., Cold Spring Harbor Press, Cold Spring Harbor, N.Y. 2001; and Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates and John Wiley & Sons, N.Y., 1994.Such peptides may be purified from the culture media or cell pellets.

[0102] In some embodiments, the peptides of the disclosure can be isolated. In some embodiments, the peptides of the disclosure may be purified. It is recognized that "purity" is a relative term, and not to be necessarily construed as absolute purity or absolute enrichment or absolute selection. In some aspects, the purity is at least or about 50%, is at least or about 60%, at least or about 70%, at least or about 80%, or at least or about 90% (e.g., at least or about 91%, at least or about 92%, at least or about 93%, at least or about 94%, at least or about 95%, at least or about 96%, at least or about 97%, at least or about 98%, at least or about 99% or is approximately 100%.

[0103] In some embodiments, the peptides described herein can be commercially synthesized by companies, such as Genscript (Piscataway, NJ), New England Peptide (Gardner, MA), and CPC Scientific (Sunnyvale, CA), Peptide Technologies Corp. (Gaithersburg, Md.), and Multiple Peptide Systems (San Diego, Calif.). In this respect, the peptides can be synthetic, recombinant, isolated, and/or purified.

[0104] The peptides of the present disclosure can be provided in accordance with one embodiment as part of a kit. Accordingly, in some embodiments, a kit for administering a peptide, to a patient in need thereof is provided wherein the kit comprises a peptide as described herein.

[0105] In one embodiment the kit is provided with a device for administering the composition to a patient, e.g., syringe needle, pen device, jet injector or another needle-free injector. The kit may alternatively or in addition include one or more containers, e.g., vials, tubes, bottles, single or multi-chambered pre-filled syringes, cartridges, infusion pumps (external or implantable), jet injectors, pre-filled pen devices and the like, optionally containing the peptide in a lyophilized form or in an aqueous solution. The kits in some embodiments comprise instructions for use. In accordance with one embodiment the device of the kit is an aerosol dispensing device, wherein the composition is prepackaged within the aerosol device. In another embodiment the kit comprises a syringe and a needle, and in one embodiment the sterile composition is prepackaged within the syringe.

[0106] A further embodiment includes a process of treating a disease comprising one or more of prescribing, selling or advertising to sell, purchasing, instructing to self-administer, or administering a peptide described herein, wherein the peptide has been approved by a regulatory agency for the treatment of a condition, to a subject in need of treatment.

[0107] A further embodiment includes a method of supplying a peptide for treating a disease, said method comprises reimbursing a physician, a formulary, a patient or an insurance company for the sale of said peptide.

Definitions

[0108] The terms "peptide" refers to a molecule comprising two or more amino acid residues joined to each other by peptide bonds. These terms encompass, e.g., native and artificial proteins, protein fragments and polypeptide analogs (such as muteins, variants, and fusion proteins) of a protein

sequence as well as post-translationally, or otherwise covalently or non-covalently, modified peptides. A peptide may be monomeric or polymeric. In certain embodiments, "peptides" are chains of amino acids whose alpha carbons may be linked through peptide bonds. The terminal amino acid at one end of the chain (amino terminal) therefore has a free amino group, while the terminal amino acid at the other end of the chain (carboxy terminal) has a free carboxyl group. As used herein, the term "amino terminus" (abbreviated N-terminus) refers to the free α-amino group on an amino acid at the amino terminal of a peptide or to the α-amino group (imino group when participating in a peptide bond) of an amino acid at any other location within the peptide. Similarly, the term "carboxy terminus" refers to the free carboxyl group on the carboxy terminus of a peptide or the carboxyl group of an amino acid at any other location within the peptide. Peptides also include essentially any polyamino acid including, but not limited to, peptide mimetics such as amino acids joined by an ether as opposed to an amide bond.

[0109] The term “therapeutic peptide" refers to peptides or analogs or fragments or variants thereof, having one or more therapeutic and/or biological activities.

[0110] The term "analog" as used herein describes a peptide comprising one or more amino acid modifications, such as but not limited to substitution and/or one or more deletion and/or one or more addition of any one of the amino acid residues for any natural or unnatural amino acid, synthetic amino acids or peptidomimetics and/or the attachment of a side chain to any one of the natural or unnatural amino acids, synthetic amino acids or peptidomimetics at any available position. The addition or deletion of amino acid residues can take place at the N-terminal of the peptide and/or at the C- terminal of the peptide.

[0111] In some embodiments, the analog has 1, 2, 3, 4, or 5 such modifications. In some embodiments, the analog retains biological activity of the original peptide. In some embodiments, the analog is a competitive or non-competitive inhibitor of the original peptide.

[0112] Peptide sequences are indicated using standard one- or three-letter abbreviations. Unless otherwise indicated, peptide sequences have their amino termini at the left and their carboxy termini at the right, A particular section of a peptide can be designated by amino acid residue number such as amino acids 3 to 6, or by the actual residue at that site such as Met3 to Gly6. A particular peptide sequence also can be described by explaining how it differs from a reference sequence.

[0113] When used herein the term "natural amino acid" is an amino acid (with the usual three letter codes & one letter codes in parenthesis) selected from the group consisting of: Glycine (Gly & G), proline (Pro & P), alanine (Ala & A), valine (Val & V), leucine (Leu & L), isoleucine (Ile & I), methionine (Met & M), cysteine (Cys & C), phenylalanine (Phe & F), tyrosine (Tyr & Y), tryptophan (Trp & W), histidine (His & H), lysine (Lys & K), arginine (Arg & R), glutamine (Gin & Q), asparagine (Asn & N), glutamic acid (Glu & E), aspartic acid (Asp & D), serine (Ser & S) and threonine (Thr & T). If anywhere herein, reference is made to a peptide, analog or derivative

or peptides comprising or not comprising G, P, A, V, L, I, M, C, F, Y, H, K, R, Q, N, E, D, S or T, without specifying further, amino acids are meant. If not otherwise indicated amino acids indicated with a single letter code in CAPITAL letters indicate the L-isoform, if however, the amino acid is indicated with a lower case letter, this amino acid is used/applied as it's D-form. Such D-forms and other non-conservative amino acid substitutions previously defined are included in a definition of unnatural amino acids.

[0114] If, due to typing errors, there are deviations from the commonly used codes, the commonly used codes apply. The amino acids present in the peptides are, preferably, amino acids which can be coded for by a nucleic acid. As is apparent from the above examples, amino acid residues may be identified by their full name, their one-letter code, and/or their three-letter code. These three ways are fully equivalent.

[0115] A "non-conservative amino acid substitution" also refers to the substitution of a member of one of these classes for a member from another class. In making such changes, according to certain embodiments, the hydropathic index of amino acids may be considered. Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics. They are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5). The importance of the hydropathic amino acid index in conferring interactive biological function on a protein is understood in the art (see, for example, Kyte et al., 1982, J. Mol. Biol. 157:105-131). It is known that certain amino acids may be substituted for other amino acids having a similar hydropathic index or score and still retain a similar biological activity. In making changes based upon the hydropathic index, in certain embodiments, the substitution of amino acids whose hydropathic indices are within $\pm$ 2 is included. In certain embodiments, those that are within $\pm$ 1 are included, and in certain embodiments, those within $\pm$ 0.5 are included. It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity, particularly where the biologically functional protein or peptide thereby created is intended for use in immunological embodiments, as disclosed herein. In certain embodiments, the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with its immunogenicity and antigenicity, i.e., with a biological property of the protein. The following hydrophilicity values have been assigned to these amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0.+-.1); glutamate (+3.0.+-.1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5.+-.1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5) and tryptophan (-3.4). In making changes based upon similar hydrophilicity values, in certain embodiments, the substitution of amino acids whose hydrophilicity values are

within ± 2 is included, in certain embodiments, those that are within ± 1 are included, and in certain embodiments, those within ± 0.5 are included.

[0116] Other amino acid substitutions are set forth in Table 3.

TABLE 3

Original Residues	Substitutions	Preferred Substitutions
Ala	Val, Leu, Ile	Val
Arg	Lys, Gln, Asn	Lys
Asn	Gln	Gln
Asp	Glu	Glu
Cys	Ser, Ala	Ser
Gln	Asn	Asn
Glu	Asp	Asp
Gly	Pro, Ala	Ala
His	Asn, Gln, Lys, Arg	Arg
Ile	Leu, Val, Met, Ala, Phe, Norleucine	Leu
Leu	Norleucine, Ile, Val, Met, Ala, Phe	Ile
Lys	Arg, Gln, Asn, 1,4-Diamino-butyric Acid	Arg
Met	Leu, Phe, Ile	Leu
Phe	Leu, Val, Ile, Ala, Tyr	Leu
Pro	Ala	Gly
Ser	Thr, Ala, Cys	Thr
Thr	Ser	Ser
Trp	Tyr, Phe	Tyr
Tyr	Trp, Phe, Thr, Ser	Phe
Val	Ile, Met, Leu, Phe, Ala, Norleucine	Leu

[0117] The term "amino acid" as used herein, alone or in combination, means a substituent of the form -R^x-NH-CH(R^y)C(=O)OH, wherein R^x is typically hydrogen, but may be cyclized with N (for example, as in the case of the amino acid proline), and R^y is selected from the group consisting of hydrogen, alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, amino, amido, cycloalkylalkyl, heterocycloalkylalkyl, arylalkyl, heteroarylalkyl, aminoalkyl, amidoalkyl, hydroxyalkyl, thiol, thioalkyl, alkylthioalkyl, and alkylthio, any of which may be optionally substituted. The term "amino acid" includes all naturally occurring amino acids as well as synthetic analogs.

[0118] As used herein the term “charged amino acid” or “charged residue” refers to an amino acid that comprises a side chain that is negative-charged (i.e., de-protonated) or positive-charged (i.e.,

protonated) in aqueous solution at physiological pH. For example, negative-charged amino acids include aspartic acid, glutamic acid, cysteic acid, homocysteic acid, and homoglutamic acid, whereas positive-charged amino acids include arginine, lysine and histidine. Charged amino acids include the charged amino acids among the 20 coded amino acids, as well as atypical or non-naturally occurring or non-coded amino acids.

[0119] As used herein the term "acidic amino acid" refers to an amino acid that comprises a second acidic moiety (other than the carboxylic acid of the amino acid), including for example, a carboxylic acid or sulfonic acid group.

[0120] As used herein, the term "acylated amino acid" refers to an amino acid comprising an acyl group which is non-native to a naturally-occurring amino acid, regardless of the means by which it is produced (e.g. acylation prior to incorporating the amino acid into a peptide, or acylation after incorporation into a peptide).

[0121] As used herein the term "alkylated amino acid" refers to an amino acid comprising an alkyl group which is non-native to a naturally-occurring amino acid, regardless of the means by which it is produced. Accordingly, the acylated amino acids and alkylated amino acids of the present disclosures are non-coded amino acids.

[0122] A skilled artisan will be able to determine active variants or analogs of peptides as set forth herein using well-known techniques. In certain embodiments, one skilled in the art may identify suitable areas of the molecule that may be changed without destroying activity by targeting regions not believed to be important for activity. In other embodiments, the skilled artisan can identify residues and portions of the molecules that are conserved among similar peptides. In further embodiments, even areas that may be important for biological activity or for structure may be subject to conservative amino acid substitutions without destroying the biological activity or without adversely affecting the peptide structure. Changes in caspase activity in cells treated with a test compounds are well known to be an indicator of potential therapeutic utility. Regardless of whether caspases have been definitively implicated in the etiology or pathological consequences of a disease, a decrease in caspase activity has been associated with amelioration of the symptoms of several conditions caused by inappropriate apoptotic cell death, including diabetes, cardiovascular disease, detrimental hepatocyte apoptosis, ischemia reperfusion injury, traumatic brain injury, organ transplant, and neurodegeneration (Choadhry, J Thorac Cardiovasc Surg. 2007 Jul;134(1):124-31; McIlwain, Cold Spring Harb Perspect Biol 2013;5:a008656). In addition, it is well known that increases in caspase activity indicates potential utility for treating diseases and disorders responsive to induction of apoptosis, including cancer, autoimmune disorders, rheumatoid arthritis, infectious diseases, inflammatory disease (Elmore, Toxicol Pathol. 2007; 35(4): 495–516). Changes in cell viability in cells treated with a test compounds are well known to be an indicator of potential therapeutic utility. A decrease in cell viability indicates potential utility for treating diseases and disorders responsive to changes in cell viability/proliferation,

including for example cancer (Boyd, Drug Dev Res 34:91-109 (1995)). An increase in cell viability indicates potential utility for treating diseases associated with decreased cell viability, including diabetes, cardiovascular disease, ischemia reperfusion injury, traumatic brain injury, organ transplant, chemotherapy, and neurodegeneration. Additionally, an increase in cell viability indicates potential utility for improving cell viability of animal cells in culture.

[0123] Additionally, one skilled in the art can review structure-function studies identifying residues in similar peptides that are important for activity or structure. In view of such a comparison, the skilled artisan can predict the importance of amino acid residues in a peptide that correspond to amino acid residues important for activity or structure in similar peptides. One skilled in the art may opt for chemically similar amino acid substitutions for such predicted important amino acid residues.

[0124] One skilled in the art can also analyze the three-dimensional structure and amino acid sequence in relation to that structure in similar peptides. In view of such information, one skilled in the art may predict the alignment of amino acid residues of a peptide with respect to its three-dimensional structure. In certain embodiments, one skilled in the art may choose to not make radical changes to amino acid residues predicted to be on the surface of the peptide, since such residues may be involved in important interactions with other molecules. Moreover, one skilled in the art may generate test variants containing a single amino acid substitution at each desired amino acid residue. The variants can then be screened using activity assays known to those skilled in the art. Such variants could be used to gather information about suitable variants. For example, if one discovered that a change to a particular amino acid residue resulted in destroyed, undesirably reduced, or unsuitable activity, variants with such a change can be avoided. In other words, based on information gathered from such routine experiments, one skilled in the art can readily determine the amino acids where further substitutions should be avoided either alone or in combination with other mutations.

[0125] The term "acyl" as used herein, alone or in combination, refers to a carbonyl attached to an alkenyl, alkyl, aryl, cycloalkyl, heteroaryl, heterocycloalkyl, or any other moiety were the atom attached to the carbonyl is carbon. An "acetyl" group, which is a type of acyl, refers to a $-C(O)CH_3$ group. An "alkylcarbonyl" or "alkanoyl" group refers to an alkyl group attached to the parent molecular moiety through a carbonyl group. Examples of such groups include methylcarbonyl and ethyl carbonyl. Examples of acyl groups include formyl, alkanoyl and aroyl.

[0126] The term "derivative" as used herein means a chemically modified peptide, in which one or more side chains have been covalently attached to the peptide. The term "side chain" may also be referred to as a "substituent". A derivative comprising such side chains will thus be "derivatized" peptide or "derivatized" analog. The term may also refer to peptides containing one or more chemical moieties not normally a part of the peptide molecule such as esters and amides of free carboxy groups, acyl and alkyl derivatives of free amino groups, phospho esters and ethers of free

hydroxy groups. Such modifications may be introduced into the molecule by reacting targeted amino acid residues of the peptide with an organic derivatizing agent that is capable of reacting with selected side chains or terminal residues. Preferred chemical derivatives include peptides that have been phosphorylated, C-termini amidated or N-termini acetylated. The term may also refer to peptides as used herein which may be prepared from the functional groups which occur as side chains on the residues or the N- or C-terminal groups, by means known in the art, and are included herein as long as they remain pharmaceutically acceptable, i.e., they do not destroy the activity of the peptide, do not confer toxic properties on compositions containing it and do not adversely affect the antigenic properties thereof. These derivatives may, for example, include aliphatic esters of the carboxyl groups, amides of the carboxyl groups produced by reaction with ammonia or with primary or secondary amines, N-acyl derivatives of free amino groups of the amino acid residues formed by reaction with acyl moieties (e.g., alkanoyl or carbocyclic aroyl groups) or O-acyl derivatives of free hydroxyl group (for example that of seryl or threonyl residues) formed by reaction with acyl moieties.

[0127] A modified amino acid residue is an amino acid residue in which any group or bond was modified by deletion, addition, or replacement with a different group or bond, as long as the functionality of the amino acid residue is preserved or if functionality changed (for example replacement of tyrosine with substituted phenylalanine) as long as the modification did not impair the activity of the peptide containing the modified residue.

[0128] The term "substituent" or "side chain" as used herein means any suitable moiety bonded, in particular covalently bonded, to an amino acid residue, in particular to any available position on an amino acid residue. Typically, the suitable moiety is a chemical moiety.

[0129] The term "fatty acid" refers to aliphatic monocarboxylic acids having from 4 to 28 carbon atoms, it is preferably un-branched, and it may be saturated or unsaturated. In the present disclosure fatty acids comprising 10 to 16 amino acids are preferred.

[0130] The term "fatty diacid" refers to fatty acids as defined above but with an additional carboxylic acid group in the omega position. Thus, fatty diacids are dicarboxylic acids. In the present disclosure fatty acids comprising 14 to 20 amino acids are preferred.

[0131] When ranges of values are disclosed, and the notation "from n_1 to n_2" is used, where n_1 and n_2 are the numbers, then unless otherwise specified, this notation is intended to include the numbers themselves and the range between them. This range may be integral or continuous between and including the end values. By way of example, the range "from 2 to 6 carbons" is intended to include two, three, four, five, and six carbons, since carbons come in integer units. Compare, by way of example, the range "from 1 to 3 μM (micromolar)" which is intended to include 1 μM, 3 μM, and everything in between to any number of significant figures (e.g., 1.255 μM, 2.1 μM, 2.9999 μM, etc.).

[0132] The term "% sequence identity" is used interchangeably herein with the term "% identity" and refers to the level of amino acid sequence identity between two or more peptide sequences or the level of nucleotide sequence identity between two or more nucleotide sequences, when aligned using a sequence alignment program. For example, as used herein, 80% identity means the same thing as 80% sequence identity determined by a defined algorithm, and means that a given sequence is at least 80% identical to another length of another sequence.

[0133] The term "% sequence homology" is used interchangeably herein with the term "% homology" and refers to the level of amino acid sequence homology between two or more peptide sequences or the level of nucleotide sequence homology between two or more nucleotide sequences, when aligned using a sequence alignment program. For example, as used herein, 80% homology means the same thing as 80% sequence homology determined by a defined algorithm, and accordingly a homologue of a given sequence has greater than 80% sequence homology over a length of the given sequence.

[0134] Exemplary computer programs which can be used to determine degrees of identity or homology between two sequences include, but are not limited to, the suite of BLAST programs, e.g., BLASTN, BLASTX, and TBLASTX, BLASTP and TBLASTN, publicly available on the Internet at the NCBI website. See also Altschul et al., 1990, J. Mol. Biol. 215:403-10 (with special reference to the published default setting, i.e., parameters w=4, t=17) and Altschul et al., 1997, Nucleic Acids Res., 25:3389-3402. Sequence searches are typically carried out using the BLASTP program when evaluating a given amino acid sequence relative to amino acid sequences in the GenBank Protein Sequences and other public databases. The BLASTX program is preferred for searching nucleic acid sequences that have been translated in all reading frames against amino acid sequences in the GenBank Protein Sequences and other public databases. Both BLASTP and BLASTX are run using default parameters of an open gap penalty of 11.0, and an extended gap penalty of 1.0, and utilize the BLOSUM-62 matrix. (Id). In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul, Proc. Nat'l. Acad. Sci. USA, 90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance.

[0135] A "pharmaceutical composition" refers to a composition suitable for pharmaceutical use in an animal or human. A pharmaceutical composition comprises a pharmacologically and/or therapeutically effective amount of an active agent and a pharmaceutically acceptable excipient or carrier. Pharmaceutical compositions and methods for their preparation will be readily apparent to those skilled in the art. Such compositions and methods for their preparation may be found, for example, in Remington's Pharmaceutical Sciences, 19th Edition (Mack Publishing Company, 1995). The pharmaceutical compositions are generally formulated as sterile, substantially isotonic

and in full compliance with all GMP regulations of the U.S. Food and Drug Administration. The term also encompasses any of the agents listed in the US Pharmacopeia for use in animals, including humans. Suitable pharmaceutical carriers and formulations are described in Remington's Pharmaceutical Sciences, 21st Ed. 2005, Mack Publishing Co, Easton.

[0136] "Pharmaceutically acceptable carrier" or "pharmaceutically acceptable excipient" refers to compositions that do not produce adverse, allergic, or other untoward reactions when administered to an animal or a human. As used herein, "pharmaceutically acceptable carrier" or "pharmaceutically acceptable excipient" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Some examples of pharmaceutically acceptable excipients are water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, the excipients will include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Additional examples of pharmaceutically acceptable excipients are wetting agents or minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the peptide.

[0137] As used herein the term "pharmaceutically acceptable salt" refers to salts of peptides that retain the biological activity of the parent peptide, and which are not biologically or otherwise undesirable. Many of the peptides disclosed herein are capable of forming acid and/or base salts by virtue of the presence of amino and/or carboxyl groups or groups similar thereto. Pharmaceutically acceptable base addition salts can be prepared from inorganic and organic bases. Salts derived from inorganic bases, include by way of example only, sodium, potassium, lithium, ammonium, calcium and magnesium salts. Salts derived from organic bases include, but are not limited to, salts of primary, secondary and tertiary amines.

[0138] It may be convenient or desirable to prepare, purify, and/or handle a corresponding solvate of the peptide. The term "solvate" is used herein in the conventional sense to refer to a complex of solute (e.g., peptide, salt of peptide) and solvent. If the solvent is water, the solvate may be conveniently referred to as a hydrate, for example, a mono-hydrate, a di-hydrate, a tri-hydrate, etc. Unless otherwise specified, a reference to a particular peptide also includes solvate and hydrate forms thereof.

[0139] The "co-crystal" or "co-crystal salt" as used herein means a crystalline material composed of two or more unique solids at room temperature, each of which has distinctive physical characteristics such as structure, melting point, and heats of fusion, hygroscopicity, solubility, and stability. A co-crystal or a co-crystal salt can be produced according to a per se known co-crystallization method. The terms co-crystal (or cocrystal) or co-crystal salt also refer to a multicomponent system in which there exists a host API (active pharmaceutical ingredient) molecule or molecules, such as a peptide of Formulas I-II, and a guest (or co-former) molecule or molecules.

[0140] As used herein, a "therapeutically effective amount" of a peptide that when provided to a subject in accordance with the disclosed and claimed methods affects biological activities such as modulating cell signaling associated with aberrant cellular proliferation and malignancy, impacting cell viability and providing neuroprotection.

[0141] The terms "treat", "treating" and "treatment" refer refers to an approach for obtaining beneficial or desired clinical results. Further, references herein to "treatment" include references to curative, palliative and prophylactic treatment. The term "treating" refers to inhibiting, preventing or arresting the development of a pathology (disease, disorder or condition) and/or causing the reduction, remission, or regression of a pathology. Those of skill in the art will understand that various methodologies and assays can be used to assess the development of a pathology, and similarly, various methodologies and assays may be used to assess the reduction, remission or regression of a pathology.

[0142] The term "disease" as used herein is intended to be generally synonymous, and is used interchangeably with, the terms "disorder" and "condition" (as in medical condition), in that all reflect an abnormal condition of the body or of one of its parts that impairs normal functioning and is typically manifested by distinguishing signs and symptoms

[0143] The term "improving cell survival" refers to an increase in the number of cells that survive a given condition, as compared to a control, e.g., the number of cells that would survive the same conditions in the absence of treatment. Conditions can be in vitro, in vivo, ex vivo, or in situ. Improved cell survival can be expressed as a comparative value, e.g., twice as many cells survive if cell survival is improved two-fold. Improved cell survival can result from a reduction in apoptosis, an increase in the life-span of the cell, or an improvement of cellular function and condition.

[0144] For clarity, the term "instructing" is meant to include information on a label approved by a regulatory agency, in addition to its commonly understood definition.

[0145] In an embodiment, the peptides may be administered as their nucleotide equivalents via gene therapy methods. The term "nucleotide equivalents" includes any nucleic acid which includes a nucleotide sequence that encodes a peptide. For example, the invention includes polynucleotides that comprise or conist of a nucleotide sequence that encodes a peptide described herein. The invention also includes vectors, including exression vectors, that comprise a nucleoide sequence that encodes a peptide described herein. Expression vectors include one or more expressin control sequences, such as a promoter, operably linked to the coding sequence such that the peptide is expressed in suitable host cells that contain the expression vector. In one embodiment, the peptide-related polynucleotide is encoded in a plasmid or vector, which may be derived from an adeno-associated virus (AAV). The AAV may be a recombinant AAV virus and may comprise a capsid serotype such as, but not limited to, of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV9.47, AAV9(hu14), AAV10, AAV11, AAV12, AAVrh8, AAVrh10,

AAV-DJ, and AAV-DJ8. As a non-limiting example, the capsid of the recombinant AAV virus is AAV2. As a non-limiting example, the capsid of the recombinant AAV virus is AAVrh10. As a non-limiting example, the capsid of the recombinant AAV virus is AAV9(hu14). As a non-limiting example, the capsid of the recombinant AAV virus is AAV-DJ. As a non-limiting example, the capsid of the recombinant AAV virus is AAV9.47. As a non-limiting example, the capsid of the recombinant AAV virus is AAV-DJ8. An embodiment comprises the nucleotide equivalents of the peptide sequences of SEQ ID NOs: 1-64.

[0146] A person skilled in the art may recognize that a target cell may require a specific promoter including but not limited to a promoter that is species specific, inducible, tissue- specific, or cell cycle-specific Parr et al, Nat. Med. 3:1145-9 (1997); the contents of which are herein incorporated by reference in its entirety).

[0147] As used herein, a "vector" is any molecule or moiety which transports, transduces or otherwise acts as a carrier of a heterologous molecule such as the polynucleotides of the invention. A "viral vector" is a vector which comprises one or more polynucleotide regions encoding or comprising payload molecule of interest, e.g., a transgene, a polynucleotide encoding a polypeptide or multi-polypeptide. Viral vectors of the present invention may be produced recombinantly and may be based on adeno-associated virus (AAV) parent or reference sequence. Serotypes which may be useful in the present invention include any of those arising from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV9.47, AAV9(hul4), AAV10, AAV11, AAV 12, AAVrh8, AAVrhlO, AAV-DJ, and AAV-DJ8.

[0148] In one embodiment, the serotype which may be useful in the present invention may be AAV-DJ8. The amino acid sequence of AAV-DJ8 may comprise two or more mutations in order to remove the heparin binding domain (HBD). As a non-limiting example, the AAV-DJ sequence described as SEQ ID NO: 1 in US Patent No. 7,588,772, the contents of which are herein incorporated by reference in its entirety, may comprise two mutations: (1) R587Q where arginine (R; arg) at amino acid 587 is changed to glutamine (Q; gln) and (2) R590T where arginine (R; arg) at amino acid 590 is changed to threonine (T; thr). As another non-limiting example, may comprise three mutations: (1) K406R where lysine (K; lys) at amino acid 406 is changed to arginine (R; arg), (2) R587Q where arginine (R; arg) at amino acid 587 is changed to glutamine (Q; gln) and (3) R590T where arginine (R; arg) at amino acid 590 is changed to threonine (T; thr).

[0149] AAV vectors may also comprise self-complementary AAV vectors (scAAVs). scAAV vectors contain both DNA strands which anneal together to form double stranded DNA. By skipping second strand synthesis, scAAVs allow for rapid expression in the cell.

[0150] In one embodiment, the pharmaceutical composition comprises a recombinant adeno- associated virus (AAV) vector comprising an AAV capsid and an AAV vector genome. The AAV vector genome may comprise at least one peptide related polynucleotide described herein, such as, but not limited to, SEQ ID NOs: 1-64 or variants having at least 95% identity thereto. The recombinant

AAV vectors in the pharmaceutical composition may have at least 70% which contain an AAV vector genome.

[0151] In one embodiment, the pharmaceutical composition comprises a recombinant adeno- associated virus (AAV) vector comprising an AAV capsid and an AAV vector genome. The AAV vector genome may comprise at least one peptide related polynucleotide described herein, such as, but not limited to, SEQ ID NOs: 1-64 or variants having at least 95% identity thereto, plus an additional N-terminal proline. The recombinant AAV vectors in the pharmaceutical composition may have at least 70% which contain an AAV vector genome.

[0152] In one embodiment, the viral vector comprising a peptide-related polynucleotide may be administered or delivered using the methods for the delivery of AAV virions described in European Patent Application No. EP1857552, the contents of which are herein incorporated by reference in its entirety.

[0153] In one embodiment, the viral vector comprising a peptide-related polynucleotide may be administered or delivered using the methods for delivering proteins using AAV vectors described in European Patent Application No. EP2678433, the contents of which are herein incorporated by reference in its entirety.

[0154] In one embodiment, the viral vector comprising a peptide-related polynucleotide may be administered or delivered using the methods for delivering DNA molecules using AAV vectors described in US Patent No. US 5858351, the contents of which are herein incorporated by reference in its entirety.

[0155] In one embodiment, the viral vector comprising a peptide-related polynucleotide may be administered or delivered using the methods for delivering DNA to the bloodstream described in US Patent No. US 6211163, the contents of which are herein incorporated by reference in its entirety.

[0156] In one embodiment, the viral vector comprising a peptide-related polynucleotide may be administered or delivered using the methods for delivering AAV virions described in US Patent No. US 6325998, the contents of which are herein incorporated by reference in its entirety.

[0157] In one embodiment, the viral vector comprising a peptide-related polynucleotide may be administered or delivered using the methods for delivering a payload to the central nervous system described in US Patent No. US 7588757, the contents of which are herein incorporated by reference in its entirety.

[0158] In one embodiment, the viral vector comprising a peptide-related polynucleotide may be administered or delivered using the methods for delivering a payload described in US Patent No. US 8283151, the contents of which are herein incorporated by reference in its entirety.

[0159] In one embodiment, the viral vector comprising a peptide-related polynucleotide may be administered or delivered using the methods for delivering a payload using a glutamic acid

decarboxylase (GAD) delivery vector described in International Patent Publication No. WO2001089583, the contents of which are herein incorporated by reference in its entirety.

[0160] In one embodiment, the viral vector comprising a peptide-related polynucleotide may be administered or delivered using the methods for delivering a payload to neural cells described in International Patent Publication No. WO2012057363, the contents of which are herein incorporated by reference in its entirety.

[0161] In one embodiment, the viral vector comprising a peptide-related polynucleotide may be administered or delivered using the methods for delivering a payload to cells described in US Patnet Number 9585971, the contents of which are herein incorporated by reference in its entirety.

[0162] In one embodiment, the viral vector comprising a peptide-related polynucleotide may be administered or delivered using the methods for delivering a payload to cells described in Deverman et al. Nature Biotechnology, 34, 204-09 (2016).

[0163] In one embodiment, the viral vector comprising a peptide-related polynucleotide may be administered or delivered using the methods for the delivery of AAV virions described in US7198951 [adeno-assoicated virus (AAV) serotype 9 sequences, vectors containing same, and uses therefor], US 9217155 [isolation of novel AAV's and uses thereof], WO2011126808 [pharmacologically induced transgene ablation system], US6015709 [transcriptional activators, and compositions and uses related thereto], US7094604 [Production of pseudotyped recombinant AAV virions], WO2016126993 [anti-tau constructs], US7094604 [recombinant AAV capsid protein], US8,292,769 [Avian adenoasssocited viru (aaav) and uses thereof], US9102949 [CNS targeting aav vectors andmethods of use thereof], US20160120960 [adeno-associated virus mediated gene transfer to the central nervous system], WO2016073693 [AADC polynucleotides for the treatment of parkinson's disease], WO2015168666 [AAV VECTORS FOR RETINAL AND CNS GENE Therapy], US20090117156 [Gene Therapy for Niemann-Pick Disease type A] or WO2005120581 [gene therapy for neurometabolic disorders].

[0164] The pharmaceutical compositions of viral vectors described herein may be characterized by one or more of bioavailability, therapeutic window and/or volume of distribution.

[0165] In some embodiments, peptide-related nucleotides and/or peptide-related nucleotide compositions of the present invention may be combined with, coated onto or embedded in a device. Devices may include, but are not limited to stents, pumps, and/or other implantable therapeutic device. Additionally, peptide-related nucleotides and/or peptide-related nucleotide compositions may be delivered to a subject while the subject is using a compression device such as, but not limited to, a compression device to reduce the chances of deep vein thrombosis (DVT) in a subject. The present invention provides for devices which may incorporate viral vectors that encode one or more peptide-related polynucleotide payload molecules. These devices contain in a stable formulation the viral vectors which may be immediately delivered to a subject in need thereof, such as a human patient.

[0166] Devices for administration may be employed to deliver the viral vectors comprising an peptide-related nucleotides of the present invention according to single, multi- or split-dosing regimens taught herein.

[0167] As used herein and in the appended claims, the singular forms "a," "an", "or," and "the" include plural referents unless the context clearly dictates otherwise. It is understood that aspects and variations of the disclosure described herein include "consisting" and/or "consisting essentially of" aspects and variation.

[0168] The term "about" as used herein means greater or lesser than the value or range of values stated by 10 percent, but is not intended to designate any value or range of values to only this broader definition. Each value or range of values preceded by the term "about" is also intended to encompass the embodiment of the stated absolute value or range of values.

[0169] As used herein, the term "preventing" refers to keeping a disease, disorder or condition from occurring in a subject who may be at risk for the disease, but has not yet been diagnosed as having the disease.

[0170] As used herein, the term "subject" includes mammals, preferably human beings at any age which suffer from the pathology. Preferably, this term encompasses individuals who are at risk to develop the pathology.

[0171] The pharmaceutical compositions are typically suitable for parenteral administration. As used herein, "parenteral administration" of a pharmaceutical composition includes any route of administration characterized by physical breaching of a tissue of a subject and administration of the pharmaceutical composition through the breach in the tissue, thus generally resulting in the direct administration into the blood stream, into muscle, or into an internal organ. Parenteral administration thus includes, but is not limited to, administration of a pharmaceutical composition by injection of the composition, by application of the composition through a surgical incision, by application of the composition through a tissue-penetrating non-surgical wound, and the like. In particular, parenteral administration is contemplated to include, but is not limited to, subcutaneous injection, intraperitoneal injection, intramuscular injection, intrasternal injection, intravenous injection, intraarterial injection, intrathecal injection, intraventricular injection, intraurethral injection, intracranial injection, intrasynovial injection or infusions; or kidney dialytic infusion techniques.

[0172] In various embodiments, the peptide is admixed with a pharmaceutically acceptable excipients to form a pharmaceutical composition that can be systemically administered to the subject orally or via intravenous injection, intramuscular injection, subcutaneous injection, intraperitoneal injection, transdermal injection, intra-arterial injection, intrasternal injection, intrathecal injection, intraventricular injection, intraurethral injection, intracranial injection, intrasynovial injection or via infusions. The pharmaceutical composition preferably contains at least one component that is not found in nature.

[0173] Formulations of a pharmaceutical composition suitable for parenteral administration typically generally comprise the active ingredient combined with a pharmaceutically acceptable excipient, such as sterile water or sterile isotonic saline. Such formulations may be prepared, packaged, or sold in a form suitable for bolus administration or for continuous administration. Injectable formulations may be prepared, packaged, or sold in unit dosage form, such as in ampoules or in multi-dose containers containing a preservative. Formulations for parenteral administration include, but are not limited to, suspensions, solutions, emulsions in oily or aqueous vehicles, pastes, and the like. Such formulations may further comprise one or more additional ingredients including, but not limited to, suspending, stabilizing, or dispersing agents. In one embodiment of a formulation for parenteral administration, the active ingredient is provided in dry (i.e. powder or granular) form for reconstitution with a suitable vehicle (e.g. sterile pyrogen-free water) prior to parenteral administration of the reconstituted composition. Parenteral formulations also include aqueous solutions which may contain carriers such as salts, carbohydrates and buffering agents (preferably to a pH of from 3 to 9), but, for some applications, they may be more suitably formulated as a sterile non-aqueous solution or as a dried form to be used in conjunction with a suitable vehicle such as sterile, pyrogen-free water. Exemplary parenteral administration forms include solutions or suspensions in sterile aqueous solutions, for example, aqueous propylene glycol or dextrose solutions. Such dosage forms can be suitably buffered, if desired. Other parentally-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form, or in a liposomal preparation. Formulations for parenteral administration may be formulated to be immediate and/or modified release. Modified release formulations include delayed-, sustained-, pulsed-, controlled-, targeted and programmed release.

[0174] The present disclosure includes compositions and methods for transdermal or topical delivery, to act locally at the point of application, or to act systemically once entering the body's blood circulation. In these systems, delivery may be achieved by techniques such as direct topical application of a substance or drug in the form of an ointment or the like, or by adhesion of a patch with a reservoir or the like that holds the drug (or other substance) and releases it to the skin in a time-controlled fashion. For topical administration, the compositions can be in the form of emulsions, lotions, gels, creams, jellies, solutions, suspensions, ointments, and transdermal patches. Some topical delivery compositions may contain polyenylphosphatidylcholine (herein abbreviated "PPC"). In some cases, PPC can be used to enhance epidermal penetration. The term "polyenylphosphatidylcholine," as used herein, means any phosphatidylcholine bearing two fatty acid moieties, wherein at least one of the two fatty acids is an unsaturated fatty acid with at least two double bonds in its structure, such as linoleic acid. Such topical formulations may comprise one or more emulsifiers, one or more surfactants, one or more polyglycols, one or more lecithins, one or more fatty acid esters, or one or more transdermal penetration enhancers. Preparations can include sterile aqueous or nonaqueous solutions, suspensions and emulsions, which can be isotonic

with the blood of the subject in certain embodiments. Examples of nonaqueous solvents are polypropylene glycol, polyethylene glycol, vegetable oil such as olive oil, sesame oil, coconut oil, arachis oil, peanut oil, mineral oil, organic esters such as ethyl oleate, or fixed oils including synthetic mono or di-glycerides. Aqueous solvents include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, 1,3-butandiol, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, antioxidants, chelating agents and inert gases and the like.

[0175] For example, in one aspect, sterile injectable solutions can be prepared by incorporating a peptide in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active peptide into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation such as vacuum drying and freeze-drying yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin. In various embodiments, the injectable compositions will be administered using commercially available disposable injectable devices.

[0176] The parenteral formulations can be presented in unit-dose or multi-dose sealed containers, such as ampoules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid excipient, for example, water, for injections, immediately prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind known in the art. Injectable formulations are in accordance with the disclosure. The requirements for effective pharmaceutical excipients for injectable compositions are well-known to those of ordinary skill in the art (see, e.g., *Pharmaceutics and Pharmacy Practice*, J. B. Lippincott Company, Philadelphia, Pa., Banker and Chalmers, eds., pages 238-250 (1982), and *ASHP Handbook on Injectable Drugs*, Toissel, 4th ed., pages 622-630 (1986)).

[0177] Additionally, the peptides of the present disclosures can be made into suppositories for rectal administration by mixing with a variety of bases, such as emulsifying bases or water-soluble bases. Formulations suitable for vaginal administration can be presented as pessaries, tampons, creams,

gels, pastes, foams, or spray formulas containing, in addition to the active ingredient, such carriers as are known in the art to be appropriate.

[0178] It will be appreciated by one of skill in the art that, in addition to the above-described pharmaceutical compositions, the peptides of the disclosure can be formulated as inclusion complexes, such as cyclodextrin inclusion complexes, or liposomes.

[0179] The peptide can be administered intranasally or by inhalation, typically in the form of a dry powder (either alone, as a mixture, or as a mixed component particle, for example, mixed with a suitable pharmaceutically acceptable carrier) from a dry powder inhaler, as an aerosol spray from a pressurized container, pump, spray, atomiser (preferably an atomiser using electrohydrodynamics to produce a fine mist), or nebulizer, with or without the use of a suitable propellant, or as nasal drops. The pressurized container, pump, spray, atomizer, or nebulizer generally contains a solution or suspension of a peptide comprising, for example, a suitable agent for dispersing, solubilizing, or extending release of the active, a propellant(s) as solvent. Prior to use in a dry powder or suspension formulation, the drug product is generally micronized to a size suitable for delivery by inhalation (typically less than 5 microns). This may be achieved by any appropriate comminuting method, such as spiral jet milling, fluid bed jet milling, supercritical fluid processing to form nanoparticles, high pressure homogenization, or spray drying. Capsules, blisters and cartridges for use in an inhaler or insufflator may be formulated to contain a powder mix of the peptide, a suitable powder base and a performance modifier. Suitable flavors, such as menthol and levomenthol, or sweeteners, such as saccharin or saccharin sodium, may be added to those formulations intended for inhaled/intranasal administration. Formulations for inhaled/intranasal administration may be formulated to be immediate and/or modified release. Modified release formulations include delayed-, sustained-, pulsed-, controlled-, targeted and programmed release. In the case of dry powder inhalers and aerosols, the dosage unit is determined by means of a valve which delivers a metered amount. Units are typically arranged to administer a metered dose or "puff" of a peptide. The overall daily dose will typically be administered in a single dose or, more usually, as divided doses throughout the day.

[0180] According to one aspect, the peptides are for use in medicine, particularly human medicine. The peptides are effective to modulate cell signaling associated with aberrant cellular proliferation and malignancy. Additionally, the disclosure provides peptides effective in impacting cell viability and cytoprotection.

[0181] In some aspects, methods are provided herein for treating a condition for which apoptotic cell death, inflammation, autoimmunity, angiogenesis, and/or metastasis is an etiological determinant.

[0182] In another aspect, there is provided a peptide, for use in in the prevention and/or treatment of bone- or cartilages disorders/diseases, cancer, autoimmune diseases, fibrotic diseases, inflammatory diseases, obesity, type I and type II diabetes, neurodegenerative diseases, bone fractures, skeletal

chondrodysplasias, infectious diseases, lung diseases, infertility, muscular disorders, aging, skin diseases, and metabolic diseases.

[0183] In some aspects, the peptides are administered to treat a condition associated with cellular stress responses, such as but not limited to, the induction of heat shock proteins and/or metabolic and oxidative stress. The cellular stress response can be responsive to any stressor, including, e.g., thermal, immunological, cytokine, oxidative, metabolic, anoxic, endoplasmic, reticulum, protein unfolding, nutritional, chemical, mechanical, osmotic and glycemic stresses.

[0184] In some aspects, peptides are administered according to a method provided herein to treat an inflammatory condition, such as but not limited to, diabetes, cardiovascular disease, kidney disease, retinopathy, obesity, metabolic disease, neurodegenerative disease, gastrointestinal disease, autoimmune disease, rheumatological disease or infectious disease.

Combination Therapy

[0185] According to another embodiment, the peptides are co-administered or co-formulated with other known therapeutic agents. According to a further aspect of the present disclosure, provided herein is a combination treatment comprising the administration of a pharmacologically effective amount of a peptide or peptide analog according to the present disclosure, or a pharmaceutically acceptable salt thereof, optionally together with a pharmaceutically acceptable diluent or carrier, with the simultaneous, sequential or separate administration of one or more of the following agents selected from: (1) insulin and insulin analogues; (2) insulin secretagogues, including sulphonylureas (e.g. glipizide) and prandial glucose regulators (sometimes called "short-acting secretagogues"), such as meglitinides (e.g. repaglinide and nateglinide); (3) agents that improve incretin action, for example dipeptidyl peptidase IV (DPP-4) inhibitors (e.g. vildagliptin, saxagliptin, and sitagliptin), and glucagon-like peptide- 1 (GLP-1) agonists (e.g. exenatide); (4) insulin sensitising agents including peroxisome proliferator activated receptor gamma (PPARy) agonists, such as thiazolidinediones (e.g. pioglitazone and rosiglitazone), and agents with any combination of PPAR alpha, gamma and delta activity; (5) agents that modulate hepatic glucose balance, for example biguanides (e.g. metformin), fructose 1 ,6- bisphosphatase inhibitors, glycogen phopsphorylase inhibitors, glycogen synthase kinase inhibitors, and glucokinase activators; (6) agents designed to reduce/slow the absorption of glucose from the intestine, such as alpha-glucosidase inhibitors (e.g. miglitol and acarbose); and (7) agents which antagonise the actions of or reduce secretion of glucagon, such as amylin analogues (e.g. pramlintide); (7) agents that prevent the reabsorption of glucose by the kidney, such as sodium-dependent glucose transporter 2 (SGLT-2) inhibitors (e.g. dapagliflozin); (8) agents designed to treat the complications of prolonged hyperglycaemia, such as aldose reductase inhibitors (e.g. epalrestat and ranirestat); and agents used to treat complications related to micro-angiopathies; (9) anti-dyslipidemia agents, such as HMG-CoA reductase inhibitors (statins, e.g. rosuvastatin) and other cholesterol-lowering agents; PPARa agonists (fibrates, e.g.

gemfibrozil and fenofibrate); bile acid sequestrants (e.g. cholestyramine); (10) cholesterol absorption inhibitors (e.g. plant sterols (i.e. phytosterols), synthetic inhibitors); cholesteryl ester transfer protein (CETP) inhibitors; inhibitors of the ileal bile acid transport system (I BAT inhibitors); bile acid binding resins; nicotinic acid (niacin) and analogues thereof; anti-oxidants, such as probucol; and omega-3 fatty acids; (1 1) antihypertensive agents, including adrenergic receptor antagonists, such as beta blockers (e.g. atenolol), alpha blockers (e.g. doxazosin), and mixed alpha/beta blockers (e.g. labetalol); adrenergic receptor agonists, including alpha-2 agonists (e.g. clonidine); angiotensin converting enzyme (ACE) inhibitors (e.g. lisinopril), calcium channel blockers, such as dihydropyridines (e.g. nifedipine), phenylalkylamines (e.g. verapamil), and benzothiazepines (e.g. diltiazem); angiotensin II receptor antagonists (e.g. candesartan); aldosterone receptor antagonists (e.g. eplerenone); centrally acting adrenergic drugs, such as central alpha agonists (e.g. clonidine); and diuretic agents (e.g. furosemide); (12) haemostasis modulators, including antithrombotics, such as activators of fibrinolysis; thrombin antagonists; factor VIIa inhibitors; anticoagulants, such as vitamin K antagonists (e.g. warfarin), heparin and low molecular weight analogues thereof, factor Xa inhibitors, and direct thrombin inhibitors (e.g. argatroban); antiplatelet agents, such as cyclooxygenase inhibitors (e.g. aspirin), adenosine diphosphate (ADP) receptor inhibitors (e.g. clopidogrel), phosphodiesterase inhibitors (e.g. cilostazol), glycoprotein I IB/I I A inhibitors (e.g. tirofiban), and adenosine reuptake inhibitors (e.g. dipyridamole); (14) anti-obesity agents, such as appetite suppressant (e.g. ephedrine), including noradrenergic agents (e.g. phentermine) and serotonergic agents (e.g. sibutramine), pancreatic lipase inhibitors (e.g. orlistat), microsomal transfer protein (MTP) modulators, diacyl glycerolacyltransferase (DGAT) inhibitors, and cannabinoid (CB1) receptor antagonists (e.g. rimonabant); (15) feeding behavior modifying agents, such as orexin receptor modulators and melanin-concentrating hormone (MCH) modulators; (16) glucagon like peptide- 1 (GLP-1) receptor modulators; (17) neuropeptideY (NPY)/NPY receptor modulators; (18) pyruvate dehydrogenase kinase (PDK) modulators; (19) serotonin receptor modulators; (20) leptin/leptin receptor modulators; (21) ghrelin/ghrelin receptor modulators; or (22) monoamine transmission-modulating agents, such as selective serotonin reuptake inhibitors (SSRI) (e.g. fluoxetine), noradrenaline reuptake inhibitors (NARI), noradrenaline- serotonin reuptake inhibitors (SNRI), triple monoamine reuptake blockers (e.g. tesofensine), and monoamine oxidase inhibitors (MAOI) (e.g. toloxatone and amiflamine), or a pharmaceutically acceptable salt, solvate, solvate of such a salt or a prodrug thereof, optionally together with a pharmaceutically acceptable carrier to a mammal, such as man, in need of such therapeutic treatment.

[0186] According to another embodiment, the peptides are co-administered or co-formulated with other known therapeutic agents for trteating NASH. According to a further aspect of the present disclosure, provided herein is a combination treatment comprising the administration of a pharmacologically effective amount of a peptide or peptide analog according to the present

disclosure, or a pharmaceutically acceptable salt thereof, optionally together with a pharmaceutically acceptable diluent or carrier, with the simultaneous, sequential or separate administration of one or more of the following agents selected from: (miR-103/107 antagonists, FXR agonists, Galectin-1/3 agonists, ACC inhibitors, CB-1 inhibitors, Ketohexakinase inhibitors, PDE4 inhbitors, PPARγ agonists, A3AR agonists, PDE inhibitors, fluoroketolide, mTOT insulin sensitizers, Caspase inhibitors, Leptin analogs, Galectin-1/3 agonists, SCD1 inhibitors, PPARαδ agonists, LOXL2 antibodies, ASK1 inhibitors, 11β-HSD1 inhibitors, PPARαδγ agonists, THR-β agonists, Aldosterone inhibitors, FGF-19 analogs, SBAT inhibitors, CCR2/CCR5 inhbitors, GLP-1 agonists, and PPARαγ agonists). Combinations together with the following compounds are also contemplated as embodiments of the current invention: Astra ZenecA AZD4076, Enanta EDP-305, Galectin Therapeutics GR-MD-02, gemcabene, Gilead GS-0976, Gilead GS-9674, Merck MK-4074, pioglitazone, Pfizer PF-06835919, Pfizer CP-945598, Astellas ASP9831, Boehringer Ingelheim BI 1467335, Bristol Myers Squibb BMS-986036, avandia, metformin, losartan, Can-Fite CF102, pentoxifylline, solithromycin, Cirius MSDC-0602K, emricasan, Conatus IDN-6556, metreleptin, aramchol, Genfit GFT505, simtuzumab, Gilead GS-4997, Gilead GS-9450, Roche TRO19622, Roche RO5093151, Immuron IMM-124E, obeticholic acid, Inventiva IVA337, Madrigal MGL-3196, MN-001, Mitsubishi Tanabe MT-3995, Mochida EPA-E, NGM Biopharma NGM282, Novartis LMB763, Novartis LJN452, Shire SHP626, cenicriviroc, liraglutide, and saroglitazar.

COMORBIDITIES LINKED TO OBESITY – MONOTHERAPY OR COMBINATION

[0187] For example, in addition to being overweight or obese, a subject or patient can further have overweight- or obesity-related comorbidities, i.e., diseases and other adverse health conditions associated with, exacerbated by, or precipitated by being overweight or obese. Contemplated herein are disclosed peptides of the current invention administered alone in combination with at least one other agent that has previously been shown to treat these overweight- or obesity- related conditions.

[0188] For example, Type II diabetes has been associated with obesity. Certain complications of Type II diabetes, e.g., disability and premature death, can be prevented, ameliorated, or eliminated by sustained weight loss (Astrup, A. Pub Health Nutr (2001) 4:499-5 15). Agents administered to treat Type II diabetes include sulfonylureas (e.g., Chlorpropamide, Glipizide, Glyburide, Glimepiride); meglitinides (e.g., Repaglinide and Nateglinide); biguanides (e.g., Metformin); thiazolidinediones (Rosiglitazone, Troglitazone, and Pioglitazone); dipeptidylpeptidase-4 inhibitors (e.g., Sitagliptin, Vildagliptin, and Saxagliptin); glucagon-like peptide-1 mimetics (e.g., Exenatide and Liraglutide); and alpha-glucosidase inhibitors (e.g., Acarbose and Miglitol.

[0189] Cardiac disorders and conditions, for example hypertension, dyslipidemia, ischemic heart disease, cardiomyopathy, cardiac infarction, stroke, venous thromboembolic disease and pulmonary

hypertension, have been linked to overweight or obesity. For example, hypertension has been linked to obesity because excess adipose tissue secretes substances that are acted on by the kidneys, resulting in hypertension. Additionally, with obesity there are generally higher amounts of insulin produced (because of the excess adipose tissue) and this excess insulin also elevates blood pressure. A major treatment option of hypertension is weight loss. Agents administered to treat hypertension include Chlorthalidone; Hydrochlorothiazide; Indapamide, Metolazone; loop diuretics (e.g., Bumetanide, Ethacrynic acid, Furosemide, Lasix, Torsemide); potassium- sparing agents (e.g., Amiloride hydrochloride, benzamil, Spironolactone, and Triamterene); peripheral agents (e.g., Reserpine); central alpha-agonists (e.g., Clonidine hydrochloride, Guanabenz acetate, Guanfacine hydrochloride, and Methyldopa); alpha-blockers (e.g., Doxazosin mesylate, Prazosin hydrochloride, and Terazosin hydrochloride); beta-blockers (e.g., Acebutolol, Atenolol, Betaxolol, Bisoprolol fumarate, Carteolol hydrochloride, Metoprolol tartrate, Metoprolol succinate, Nadolol, Penbutolol sulfate, Pindolol, Propranolol hydrochloride, and Timolol maleate); combined alpha- and beta-blockers (e.g., Carvedilol and Labetalol hydrochloride); direct vasodilators (e.g., Hydralazine hydrochloride and Minoxidil); calcium antagonists (e.g., Diltiazem hydrochloride and Verapamil hydrochloride); dihydropyridines (e.g., Amlodipine besylate, Felodipine, Isradipine, Nicardipine, Nifedipine, and Nisoldipine); ACE inhibitors (benazepril hydrochloride, Captopril, Enalapril maleate, Fosinopril sodium, Lisinopril, Moexipril, Quinapril hydrochloride, Ramipril, Trandolapril); Angiotensin II receptor blockers (e.g., Losartan potassium, Valsartan, and Irbesartan); Renin inhibitors (e.g., Aliskiren); and combinations thereof. These compounds are administered in regimens and at dosages known in the art.

[0190] Carr et al. (The Journal of Clinical Endocrinology & Metabolism (2004) Vol. 89, No. 6 2601-2607) discusses a link between being overweight or obese and dyslipidemia. Dyslipidemia is typically treated with statins. Statins, HMG-CoA reductase inhibitors, slow down production of cholesterol in a subject and/or remove cholesterol buildup from arteries. Statins include mevastatin, lovastatin, pravastatin, simvastatin, velostatin, dihydrocompactin, fluvastatin, atorvastatin, dalvastatin, carvastatin, crilvastatin, bevastatin, cefvastatin, rosuvastatin, pitavastatin, and glenvastatin. These compounds are administered in regimens and at dosages known in the art. Eckel (Circulation (1997) 96:3248-3250) discusses a link between being overweight or obese and ischemic heart disease. Agents administered to treat ischemic heart disease include statins, nitrates (e.g., Isosorbide Dinitrate and Isosorbide Mononitrate), beta-blockers, and calcium channel antagonists. These compounds are administered in regimens and at dosages known in the art.

[0191] Wong et al. (Nature Clinical Practice Cardiovascular Medicine (2007) 4:436-443) discusses a link between being overweight or obese and cardiomyopathy. Agents administered to treat cardiomyopathy include inotropic agents (e.g., Digoxin),. diuretics (e.g., Furosemide), ACE inhibitors, calcium antagonists, anti- arrhythmic agents (e.g., Sotolol, Amiodarone and Disopyramide), and beta-blockers. These compounds are administered in regimens and at dosages

known in the art. Yusef et al. (Lancet (2005) 366(9497): 1640-1649) discusses a link between being overweight or obese and cardiac infarction. Agents administered to treat cardiac infarction include ACE inhibitors, Angiotensin II receptor blockers, direct vasodilators, beta blockers, anti-arrhythmic agents and thrombolytic agents (e.g., Alteplase, Retaplase, Tenecteplase, Anistreplase, and Urokinase). These compounds are administered in regimens and at dosages known in the art.

[0192] Suk et al. (Stroke (2003) 34: 1586- 1592) discusses a link between being overweight or obese and strokes. Agents administered to treat strokes include anti-platelet agents (e.g., Aspirin, Clopidogrel, Dipyridamole, and Ticlopidine), anticoagulant agents (e.g., Heparin), and thrombolytic agents. Stein et al. (The American Journal of Medicine (2005) 18(9):978-980) discusses a link between being overweight or obese and venous thromboembolic disease. Agents administered to treat venous thromboembolic disease include anti-platelet agents, anticoagulant agents, and thrombolytic agents. Sztrymf et al. (Rev Pneumol Clin (2002) 58(2): 104-10) discusses a link between being overweight or obese and pulmonary hypertension. Agents administered to treat pulmonary hypertension include inotropic agents, anticoagulant agents, diuretics, potassium (e.g., K-dur), vasodilators (e.g., Nifedipine and Diltiazem), Bosentan, Epoprostenol, and Sildenafil. Respiratory disorders and conditions such as obesity-hypoventilation syndrome, asthma, and obstructive sleep apnea, have been linked to being overweight or obese. Elamin (Chest (2004) 125: 1972-1974) discusses a link between being overweight or obese and asthma. Agents administered to treat asthma include bronchodilators, anti-inflammatory agents, leukotriene blockers, and anti-Ige agents. Particular asthma agents include Zafirlukast, Flunisolide, Triamcinolone, Beclomethasone, Terbutaline, Fluticasone, Formoterol, Beclomethasone, Salmeterol, Theophylline, and Xopenex.

[0193] Kessler et al. (Eur Respir J (1996) 9:787-794) discusses a link between being overweight or obese and obstructive sleep apnea. Agents administered to treat sleep apnea include Modafinil and amphetamines.

[0194] Hepatic disorders and conditions, such as nonalcoholic fatty liver disease, have been linked to being overweight or obese. Tolman et al. (Ther Clin Risk Manag (2007) 6: 1153- 1163) discusses a link between being overweight or obese and nonalcoholic fatty liver disease. Agents administered to treat nonalcoholic fatty liver disease include antioxidants (e.g., Vitamins E and C), insulin sensitizers (Metformin, Pioglitazone, Rosiglitazone, and Betaine), hepatoprotectants, and lipid-lowering agents.

[0195] Skeletal disorders and conditions, such as, back pain and osteoarthritis of weight-bearing joints, have been linked to being overweight or obese, van Saase (J Rheumatol (1988) 15(7): 1152- 1158) discusses a link between being overweight or obese and osteoarthritis of weight-bearing joints. Agents administered to treat osteoarthritis of weight-bearing joints include Acetaminophen, non-steroidal anti-inflammatory agents (e.g., Ibuprofen, Etodolac, Oxaprozin, Naproxen, Diclofenac,

and Nabumetone), COX-2 inhibitors (e.g., Celecoxib), steroids, supplements (e.g. glucosamine and chondroitin sulfate), and artificial joint fluid.

[0196] Metabolic disorders and conditions, for example, Prader-Willi Syndrome and polycystic ovary syndrome, have been linked to being overweight or obese. Cassidy (Journal of Medical Genetics (1997) 34:917-923) discusses a link between being overweight or obese and Prader-Willi Syndrome. Agents administered to treat Prader-Willi Syndrome include human growth hormone (HGH), somatropin, and weight loss agents (e.g., Orlistat, Sibutramine, Methamphetamine, Ionamin, Phentermine, Bupropion, Diethylpropion, Phendimetrazine, Benzphetermine, and Topamax).

[0197] Hoeger (Obstetrics and Gynecology Clinics of North America (2001) 28(1):85-97) discusses a link between being overweight or obese and polycystic ovary syndrome. Agents administered to treat polycystic ovary syndrome include insulin-sensitizers, combinations of synthetic estrogen and progesterone, Spironolactone, Eflornithine, and Clomiphene. Reproductive disorders and conditions such as sexual dysfunction, erectile dysfunction, infertility, obstetric complications, and fetal abnormalities, have been linked to being overweight or obese. Larsen et al. (Int J Obes (Lond) (2007) 8: 1189-1198) discusses a link between being overweight or obese and sexual dysfunction. Chung et al. (Eur Urol (1999) 36(l):68-70) discusses a link between being overweight or obese and erectile dysfunction. Agents administered to treat erectile dysfunction include phosphodiesterase inhibitors (e.g., Tadalafil, Sildenafil citrate, and Vardenafil), prostaglandin E analogs (e.g., Alprostadil), alkaloids (e.g., Yohimbine), and testosterone. Pasquali et al. (Hum Reprod (1997) 1:82-87) discusses a link between being overweight or obese and infertility. Agents administered to treat infertility include Clomiphene, Clomiphene citrate, Bromocriptine, Gonadotropin-releasing Hormone (GnRH), GnRH agonist, GnRH antagonist, Tamoxifen/nolvadex, gonadotropins, Human Chorionic Gonadotropin (HCG), Human Menopausal Gonadotropin (HmG), progesterone, recombinant follicle stimulating hormone (FSH), UrofoUitropin, Heparin, Follitropin alfa, and Follitropin beta.

[0198] Weiss et al. (American Journal of Obstetrics and Gynecology (2004) 190(4): 1091-1097) discusses a link between being overweight or obese and obstetric complications. Agents administered to treat obstetric complications include Bupivacaine hydrochloride, Dinoprostone PGE2, Meperidine HC1, Ferro-folic-500/iberet-folic-500, Meperidine, Methylergonovine maleate, Ropivacaine HC1, Nalbuphine HC1, Oxymorphone HC1, Oxytocin, Dinoprostone, Ritodrine, Scopolamine hydrobromide, Sufentanil citrate, and Oxytocic.

[0199] Psychiatric disorders and conditions, for example, weight-associated depression and anxiety, have been linked to being overweight or obese. Dixson et al. (Arch Intern Med (2003) 163:2058-2065) discusses a link between being overweight or obese and depression. Agents administered to treat depression include serotonin reuptake inhibitors (e.g., Fluoxetine, Escitalopram, Citalopram, Paroxetine, Sertraline, and Venlafaxine); tricyclic antidepressants (e.g., Amitriptyline, Amoxapine,

Clomipramine, Desipramine, Dosulepin hydrochloride, Doxepin, Imipramine, Iprindole, Lofepramine, Nortriptyline, Opipramol, Protriptyline, and Trimipramine); monoamine oxidase inhibitors (e.g., Isocarboxazid, Moclobemide, Phenelzine, Tranylcypromine, Selegiline, Rasagiline, Nialamide, Iproniazid, Iproclozide, Toloxatone, Linezolid, Dienolide kavapyrone desmethoxyyangonin, and Dextroamphetamine); psychostimulants (e.g., Amphetamine, Methamphetamine, Methylphenidate, and Arecoline); antipsychotics (e.g., Butyrophenones, Phenothiazines, Thioxanthenes, Clozapine, Olanzapine, Risperidone, Quetiapine, Ziprasidone, Amisulpride, Paliperidone, Symbyax, Tetrabenazine, and Cannabidiol); and mood stabilizers (e.g., Lithium carbonate, Valproic acid, Divalproex sodium, Sodium valproate, Lamotrigine, Carbamazepine, Gabapentin, Oxcarbazepine, and Topiramate).

[0200] Simon et al. (Archives of General Psychiatry (2006) 63(7):824-830) discusses a link between being overweight or obese and anxiety. Agents administered to treat anxiety include serotonin reuptake inhibitors, mood stabilizers, benzodiazepines (e.g., Alprazolam, Clonazepam, Diazepam, and Lorazepam), tricyclic antidepressants, monoamine oxidase inhibitors, and beta-blockers.

[0201] Another aspect of the invention provides methods for facilitating and maintaining weight loss in a subject involving administering to the subject an amount of a disclosed compound effective to result in weight loss in the subject; and administering a therapeutically effective amount of a different weight loss agent to maintain a reduced weight in the subject. Weight loss agents include serotonin and noradrenergic re-uptake inhibitors; noradrenergic re-uptake inhibitors; selective serotonin re-uptake inhibitors; and intestinal lipase inhibitors. Particular weight loss agents include liraglutide, orlistat, sibutramine, methamphetamine, ionamin, phentermine, bupropion, diethylpropion, phendimetrazine, benzphetermine, bromocriptine, lorcaserin, topiramate, or agents acting to modulate food intake by blocking ghrelin action, inhibiting diacylglycerol acyltransferase 1 (DGATl) activity, inhibiting stearoyl Co A desaturase 1 (SCD1) activity, inhibiting neuropeptide Y receptor 1 function, activating neuropeptide Y receptor 2 or 4 function, or inhibiting activity of sodium-glucose cotransporters 1 or 2. These compounds are administered in regimens and at dosages known in the art.

[0202] Without being bound by a specific theory, free fatty acids (FFA) in cell culture media after treatment of adipocytes with the peptides indicates a modulation of pathways involved in cellular regulation of lipid or fatty acid levels. Decreases in fatty acid levels in the media may result from a number of processes, including but not limited to inhibition of signaling pathways, reduction in cellular lipogenesis, reduction in lipolysis, or increase in fatty acid oxidation. Peptides that have an effect on the net concentration of free fatty acids have potential utility for treatment of metabolic disorders.

[0203] Lipodystrophy is a common name for disorders characterized by selective loss of adipose tissue (body fat) from various body regions and/or accumulation of excess fat in other areas. Localized fat loss from one area, such as the face, is called lipoatrophy. The extent of fat loss can range from

very small areas on one part of the body to near total absence of adipose tissue from the entire body. In addition, patients may have either severe metabolic complications or mere cosmetic problems. Lipodystrophy associated with severe fat loss may contribute to metabolic complications related to insulin resistance, such as diabetes mellitus, high levels of serum triglycerides and fatty liver (hepatic steatosis). Lipodystrophy may be either congenital (such as familial partial lipodystrophy or Beradinelli-Seip syndrome) or acquired (e.g. associated with various types of illnesses or drugs). Acquired lipodystrophies are caused by medications, autoimmune mechanisms or may be idiopathic. Acquired lipodystrophies include lipodystrophy in HIV-infected patients (LD-HIV) which may be induced by highly active antiretroviral therapy (HAART), acquired generalized lipodystrophy (AGL), acquired partial lipodystrophy (APL) and localized lipodystrophy. Acquired lipodystrophies do not have a direct genetic basis. According to some embodiments, the present invention provides a method for reducing, ameliorating or preventing lipodystrophy.

[0204] The peptides are useful in the treatment of conditions associated with an unbalanced metabolic state manifested by abnormal blood levels of glucose, reactive oxygen species (ROS) and/or free fatty acids (FFA). A favorable metabolic status is defined as a balanced energy homeostasis, characterized by blood levels of glucose, ROS and FFA that are equivalent to those of healthy subjects (within the range of average levels for the healthy population). Accordingly, an unfavorable metabolic status as used herein refers to blood levels of glucose, ROS and/or FFA that are abnormal, i.e. significantly altered compared to their respective levels in healthy control subjects (e.g. as evaluated by a physician or skilled artisan). The term unfavorable metabolic status refers in some embodiments to blood levels of glucose, ROS and/or FFA that are significantly enhanced compared to their respective levels in healthy control subjects (e.g. as evaluated by a physician or skilled artisan). An unfavorable metabolic status may result from abnormal metabolism which may involve glucose (carbohydrate) and/or fatty acid oxidation pathways. When aberrations in fatty acid oxidation pathways are involved, the unfavorable metabolic status is typically manifested by ROS blood levels that are significantly enhanced compared to healthy control subjects and/or by abnormal FFA blood levels. These aberrations may also be manifested by elevated blood levels of oxidized low density lipoproteins (LDL). When aberrations in glucose metabolism are involved, glucose blood levels are typically significantly enhanced compared to healthy control subjects. As used herein, a patient with significantly enhanced blood glucose levels that do not exceed the threshold for unbalanced glycemic control will be defined as having an unfavorable metabolic status if said enhancement is accompanied by abnormal blood ROS and/or FFA values, as described herein. An unbalanced metabolic state may also be evaluated by said physician or skilled artisan by considering the energy intake and various energy consumption and utilization parameters, as known in the art. For example, without limitation, parameters at the cellular level such as cellular (e.g. platelet) ATP production and cellular oxidation, and parameters

at the whole body level such as respiratory quotient (RQ) may be evaluated to determine the metabolic status of the subject. For example, by comparing the relative ratio of such parameters between healthy and sick patients the skilled artisan may evaluate the metabolic status of the subject compared to healthy controls. An unfavorable metabolic status may be found in patients afflicted with chronic metabolic and/or inflammatory disorders that are not adequately treated or balanced by a suitable therapeutic regimen.

[0205] The term "metabolic disease" or "metabolic disorder" refers to a group of identified disorders in which errors of metabolism, imbalances in metabolism, or sub-optimal metabolism occur, which may involve glucose (carbohydrate), fatty acid and/or protein oxidation pathways. Accordingly, when unbalanced, these disorders are typically manifested by an unfavorable metabolic status characterized by abnormal blood levels of glucose, ROS and/or FFA compared to their respective levels in healthy control subjects, as described herein. Such disorders include without limitation diabetes and disorders associated with nutritional or endocrine imbalance.

[0206] An unfavorable metabolic status may also occur as a result of chronic inflammatory disorders, in which a non-resolving, unbalanced inflammatory process is accompanied by secondary metabolic complications manifested by abnormal blood levels of glucose, ROS and/or FFA compared to their respective levels in healthy control subjects. Non-limitative examples of such disorders are sepsis and autoimmune diseases.

[0207] Syndrome X (or metabolic syndrome) denotes a set of signs and symptoms associated with the accumulation of fat in the abdomen. This form of fat distribution is common in middle-aged men and is often visible as a pot belly or paunch. Syndrome X is characterized by a number of disorders including gout, impaired glucose metabolism (increasing susceptibility to diabetes), raised blood pressure, and elevated blood cholesterol levels. People with Syndrome X have a high risk of heart disease. Syndrome X is defined as a constellation of metabolic abnormalities in serum or plasma insulin/glucose level ratios, lipids, uric acid levels, vascular physiology, and coagulation factor imbalances by the American Association of Clinical Endocrinologists. The term "syndrome X" as used herein thus refers to a condition characterized by positive diagnosis of at least two of the following: Non-insulin-dependent diabetes, blood pressure above a level considered normal, insulin level above a level considered normal, dyslipidemia, and obesity.

[0208] A peptide may be useful in the following metabolic diseases

(a) prevention and/or treatment of all forms of diabetes, such as hyperglycemia, type 2 diabetes, impaired glucose tolerance, type 1 diabetes, non-insulin dependent diabetes, MODY (maturity onset diabetes of the young), gestational diabetes, and/or for reduction of HbAlC;

(b) delaying or preventing diabetic disease progression, such as progression in type 2 diabetes, delaying the progression of impaired glucose tolerance (IGT) to insulin requiring type 2

diabetes, delaying or preventing insulin resistance, and/or delaying the progression of non-insulin requiring type 2 diabetes to insulin requiring type 2 diabetes;

(c) improving β-cell function, such as decreasing β-cell apoptosis, increasing β-cell function and/or β-cell mass, and/or for restoring glucose sensitivity to β-cells;

(d) prevention and/or treatment of cognitive disorders and/or neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, and/or multiple sclerosis;

(e) prevention and/or treatment of eating disorders, such as obesity, e.g. by decreasing food intake, reducing body weight, suppressing appetite, inducing satiety; treating or preventing binge eating disorder, bulimia nervosa, and/or obesity induced by administration of an antipsychotic or a steroid; reduction of gastric motility; delaying gastric emptying; increasing physical mobility; and/or prevention and/or treatment of comorbidities to obesity, such as osteoarthritis and/or urine incontinence;

(f) prevention and/or treatment of diabetic complications, such as angiopathy; neuropathy, including peripheral neuropathy; nephropathy; and/or retinopathy;

(g) improving lipid parameters, such as prevention and/or treatment of dyslipidemia, lowering total serum lipids; increasing HDL; lowering small, dense LDL; lowering VLDL; lowering triglycerides; lowering cholesterol; lowering plasma levels of lipoprotein a (Lp(a)) in a human; inhibiting generation of apolipoprotein a (apo(a)) in vitro and/or in vivo;

(h) prevention and/or treatment of cardiovascular diseases, such as syndrome X, atherosclerosis, myocardial infarction, coronary heart disease, reperfusion injury, stroke, hypoxia, cerebral ischemia, an early cardiac or early cardiovascular disease, left ventricular hypertrophy, coronary artery disease, hypertension, essential hypertension, acute hypertensive emergency, cardiomyopathy, heart insufficiency, exercise intolerance, acute and/or chronic heart failure, arrhythmia, cardiac dysrhythmia, syncopy, angina pectoris, cardiac bypass and/or stent reocclusion, intermittent claudication (atherosclerosis obliterans), diastolic dysfunction, and/or systolic dysfunction; and/or reduction of blood pressure, such as reduction of systolic blood pressure;

(i) prevention and/or treatment of gastrointestinal diseases, such as inflammatory bowel disease, short bowel syndrome, or Crohn's disease or colitis; dyspepsia, and/or gastric ulcers; and/or inflammation, such as psoriasis, psoriatic arthritis, rheumatoid arthritis, and/or systemic lupus erythematosus;

(j) prevention and/or treatment of critical illness, such as treatment of a critically ill patient, a critical illness poly-nephropathy (CIPNP) patient, and/or a potential CIPNP patient; prevention of development of critical illness or CIPNP; prevention, treatment and/or cure of systemic

inflammatory response syndrome (SIRS) in a patient; prevention or reduction of the likelihood of a patient suffering from bacteremia, septicemia, and/or septic shock during hospitalization; and/or stabilizing blood glucose, insulin balance and optionally metabolism in intensive care unit patients with acute illness;

(k) prevention and/or treatment of polycystic ovary syndrome (PCOS);

(l) prevention and/or treatment of cerebral disease, such as cerebral ischemia, cerebral hemorrhage, and/or traumatic brain injury;

(m) prevention and/or treatment of sleep apnea;

(n) prevention and/or treatment of abuse, such as alcohol abuse and/or drug abuse;

(o) prevention or treatment of fatty liver conditions, including but not limited to Fatty Liver Disease (FLD), nonalcoholic fatty liver disease (NAFLD), and nonalcoholic steatohepatitis (NASH); and/or

(p) treatment of intracellular production of reactive oxygen species (ROS).

[0209] In further aspects, methods are provided herein for treating diabetes and/or diabetes related complications by administering an effective amount, of the peptides to a patient in need of treatment. Advantageously, the peptides used for treating diabetes and/or related complications according to methods provided herein have anti-apoptotic activity against and/or stimulate proliferation of pancreatic β cells, such that administering the peptides increases the number of insulin producing β cells and the level of insulin produced by the patient.

[0210] The present disclosure also includes methods of treating cancer comprising administering an effective amount of a peptide or analog or variant thereof to a subject in need of treatment. The peptides provided herein exert a variety of anticancer effects and can be used to treat a wide range of cancers and other proliferative disorders. Peptides provided herein can have a variety of anticancer activities, such as but not limited to, inducing apoptosis in cancerous cells, inhibiting tumor angiogenesis, inhibiting tumor metastasis, modulating the cell cycle, inhibiting cancer cell proliferation, promoting cancer cell differentiation, inhibiting production of and/or protecting against reactive oxygen species, and enhancing stress resistance. A "cancer" refers generally to a disease characterized by uncontrolled, abnormal cell growth and proliferation. A "tumor" or "neoplasm" is an abnormal mass of tissue that results from excessive, un controlled, and progressive cell division. Methods described herein are useful for treating cancers and proliferative disorders of any type, including but not limited to, carcinomas, sarcomas, soft tissue sarcomas, lymphomas, hematological cancers, leukemias, germ cell tumors, and cancers without solid tumors (e.g., hematopoietic cancers). In various aspects, the peptides can be used to treat cancers and/or tumors originating from and/or effecting any tissue, including but not limited to,

lung, breast, epithelium, large bowel, rectum, testicle, bladder, thyroid, gallbladder, bile duct, biliary tract, prostate, colon, stomach, esophagus, pancreas, liver, kidney, uterus, cervix, ovary, and brain tissues. Non-limiting examples of specific cancers treatable with the peptides include, but are not limited to, acute lymphoblastic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, chronic myelogenous leukemia, adrenocortical carcinoma, AIDS-related lymphoma, anal cancer, astrocytoma, cerebral basal cell carcinoma, bile duct cancer, extrahepatic bladder cancer, bladder cancer, bone cancer, osteosarcoma/malignant fibrous histiocytoma, brain stem glioma, brain tumor, brain stem glioma, cerebral astrocytoma/malignant glioma, ependymoma, medulloblastoma, supratentorial primitive neuroectodermal tumor, visual pathway and hypothalamic glioma, breast cancer, male bronchial adenomas/carcinoids, Burkitt's lymphoma, carcinoid tumor, gastrointestinal carcinoma of unknown primary central nervous system lymphoma, cervical cancer, chronic lymphocytic leukemia, chronic myelogenous leukemia, chronic myeloproliferative disorders, colon cancer, colorectal cancer, cutaneous t-cell lymphoma, mycosis fungoides and sezary syndrome, endometrial cancer, ependymoma, esophageal cancer, Ewing's family tumors, germ cell tumors, extrahepatic bile duct cancer, eye cancer, intraocular melanoma, retinoblastoma, gallbladder cancer, gastric (stomach) cancer, gastrointestinal carcinoid tumors, ovarian gestational, trophoblastic tumors, glioma, hypothalamic skin cancer (melanoma), skin cancer (non-melanoma), skin carcinoma, small cell lung cancer, small intestine cancer, soft tissue sarcoma, squamous cell carcinoma, squamous neck cancer with occult primary, metastatic stomach (gastric) cancer, stomach (gastric) cancer, t-cell lymphoma, testicular cancer, thymoma, thymic carcinoma, thyroid cancer, transitional cell cancer of the renal pelvis, ureter trophoblastic tumors, transitional cell cancer, urethral cancer, uterine cancer, uterine sarcoma, vaginal cancer, hypothalamic glioma, vulvar cancer, Waldenstrom's macroglobulinemia, Wilms' tumor, hairy cell leukemia, head and neck cancer, hepatocellular (liver) cancer, Hodgkin's lymphoma, hypopharyngeal cancer, islet cell carcinoma (endocrine pancreas), Kaposi's sarcoma, kidney (renal cell) cancer, kidney cancer, laryngeal cancer, hairy cell lip and oral cavity cancer, liver cancer, lung cancer, non-small cell lung cancer, small cell lymphoma, Burkitt's lymphoma, cutaneous t-cell, Hodgkin's lymphoma, non-Hodgkin's lymphoma, Waldenstrom's malignant fibrous histiocytoma of bone/osteosarcoma medulloblastoma, intraocular (eye) merkel cell carcinoma, mesothelioma, malignant mesothelioma, metastatic squamous neck cancer with occult primary multiple endocrine neoplasia syndrome, multiple myeloma/plasma cell neoplasm, mycosis fungoides myelodysplastic syndromes, myelodysplastic/myeloproliferative diseases, myelogenous leukemia, multiple myeloproliferative disorders, chronic nasal cavity and paranasal sinus cancer, nasopharyngeal cancer, pleoropulmonary blastoma, osteosarcoma/malignant fibrous histiocytoma of bone, pheochromocytoma, pineoblastoma, and supratentorial primitive neuroectodermal tumors. In some preferred aspects, the cancer is breast cancer. In some preferred aspects, the cancer is prostate cancer.

[0211] In some aspects, administering a peptide according to a method provided herein enhances efficacy of an established cancer therapy. In further aspects, administering a peptide according to a method provided herein enhances the anticancer activity of another cancer therapy, such as radiation or chemotherapy. In some aspects, methods are provided herein for inducing cell death in cancer cells and/or tumor cells, the methods comprising administering a peptide described herein in an amount sufficient to induce cancer cell death and/or tumor cell death.

[0212] In some embodiments, the peptides have one or more cell protective or cytoprotective activities. For example, in some aspects, the peptides are capable of preventing cell damage, improving cell survival, and/or enhancing resistance to environmental stress, such as but not limited to, heat shock, serum withdrawal, chemotherapy, and/or radiation.

[0213] In some aspects, administering a peptide according to a method provided herein decreases adverse effects of an established cancer therapy.

[0214] The methods disclosed herein include neuroprotection, treating conditions associated with the integrity and function of, or treat damage to, any of the tissues or cells of the CNS, and particularly the neurons, glial cells, or endothelial cells, from a condition, disease or event that would otherwise result in damage to such tissues or cells or to the integrity of the blood-brain barrier. Such neuroprotection serves to prevent, reduce or treat the damage that would otherwise occur to such tissues or cells caused by such condition, disease or event. Such methods include treatment of traumatic spinal cord injury, traumatic brain injury, multiple sclerosis, peripheral nerve injury, and ischemic or hemorrhagic stroke.

[0215] In particular, the peptides may be effective in the protection of white blood cells from suppression, protecting germ cells from cell death induced by a chemotherapeutic agent and inhibiting a reduction or decrease in fertility induced by a chemotherapeutic agent.

[0216] For example, in some aspects, administering a peptide according to a method provided herein protects non-cancerous cells against the adverse effects of a non-specific cancer therapy, such as radiation or chemotherapy.

[0217] In some embodiments, the peptides provided herein have neuroprotective activity against neurotoxicity in the peripheral nervous system, such as but not limited to, neurotoxicity associated with chemotherapeutic agents, radiation therapy, anti-infective agents, and/or other therapeutics. For example, the peptides provided herein may exert neuroprotective activity against peripheral neurotoxicity associated with Vinca alkaloids, platinum compounds, suramin, taxanes, and/or other chemotherapeutic agents.

[0218] In some embodiments, the peptides exhibit cell survival promoting (e.g., anti-apoptotic) activity against disease-associated cells and/or stimuli, such as but not flouted to, cells of subjects suffering from diabetes, kidney disease, and/or cancer. For example, in some aspects, the peptides have anti-apoptotic activity against pancreatic β-cells of diabetic subjects and/or tumor cells.

[0219] Advantageously, administering a peptide according to methods provided herein provides a protective effect against neurodegenerative effects, including for example, cell death induced by the SOD1 mutant in amyotrophic lateral sclerosis subjects, mutant APP, PS-1, PS-22, or amyloid-beta (Aβ) peptides in Alzheimer's disease subjects, and/or polyglutamine repeat mutations in Huntington's disease subjects.

[0220] In some embodiments, the peptides provided herein have cell growth-stimulating activity against disease-associated cells, such as but not limited to, pancreatic β-cells of diabetic subjects.

[0221] In some embodiments, the peptides provided herein have differentiation-stimulating activity against disease-associated cells. For example, in some aspects, the peptides stimulate insulin-induced differentiation of adipocytes front diabetic patients.

[0222] In some embodiments, the peptides have anticancer activity. For example, in some aspects, the peptides have pro-apoptotic activity against cancer cells, such as but not limited to, prostate cancer cells and/or breast cancer cells. In further aspects, the peptides have anti-proliferative activity against cancer cells, such as but not limited to, prostate cancer cells and/or breast cancer cells.

[0223] Further preferred medical uses include treatment or prevention of degenerative disorders, particularly neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, Huntington's disease, ataxia, e.g. spinocerebellar ataxia, Kennedy disease, myotonic dystrophy, Lewy body dementia, multi-systemic atrophy, amyotrophic lateral sclerosis, primary lateral sclerosis, spinal muscular atrophy, prion-associated diseases, e.g. Creutzfeldt-Jacob disease, multiple sclerosis, telangiectasia, Batten disease, corticobasal degeneration, corticobasal degeneration, subacute combined degeneration of spinal cord, Tabes dorsalis, Tay-Sachs disease, toxic encephalopathy, infantile Refsum disease, Refsum disease, neuroacanthocytosis, Niemann-Pick disease, Lyme disease, Machado-Joseph disease, Sandhoff disease, Shy-Drager syndrome, wobbly hedgehog syndrome, proteopathy, cerebral β-amyloid angiopathy, retinal ganglion cell degeneration in glaucoma, synucleinopathies, tauopathies, frontotemporal lobar degeneration (FTLD), dementia, cadasil syndrome, hereditary cerebral hemorrhage with amyloidosis, Alexander disease, seipinopathies, familial amyloidotic neuropathy, senile systemic amyloidosis, serpinopathies, AL (light chain) amyloidosis (primary systemic amyloidosis), AH (heavy chain) amyloidosis, AA (secondary) amyloidosis, aortic medial amyloidosis, ApoAI amyloidosis, ApoAII amyloidosis, ApoAIV amyloidosis, familial amyloidosis of the Finnish type (FAF), Lysozyme amyloidosis, Fibrinogen amyloidosis, Dialysis amyloidosis, Inclusion body myositis/myopathy, Cataracts, Retinitis pigmentosa with rhodopsin mutations, medullary thyroid carcinoma, cardiac atrial amyloidosis, pituitary prolactinoma, Hereditary lattice corneal dystrophy, Cutaneous lichen amyloidosis, Mallory bodies, corneal lactoferrin amyloidosis, pulmonary alveolar proteinosis, odontogenic (Pindborg) tumor amyloid, cystic fibrosis, sickle cell disease or critical illness myopathy (CIM). Without being limited by a particular theory, it is believed that the peptides provided herein have one or more activities capable of repairing and/or preventing

neurodegenerative damage of neural cells and/or other cell types. "Neurodegenerative diseases" treatable according to methods provided herein are progressive diseases resulting in the degeneration and/or loss of neurons, for example due to neuronal cell death (apoptosis). Examples of neurodegenerative diseases include, but are not limited to, cerebral degenerative diseases (e.g., Alzheimer's disease (AD), Parkinson's disease, progressive supranuclear palsy, and Huntington's disease (HD)), and spinal degenerative disease/motor neuron degenerative diseases (e.g., amyotrophic lateral sclerosis (ALS), (SMA: Werdnig-Hoffmann disease or Kugelberg-Welander syndrome), spinocerebellar ataxia, bulbospinal muscular atrophy (BSMA; Kennedy-Alter-Sung syndrome)). A "motor neuron degenerative disease" is a neurodegenerative disease characterized by a progressive, retrograde disorder of upper and lower motor neurons that control motion in the body. In further aspects, the peptides and compositions thereof are also effective in ameliorating conditions resulting from motor neuron degenerative disease, such as muscular atrophy, muscular weakness, bulbar palsy (muscular atrophy or weakness in the face, pharynx, and tongue, and aphasia or dysphagia caused thereby), muscular fasciculation, and respiratory disorder.

[0224] Further uses include the prevention and treatment of diseases or conditions associated with mitochondrial dysfunction. Mitochondria, central to metabolic processes, are involved with energy production, programmed cell death, and reactive oxygen species (ROS) generation. Traditionally, mitochondria have been considered as "end-function" organelles, receiving and processing vast amounts of cellular signals to regulate energy production and cell death. The peptides and pharmaceutical formulations thereof can be used to treat various age-related disease with much metabolic implications. Also they have an impact on has also been tested in various ways in vitro and in vivo to affect mitochondrial respiration, glucose transport, glucose utilization, glycolysis, insulin regulation and cellular proliferation/survival. Mitochondrial dysfunction is associated with but not limited to metabolic disorders, neurodegenerative diseases, chronic inflammatory diseases, and diseases of aging. Some mitochondrial diseases are due to mutations or deletions in the mitochondrial genome. Mitochondria divide and proliferate with a faster turnover rate than their host cells, and their replication is under control of the nuclear genome. If a threshold proportion of mitochondria in a cell is defective, and if a threshold proportion of such cells within a tissue have defective mitochondria, symptoms of tissue or organ dysfunction can result. Practically any tissue can be affected, and a large variety of symptoms may be present, depending on the extent to which different tissues are involved. In addition to congenital disorders involving inherited defective mitochondria, acquired mitochondrial dysfunction contributes to diseases, particularly neurodegenerative disorders associated with aging like Parkinson's, Alzheimer's, and Huntington's Diseases. The incidence of somatic mutations in mitochondrial DNA rises exponentially with age; diminished respiratory chain activity is found universally in aging people. Mitochondrial dysfunction is also implicated in excitotoxic neuronal injury, such as

that associated with seizures or ischemia. Other disorders associated with mitochondrial dysfunction include chronic inflammatory disorders and metabolic disorders.

[0225] Peptides that are cytoprotective have potential utility to extend the viability of cells in culture. The peptides are useful for manufacture of biological products, including proteins, antibodies and the like. The present disclosure relates generally to peptides and processes for modulating one or more properties of a cell culture, including mammalian cell cultures such as CHO cell cultures, or e. coli cell cultures. In one embodiment, there is provided a method of increasing specific productivity in a mammalian cell culture expressing a recombinant protein comprising establishing a mammalian cell culture in a culture medium; increasing cell growth viability by contacting the cell culture with a culture medium comprising a peptide; and maintaining the cell culture by contacting the culture with a culture medium comprising a peptide.

[0226] According to another embodiment, the peptides are coadministered or co-formulated with other known chemotherapeutic agents and/or anti-inflammatory agents.

[0227] Both human apelin receptor (APJ) and apelin have been implicated as the key mediators of physiological responses to multiple homeostatic perturbations, including cardiovascular control, water balance, hypothalamic-pituitary-adrenal (HP A) axis regulation and metabolic homeostasis. Elevated levels of apelin have been detected in many pathological states or disease processes, such as heart disease, atherosclerosis, tumor angiogenesis, cerebral ischemic injury and diabetes. The apelinergic system has been implicated in tumor neoangiogenesis. Apelin agonists may have therapeutic effects in ischemia recovery due to vessel regeneration and endothelial proliferation and blood vessel diameter regulation. It has also been associated with sepsis related injury, cerebral ischemic events, thromibin related aggregation and UVB radiation recovery. See Tian etal, Fronteirs in Neurology, 11:75 (2020); Sawane et al, AJP, 179(6), 2691-2697 (2011); Luo, et al ., Int. J of Molecular Med., 42, 1161-1167 (2018); and Adam et al. Blood, 127, (7) 908-920, Feb 2016.

[0228] APJ is localized in the hypothalamic pPVN and the anterior pituitary gland, key areas involved in the stress response. The presence of APJ and apelin in VP- and CRH-containing hypothalamic nuclei, which are pivotal to the HPA axis responses to stress, suggests a role for apelin/APJ in neuroadenohypophysial hormone release.

[0229] Apelin and APJ are regulators of central and peripheral responses to multiple homeostatic perturbations such as cardiovascular control and function; angiogenesis; fluid homeostasis; water balance; hypothalamic-pituitary-adrenal (HPA) axis regulation; metabolic homeostasis; energy metabolism; and kidney function. APJ-apelin signaling plays a role in the maintenance of pulmonary vascular homeostasis (see, e.g., Kim supra). Evidence also points to a nexus between apelinergic system (e.g., apelin and APJ receptor) and the treatment of conditions such as sepsis, septic shock, and renal failure (see, e.g., Coquerel, D., et al., Critical Care 2018, 22: 10). As another example, apelin, synthesized and secreted by adipocytes, has been described as a

beneficial adipokine. Therefore, the peptides of Formula I-II are effective as treatment of pulmonary hypertension (e.g., PAH); heart failure; type II diabetes; renal failure; sepsis; and systemic hypertension.

[0230] The present invention is based on the discovery of a series of potent agonists of the apelin receptor (APJ). In further aspects, the peptides of the current invention are used for the treatment of apelin mediated diseases or disorders. In further aspects, the peptides of the current invention are used for the treatment of diseases including heart failure, chronic kidney disease, hypertension, and metabolic disorders.

[0231] One aspect of the invention is a method of preventing or treating in a subject an apelin-mediated disease or disorder, comprising administering to the subject a peptide listed herein, thereby preventing or treating the disease or disorder is also provided herein.

[0232] In further aspects the disease or disorder is caused by CNS-dependent or CNS-independent disturbed fluid homeostasis, acute or chronic renal failure, hypertension, pulmonary hypertension, portal hypertension or systolic hypertension.

[0233] In other aspects, the disease or disorder is a vascular disease or disorder, vascular permeability, nonfunctional blood vessels, vascular hypertrophy, vascular remodeling, vascular stiffness, atherosclerosis, peripheral arterial occlusive disease (PAOD), restenosis, thrombosis, vascular permeability disorders, ischemia, reperfusion damage, ischemia or reperfusion damage of the heart, kidney or retina, or a combination thereof.

[0234] In certain aspects, the disease or disorder is thrombosis or thrombin-mediated platelet aggregation. The present apelin agonists can be used to maintain hemostasis and regulation of platelet function. The agonists can inhibit thrombin-mediated and collagen-mediated platelet activation. The peptides of the invention are anti-aggregation agents and anti-thrombotic agents. The peptides of the invention are useful for the prevention of platelet aggregation and thrombin mediated events.

[0235] In certain aspects, the disease or disorder is a cardiovascular disease or disorder, coronary heart disease, stroke, heart failure, systolic heart failure, diastolic heart failure, diabetic heart failure, heart failure with preserved ejection fraction, cardiomyopathy, myocardial infarction, left ventricular dysfunction, left ventricular dysfunction after myocardial infarction, cardiac hypertrophy, myocardial remodeling, myocardial remodeling after infarction, myocardial remodeling after cardiac surgery or valvular heart disease.

[0236] In other aspects the disease or disorder is a metabolic disease or disorder, metabolic syndrome, insulin resistance, diabetes mellitus, diabetic late complications, diabetic macro-and micro-vasculopathies, diabetic nephropathy, diabetic retinopathy, diabetic neuropathies or cardiac autonomic neuropathy.

[0237] In further aspects, the invention includes a method of treating and/or preventing a disease or disorder selected from hypertension, endothelial dysfunction, damages to cardiovascular tissues, heart failure, coronary heart disease, ischemic and/or hemorrhagic stroke, macrovascular disease,

microvascular disease, diabetic heart (including diabetic cardiomyopathy and heart failure as a diabetic complication) coronary heart disease, peripheral artery disease, peripheral arterial occlusive disease, pre-eclampsia, resistant hypertension, refractory hypertension, hypertensive crisis, blood or fetal-placental circulation, edematous diseases, pulmonary dysfunction, acute lung injury (ALI), acute respiratory distress syndrome (ARDS), trauma and/or burns, and/or ventilator induced lung injury (VI LI), pulmonary fibrosis, mountain sickness, chronic kidney diseases, acute kidney injury, lymphedema, lymphatic vessel regeneration, inflammatory bowel disease, inflammatory disease, or ocular disorders associated with disturbed vascular function, topical wounds, migraine, angiogenesis, degeneration of cartilage, osteoarthritis, and cancers.

[0238] In further aspects the APJ agonists reduce extravascular lung water accumulation, capillary-alveolar leakage, and hypoxemia. In further aspects the APJ agonists act as key regulators of central and peripheral responses to multiple homeostatic perturbations. In further aspects the APJ agonists regulate angiogenesis, fluid homeostasis or energy metabolism. In further aspects, the APJ agonists act as neuroendocrine modulators of the FIPA axis responses to stress. In further aspects the APJ agonists benefit cardiovascular function.

[0239] The term "apelin mediated disease or disorder" as used herein includes any disease or disorder that is mediated by apelin. Examples of apelin mediated diseases or disorders include, but are not limited to, a cardiovascular disease or disorder, coronary heart disease, stroke, heart failure, systolic heart failure, diastolic heart failure, diabetic heart failure, heart failure with preserved ejection fraction, cardiomyopathy, myocardial infarction, left ventricular dysfunction, left ventricular dysfunction after myocardial infarction, cardiac hypertrophy, myocardial remodeling, myocardial remodeling after infarction, myocardial remodeling after cardiac surgery, valvular heart disease; a metabolic disease or disorder, metabolic syndrome, insulin resistance, diabetes mellitus, diabetic late complications, diabetic macro- and micro-vasculopathies, diabetic nephropathy, diabetic retinopathy, diabetic neuropathies, cardiac autonomic neuropathy; a disease or disorder is caused by CNS-dependent or CNS-independent disturbed fluid homeostasis, acute or chronic renal failure, hypertension, pulmonary hypertension, portal hypertension, systolic hypertension; a vascular disease or disorder, vascular permeability, nonfunctional blood vessels, vascular hypertrophy, vascular remodeling, vascular stiffness, atherosclerosis, peripheral arterial occlusive disease (PAOD), restenosis, thrombosis, vascular permeability disorders, ischemia, reperfusion damage, ischemia, reperfusion damage of the heart, kidney or retina, or a combination thereof.

[0240] In one aspect, a treatment is disclosed that can reduce the incidence of a cytokine storm in a subject with a pathogenic infection, whether cytokines were induced by the pathogen itself or as a consequence of priming of cells and subsequent bacterial infection.

[0241] The peptides of the invention are useful for treatment and/or prophylaxis of bacterial infection in humans or other animals by administering to the subject in need of a therapeutically effective

amount of peptide of Formula I-II, or a pharmaceutically acceptable salt, or thereof. The peptides and methods of the invention are particularly well suited for human patients infected by pathogens that include Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii and Pseudomonas aeruginosa.

[0242] Examples of bacterial infections may include, but not limited to, upper respiratory infections, lower respiratory infections, ear infections, pleuropulmonary and bronchial infections, complicated urinary tract infections, uncomplicated urinary tract infections, intra-abdominal infections, cardiovascular infections, a blood stream infection, sepsis, bacteremia, CNS infections, skin and soft tissue infections, GI infections, bone and joint infections, genital infections, eye infections, or granulomatous infections. Examples of specific bacterial infections include, but not limited to, uncomplicated skin and skin structure infections (uSSSI), complicated skin and skin structure infections (cSSSI), catheter infections, pharyngitis, sinusitis, otitis externa, otitis media, bronchitis, empyema, pneumonia, community-acquired bacterial pneumoniae (CABP), hospital-acquired pneumonia (HAP), hospital-acquired bacterial pneumonia, ventilator-associated pneumonia (VAP), diabetic foot infections, vancomycin resistant enterococci infections, cystitis and pyelonephritis, renal calculi, prostatitis, peritonitis, complicated intra-abdominal infections (cIAI) and other inter-abdominal infections, dialysis-associated peritonitis, visceral abscesses, endocarditis, myocarditis, pericarditis, transfusion-associated sepsis, meningitis, encephalitis, brain abscess, osteomyelitis, arthritis, genital ulcers, urethritis, vaginitis, cervicitis, gingivitis, conjunctivitis, keratitis, endophthalmitisa, an infection in cystic fibrosis patients or an infection of febrile neutropenic patients.

[0243] In one aspect disclosed herein is a method of treating, preventing, inhibiting, reducing the incidence of, ameliorating, or alleviating sepsis, or any combination thereof, in a subject in need, comprising the step of administering a composition comprising an early apoptotic cell population to said subject, wherein said administering treats, prevents, inhibits, reduces the incidence of, ameliorates, or alleviates sepsis in said subject.

[0244] In a related aspect, the sepsis comprises mild or severe sepsis. In some embodiments, the source of sepsis comprises pneumonia, an endovascular methicillin-resistant Staphylococcus aureus (MRS A) infection, sepsis-induced cardiomyopathy or a urinary tract infection (UTI).

[0245] In another related aspect, the method results in increased survival of said subject. In another related aspect, the incidence of organ failure or organ dysfunction, or organ damage, or a combination thereof, in a subject treated by the method, is reduced. In a further related aspect, the organ failure comprises acute multiple organ failure.

[0246] The present invention relates to methods of using a peptide of Formula I-II as a pharmaceutical agent for the treatment and prevention of radiation and/or chemotherapy related injuries and/or afflictions, such as myelosuppression and decreased macrophage activity. The present invention

relates to methods of using a peptide of Formula I-II as a radioprotective agent. The peptides can also be used for the treatment of skin injury from UVB irradiation.

[0247] The person skilled in the art can easily determine whether the peptide is biologically active. For example, the capacity to activate the apelin/apelin receptor pathway can be determined by assessing inhibition of cAMP production induced by forskolin, ERK phosphorylation and towards apelin receptor internalization (e.g. as described in Example). Agonistic activities of an apelin analogue toward APJ may be determined by any well-known method in the art. For example, since the peptides of the present invention can promote the function of the apelin receptor, the agonist can be screened by using apelin, the natural agonist of APJ in a competitive binding test and test associated with the biological activity.

[0248] Thus, the skilled artisan would appreciate, based upon the disclosure provided herein, that the dose and dosing regimen is adjusted in accordance with methods well-known in the therapeutic arts. That is, the maximum tolerable dose can be readily established, and the effective amount providing a detectable therapeutic benefit to a subject may also be determined, as can the temporal requirements for administering each agent to provide a detectable therapeutic benefit to the subject. Accordingly, while certain dose and administration regimens are exemplified herein, these examples in no way limit the dose and administration regimen that may be provided to a subject in practicing the present disclosure.

[0249] It is to be noted that dosage values may vary with the type and severity of the condition to be ameliorated, and may include single or multiple doses. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition. Further, the dosage regimen with the compositions of this disclosure may be based on a variety of factors, including the type of disease, the age, weight, sex, medical condition of the subject, the severity of the condition, the route of administration, and the particular peptide employed. Thus, the dosage regimen can vary widely, but can be determined routinely using standard methods. For example, doses may be adjusted based on pharmacokinetic or pharmacodynamic parameters, which may include clinical effects such as toxic effects and/or laboratory values. Thus, the present disclosure encompasses intra-subject dose-escalation as determined by the skilled artisan. Determining appropriate dosages and regimens are well-known in the relevant art and would be understood to be encompassed by the skilled artisan once provided the teachings disclosed herein.

[0250] The dose of the peptide of the present disclosure also will be determined by the existence, nature and extent of any adverse side effects that might accompany the administration of a particular peptide of the present disclosure. Typically, the attending physician will decide the dosage of the peptide of the present disclosure with which to treat each individual patient, taking into

consideration a variety of factors, such as age, body weight, general health, diet, sex, peptide of the present disclosure to be administered, route of administration, and the severity of the condition being treated. By way of example and not intending to be limiting, the dose of the peptide of the present disclosure can be about 0.0001 to about 100 mg/kg body weight of the subject being treated/day, from about 0.001 to about 10 mg/kg body weight/day, or about 0.01 mg to about 1 mg/kg body weight/day. The peptide can be administered in one or more doses, such as from 1 to 3 doses.

[0251] In some embodiments, the pharmaceutical composition comprises any of the analogs disclosed herein at a purity level suitable for administration to a patient. In some embodiments, the analog has a purity level of at least about 90%, preferably above about 95%, more preferably above about 99%, and a pharmaceutically acceptable diluent, carrier or excipient.

[0252] The pharmaceutical compositions may be formulated to achieve a physiologically compatible pH. In some embodiments, the pH of the pharmaceutical composition may be at least 5, or at least 6, or at least 7, depending on the formulation and route of administration.

[0253] In various embodiments, single or multiple administrations of the pharmaceutical compositions are administered depending on the dosage and frequency as required and tolerated by the subject. In any event, the composition should provide a sufficient quantity of at least one of the peptide disclosed herein to effectively treat the subject. The dosage can be administered once but may be applied periodically until either a therapeutic result is achieved or until side effects warrant discontinuation of therapy.

[0254] The dosing frequency of the administration of the peptide pharmaceutical composition depends on the nature of the therapy and the particular disease being treated. The administration may be once, twice, three times or four times daily, for the peptide. Treatment of a subject with a therapeutically effective amount of a peptide, can include a single treatment or, preferably, can include a series of treatments. In a preferred example, a subject is treated with peptide daily, one time per week or biweekly.

[0255] Reference will now be made in detail to embodiments of the present disclosure. While certain embodiments of the present disclosure will be described, it will be understood that it is not intended to limit the embodiments of the present disclosure to those described embodiments. To the contrary, reference to embodiments of the present disclosure is intended to cover alternatives, modifications, and equivalents as may be included within the spirit and scope of the embodiments of the present disclosure as defined by the appended claims.

EMBODIMENTS

[0256] The embodiments listed below are presented in numbered form for convenience and for ease and clarity of reference in referring back to multiple embodiments.

[0257] 1. A peptide comprising an amino acid sequence of Formula I:

X^1- RX^2-X^3-X^4-X^5-X^6-Q-X^7-L-X^8-X^9 (I) (SEQ ID NO: 1)

wherein X^1 is absent or if present is an amino acid having a polar side chain or a non-polar side chain; X^2 is an amino acid having a polar side chain or a non-polar side chain; X^3 is absent or if present is one to three amino acids, each amino acid independently having a polar side chain or a non-polar side chain; X^4 is an amino acid having a polar side chain or a non-polar side chain; X^5 is an amino acid having a non-polar side chain; X^6 is an amino acid having a polar side chain or a non-polar side chain; X^7 is an amino acid having a polar side chain; X^8 is an amino acid having a polar side chain; and X^9 is absent or if present is one to three amino acids, each amino acid independently having a polar side chain or a non-polar side chain; or an analog of said peptide having a deletion, insertion or substitution of one, two, three, or four amino acids; or C-terminal acids or amides, or N-acetyl derivatives thereof; or a pharmaceutically acceptable salt thereof.

[0258] 2. The peptide or analog of Embodiment 1 wherein X^3 is absent, or if present is -$X^{12}X^{11}X^{10}$-; wherein X^{10} is absent, or if present is an amino acid having a non-polar side chain; X^{11} is absent, or if present is an amino acid having a non-polar side chain; and X^{12} is an amino acid having a polar side chain or a non-polar side chain; or C-terminal acids or amides, or N-acetyl derivatives thereof; or a pharmaceutically acceptable salt thereof.

[0259] 3. The peptide or analog of Embodiment 1 wherein X^9 is absent, or if present is -$X^{13}X^{14}X^{15}$; wherein X^{13} is an amino acid having a non-polar side chain; X^{14} is absent, or if present is an amino acid having a non-polar side chain; and X^{15} is absent, or if present is an amino acid having a polar side chain; or C-terminal acids or amides, or N-acetyl derivatives thereof; or a pharmaceutically acceptable salt thereof.

[0260] 4. The peptide or analog of Embodiment 1 wherein:

X^1 is absent, or if present is selected from D, (dD), E, (dE), K, (dK), R, (dR), H, (dH), N, (dN), Q, (dQ), S, (dS), T, (dT), Y, (dY), C, (dC), G, A, (dA), V, (dV), L, (dL), I, (dI), F, (dF), W, (dW), P (dP), M and (dM);

X^2 is selected from D, (dD), E, (dE), K, (dK), R, (dR), H, (dH), N, (dN), Q, (dQ), S, (dS), T, (dT), Y, (dY), C, (dC), G, A, (dA), V, (dV), L, (dL), I, (dI), F, (dF), W, (dW), P (dP), M and (dM);

X^3 is absent or if present is D, (dD), E, (dE), K, (dK), R, (dR), H, (dH), N, (dN), Q, (dQ), S, (dS), T, (dT), Y, (dY), C, (dC), G, A, (dA), V, (dV), L, (dL), I, (dI), F, (dF), W, (dW), P (dP), M, (dM) or-$X^{12}X^{11}X^{10}$-;

X^4 is an amino acid selected from D, (dD), E, (dE), K, (dK), R, (dR), H, (dH), N, (dN), Q, (dQ), S, (dS), T, (dT), Y, (dY), C, (dC), G, A, (dA), V, (dV), L, (dL), I, (dI), F, (dF), W, (dW), P (dP), M and (dM);

X^5 is an amino acid selected from G, A, (dA), V, (dV), L, (dL), I, (dI), F, (dF), W, (dW), P (dP), M and (dM);

X^6 is an amino acid selected from D, (dD), E, (dE), K, (dK), R, (dR), H, (dH), N, (dN), Q, (dQ), S, (dS), T, (dT), Y, (dY), C, (dC), G, A, (dA), V, (dV), L, (dL), I, (dI), F, (dF), W, (dW), P (dP), M and (dM);

X^7 is an amino acid selected from D, (dD), E, (dE), K, (dK), R, (dR), H, (dH), N, (dN), Q, (dQ), S, (dS), T, (dT), Y, (dY), C, and (dC);

X^8 is an amino acid selected from D, (dD), E, (dE), K, (dK), R, (dR), H, (dH), N, (dN), Q, (dQ), S, (dS), T, (dT), Y, (dY), C, and (dC);

X^9 is absent or if present is an amino acid independently selected from G, A, (dA), V, (dV), L, (dL), I, (dI), F, (dF), W, (dW), P (dP), M and (dM) or $-X^{12}X^{13}X^{14}$;

X^{10} is absent, or if present is an amino acid selected from G, A, (dA), V, (dV), L, (dL), I, (dI), F, (dF), W, (dW), P (dP), M and (dM);

X^{11} is absent, or if present is an amino acid selected from G, A, (dA), V, (dV), L, (dL), I, (dI), F, (dF), W, (dW), P (dP), M and (dM);

X^{12} is an amino acid selected from G, A, (dA), V, (dV), L, (dL), I, (dI), F, (dF), W, (dW), P (dP), M and (dM);

X^{13} is an amino acid selected from G, A, (dA), V, (dV), L, (dL), I, (dI), F, (dF), W, (dW), P (dP), M and (dM);

X^{14} is absent, or if present is an amino acid selected from G, A, (dA), V, (dV), L, (dL), I, (dI), F, (dF), W, (dW), P (dP), M and (dM); and

X^{15} is absent, or if present is an amino acid selected from D, (dD), E, (dE), K, (dK), R, (dR), H, (dH), N, (dN), Q, (dQ), S, (dS), T, (dT), Y, (dY), C, and (dC);

or C-terminal acids or amides, or N-acetyl derivatives thereof; or a pharmaceutically acceptable salt thereof.

[0261] 5. The peptide or analog of Embodiment 1 wherein X^1 is M, K, or absent; X^2 is R or Aib; X^3 is absent or if present is M, E, -MMG-, -II(dA)-, -Nle-Nle-G- or -IIG-; X^4 is M, E, I or Nle; X^5 is V, A or G; X^6 is F, Y, A or E; X^7 is C, S or E; X^8 is C, S or E; and X^9 is -GL, -G(dA), -G(dA)K, -(dA)L, G or absent; C-terminal acids or amides, or N-acetyl derivatives thereof; or a pharmaceutically acceptable salt thereof.

[0262] 6. The peptide or analog of embodiment 5, wherein X^1 is (PEG12)-K, and/or wherein X^9 is -G(dA)-K(PEG12).

[0263] 7. The peptide or analog of Embodiment 1 comprising or consisting of an amino acid sequence selected from a peptide sequence of Table 1; or a pharmaceutically acceptable salt thereof.

[0264] 8. A peptide comprising an amino acid sequence of Formula II:

X^{16}-M-M-G-M-X^{17}- (II) (SEQ ID NO: 64)

wherein X^{16} is absent or if present is R- or R-R-; and X^{17} is absent or if present is selected from -V, -VF, -VFQ, -VFQS, -VFQSL, and -VFQSLCG(dA); or C-terminal acids or amides, or N-acetyl derivatives thereof; or a pharmaceutically acceptable salt thereof.

[0265] 9. The peptide of embodiment 8 wherein X^{16} is R- or RR-; and X^{17} is selected from VF, -VFQ, -VFQS, -VFQSL, and -VFQSLCG(dA); or C-terminal acids or amides, or N-acetyl derivatives thereof; or a pharmaceutically acceptable salt thereof.

[0266] 10. A peptide or analog comprising or consisting of an amino acid sequence selected from MMGMVF (SEQ ID NO: 47) ; RMMGMVFQ (SEQ ID NO: 51); RMMGMVFQS (SEQ ID NO: 52); RMMGMVFQSL (SEQ ID NO: 53); RMMGMVFQSLCG(dA) (SEQ ID NO: 54); RRMMGMVF (SEQ ID NO: 57); Acetyl-RRMMGMVFQSLCG(dA) (SEQ ID NO: 61); RRMMGMVFQSLCG(dA)-Amide (SEQ ID NO: 62); and Acetyl-RRMMGMVFQSLCG(dA)-Amide (SEQ ID NO: 63); or a pharmaceutically acceptable salt thereof.

[0267] 11. The peptide or analog according to any one of embodiments 1-10, that is an isolated or a non-naturally occurring peptide, or a pharmaceutically acceptable salt thereof.

[0268] 12. The peptide according to any one of embodiments 1-11, or a pharmaceutically acceptable salt thereof.

[0269] 13. A peptide analog of any one of embodiments 1-11, wherein the peptide comprises substitution with at least one amino acid selected from (i) an amino acid having a D-configuration, and (ii) a non-naturally occurring amino acid residue; or a pharmaceutically acceptable salt thereof.

[0270] 14. A peptide or analog of any one of embodiments 1-13, further comprising a duration enhancing moiety attached to the peptide or analog, and optionally further comprising a metabolically cleavable linker coupling the peptide or analog to the duration enhancing moiety.

[0271] 15. A composition comprising a peptide or analog of any one of embodiments 1-14 and a pharmaceutically acceptable excipient.

[0272] 16. The composition of embodiment 15, wherein the excipient is not found in nature.

[0273] 17. A pharmaceutical composition comprising a peptide or analog of any one of embodiments 1-14.

[0274] 18. A method of modulating cell viability comprising administering a peptide or analog of any one of embodiments 1-11, or a composition according to any one of embodiments 15-17.

[0275] 19. A method of treating cancer in patient in need of such treatment, comprising administering to the patient a pharmacologically effective amount of a peptide or analog of any one of embodiments 1-14, or a composition according to any one of embodiments 15-17.

[0276] 20. A method of treating cell proliferation in patient in need of such treatment, comprising administering to the patient a pharmacologically effective amount of a peptide or analog of any one of embodiments 1-14 or a composition according to any one of embodiments 15-17.

[0277] 21. A method of treating an apoptotic disease in a patient in need of such treatment, comprising administering to the patient a pharmacologically effect amount of a peptide or analog of any one of embodiments 1-14, or a composition according to any one of embodiments 15-17.

[0278] 22. A method of treating a metabolic disease in a patient in need of such treatment, comprising administering to the patient a pharmacologically effect amount of a peptide or analog of any one of embodiments 1-14, or a composition according to any one of embodiments 15-17.

[0279] 23. A method of providing cytoprotection in a patient in need of such treatment, comprising administering to the patient a pharmacologically effect amount of a peptide or analog of any one of embodiments 1-14, or a composition according to any one of embodiments 15-17.

[0280] 24. An isolated nucleic acid that comprises a nucleotide sequence that encodes a peptide or analog of any one of Embodiments 1-14.

[0281] 25. A vector or expression vector that comprises an isolated nucleic acid according to embodiment 24.

[0282] 26. A host cell that comprises a nucleic acid according to embodiment 24 or a vector or expression vector according to embodiment 25.

[0283] 27. A composition comprising the nucleic acid of embodiment 24, the vector or expression vector of embodiment 25, or the host cell of embodiment 26, and a pharmaceutically acceptable excipient.

[0284] 28. A method of treating a metabolic disease in a subject in need thereof, comprising administering to the subject a peptide, peptide analog, composition, nucleic acid, vector, expression vector, or host cell of any one of embodiments 1-17 and 24-27, in an amount effective to treat the metabolic disease.

[0285] 29. The method of embodiment 28, wherein the disease is selected from the group consisting of obesity, diabetes (e.g., Type 2 diabetes), cognitive disorders and/or neurodegenerative disorders, cardiovascular disease, fatty liver disease, and gastrointestinal disease.

[0286] 30. A method of treating a cancer in a subject in need thereof, comprising administering to the subject a peptide, peptide analog, composition, nucleic acid, vector, expression vector, or host cell of any one of embodiments 1-17 and 24-27, in an amount effective to treat the cancer.

[0287] 31. The method of embodiment 30, wherein the cancer is lung cancer, pancreatic cancer, breast cancer, prostate cancer, ovarian cancer, or hepatocellular cancer.

[0288] 32. A method of treating a liver disease in a subject in need thereof, comprising administering to the subject a peptide, peptide analog, composition, nucleic acid, vector, expression vector, or host cell of any one of embodiments 1-17 and 24-27, in an amount effective to treat the liver disease.

[0289] 33. The method of embodiment 32, wherein the liver disease is a fatty liver disease.

[0290] 34. The method of embodiment 33, wherein the fatty liver disease is NAFLD or NASH.

[0291] 35. A method of modulating fatty acid metabolism in a subject in need thereof, comprising administering to the subject a peptide, peptide analog, composition, nucleic acid, vector, expression vector, or host cell of any one of embodiments 1-17 and 24-27, in an amount effective to modulate fatty acid metabolism.

[0292] 36. The method of embodiment 35, wherein fatty acid metabolism is increased in the subject after the peptide, peptide analog, composition, nucleic acid, vector, expression vector, or host cell of any one of embodiments 1-17 and 24-27, is administered to the subject.

[0293] 37. A method of reducing body weight in a subject in need thereof, comprising administering to the subject a peptide, peptide analog, composition, nucleic acid, vector, expression vector, or host cell of any one of embodiments 1-17 and 24-27, in an amount effective to reduce body weight in the subject.

[0294] 38. Use of a peptide, peptide analog, composition, nucleic acid, vector, expression vector, or host cell of any one of embodiments 1-17 and 24-27, in therapeutic treatment of a metabolic disease, cancer, liver disease, or any disease, disorder, or medical condition described herein.

[0295] 39. Use of a peptide, peptide analog, composition, nucleic acid, vector, expression vector, or host cell of any one of embodiments 1-17 and 24-27, in the manufacture of a medicament for treating a metabolic disease, cancer, liver disease, or any disease, disorder, or medical condition described herein.

[0296] 40. A peptide, peptide analog, composition, nucleic acid, vector, expression vector, or host cell of any one of embodiments 1-17 and 24-27 for use in therapeutic treatment of a metabolic disease, cancer, liver disease, or any disease, disorder, or medical condition described herein.

[0297] 41. A method of treating an apelin-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a peptide, peptide analog, composition, nucleic acid, vector, expression vector, or host cell of any one of embodiments 1-17 and 24-27, in an amount effective to treat the apelin-mediated disease or disorder.

[0298] 42. The method of embodiment 41, wherein the disease is related to UVB radiation.

[0299] 43. The method of embodiment 41 wherein the a disease or disorder is selected from hypertension, endothelial dysfunction, damages to cardiovascular tissues, heart failure, coronary heart disease, ischemic and/or hemorrhagic stroke, macrovascular disease, microvascular disease, diabetic heart (including diabetic cardiomyopathy and heart failure as a diabetic complication) coronary heart disease, peripheral artery disease, peripheral arterial occlusive disease, pre-eclampsia, resistant hypertension, refractory hypertension, hypertensive crisis, blood or fetal-placental circulation, edematous diseases, pulmonary dysfunction, acute lung injury (ALI), acute respiratory distress syndrome (ARDS), trauma and/or burns, and/or ventilator induced lung injury (VI LI), pulmonary fibrosis, mountain sickness, chronic kidney diseases, acute kidney injury, lymphedema, lymphatic vessel regeneration, inflammatory bowel disease, inflammatory disease,

or ocular disorders associated with disturbed vascular function, topical wounds, migraine, tumors, metastasis, angiogenesis, degeneration of cartilage, osteoarthritis, and cancers.

[0300] 44. The method of embodiment 41, wherein the disease is sepsis or sepsis shock.

[0301] 45. The method of embodiment 41, wherein the disease is thrombosis or microthrombosis.

[0302] 46. The method of embodiment 41, wherein the disease is thrombin-related aggregation.

[0303] 47. The method of embodiment 41, wherein the disease is ischemic shock.

[0304] 48. The method of embodiment 41, wherein the disease is organ failure or multiple organ failure.

[0305] The peptides and their uses having been described, the following examples are offered by way of illustration, and not limitation.

EXAMPLES

Example 1 - Synthesis

[0306] The peptides are prepared via solid phase synthesis on a suitable resin using t-Boc or Fmoc chemistry or other well established techniques, (see for example: Stewart and Young, Solid Phase Peptide Synthesis, Pierce Chemical Co., Rockford, III., 1984; E. Atherton and R. C. Sheppard, Solid Phase Peptide Synthesis. A Practical Approach, Oxford-IRL Press, New York, 1989; Greene and Wuts, "Protective Groups in Organic Synthesis", John Wiley & Sons, 1999, Florencio Zaragoza Dorwald, "Organic Synthesis on solid Phase", Wiley-VCH Verlag GmbH, 2000, and "Fmoc Solid Phase Peptide Synthesis", Edited by W.C. Chan and P.D. White, Oxford University Press, 2000) by a method similar to that described below, unless specified otherwise.

[0307] Solid phase synthesis is initiated by attaching an N-terminally protected amino acid with its carboxy terminus to an inert solid support carrying a cleavable linker. This solid support can be any polymer that allows coupling of the initial amino acid, e.g. a Pam resin, trityl resin, a chlorotrityl resin, a Wang resin or a Rink resin in which the linkage of the carboxy group (or carboxamide for Rink resin) to the resin is sensitive to acid (when Fmoc strategy is used). The polymer support is stable under the conditions used to deprotect the □-amino group during the peptide synthesis. After the first amino acid has been coupled to the solid support, the □-amino protecting group of this amino acid is removed. The remaining protected amino acids are then coupled one after the other in the order represented by the peptide sequence using appropriate amide coupling reagents, for example BOP (benzotriazol-l -yl-oxy-tris-(dimethylamino)-phosphonium), HBTU (2-(1 H-benzotriazol-1 -yl)-1 ,1 ,3,3-tetramethyl-uronium), HATU (O-(7-azabenztriazol-1 -yl-oxy-tris-(dimethylamino)-phosphonium) or DIC (N,N'-diisopropylcarbodiimide) / HOBt (1 -hydroxybenzotriazol), wherein BOP, HBTU and HATU are used with tertiary amine bases. Alternatively, the liberated N-terminus can be functionalized with groups other than amino acids, for example carboxylic acids, etc. Usually, reactive side-chain groups of the amino acids are protected with suitable blocking groups. These protecting groups are

removed after the desired peptides have been assembled. They are removed concomitantly with the cleavage of the desired product from the resin under the same conditions. Protecting groups and the procedures to introduce protecting groups can be found in Protective Groups in Organic Synthesis, 3d ed., Greene, T. W. and Wuts, P. G. M., Wiley & Sons (New York: 1999). In some cases, it might be desirable to have side-chain protecting groups that can selectively be removed while other side-chain protecting groups remain intact. In this case the liberated functionality can be selectively functionalized. For example, a lysine may be protected with an ivDde protecting group (S.R. Chhabra et al., Tetrahedron Lett. 39, (1998), 1603) which is labile to a very nucleophilic base, for example 4% hydrazine in DMF (dimethyl formamide). Thus, if the N-terminal amino group and all side-chain functionalities are protected with acid labile protecting groups, the ivDde ([1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl) group can be selectively removed using 4% hydrazine in DMF and the corresponding free amino group can then be further modified, e.g. by acylation. The lysine can alternatively be coupled to a protected amino acid and the amino group of this amino acid can then be deprotected resulting in another free amino group which can be acylated or attached to further amino acids. Finally, the peptide is cleaved from the resin. This can be achieved by using HF or King's cocktail (D. S. King, C. G. Fields, G. B. Fields, Int. J. Peptide Protein Res. 36, 1990, 255-266). The raw material can then be purified by chromatography, e.g. preparative RP-HPLC, if necessary.

[0308] Those peptides, analogs or derivatives which include non-natural amino acids and/or a covalently attached N-terminal mono- or dipeptide mimetic may be produced as described in the experimental part. Or see e.g., Hodgson et al: "The synthesis of peptides and proteins containing non-natural amino acids", and Chemical Society Reviews, vol. 33, no. 7 (2004), p. 422-430.

[0309] The peptides are prepared according to the below-mentioned peptide synthesis and the sequences as presented in the Table 1 can be prepared similar to the below-mentioned synthesis, unless specified otherwise.

[0310] One method of peptide synthesis is by Fmoc chemistry on a microwave-based Liberty peptide synthesizer (CEM Corp., North Carolina). The resin is Tentagel S RAM with a loading of about 0.25 mmol/g or PAL-ChemMatrix with a loading of about 0.43 mmol/g or PAL AM matrix with a loading of 0.5-0.75 mmol/g. The coupling chemistry is DIC/HOAt or DIC/Oxyma in NMP or DMF using amino acid solutions of 0.3 M and a molar excess of 6-8 fold. Coupling conditions are 5 minutes at up to 70°C. Deprotection is with 10% piperidine in NMP at up to 70°C. The protected amino acids used are standard Fmoc-amino acids (supplied from e.g. Anaspec or Novabiochem or Protein Technologies).

[0311] Another method of peptide synthesis is by Fmoc chemistry on a Prelude peptide synthesizer (Protein Technologies, Arizona). The resin is Tentagel S RAM with a loading of about 0.25 mmol/g or PAL-ChemMatrix with a loading of about 0.43 mmol/g or PAL AM with a loading of 0.5-0.75 mmol/g. The coupling chemistry is DIC/HOAt or DIC/Oxyma in NMP or DMF using

amino acid solutions of 0.3 M and a molar excess of 6-8 fold. Coupling conditions are single or double couplings for 1 or 2 hours at room temperature. Deprotection is with 20% piperidine in NMP. The protected amino acids used are standard Fmoc-amino acids (supplied from e.g. Anaspec or Novabiochem or Protein Technologies). The crude peptides are purified such as by semipreparative HPLC on a 20 mm x 250 mm column packed with either 5um or 7um C-18 silica. Peptide solutions are pumped onto the HPLC column and precipitated peptides are dissolved in 5 ml 50% acetic acid H_2O and diluted to 20 ml with H_2O and injected on the column which then is eluted with a gradient of 40-60 % CH_3CN in 0.1% TFA 10 ml/min during 50 min at 40°C. The peptide containing fractions are collected. The purified peptide is lyophilized after dilution of the eluate with water.

[0312] All peptides with C terminal amides described herein are prepared by a method similar to that described below unless specified otherwise. MBHA resin (4-methylbenzhydrylamine polystyrene resin is used during peptide synthesis. MBHA resin, 100-180 mesh, 1% DVB cross-linked polystyrene; loading of 0.7-1.0 mmol/g), Boc-protected and Fmoc protected amino acids can be purchased from Midwest Biotech. The solid phase peptide syntheses using Boc-protected amino acids are performed on an Applied Biosystem 430A Peptide Synthesizer. Fmoc protected amino acid synthesis is performed using the Applied Biosystems Model 433 Peptide Synthesizer.

[0313] Synthesis of the peptides is performed on the Applied Biosystem Model 430A Peptide Synthesizer. Synthetic peptides are constructed by sequential addition of amino acids to a cartridge containing 2 mmol of Boc protected amino acid. Specifically, the synthesis is carried out using Boc DEPBT-activated single couplings. At the end of the coupling step, the peptidyl-resin is treated with TFA to remove the N-terminal Boc protecting group. It is washed repeatedly with DMF and this repetitive cycle is repeated for the desired number of coupling steps. After the assembly, the sidechain protection, Fmoc, is removed by 20% piperidine treatment and acylation was conducted using DIC. The peptidyl-resin at the end of the entire synthesis is dried by using DCM, and the peptide is cleaved from the resin with anhydrous HF. The peptidyl-resin is treated with anhydrous HF, and this typically yielded approximately 350 mg (~50% yield) of a crude deprotected-peptide. Specifically, the peptidyl-resin (30 mg to 200 mg) is placed in the hydrogen fluoride (HF) reaction vessel for cleavage. 500 μL of p-cresol was added to the vessel as a carbonium ion scavenger. The vessel is attached to the HF system and submerged in the methanol/dry ice mixture. The vessel is evacuated with a vacuum pump and 10 ml of HF is distilled to the reaction vessel. This reaction mixture of the peptidyl-resin and the HF is stirred for one hour at 0° C., after which a vacuum is established and the HF is quickly evacuated (10-15 min). The vessel is removed carefully and filled with approximately 35 ml of ether to precipitate the peptide and to extract the p-cresol and small molecule organic protecting groups resulting from HF treatment. This mixture is filtered utilizing a Teflon filter and repeated twice to remove all excess cresol. This filtrate is discarded.

The precipitated peptide dissolves in approximately 20 ml of 10% acetic acid (aq). This filtrate, which contained the desired peptide, is collected and lyophilized.

EXAMPLE 2 - Caspase 3/7 Activity

[0314] The effect of the peptides on cell death/survival can be assessed using a caspase-3 assay. Peptides were dissolved with DMSO at 10 mM stock. Staurosporine was used as a highly potent positive control for caspase induction. Staurosporine (Selleckchem) was dissolved with DMSO at 1 mM stock. Caspase-Glo 3/7 Assay Reagent was purchased from Promega (Madison, WI). MDA-MB-231 human breast cancer cell line was purchased from American Type Culture Collection (Manassas, VA). MDA-MB-231 cells were grown in DMEM Medium supplemented with 10% FBS. 100 µg/ml penicillin and 100 µg/ml streptomycin were added to the culture medium. Cultures were maintained at 37°C in a humidified atmosphere of 5% CO2 and 95% air. MDA-MB-231 cells were incubated with test peptides in duplicate at 10 µM at 37°C in a humidified atmosphere of 5% CO2 and 95% air for 18 hours. 25 µl of Caspase-Glo 3/7 Assay Reagent was added to each well and incubated at 37°C, 5% CO2 for 1 hour. Luminescence for each sample well on the plate was measured by Envision 2104 Multilabel Reader (PerkinElmer, Santa Clara, CA). Activity was calculated relative to DMSO control. The relative standard deviation of the DMSO control was 1%. Caspase activity of staurosporine (0.05 nM) treatment was 130% of the background-corrected DMSO control value. The results are reported in Table 4.

TABLE 4.

Caspase 3/7 Activity in MDA-MB-231 Cells

SEQ ID NO:	Percent of Control Activity
2	62
DMSO Control	100

EXAMPLE 3 - Cell Viability

[0315] Peptides and reference compound staurosporine (Selleckchem) were dissolved with DMSO at 10 mM stock. CellTiter 96® AQueous One Solution Reagent (MTS assay reagent) was purchased from Promega (Madison, WI). MCF-7 human breast cancer cell line was purchased from American Type Culture Collection (Manassas, VA). MCF-7 cells were grown in EMEM Medium supplemented with 10% FBS and 0.01 mg/ml human recombinant insulin. 100 µg/ml penicillin and 100 µg/ml streptomycin were added to the culture medium. Cells were incubated with test peptides at 10 µM at 37°C in a humidified atmosphere of 5% CO2 and 95% air for 72 hours. 5 µl of CellTiter 96® AQueous One Solution Reagent (MTS assay reagent) was added to each well and incubated at 37°C, 5% CO2 for 5 hours. Absorbance at 492 nm was recorded by Envision 2104 Multilabel Reader. Treatment with DMSO alone was used as the cell viability activity control.

The relative standard deviation of the DMSO control was 3%. Staurosporine was used as a highly potent positive control for decreasing cell viability. Cell viability for staurosporine (10 μM) treatment was <5% of the background corrected DMSO control value. The results are reported in Table 5.

TABLE 5.

MTS Assay in MCF-7 Cells

SEQ ID NO:	Percent of Control Activity
2	67
DMSO Control	100

EXAMPLE 4. - Free Fatty Acid Levels in Cultured Mouse Adipocytes

[0316] Mouse 3T3-L1 adipocytes were seeded at 3,000 cells per well in 96-well plates in Pre-adipocyte Medium (Zen-Bio, Durham, NC) and grown to confluence at 37°C in a humidified atmosphere of 5% CO2/95% air. Two days after confluence, cells were placed in Adipocyte Differentiation Medium (Zen-Bio, Durham, NC) and cultured for three additional days at 37°C in a humidified atmosphere of 5% CO2/95% air. The culture media was then replaced with Adipocyte Maintenance Medium (Zen-Bio) and the cells maintained for an additional 9-12 days at 37°C in a humidified atmosphere of 5% CO2/95% air with partial medium replacement every other day. Following 12-15 days' differentiation, test peptides were added to a final concentration of 25 μM and incubated for 20-22 hours in Adipocyte Maintenance Medium. After 20-22 hours, isoproterenol (1 nM) was added to all wells except untreated controls and test peptides were replenished. Cells were incubated for a further 3 hours in Assay Buffer (Zen-Bio). Free fatty acid concentrations in the media were determined using a Free Fatty Acid Assay Kit (Zen-Bio) according to the manufacturer's instructions using a plate reader (540 nm). Absorbance values were corrected for untreated background and expressed relative to isoproterenol treated cells. Treatment with isoproterenol (1 nM) alone was used as the free fatty acid level stimulatory control. The relative standard deviation of the isoproterenol control was <10%. Insulin was used as a highly potent positive control for decreasing free fatty acid levels. Free fatty acid levels for insulin (100 nM) treatment were <5% of the isoproterenol control value. The results are reported in Table 6.

TABLE 6

Free Fatty Acid Levels in 3T3-L1 Adipocytes Expressed as a Percent Isoproterenol Control

SEQ ID NO:	Percent of Control Value
3	89
2	97
4	100

5 115

EXAMPLE 5. - Cell Viability in MDA-MB-231 Cells

[0317] Test compounds and reference compound staurosporine (Selleckchem) were all dissolved with DMSO at 10 mM stock. CellTiter 96® AQueous One Solution Reagent (MTS assay reagent) was purchased from Promega (Madison, WI). MDA-MB-231 human breast cancer cell line was purchased from American Type Culture Collection (Manassas, VA). MDA-MB-231 cells were grown in EMEM Medium supplemented with 10% FBS and 0.01 mg/ml human recombinant insulin. 100 µg/ml penicillin and 100 µg/ml streptomycin were added to the culture medium. Cells were incubated with test compounds at 37°C in a humidified atmosphere of 5% CO2 and 95% air for 72 hours. 5 µl of CellTiter 96® AQueous One Solution Reagent (MTS assay reagent) was added to each well and incubated at 37°C, 5% CO2 for 5 hours. Absorbance at 492 nm was recorded by Envision 2104 Multilabel Reader. Activity was calculated relative to DMSO control. Treatment with DMSO alone was used as the cell viability activity control. The relative standard deviation of the DMSO control was 3%. Staurosporine was used as a highly potent positive control for decreasing cell viability. Cell viability was 3% of the DMSO control value for Staurosporine (10 µM). The results are reported in Table 7.

TABLE 7

MTS Assay in MDA-MB-231 Cells

SEQ ID NO:	Percent of Control Activity
2	79
DMSO Control	100

EXAMPLE 6 - Effects on Metabolic Parameters in Diet Induced Obese (DIO) Mice

[0318] DIO mouse studies are conducted by methods well known in the art. C57BL/6 mice are maintained on a high fat diet for 6 to 48 weeks to develop diet induced obesity. Animals are randomized to treatment groups based on blood glucose levels and/or body weight. The peptides of the invention or vehicle control are administered daily or twice daily by intraperitoneal or subcutaneous injection for 5 to 21 days. Body weight, blood glucose levels and food intake are monitored. Glucose tolerance is assessed by intraperitoneal administration of glucose (1 to 3 g/kg) followed by measurement of blood glucose levels over 2 hours. Administration of the peptides of the invention results in one or more effects selected from greater body weight loss, greater reduction in blood glucose, and improved glucose tolerance, when compared to animals treated with vehicle control.

EXAMPLE 7 - Mouse Xenograft Models

[0319] Mouse xenograft models are prepared by methods well known in the art. For example, SCID mice are injected with human tumor cells (for example, MCF-7, MDA-MB-231, PC-3, or the like) and tumor growth is monitored. When tumors are of sufficient size, animals are randomized to treatment groups and dosed daily, every other day, or weekly with the peptides of the invention, vehicle control, positive control (e.g., gemcitabine or paclitaxel) or the combination of the peptides of the invention + positive control. Tumor growth, body weight, and survival are monitored over 14 to 28 days. Administration of the peptides of the invention alone and/or in combination with positive control results in decreased tumor growth and/or extension of survival when compared to animals treated with vehicle control.

EXAMPLE 8 - Protection of Cells from Cytotoxic Insults

[0320] Cells (for example primary cultures of rodent cerebral cells, rodent or human nerve-derived cell lines, and the like) are cultured by methods well known in the art. Cells are treated with the peptides of the invention, vehicle control, or positive controls and cells are exposed to a cytotoxic condition, for example, addition of glutamic acid, removal of serum, generation of reactive oxygen species, addition of beta-amyloid protein, exposure to a cytotoxic agent (e.g., MPTP, staurosporine, oligomycin, etc.), exposure to a chemotherapeutic agent (e.g., cisplatin, etc.), and the like. Cell survival is measured by methods well known in the art (for example measurement of lactate dehydrogenase (LDH) activity in cell extracts; measurement of intracellular ATP, MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) assay; MTS (3 (4,5-dimethylthiaZol-2-yl)-5-(3-carboxymethoxyphenyl)-2 (4-sulfophenyl)-2H-tetrazolium) assay; trypan blue staining; calcein staining; etc.). Treatment of cells with the peptides of the invention prior to and/or during exposure to the cytotoxic condition produces an increase in cell survival when compared to cells treated with vehicle control.

EXAMPLE 9 - Levels of Reactive Oxygen Species

[0321] The protective or synergistic effect of the peptides on cellular levels of reactive oxygen species (ROS) induced by oxidative stress can be assessed using an assay of ROS in cultured cells exposed to a suitable oxidative stress. Peptides are initially prepared as 10 mM stock in DMSO and diluted to 1 mM in H2O and added at a final concentration of 10 μM (0.1% DMSO). Tert-butyl hydrogen peroxide (TBHP) is used as a highly potent inducer of ROS. TBHP is used at final concentration of 100 μM. Glutathione ethyl ester (GEE) at final concentration of 5 mM or sulforaphane at final concentration of 10 μM are used as protective controls against TBHP induced ROS production. C2C12 mouse muscle myoblast cell line is purchased from American Type Culture Collection (Manassas, VA). C2C12 cells are grown in DMEM supplemented with 10% FBS with 100 IU/ml penicillin and 100 μg/ml streptomycin. Cultures are maintained at 37°C in a humidified

atmosphere of 5% CO2/95% air. C2C12 cells are seeded at 7,500 cells per well on 96-well plates. Two days after seeding cells are incubated with test peptides at 10 µM in 0.1% DMSO or sulforaphane at 10 µM and maintained at 37°C in a humidified atmosphere of 5% CO2/95% air for 18-20 hours. After 18-20 hours of incubation the cells are loaded with DCFDA for 45 min. TBHP at 100 µM and GEE at 5 mM are then added to the appropriate wells for 1 hour. ROS acivity is determined using a DCFDA Cellular ROS Detection Assay kit (Abcam, Cambridge, MA) according to the manufacturer's instructions. Fluorescence in each sample well on the plate is measured using a Cytation 3 plate reader at Ex/Em= 485/535nm (BioTek, Winooski, VT). Activity is calculated relative to TBHP control. Administration of the peptides of the invention alone and/or in combination with positive control results in increased or decreased cellular ROS levels induced by TBHP in C2C12 cells than those treated with vehicle control.

EXAMPLE 10 - Effects in Diet Induced Obese (DIO) Mice

[0322] DIO mouse studies are conducted by methods well known in the art. C57BL/6 mice are maintained on a high fat diet for 6 to 48 weeks to develop diet induced obesity. Animals are randomized to treatment groups based on blood glucose levels and/or body weight. The peptides of the invention or vehicle control are administered daily or twice daily by intraperitoneal or subcutaneous injection for 5 to 21 days. Body weight, blood glucose levels and food intake are monitored. Glucose tolerance is assessed by intraperitoneal administration of glucose (1 to 3 g/kg) followed by measurement of blood glucose levels over 2 hours. Administration of the peptides of the invention results in one or more effects selected from greater body weight loss, greater reduction in blood glucose, and improved glucose tolerance, when compared to animals treated with vehicle control.

EXAMPLE 11 - Caspase 3/7 Activity

[0323] The effect of the peptides on cell death/survival can be assessed using a caspase-3/7 assay in cultured cells such as mouse myoblast cells. Peptides were initially prepared as 10mM stock in DMSO, diluted to 1 mM in H2O, and used at a final concentration of 10 µM (0.1% DMSO). Staurosporine was used as a highly potent positive control for caspase induction. Staurosporine (Abcam, Cambridge, MA) was dissolved at 10 mM in DMSO as a stock solution. Caspase-Glo 3/7 Assay Reagent was purchased from Promega (Madison, WI). C2C12 cell line was purchased from American Type Culture Collection (Manassas, VA). C2C12 cells were grown in DMEM supplemented with 20% FBS with 100 IU/ml penicillin and 100 µg/ml streptomycin. Cultures were maintained at 37°C in a humidified atmosphere of 5% CO2/95% air. C2C12 cells were seeded at 4,000 cells per well on 96-well plates. The next day cells were incubated with test peptides at 10µM or staurosporine at concentrations between 100 nM and 5 nM using a final concentration of 0.1% DMSO and maintained at 37°C in a humidified atmosphere of 5%

CO2/95% air for 24 hours. Caspase 3/7 activity was determined using a Caspase-Glo 3/7 Assay kit according to the manufacturer's instructions. Luminescence for each sample well on the plate was measured using a Cytation 3 plate reader (BioTek, Winooski, VT). Activity was calculated relative to 0.1% DMSO control. The relative standard deviation of the DMSO control was <10%. Caspase 3/7 activity of staurosporine (100 nM) treatment was 670% of the background-corrected DMSO control value. The results are reported in Table 8.

TABLE 8

Caspase 3/7 Activity in C2C12 Cells

SEQ ID NO:	Percent of Control Activity
7	109.4
8	97.6
9	107.9
10	96.6
11	99.1
12	105.1
13	99.0
14	100.1
15	101.6
16	93.1
17	92.0
18	93.8
19	100.1

EXAMPLE 12 - Free Fatty Acid Levels in Cultured Mouse Adipocytes

[0324] The effect of the peptides on fatty acid metabolism can be assessed using an assay of free fatty acid levels in cultured cells such as mouse adipocytes. Peptides were initially prepared as 10mM stock in DMSO, diluted to 1 mM in H2O, and used at a final concentration of 10 μM (0.1% DMSO). Isoproterenol was used as a highly potent inducer of fatty acid production. Mouse 3T3-L1 cells purchased from ZenBio were seeded at 3,000 cells per well in 96-well plates in Pre-adipocyte Medium (Zen-Bio) and grown to confluence at 37°C in a humidified atmosphere of 5% CO2/95% air. Two days after confluence, cells were placed in Adipocyte Differentiation Medium (Zen-Bio) and cultured for three additional days at 37°C in a humidified atmosphere of 5% CO2/95% air. The culture media was then replaced with Adipocyte Maintenance Medium (Zen-Bio) and the cells maintained for an additional 9-12 days at 37°C in a humidified atmosphere of 5% CO2/95% air with partial medium replacement every other day. Following 12-15 days of differentiation, test

peptides were added at a final concentration of 10 µM in 0.1% DMSO and incubated for 20-22 hours in Adipocyte Maintenance Medium at 37°C in a humidified atmosphere of 5% CO2/95% air. After 20-22 hours, 1 nM isoproterenol was added to all wells except untreated controls and test peptides were replenished. Insulin at 100 nM was added to control wells. Cells were incubated for 3 hours in Assay Buffer (Zen-Bio) at 37°C in a humidified atmosphere of 5% CO2/95% air. Free fatty acid concentrations in the media were determined using a Free Fatty Acid Assay kit (Zen-Bio) according to the manufacturer's instructions using a Cytation 3 plate reader at 540nm (BioTek, Winooski, VT). Absorbance values were corrected for untreated background and expressed relative to isoproterenol treated cells. Treatment with isoproterenol (1 nM) alone was used as the free fatty acid level stimulatory control. The relative standard deviation of the isoproterenol control was <10%. Insulin was used as a highly potent positive control for decreasing free fatty acid levels. Free fatty acid levels for insulin (100 nM) treatment were <5% of the isoproterenol control value. The results are reported in Table 9.

TABLE 9

Free Fatty Acid Levels in 3T3-L1 Mouse Adipocytes

SEQ ID NO:	Percent of Isoproterenol Control Activity
7	34.6
8	34.7
9	100.3
10	24.9
11	59.2
12	51.3
13	66.0
14	46.5
15	90.8
16	58.8
17	77.9
18	77.3
19	31.0

EXAMPLE 13 - ATP Levels

[0325] The effect of the peptides on cellular metabolism can be assessed using an assay of ATP levels in cultured cells such as mouse myoblast cells. Peptides were initially prepared as 10 mM stock in DMSO, diluted to 1 mM in H2O, and used at a final concentration of 10 µM (0.1% DMSO). Lovastatin was used as a highly potent control for inhibiting cell growth/proliferation resulting in reduction of ATP levels. Lovastatin was prepared as 10 mM stock in 70% Ethanol and used at

final concentration of 10 µM containing 0.1% DMSO. CellTiter-Glo® Assay kit was purchased from Promega (Madison, WI). C2C12 cell line was purchased from American Type Culture Collection (Manassas, VA). C2C12 cells were grown in DMEM medium supplemented with 20% FBS with 100 IU/ml penicillin and 100 µg/ml streptomycin. Cultures were maintained at 37°C in a humidified atmosphere of 5% CO2/95% air. C2C12 cells were seeded at 800 cells per well on 96-well plates. For each of the next 3 days, cells were incubated with test peptides or lovastatin at 10 µM concentration in 0.1% DMSO and maintained at 37°C in a humidified atmosphere of 5% CO2/95% air with readdition of peptides/lovastatin at 24 hours intervals. ATP levels were determined using a CellTiter-Glo Assay kit according to the manufacturer's instructions. Luminescence for each sample well on the plate was measured using a Cytation 3 plate reader (BioTek, Winooski, VT). Activity was calculated relative to 0.1% DMSO treated control. The relative standard deviation of the result for the 0.1% DMSO treated control was <5%. Lovastatin was used as a highly potent positive control for reduction of ATP levels. ATP levels for treatment with lovastatin (10 µM) were <50% of the 0.1% DMSO treated control value. The results are reported in Table 10.

TABLE 10

ATP Levels in Cultured C2C12 Cells

SEQ ID NO:	Percent of Control Activity
7	106.6
8	99.5
9	102.0
10	100.9
11	98.0
12	102.6
13	102.8
14	102.1
15	100.4
16	94.7
17	100.0
18	95.5
19	93.6

EXAMPLE 14 - Cell Proliferation

[0326] The effect of the peptides on cell proliferation was assessed using a DNA dye binding assay in cultured cells such as mouse myoblast cells. Peptides were initially prepared as 10 mM stock in DMSO, diluted to 1 mM in H2O, and added at a final concentration of 10 µM (0.1% DMSO).

Lovastatin was used as a growth arrest control at final concentration of 10 µM. C2C12 mouse muscle myoblast cell line was purchased from American Type Culture Collection (Manassas, VA). C2C12 cells were grown in DMEM supplemented with 20% FBS with 100 IU/ml penicillin and 100 µg/ml streptomycin. Cultures were maintained at 37°C in a humidified atmosphere of 5% CO2/95% air. C2C12 cells were seeded at 800 cells per well on 96-well plates. The next day cells were incubated with test peptides or lovastatin at 10 µM in 0.1% DMSO and maintained at 37°C in a humidified atmosphere of 5% CO2/95% air for 72 hours with readdition of peptides/lovastatin at 24 hour intervals. Cell proliferation was determined using Cyquant Direct Nucleic Acid Stain (Thermo Fisher Scientific, Waltham, MA) according to the manufacturer's instructions. Fluorescence for each sample well on the plate was measured using a Cytation 3 plate reader at at Ex/Em= 495/535nm (BioTek, Winooski, VT). Activity was calculated relative to 0.1% DMSO untreated control. The relative standard deviation for the lovastatin control was <27%. The cell proliferation for the lovastatin control was <25% of the value for untreated control. The results are shown in Table 11.

TABLE 11

Effect of Peptides on Cell Proliferation in C2C12 Cells

SEQ ID NO.	Percent of Control
7	94.0
8	98.1
9	99.7
10	101.5
11	88.1
12	119.3
13	90.0
14	97.2
15	93.5
16	89.6
17	89.1
18	74.9
19	92.3

EXAMPLE 15 - Levels of Reactive Oxygen Species

[0327] The protective or synergistic effect of the peptides on cellular levels of reactive oxygen species (ROS) induced by oxidative stress can be assessed using an assay of ROS in cultured cells such as mouse myoblasts exposed to a suitable oxidative stress. Peptides were initially prepared as 10 mM stock in DMSO, diluted to 1 mM in H2O, and added at a final concentration of 10 µM (0.1%

DMSO). Tert-butyl hydrogen peroxide (TBHP) was used as a highly potent inducer of ROS. TBHP was used at final concentration of 100 µM. Sulforaphane at final concentration of 10 µM was used as a protective control against TBHP induced ROS production. C2C12 mouse muscle myoblast cell line was purchased from American Type Culture Collection (Manassas, VA). C2C12 cells were grown in DMEM supplemented with 20% FBS with 100 IU/ml penicillin and 100 µg/ml streptomycin. Cultures were maintained at 37°C in a humidified atmosphere of 5% CO2/95% air. C2C12 cells were seeded at 7,500 cells per well on 96-well plates. Two days after seeding cells were incubated with test peptides at 10 µM in 0.1% DMSO or sulforaphane at 10 µM and maintained at 37°C in a humidified atmosphere of 5% CO2/95% air for 18-20 hours. After 18-20 hours of incubation the cells were loaded with DCFDA for 45 min. TBHP at 100 µM was then added to the appropriate wells for 1 hour. ROS activity was determined using a DCFDA Cellular ROS Detection Assay kit (Abcam, Cambridge, MA) according to the manufacturer's instructions. Fluorescence in each sample well on the plate was measured using a Cytation 3 plate reader at Ex/Em= 485/535nm (BioTek, Winooski, VT). Activity was calculated relative to TBHP control. The relative standard deviation of the sulforaphane control was <20%. The ROS level produced by the sulforaphane control in the presence of TBHP was 52% of the TBHP control. The results are reported in Table 12.

TABLE 12

Effect of Peptides on Cellular ROS Levels Induced by TBHP in C2C12 Cells

SEQ ID NO.	Percent of Control
7	98.6
8	102.1
9	105
10	102.3
11	100.6
12	105
13	106.8
14	105.1
15	105.9
16	106.6
17	113.6
18	109.4
19	92

EXAMPLE 16 - Effects on Metabolic Parameters in Diet Induced Obese (DIO) Mice

[0328] Male C57BL/6 mice were maintained on a high fat diet for 18 weeks to develop diet induced obesity. Animals were randomized to treatment groups based on blood glucose levels and body weight. The peptides of the invention were administered to groups of male DIO mice twice daily by intraperitoneal injection at a dose of 5 mg/kg/dose for 10 days (N = 8 animals per treatment group). An additional group of male DIO mice (n = 8) received vehicle (water) alone administered twice daily by intraperitoneal injection. Body weight, blood glucose levels and food intake were monitored. Body mass distribution (fat vs lean) was determined by quantitative whole body NMR prior to dosing and at the end of dosing. Administration of the peptides of the invention produced greater body weight loss, greater reduction in blood glucose, and greater decrease in fat mass from baseline values when compared to animals treated with vehicle alone (Table 13).

TABLE 13

Mean Difference from Vehicle Control for the Decrease from Baseline in Metabolic Parameters in Male DIO Mice Following 10 Days of Twice Daily Intraperitoneal Treatment at 5 mg/kg (N = 8)

Treatment SEQ ID NO:	Dose (mg/kg)	Difference from Vehicle Control: Decrease from Baseline in Body Weight (%)	Difference from Vehicle Control: Decrease from Baseline in Blood Glucose (mg/dL)	Difference from Vehicle Control: Decrease from Baseline in Fat Mass (g)
7	5	-3.19	-20.0	-0.84
8	5	-8.01	-16.9	-3.88
10	5	-5.47	-8.37	-2.16
11	5	-4.05	-4.91	-2.34
12	5	-2.89	-6.75	-1.73

EXAMPLE 17

Free Fatty Acid Levels in Cultured Mouse Adipocytes and Insulin Dependence

[0329] The effect of the peptides on fatty acid metabolism can be assessed using an assay to monitor free fatty acid levels produced by cultured cells such as mouse adipocytes. Peptides were initially prepared either as 10 mM stock in DMSO and diluted to 1 mM in H2O or directly as 1 mM stock in H2O, based on solubility; peptides were used at a final concentration of 10 µM (0-0.1% DMSO). Isoproterenol was used as a highly potent inducer of fatty acid production. Mouse 3T3-L1 cells purchased from ZenBio (Research Triangle Park, NC) were seeded at 3,000 cells per well in 96-well plates in Pre-adipocyte Medium (Zen-Bio) and grown to confluence at 37°C in a humidified atmosphere of 5% CO2/95% air. Two days after confluence, cells were placed in Adipocyte Differentiation Medium (Zen-Bio) and cultured for three additional days at 37°C in a humidified atmosphere of 5% CO2/95% air. The culture medium was then replaced with

Adipocyte Maintenance Medium (Zen-Bio) and the cells maintained for an additional 9 days at 37°C in a humidified atmosphere of 5% CO2/95% air with partial medium replacement every other day. Following 12 days of differentiation, medium was replaced Adipocyte Maintenance Medium without insulin and the cultures placed at 37°C in a humidified atmosphere of 5% CO_2/95% air. On day 13 of differentiation, test peptides were added directly to each well (without replacing the medium) at a final concentration of 10 µM in 0-0.1% DMSO and incubated for 20-22 hours at 37°C in a humidified atmosphere of 5% CO2/95% air. After 20-22 hours, the medium was removed and replaced with Assay Buffer (ZenBio) containing the appropriate compounds; 0.1 nM isoproterenol was added to all wells except untreated controls, test peptides were reapplied at 10 µM in either the absence or presence of 0.25 nM insulin, 0.25 nM insulin as a partial inhibitor of free fatty acid production, or 10 nM insulin as a highly potent inhibitor of free fatty acid production. Cells were incubated for 3 hours in Assay Buffer (Zen-Bio) at 37°C in a humidified atmosphere of 5% CO2/95% air. Following 3 h incubation, conditioned medium from each well was transferred to a fresh 96-well plate. Free fatty acid concentrations in the medium were determined using a Free Fatty Acid Assay kit (Zen-Bio) according to the manufacturer's instructions; absorbance measured using a Cytation 3 plate reader at 540 nm (BioTek, Winooski, VT). Absorbance values were expressed as percent control activity relative to 0.1 nM isoproterenol treated cells (no insulin) for 10 µM peptide alone or 0.25 nM insulin treated cells (with insulin) for 10 µM peptide in presence of 0.25 nM insulin. Treatment with isoproterenol (0.1 nM) alone was used as the free fatty acid level stimulatory control. The relative standard deviation of the isoproterenol control was <10%. Insulin at 10 nM was used as a highly potent positive control for decreasing free fatty acid levels. Free fatty acid levels for insulin (10 nM) treatment were <5% of the isoproterenol control value. Data are presented as averages values from 2-3 independent experiments with each data point performed in triplicate. The results are reported in Table 16.

TABLE 16

Free Fatty Acid Levels in Cultured 3T3-L1 Mouse Adipocytes

SEQ ID NO:	Percent of Isoproterenol Control Activity No Insulin	Percent of Isoproterenol Control Activity With Insulin
7	102	71.3
8	106	57.8
9	106	93.1
10	97.9	48.7
11	104	58.5
12	103	54.6
13	105	56.4

14	100	51.0
15	109	89.0
16	110	63.0
17	98.4	87.7
18	110	81.1
19	105	79.0
20	101	90.7
21	109	59.1
22	107	61.9
23	104	57.7
24	108	59.2
25	110	67.4
26	104	52.8
27	108	74.2
28	109	57.8
29	110	52.8
30	102	56.0
31	113	94.6
32	108	62.9
33	98	80
34	104	122
35	101	112
36	102	114
37	103	111
38	101	68
39	97	67
40	101	76
41	97	73
42	103	134
43	103	91
44	103	96
45	105	89
46	98	104
47	101	93
48	92	93
49	101	103
50	103	101

51	99	79
52	102	79
53	101	81
54	102	75
55	100	90
56	99	94
57	96	87
58	110	114
59	111	114
60	110	101
61	112	143
62	112	109
63	112	92

EXAMPLE 18

Glucose Utilization in Cultured C2C12 Cells and Insulin Dependence

[0330] The effect of the peptides on glucose homeostasis can be assessed using an assay to monitor glucose utilization by cultured cells such as mouse myotubes. Peptides were initially prepared either as 10 mM stock in DMSO and diluted to 1 mM in H2O or directly as 1 mM stock in H2O, based on solubility; peptides were used at a final concentration of 10 μM (0-0.1% DMSO). C2C12 mouse muscle cell line was purchased from Millipore Sigma (Saint Louis, MO). C2C12 cells were seeded in standard medium (DMEM/low glucose (1g/L) + 10% Fetal Bovine Serum + antibiotics) at 7,500 cells/well on 96-well plates and grown to confluence at 37°C in a humidified atmosphere of 5% CO_2/95% air. Three days after plating the medium was removed and with Differentiation Medium (DMEM/low glucose (1g/L) + 2% Horse Serum + antibiotics) added. The cultures were placed at 37°C in a humidified atmosphere of 5% CO_2/95% air for 5 days with partially replacement of medium daily. After 5 days of differentiation, the medium was removed from myotube cultures and replaced with Assay Medium (DMEM/low glucose (1g/L) + antibiotics). Culture were placed at 37°C in a humidified atmosphere of 5% CO_2/95% air for 5 h. Following the 5 h incubation the medium was removed and fresh Assay Medium containing compounds added; 10 μM peptide in absence or presence of 5-20 nM insulin, or 1 mM metformin as a potent stimulator of glucose utilization. Cells were incubated for 22 h at 37°C in a humidified atmosphere of 5% CO_2/95% air. Following 22 h incubation, conditioned medium from each well was transferred to a fresh 96-well plate. Glucose concentrations in the medium were determined using a Glucose Assay kit (Abcam; Cambridge, MA) according to the manufacturer's instructions; absorbance measured using a Cytation 3 plate reader at 570 nm (BioTek, Winooski, VT).

Absorbance values were expressed as percent control activity relative to untreated treated cells (no insulin) for 10 μM peptide alone or 5-20 nM insulin treated cells (with insulin) for 10 μM peptide in presence of 5-20 nM insulin. Untreated cells were used as reference for basal glucose utilization. The relative standard deviation from plate to plate of untreated control was <10%. Metformin at 1 mM was used as a potent control for increasing glucose utilization. Glucose levels for metformin (1 mM) treatment were <10% of the untreated control value. Data are presented as averages values from 2-3 independent experiments with each data point performed in triplicate. The results are reported in Table 19.

TABLE 19

Glucose Utilization in Cultured C2C12 Cells

SEQ ID NO:	Percent of Control Activity No Insulin	Percent of Control Activity With Insulin
7	98.1	100.7
8	90.8	85.5
9	95.8	106.1
10	102.6	76.3
11	107.2	84.5
12	102.3	84.5
13	104.4	97.8
14	102.4	112.3
15	103	106.9
16	99.7	88.4
17	102.6	96.2
18	103.6	122.1
19	100.1	76.5
20	108	91
21	107.5	86
22	100.9	79.8
23	102	86.6
24	98.8	85.8
25	99.9	77.4
26	102.2	98.4
27	101.2	106.2
28	103.3	88.2
29	99.2	92.3
30	97.7	88.3

31	103.7	99.2
32	103.8	85.0

EXAMPLE 19

Glucose Production in Cultured H4-IIE Cells and Insulin Dependence

[0331] The effect of the peptides on glucose homeostasis can be assessed using an assay to monitor glucose production by cultured cells such as rat hepatocytes. Peptides were initially prepared either as 10 mM stock in DMSO and diluted to 1 mM in H2O or directly as 1 mM stock in H2O, based on solubility; peptides were used at a final concentration of 10 μM (0-0.1% DMSO). H4-IIE rat hepatic cell line was purchased from American Type Culture Collection (Manassas, VA). H4-IIE cells were seeded in standard medium (DMEM/high glucose + 10% Fetal Bovine Serum + antibiotics) at 100,000 cells/well on 96-well plates and allowed to adhere overnight at 37°C in a humidified atmosphere of 5% CO_2/95% air. 24 h after seeding, the medium was removed, cells were rinsed with glucose free DMEM, and the medium replaced with Glucose Production Medium (glucose free DMEM + 2 mM sodium pyruvate + 10 mM sodium lactate + antibiotics). The cultures were placed at 37°C in a humidified atmosphere of 5% CO_2/95% air overnight. The next morning, the medium was removed and fresh Glucose Production Medium containing compounds was added; 10 uM peptide in absence or presence of 800 pM insulin. Cells were incubated for 24 h at 37°C in a humidified atmosphere of 5% CO_2/95% air. Following 24 h incubation, conditioned medium from each well was transferred to a fresh 96-well plate. Glucose concentrations in the medium were determined using a Glucose Assay kit (Abcam; Cambridge, MA) according to the manufacturer's instructions; absorbance measured using a Cytation 3 plate reader at 570 nm (BioTek, Winooski, VT). Absorbance values were expressed as percent control activity relative to untreated treated cells (no insulin) for 10 μM peptide alone or 800 pM insulin treated cells (with insulin) for 10 μM peptide in presence of 800 pM insulin. Untreated cells were used as reference for maximal glucose production. The relative standard deviation from plate to plate of untreated control was <25%. Insulin at 800 pM was used as a modest inhibitor glucose production. Glucose levels for insulin (800 pM) treatment were <20-50% of the untreated control value. Data are presented as averages values from 2-4 independent experiments with each data point performed in triplicate. The results are shown in Table 20.

TABLE 20

Glucose Production in Cultured H4-IIE Cells

SEQ ID NO:	Percent of Control Activity No Insulin	Percent of Control Activity With Insulin
7	54.2	64.2
8	78.9	102.8
9	74.9	93.7

10	92.2	38.2
11	96.6	57.5
12	96.4	44.8
13	100.9	68
14	104.4	159.6
15	101.4	63.9
16	99.8	56.8
17	101.7	95.5
18	103.1	140.4
19	100.8	68.9
20	98.4	108.6
21	98.4	72.7
22	109.8	49.1
23	104.3	57.7
24	107.6	61.9
25	108.2	65.2
26	111.5	103.1
27	101.7	98.9
28	107.4	91.4
29	113.9	148.6
30	105.7	65.7
31	95.5	108.3
32	99.5	81.9
33	113.8	
34	96.9	
35	98.1	
36	94.4	
37	105.5	
38	98.5	
39	103.9	
40	109.6	
41	122.7	
42	95.3	
43	115.8	
44	98.8	
45	106.9	
46	105.0	

47	108.0
48	110.0
49	120.1
50	99.8
51	106.1
52	108.4
53	91.9
54	112.8
55	92.6
56	97.1
57	98.3
58	114.5
59	114.0
60	115.9
61	122.7
62	105.6
63	107.5

EXAMPLE 20

Effects on Metabolic Parameters in Diet Induced Obese (DIO) Mice

[0332] Male C57BL/6 mice were maintained on a high fat diet for 18 weeks to develop diet induced obesity. Animals were randomized to treatment groups based on blood glucose levels and body weight. The peptides of the invention were administered to groups of male DIO mice once or twice daily by intraperitoneal injection at a dose of 5 mg/kg/dose for 8 to 10 days (N = 8 animals per treatment group). Additional groups of male DIO mice (n = 8) received vehicle (water) alone administered once or twice daily by intraperitoneal injection. Body weight, blood glucose levels and food intake were monitored. Body mass distribution (fat vs lean) was determined by quantitative whole body NMR prior to dosing and at the end of dosing. Administration of the peptides of the invention produced greater body weight loss, greater reduction in blood glucose, and greater decrease in fat mass from baseline values when compared to animals treated with vehicle alone (Table 21).

TABLE 21

Mean Difference from Vehicle Control for the Decrease from Baseline in Metabolic Parameters in Male DIO Mice Following Repeated Intraperitoneal Treatment at 5 mg/kg/dose (N = 8)

Treatment	Dose (mg/kg/dose) and Frequency	Duration (days)	Difference from Vehicle Control: Decrease from Baseline in Body Weight (%)	Difference from Vehicle Control: Decrease from Baseline in Blood Glucose (mg/dL)	Difference from Vehicle Control: Decrease from Baseline in Fat Mass (g)
SEQ ID NO: 8	5 BID	8	-5.66	-14.3	-2.15
SEQ ID NO: 15	5 QD	10	-4.97	-3.38	-2.19
SEQ ID NO: 20	5 QD	10	-3.59	-0.75	-1.78
SEQ ID NO: 38	5 QD	10	-3.12	-24.1	ND
SEQ ID NO: 42	5 QD	10	-2.94	-10.7	ND

EXAMPLE 21 - Pharmacokinetics in Cynomolgus Monkeys.

[0333] Male cynomolgus monkeys (2 to 6 kg) are fasted for 8 hours prior to dosing. Groups of animals are injected with a single dose of the test peptide (0.1 to 15 mg/kg) by a suitable route. Blood samples are withdrawn at intervals over 24 hours and processed for plasma. Food is returned at four hours post-injection. Concentrations of peptides and/or metabolites in plasma samples are determined by suitable analytical methods (e.g., LC/MS-MS) and pharmacokinetic parameters are calculated by non-compartmental methods.

EXAMPLE 22 - Effects in a Non-Human Primate Model of Obesity.

[0334] Spontaneously obese male cynomolgus monkeys are acclimated to dosing and handling for at least 3 weeks. Baseline animal characteristics are determined and animals are randomized into treatment groups based upon body weight and baseline metabolic parameters such as triglyceride levels. Following randomization, groups of monkeys receive daily or twice daily doses of the peptides of the present invention administered by a suitable route for 4 or more weeks. Control groups of monkeys receive daily doses of vehicle or positive control. Food consumption and body weight are measured at intervals during the study. Effects of the administered peptides on body weight, food intake, BMI and/or metabolic parameters are compared to control animals treated with vehicle.

EXAMPLE 23

Effects in the STAM® Mouse Model of Non-alcoholic Steatohepatitis (NASH).

[0335] In the STAM model of NASH, C57/bl6 mice are injected with a singlesubcutaneous dose of streptotoxin, three days after birth to destroy pancreatic β-cells. At the age of 4 weeks, animals are put on a high fat diet. This combined treatment results in the development of steatosis, fibrosis,

cirrhosis and finally hepatocellularcarcinoma (HCC) along with hyperglycemia and moderate hyperlipidemia thus closely resembling human NASH. Beginning at 5 weeks of age, groups of STAM animals (8animals per group) are treated with the peptides of the present invention administered daily or twice daily by an appropriate route, until study termination. A control group of animals receive daily administration of a suitable positive control compound (e.g. telmisartan). At approximately 10 weeks of age, metabolic parameters are determined and animals are sacrificed. Liver samples are obtained and fixed, embedded in paraffin, stained with hematoxylin and eosin or Masson's trichrome, and examined by light microscopy. The extent of steatosis and the non-alcoholic fatty liver disease (NAFLD) activity score (NAS) are determined histopathologically according to methods known in the art.

EXAMPLE 24

β -Arrestin Recruitment in Cultured Apelin Receptor Overexpressing CHO-K1 Cells

[0336] The effect of the peptides on activation of Apelin Receptor (APJ) can be assessed using an assay to monitor β-Arrestin recruitment in cultured cells overexpressing APJ such as CHO-K1, derived from Chinese hamster ovary. β-Arrestin recruitment assays were performed by Eurofins-DiscoverX (Fremont, CA) using CHO-K1 AGTRL1 β-Arrestin cell line (co-expressing ProLink tagged human APJ and Enzyme Acceptor tagged β-Arrestin) and PathHunter detection kit. Peptides were initially prepared either as 10 mM stock in DMSO and used at a final concentration of 10 μM (0.1% DMSO). CHO-K1 AGTRL1 β-Arrestin cells were seed onto 384-well plates in standard medium. After overnight culture, the medium was replaced with buffer containing 500 nM Apelin-13 (positive control) or 10 μM peptide. Following 90 min incubation at 37°C, β-Arrestin recruitment in response to various treatments was quantified using a chemiluminescent complementation reporter assay to measure association of tagged human APJ (ProLink tag) and tagged β-Arrestin (Enzyme Acceptor tag). Data are presented as percent of Apelin-13 response (100%) with each data point representing the average of duplicates. The results are shown in Table 22. This example illustrates the activity of various peptides as APJ agonists

TABLE 22

β -Arrestin Recruitment in Cultured CHO-K1 AGTRL1 β-Arrestin Cells

SEQ ID NO:	Percent of Apelin-13 Control Activity
7	91
8	17
9	
10	3
11	0

12	-1
13	1
14	0
15	40
16	0
17	10
18	1
19	1
20	1
21	1
22	0
23	0
24	-1
25	-1
26	0
27	-1
28	-1
29	0
30	-1
31	0
32	0
33	9
34	8
35	17
36	63
37	12
38	0
39	0
40	0
41	0
42	8
43	82
44	0
45	0
46	0
47	1
48	1

49	1
50	0
51	0
52	0
53	1
54	1
55	1
56	0
57	0
58	0
59	0
60	0
61	5
62	2
63	9

EXAMPLE 25

cAMP Levels in Cultured Apelin Receptor Overexpressing CHO-K1 Cells

[0337] The effect of the peptides on activation of Apelin Receptor (APJ) can be assessed using an assay to monitor inhibition of cAMP expression in cultured cells overexpressing APJ such as CHO-K1, derived from Chinese hamster ovary. Peptides were initially prepared as 30 mM stock in DMSO and diluted to 3 mM in H_2O or directly as 3 mM stock in H_2O; used at a final concentration of 10 µM (0-0.1% DMSO). Forskolin was used as a highly potent inducer of cAMP expression. CHO-K1 AGTRL1 Gi cells stably overexpressing APJ were purchased from Eurofins-DiscoverX (Fremont, CA). CHO-K1 AGTRL1 Gi cells were seeded onto 384-well plates in standard culture medium (F12K + 10% Fetal Bovine Serum + antibiotics) at 10,000 cells/well and allowed to adhere overnight at 37°C in a humidified atmosphere of 5% CO_2/95% air. After overnight culture, the medium was replaced with buffer containing 10 µM forskolin (to increase cAMP expression) and either 500 nM Pyr-Apelin-13 (inhibits cAMP accumulation) or 10 µM peptide. Following 30 min incubation at 37°C, cAMP levels in response to various treatments were quantified using HitHunter cAMP kit according to manufactures protocol (Eurofins-DiscoverX); chemiluminescent signal was measured using a Cytation 3 plate reader (BioTek, Winooski, VT). Data are presented as percent of Pyr-Apelin-13 response (100%) with each data point representing the average of triplicates. The results are shown in Table 23. This example illustrates the activity of various peptides as APJ agonists.

TABLE 23

cAMP Levels in Cultured CHO-K1 AGTRL1 Gi Cells

SEQ ID NO:	Percent of Pyr-Apelin-13 Control Activity
7	109
8	64
10	70
11	49
12	24
13	49
14	39
15	105
17	26
18	30
19	27
20	37
21	34
22	62
23	-4
24	16
25	3
26	29
27	9
28	3
29	21
30	31
31	2
32	4
33	27
34	61
35	8
36	101
37	53
38	12
39	-13
40	19
41	15

42	33
43	44
44	5
45	10
46	11
47	-6
48	15
49	14
50	8
51	24
52	15
53	6
54	20
55	-7
56	-2
57	-1
58	5
59	-2
60	16
61	1
62	27
63	21

EXAMPLE 26

cAMP Levels in Cultured Apelin Receptor Overexpressing CHO-K1 Cells

[0338] The effect of the peptides on activation of Apelin Receptor (APJ) can be assessed using an assay to measure inhibition of forskolin-stimulated cAMP accumulation in cultured cells overexpressing APJ such as CHO-K1 cells. CHO-K1 AGTRL1 Gi cells stably overexpressing APJ were purchased from Eurofins-DiscoverX (Fremont, CA), seeded onto 384-well plates in standard culture medium at 10,000 cells/well, and allowed to adhere overnight at 37°C in a humidified atmosphere of 5% CO_2/95% air. After overnight culture, the medium was replaced with buffer containing 10 μM forskolin to increase cAMP expression together with either Pyr-Apelin-13 (0.025-167 nM) or peptides of the invention (0.005-30 μM). Following 30 min incubation at 37°C, cAMP levels in response to various treatments were quantified using HitHunter cAMP kit according to manufacturer's protocol (Eurofins-DiscoverX); chemiluminescent signal was measured using a Cytation 3 plate reader (BioTek, Winooski, VT). Data were plotted as mean

(SD) percent of Pyr-Apelin-13 response (100%) based on the average of 2-3 values. IC50 values were determined by GraphPad Prism software (GraphPad Software, San Diego, CA). Data are mean (SD) n=2-3 for all data points. The IC50 values are shown in Table 24.

TABLE 24

SEQ ID NO	IC50 (M)
Apelin-13	1.763x10-9
15	4.492x10-6
36	1.602 x10-6
37	2.499 x10-6
42	4.382 x10-6
43	2.069 x10-6
7	1.243 x10-6

[0339] All of the articles and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the articles and methods of this disclosure have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the articles and methods without departing from the spirit and scope of the disclosure. All such variations and equivalents apparent to those skilled in the art, whether now existing or later developed, are deemed to be within the spirit and scope of the disclosure as defined by the appended claims. All patents, patent applications, and publications mentioned in the specification are indicative of the levels of those of ordinary skill in the art to which the disclosure pertains. The disclosure illustratively described herein suitably may be practiced in the absence of any element(s) not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of", and "consisting of" may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the disclosure claimed. Thus, it should be understood that although the present disclosure has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this disclosure as defined by the appended claims.

[0340] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference in their entirety and to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its

entirety herein (to the maximum extent permitted by law). All headings and sub-headings are used herein for convenience only and should not be construed as being limiting in any way. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the disclosure and does not pose a limitation on the scope of the disclosure unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the disclosure. The citation and incorporation of patent documents herein is done for convenience only and does not reflect any view of the validity, patentability, and/or enforceability of such patent documents.

[0341] This disclosure includes all modifications and equivalents of the subject matter recited in the aspects appended hereto as permitted by applicable law.

[0342] The present application includes a Sequence Listing. To the extent differences exist between information/description of sequences in the specification and information in the Sequence Listing, the specification is controlling.

What is claimed:

1. A peptide comprising an amino acid sequence of Formula I:

$$X^1\text{- }RX^2\text{-}X^3\text{-}X^4\text{-}X^5\text{-}X^6\text{-Q-}X^7\text{-L-}X^8\text{-}X^9 \quad \text{(I) (SEQ ID NO: 1)}$$

wherein:

X^1 is absent or if present is an amino acid having a polar side chain or a non-polar side chain;

X^2 is an amino acid having a polar side chain or a non-polar side chain;

X^3 is absent or if present is one to three amino acids, each amino acid independently having a polar side chain or a non-polar side chain;

X^4 is an amino acid having a polar side chain or a non-polar side chain;

X^5 is an amino acid having a non-polar side chain;

X^6 is an amino acid having a polar side chain or a non-polar side chain;

X^7 is an amino acid having a polar side chain;

X^8 is an amino acid having a polar side chain; and

X^9 is absent or if present is one to three amino acids, each amino acid independently having a polar side chain or a non-polar side chain;

or an analog of said peptide having a deletion, insertion or substitution of one, two, three, or four amino acids;

or C-terminal acids or amides, or N-acetyl derivatives thereof;

or a pharmaceutically acceptable salt thereof.

2. The peptide or analog of Claim 1 wherein X^3 is absent, or if present is $-X^{12}X^{11}X^{10}-$; wherein:

X^{10} is absent, or if present is an amino acid having a non-polar side chain;

X^{11} is absent, or if present is an amino acid having a non-polar side chain; and

X^{12} is an amino acid having a polar side chain or a non-polar side chain;

or C-terminal acids or amides, or N-acetyl derivatives thereof;

or a pharmaceutically acceptable salt thereof.

3. The peptide or analog of Claim 1 wherein X^9 is absent, or if present is $-X^{13}X^{14}X^{15}$; wherein:

X^{13} is an amino acid having a non-polar side chain;

X^{14} is absent, or if present is an amino acid having a non-polar side chain; and

X^{15} is absent, or if present is an amino acid having a polar side chain;

or C-terminal acids or amides, or N-acetyl derivatives thereof;

or a pharmaceutically acceptable salt thereof.

4. The peptide or analog of Claim 1 wherein:

X^1 is absent, or if present is selected from D, (dD), E, (dE), K, (dK), R, (dR), H, (dH), N, (dN), Q, (dQ), S, (dS), T, (dT), Y, (dY), C, (dC), G, A, (dA), V, (dV), L, (dL), I, (dI), F, (dF), W, (dW), P (dP), M and (dM);

X^2 is selected from D, (dD), E, (dE), K, (dK), R, (dR), H, (dH), N, (dN), Q, (dQ), S, (dS), T, (dT), Y, (dY), C, (dC), G, A, (dA), V, (dV), L, (dL), I, (dI), F, (dF), W, (dW), P (dP), M and (dM);

X^3 is absent or if present is D, (dD), E, (dE), K, (dK), R, (dR), H, (dH), N, (dN), Q, (dQ), S, (dS), T, (dT), Y, (dY), C, (dC), G, A, (dA), V, (dV), L, (dL), I, (dI), F, (dF), W, (dW), P (dP), M, (dM) or-$X^{12}X^{11}X^{10}$-;

X^4 is an amino acid selected from D, (dD), E, (dE), K, (dK), R, (dR), H, (dH), N, (dN), Q, (dQ), S, (dS), T, (dT), Y, (dY), C, (dC), G, A, (dA), V, (dV), L, (dL), I, (dI), F, (dF), W, (dW), P (dP), M and (dM);

X^5 is an amino acid selected from G, A, (dA), V, (dV), L, (dL), I, (dI), F, (dF), W, (dW), P (dP), M and (dM);

X^6 is an amino acid selected from D, (dD), E, (dE), K, (dK), R, (dR), H, (dH), N, (dN), Q, (dQ), S, (dS), T, (dT), Y, (dY), C, (dC), G, A, (dA), V, (dV), L, (dL), I, (dI), F, (dF), W, (dW), P (dP), M and (dM);

X^7 is an amino acid selected from D, (dD), E, (dE), K, (dK), R, (dR), H, (dH), N, (dN), Q, (dQ), S, (dS), T, (dT), Y, (dY), C, and (dC);

X^8 is an amino acid selected from D, (dD), E, (dE), K, (dK), R, (dR), H, (dH), N, (dN), Q, (dQ), S, (dS), T, (dT), Y, (dY), C, and (dC);

X^9 is absent or if present is an amino acid independently selected from G, A, (dA), V, (dV), L, (dL), I, (dI), F, (dF), W, (dW), P (dP), M and (dM) or -$X^{12}X^{13}X^{14}$;

X^{10} is absent, or if present is an amino acid selected from G, A, (dA), V, (dV), L, (dL), I, (dI), F, (dF), W, (dW), P (dP), M and (dM);

X^{11} is absent, or if present is an amino acid selected from G, A, (dA), V, (dV), L, (dL), I, (dI), F, (dF), W, (dW), P (dP), M and (dM);

X^{12} is an amino acid selected from G, A, (dA), V, (dV), L, (dL), I, (dI), F, (dF), W, (dW), P (dP), M and (dM);

X^{13} is an amino acid selected from G, A, (dA), V, (dV), L, (dL), I, (dI), F, (dF), W, (dW), P (dP), M and (dM);

X^{14} is absent, or if present is an amino acid selected from G, A, (dA), V, (dV), L, (dL), I, (dI), F, (dF), W, (dW), P (dP), M and (dM); and

X^{15} is absent, or if present is an amino acid selected from D, (dD), E, (dE), K, (dK), R, (dR), H, (dH), N, (dN), Q, (dQ), S, (dS), T, (dT), Y, (dY), C, and (dC);

or C-terminal acids or amides, or N-acetyl derivatives thereof;

or a pharmaceutically acceptable salt thereof.

5. The peptide or analog of Claim 1 wherein:

X^1 is M, K, or absent;

X^2 is R or Aib;

X^3 is absent or if present is M, E, -MMG-, -II(dA)-, -Nle-Nle-G- or -IIG-;

X^4 is M, E, I or Nle;

X^5 is V, A or G;

X^6 is F, Y, A or E;

X^7 is C, S or E;

X^8 is C, S or E; and X^9 is -GL, -G(dA), -G(dA)K, -(dA)L, G or absent;

or C-terminal acids or amides, or N-acetyl derivatives thereof;

or a pharmaceutically acceptable salt thereof.

6. The peptide or analog of claim 5, wherein X^1 is (PEG12)-K, and/or wherein X^9 is -G(dA)-K(PEG12).

7. The peptide or analog of Claim 1 comprising or consisting of an amino acid sequence selected from a peptide sequence of Table 1; or a pharmaceutically acceptable salt thereof.

8. A peptide comprising an amino acid sequence of Formula II:

X^{16}-M-M-G-M-X^{17} (II) (SEQ ID NO: 64)

wherein **X^{16}** is absent or if present is R- or R-R-; and **X^{17}** is absent or if present is selected from -V, -VF, -VFQ, -VFQS, -VFQSL, and -VFQSLCG(dA);

or C-terminal acids or amides, or N-acetyl derivatives thereof;

or a pharmaceutically acceptable salt thereof.

9. The peptide of claim 8 wherein **X^{16}** is R- or RR-; and **X^{17}** is selected from VF, -VFQ, -VFQS, -VFQSL, and -VFQSLCG(dA);

or C-terminal acids or amides, or N-acetyl derivatives thereof;

or a pharmaceutically acceptable salt thereof.

10. A peptide or analog comprising or consisting of an amino acid sequence selected from MMGMVF (SEQ ID NO: 47) ; RMMGMVFQ (SEQ ID NO: 51); RMMGMVFQS (SEQ ID NO: 52); RMMGMVFQSL (SEQ ID NO: 53); RMMGMVFQSLCG(dA) (SEQ ID NO: 54); RRMMGMVF (SEQ

ID NO: 57); Acetyl-RRMMGMVFQSLCG(dA) (SEQ ID NO: 61); RRMMGMVFQSLCG(dA)-Amide (SEQ ID NO: 62); and Acetyl-RRMMGMVFQSLCG(dA)-Amide (SEQ ID NO: 63); or a pharmaceutically acceptable salt thereof.

11. The peptide or analog according to any one of claims 1-10, that is an isolated or a non-naturally occurring peptide, or a pharmaceutically acceptable salt thereof.

12. The peptide according to any one of claims 1-11, or a pharmaceutically acceptable salt thereof.

13. A peptide analog of any one of claims 1-11, wherein the peptide comprises substitution with at least one amino acid selected from (i) an amino acid having a D-configuration, and (ii) a non-naturally occurring amino acid residue; or a pharmaceutically acceptable salt thereof.

14. A peptide or analog of any one of claims 1-13, further comprising a duration enhancing moiety attached to the peptide or analog, and optionally further comprising a metabolically cleavable linker coupling the peptide or analog to the duration enhancing moiety.

15. A composition comprising a peptide or analog of any one of claims 1-14 and a pharmaceutically acceptable excipient.

16. The composition of claim 15, wherein the excipient is not found in nature.

17. A pharmaceutical composition comprising a peptide or analog of any one of claims 1-14.

18. A method of modulating cell viability comprising administering a peptide or analog of any one of claims 1-14, or a composition according to any one of claims 15-17.

19. A method of treating cancer in patient in need of such treatment, comprising administering to the patient a pharmacologically effective amount of a peptide or analog of any one of claims 1-14, or a composition according to any one of claims 15-17.

20. A method of treating cell proliferation in patient in need of such treatment, comprising administering to the patient a pharmacologically effective amount of a peptide or analog of any one of claims 1-14 or a composition according to any one of claims 15-17.

21. A method of treating an apoptotic disease in a patient in need of such treatment, comprising administering to the patient a pharmacologically effect amount of a peptide or analog of any one of claims 1-14, or a composition according to any one of claims 15-17.

22. A method of treating a metabolic disease in a patient in need of such treatment, comprising administering to the patient a pharmacologically effect amount of a peptide or analog of any one of claims 1-14, or a composition according to any one of claims 15-17.

23. A method of providing cytoprotection in a patient in need of such treatment, comprising administering to the patient a pharmacologically effect amount of a peptide or analog of any one of claims 1-14, or a composition according to any one of claims 15-17.

24. An isolated nucleic acid that comprises a nucleotide sequence that encodes a peptide or analog of any one of Claims 1-14.

25. A vector or expression vector that comprises an isolated nucleic acid according to claim 24.

26. A host cell that comprises a nucleic acid according to claim 24 or a vector or expression vector according to claim 25.

27. A composition comprising the nucleic acid of claim 24, the vector or expression vector of claim 25, or the host cell of claim 26, and a pharmaceutically acceptable excipient.

28. A method of treating a metabolic disease in a subject in need thereof, comprising administering to the subject a peptide, peptide analog, composition, nucleic acid, vector, expression vector, or host cell of any one of claims 1-17 and 24-27, in an amount effective to treat the metabolic disease.

29. The method of claim 28, wherein the disease is selected from the group consisting of obesity, diabetes (e.g., Type 2 diabetes), cognitive disorders and/or neurodegenerative disorders, cardiovascular disease, fatty liver disease, and gastrointestinal disease.

30. A method of treating a cancer in a subject in need thereof, comprising administering to the subject a peptide, peptide analog, composition, nucleic acid, vector, expression vector, or host cell of any one of claims 1-17 and 24-27, in an amount effective to treat the cancer.

31. The method of claim 30, wherein the cancer is lung cancer, pancreatic cancer, breast cancer, prostate cancer, ovarian cancer, or hepatocellular cancer.

32. A method of treating a liver disease in a subject in need thereof, comprising administering to the subject a peptide, peptide analog, composition, nucleic acid, vector, expression vector, or host cell of any one of claims 1-17 and 24-27, in an amount effective to treat the liver disease.

33. The method of claim 32, wherein the liver disease is a fatty liver disease.

34. The method of claim 33, wherein the fatty liver disease is NAFLD or NASH.

35. A method of modulating fatty acid metabolism in a subject in need thereof, comprising administering to the subject a peptide, peptide analog, composition, nucleic acid, vector, expression vector, or host cell of any one of claims 1-17 and 24-27, in an amount effective to modulate fatty acid metabolism.

36. The method of claim 35, wherein fatty acid metabolism is increased in the subject after the peptide, peptide analog, composition, nucleic acid, vector, expression vector, or host cell of any one of claims 1-17 and 24-27, is administered to the subject.

37. A method of reducing body weight in a subject in need thereof, comprising administering to the subject a peptide, peptide analog, composition, nucleic acid, vector, expression vector, or host cell of any one of claims 1-17 and 24-27, in an amount effective to reduce body weight in the subject.

38. Use of a peptide, peptide analog, composition, nucleic acid, vector, expression vector, or host cell of any one of claims 1-17 and 24-27, in therapeutic treatment of a metabolic disease, cancer, liver disease, or any disease, disorder, or medical condition described herein.

39. Use of a peptide, peptide analog, composition, nucleic acid, vector, expression vector, or host cell of any one of claims 1-17 and 24-27, in the manufacture of a medicament for treating a metabolic disease, cancer, liver disease, or any disease, disorder, or medical condition described herein.

40. A peptide, peptide analog, composition, nucleic acid, vector, expression vector, or host cell of any one of claims 1-17 and 24-27 for use in therapeutic treatment of a metabolic disease, cancer, liver disease, or any disease, disorder, or medical condition described herein.

41. A method of treating an apelin-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a peptide, peptide analog, composition, nucleic acid, vector, expression vector, or host cell of any one of claims 1-17 and 24-27, in an amount effective to treat the apelin-mediated disease or disorder.

42. The method of claim 41, wherein the disease is related to UVB radiation.

43. The method of Claim 41 wherein the a disease or disorder is selected from hypertension, endothelial dysfunction, damages to cardiovascular tissues, heart failure, coronary heart disease, ischemic and/or hemorrhagic stroke, macrovascular disease, microvascular disease, diabetic heart (including diabetic cardiomyopathy and heart failure as a diabetic complication) coronary heart disease, peripheral artery disease, peripheral arterial occlusive disease, pre-eclampsia, resistant hypertension, refractory hypertension, hypertensive crisis, blood or fetal-placental circulation, edematous diseases, pulmonary dysfunction, acute lung injury (ALI), acute respiratory distress syndrome (ARDS), trauma and/or burns, and/or ventilator induced lung injury (VI LI), pulmonary fibrosis, mountain sickness, chronic kidney diseases, acute kidney injury, lymphedema, lymphatic vessel regeneration, inflammatory bowel disease, inflammatory disease, or ocular disorders associated with disturbed vascular function, topical wounds, migraine, tumors, metastasis, angiogenesis, degeneration of cartilage, osteoarthritis, and cancers.

44. The method of claim 41, wherein the disease is sepsis or sepsis shock.

45. The method of claim 41, wherein the disease is thrombosis or microthrombosis.

46. The method of claim 41, wherein the disease is thrombin-related aggregation.

47. The method of claim 41, wherein the disease is ischemic shock.

48. The method of claim 41, wherein the disease is organ failure or multiple organ failure.