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(54) Title: DIETARY PEPTIDES

(57) Abstract: The present invention relates to novel peptides, composition comprising such peptides including nutritional supplements and methods for inducing satiation and satiety, for weight management and preventing or reducing the incidence of obesity, or for preventing or reducing cardiovascular diseases, atherosclerosis, hypertension, hepatosteatosis, cancer and/or diabetes.

DIETARY PEPTIDES

FIELD OF THE INVENTION

The present invention relates to novel peptides, composition comprising such peptides including nutritional supplements and methods for inducing satiation and satiety, for preventing or reducing the incidence of metabolic syndrome comprising overweight and obesity, cardiovascular diseases, atherosclerosis, hypertension, hepatosteatosis, diabetes and/or cancer.

BACKGROUND OF THE INVENTION

Obesity is a common medical condition affecting numerous humans throughout the world and is associated with, induces or increases the risk of developing conditions such as cardiovascular diseases, atherosclerosis, hypertension, hepatosteatosis, cancer and/or diabetes.

Some regulators of obesity have been identified. However, despite intensive study, the regulation of obesity is still poorly understood.

Protein is more satiating than carbohydrate and fat, and its effect on food intake is more than can be accounted for by its energy content alone. The mechanism by which proteins trigger food intake regulatory systems is unclear. However, it seems likely that satiety signals arising from protein ingestion begin in the gastrointestinal tract upon proteolytic digestion.

Accordingly, dietary proteolytic products (peptides and amino acids) induce signalling in enteroendocrine cells of the intestine, which leads to secretion of various gut hormones, e.g. glucagon-like peptide-1 (GLP-1) (fig 1) with neuronal, local (auto- and paracrine) and systemic (endocrine) effects (fig 2), ultimately leading to satiation (amount of food ingested as a meal) and satiety (length of time between meals). It is well-known that (some) enteroendocrine cells respond to free amino acids and small peptides (di- and tripeptides), which are readily taken up by the enterocytes and metabolized and/or transported into systemic circulation. Rate of digestion, i.e. transit time in the GI tract, secretion of digestive enzymes, etc, is a highly regulated process, where cellular responses to undigested proteins and/or increases in amino acids and peptides in the gut leads to secretion of gut hormones, e.g. GLP-1, peptide tyrosine-tyrosine (PYY), neurotensin (NT), which induces satiation. If these signals persist in the gut because of slow and prolonged release, satiety is enhanced. One such mechanism is the ileal brake, where unknown components in partly digested food

reaches the distal small intestine and invokes a response in the form of secretion of the gut hormones GLP-1, PYY, NT and possibly others, as yet unknown hormones. However, the precise mechanism behind the ileal brake is unknown.

The specific peptide(s) responsible for this satiety inducing signal(s) is largely unknown and it would be of great importance if any of these peptides could be identified.

OBJECT OF THE INVENTION

It is an object of embodiments of the invention to provide new polypeptides that induce or signals satiety in a subject.

The polypeptides of the invention may be used to treat conditions associated with a wide variety of metabolic diseases, for use in weight management, and/or for preventing or reducing the incidence of overweight and/or obesity, or for preventing or reducing cardiovascular diseases, atherosclerosis, hypertension, hepatosteatosis, cancer and/or diabetes.

SUMMARY OF THE INVENTION

Dietary proteolytic products (peptides and amino acids) induce signalling in enteroendocrine cells of the intestine, which leads to secretion of various gut hormones, e.g. GLP-1 (fig 1) with both central (CNS), local (auto- and paracrine) and systemic (endocrine) effects (fig 2), ultimately leading to satiation and satiety.

It has been found by the present inventor(s) that novel meat-derived polypeptides are superior in signalling of intestinal cell lines (fig 3) and that only very specific peptides are capable of signalling (fig 4). The inventors of the present invention have identified polypeptides including an octapeptide (ASDKPYIL, SEQ ID NO:6) present in proteolytic digests (fig 5) and resistant to pepsin degradation, of which a pentapeptide (KPYIL, SEQ ID NO:9) is the minimal sequence with significant biologic activity (fig 6). The octapeptide sequence is unique for the muscle-specific alpha-actinin-2 protein, and the sequence is conserved between all animal species. This peptide would be applicable as a novel, but natural nutritional supplement to induce satiation and satiety.

So, in a first aspect the present invention relates to an isolated polypeptide comprising the amino acid sequence

AA1-AA2-AA3-K-AA5-AA6-AA7-AA8 (formula I, SEQ ID NO:1),

wherein AA1 is an optional amino acid selected from A, L, I, and V; AA2 is an optional amino acid selected from S, T, G, A, N, E and D; AA3 is an optional amino acid selected from D, E, and G; AA5 is selected from P, N, S, D, A, T, K, and G; AA6 is selected from Y, N, I, W, and F; AA7 is selected from I, L, R, and V; AA8 is selected from L, I, V, S, M, and T; which polypeptide is not more than 50 amino acids in length; or a variant thereof with a sequence identity of at least 80%.

In a second aspect the present invention relates to an isolated polypeptide consisting of the amino acid sequence

R1-AA1-AA2-AA3-K-AA5-AA6-AA7-AA8-R2 (formula II, SEQ ID NO:2),

wherein AA1 is an optional amino acid selected from A, L, I, and V; AA2 is an optional amino acid selected from S, T, G, A, N, E and D; AA3 is an optional amino acid selected from D, E, and G; AA5 is selected from P, N, S, D, A, T, K, and G; AA6 is selected from Y, N, I, W, and F; AA7 is selected from I, L, R, and V; AA8 is selected from L, I, V, S, M, and T; R1 defines the N-term (-NH2) or a protection group; R2 defines the C-term (-COOH).

In a third aspect the present invention relates to a polypeptide having or comprising a sequence selected from ASDKPYIL (SEQ ID NO:6), SDKPYIL (SEQ ID NO:7), DKPYIL (SEQ ID NO:8), KPYIL (SEQ ID NO:9), AGDKNYIL (SEQ ID NO:10), AGDKNYIT (SEQ ID NO:11), AGDKSYIT (SEQ ID NO:12), ADGKPYIV (SEQ ID NO:13), AEDKDFIT (SEQ ID NO:14), AADKPYIL (SEQ ID NO:15), ATDKPYIL (SEQ ID NO:16), AGDKPYIT (SEQ ID NO:17), ASEKPYIL (SEQ ID NO:18), ADGKPYVT (SEQ ID NO:19), AGDKPYIL (SEQ ID NO:20), ASDKPNIL (SEQ ID NO:21), ASDKPYIT (SEQ ID NO:22), AADKPFIL (SEQ ID NO:23), ASDKAYIT (SEQ ID NO:24), AGDKAYIT (SEQ ID NO:25), ANGKPFIT (SEQ ID NO:26), AGDKNFIT (SEQ ID NO:27), ASDKSYIT (SEQ ID NO:28), ASDKTYIT (SEQ ID NO:29), ASDKNYIT (SEQ ID NO:30), AGDKKYIT (SEQ ID NO:31), AGDKNYIS (SEQ ID NO:32), AADKNYIT (SEQ ID NO:33), AGDKNYIM (SEQ ID NO:34), AADKNFIM (SEQ ID NO:35), AADKNFIT (SEQ ID NO:36), and AGDKGIRS (SEQ ID NO:37).

In a fourth aspect the present invention relates to a composition comprising a polypeptide of the invention.

In a further aspect the present invention relates to a polypeptide according to the invention for use in promoting satiety in a subject, for use in weight management, and/or for preventing or reducing the incidence of overweight and/or obesity in a subject, or for

preventing or reducing cardiovascular diseases, atherosclerosis, hypertension, hepatosteatosis, cancer and/or diabetes.

In a further aspect the present invention relates to a method of preventing or reducing the incidence of obesity in a subject, and/or of promoting satiety in a subject, and/or to reduce or treat cardiovascular diseases, atherosclerosis, hypertension, hepatosteatosis, cancer and/or diabetes comprising enteral administering to a subject in need thereof a polypeptide comprising or consisting of the amino acid sequence

AA1-AA2-AA3-AA4-AA5-AA6-AA7-AA8 (formula III, SEQ ID NO:3),

wherein AA1 is an optional amino acid selected from A, L, I, and V; AA2 is an optional amino acid selected from S, T, G, A, N, E and D; AA3 is an optional amino acid selected from D, R, K, E, and G; AA4 is an amino acid selected from K and R; AA5 is selected from P, N, S, D, A, T, K, and G; AA6 is selected from Y, N, I, W, and F; AA7 is selected from I, L, R, and V; AA8 is selected from L, I, V, S, M, and T; which polypeptide is not more than 50 amino acids in length; or a variant thereof with a sequence identity of at least 80%.

In a further aspect the present invention relates to a composition according to the invention for use in promoting satiety in a subject, and/or for use in weight management, and/or for preventing or reducing the incidence of obesity in a subject and/or for use in preventing or reducing cardiovascular diseases, atherosclerosis, hypertension, cardiovascular diseases, high blood pressure, cancer and/or diabetes.

In a further aspect the present invention relates to a method of promoting satiety in a subject, and/or of preventing or reducing the incidence of obesity in a subject, and/or to reduce or treat cardiovascular diseases, atherosclerosis, hypertension, cardiovascular diseases, high blood pressure, cancer and/or diabetes, comprising administering to a subject in need thereof a composition according to the invention.

LEGENDS TO THE FIGURES

Fig. 1. Dose-response curve for effect of protein hydrolysate on release of GLP-1 from GLUTag cells (open circles) or a control cell line (closed symbols) that does not produce GLP-1. Cells (~5x10^5 per sample) were incubated for up to 90 min in Dulbeccos Modified Eagle Medium (DMEM) containing 5,56 mM glucose in absence or presence of different amounts (weight/volume) of meat protein hydrolysate. Supernatant was filtered through 0,45 micron

filters and assayed for content of GLP-1 as described in ELISA protocol. Data are mean + SEM from quadruplicate samples.

Fig. 2. Signaling by dietary nutrients in enteroendocrine cells. Illustration from Horm Res Paediatr. 2015;83(1):1-10.

Fig. 3. Stimulation of cell signaling (measured as increase in intracellular fluorescence) by meat protein hydrolysates (filled symbols) or whey protein hydrolysates (open symbols) in three different intestinal cell lines: Top) a murine intestinal cell line; middle) GLUTag cells; bottom) CaCo2 cells.

Fig. 4. Size exclusion fractionation of protein hydrolysate and test of biologic activity. Absorbance at 280 nm shown by thick, solid line, activity of fractions by filled circles.

Fig. 5. Verification of identified sequence ASDKPYIL by synthetic peptide. Comparison of dose-response relationship of meat hydrolysate and pure, synthetic peptide identified by sequencing of purified fractions.

Fig. 6. Identification of minimal active sequence in ASDKPYIL in murine (mIC) and human (hIC) intestinal cells.
Truncation from the amino-terminal or from the carboxy-terminal end of ASDKPYIL has different consequences. Deleting the carboxy-terminal leucine reduces potency more than two orders of magnitude in mIC cells and abrogates activity in hIC. Peptides with further deletions of 2, 3 or 4 amino acids from the carboxy-terminus are without activity. Deleting the first three amino acids from the amino-terminus has no big impact on activity. However, the fourth amino acid, lysine, is critical, since PYIL has two orders of magnitude lower activity compared with the full sequence in mIC and no activity in hIC.

Fig. 7. Identification of critical residues in ASDKPYIL (d-Ala (A_D) scan). Systematic replacement of all residues in ASDKPYIL with the d-isomer of alanine and corresponding biological activity. Results show that 1) the last four amino acids (PYIL, SEQ ID NO:4) are critical, 2) replacing K reduces potency more than 30-fold, 3) replacing the aspartic residue improves potency almost 10-fold, and 4) alanine and serine on the first two positions are without importance.

Fig. 8. Stability of peptides in rodent intestine.
0,001 mg/ml of the indicated peptides were incubated with pieces of rodent intestine (mouse and rat intestine gave similar results) for up to 10 minutes at 37 °C. Recovery of activity was tested with dose-response curves as indicated.

Fig.9. Stability of peptides in rodent intestine. EC_{50} values for different peptides and different incubation times were calculated from figure 8 and recovered activity plotted as a function of time.

Figure 10. Comparison of the sequences of three known gut hormones, neurotensin, neuromedin N and xenin with that of DC7-2 (ASDKPYIL). The PYIL sequence is conserved, although Y is replaced by W in xenin.

Figure 11. Comparison of the DC7-2 sequence (aa 891-898) in isoforms of a-actinin 2 (Hs: Homo sapiens ACTN1-4) and conservation between species (Dm: Drosophila melanogaster; Ce: Caenorhabditis elegans; Dd: Dictyostelium discoideum; Sp: Schizosaccharomyces pombe; Dr: Danio rerio)

Figure 12. 24 Balb/c female mice, 10-11 weeks, 20-22 g, were acclimatized to 12 h dark light cycle and placed single-housed in metabolic cages. Following administration of the indicated doses of DC7-2, feed and water intake was monitored for 6 h.

Figure 13. Summary of cell signaling activities of N-terminal substitutions in octa-, hepta-, hexa- and pentapeptides based on the sequence of DC7-2. Single-letter abbreviations for the 20 amino acids are shown on the plot centered at the corresponding EC50. The native amino acid in DC7-2 is marked with a grey circle for each of the peptides.

Figure 14. Stability of DC7-2 families of peptides in intestine homogenates. Single-letter abbreviations for the 20 amino acids are shown on the plot with the corresponding stability expressed as the logarithm to the concentration of intestine homogenate that degrades half of the activity of peptide. All peptides were incubated at 10-5 M with various dilutions of a homogenate of the entire small intestine (pool from 20 mice). After incubation for 90 min at 37 oC, degradation was stopped by addition of 1 M phosphoric acid (final 0,4 M, pH ~1.2). Each peptide incubation mix was neutralized with NaOH and immediately tested for activity in intestinal cells. Control for zero degradation, i.e. addition of phosphoric acid before addition of intestine homogenate, was included for each peptide. The native amino acid in DC7-2 is marked with a grey circle for each of the peptides.

Figure 15. Stability of DC7-2 families of peptides in serum.

Figure 16. Stability of X-KPYIL hexapeptides in intestine homogenate and serum.

Figure 17. 24 Balb/c female mice, 10-11 weeks, 20-22 g, were acclimatized to 12 h dark light cycle. Mice were divided into four groups each of six mice and placed single-housed in

metabolic cages. Mice were then administered vehicle alone (day 1) for monitoring of feed and water intake for 6 h. On day 3, the same groups received the indicated doses of DC7-2,and feed and water intake was monitored for 6 h.

Figure 18. Swiss Webster male mice, 25-30 g, were acclimatized to 12 h dark/light cycle and placed single-housed in cages. Following administration just prior to onset of dark cycle of vehicle alone (0.5 ml of PBS w 1% of BSA) or vehicle + DC7-2, feed intake was monitored every hour for 6 h (during dark cycle). Mean and SEM from four experiments, each with 6-8 mice per treatment. Data were fitted with linear regression (R2>0.99) and 95 % confidence intervals are shown as grey lines. Accumulated feed intake for treatment with DC7-2 was 64 % +/- 5 % compared with control for these four experiments.

Figure 19. Swiss Webster male mice, 25-30 g, were acclimatized to 12 h dark/light cycle and placed single-housed in cages. Following administration just prior to onset of dark cycle of vehicle alone (0.5 ml of PBS w 1% of BSA) or vehicle + DC7-2, feed intake was monitored every hour for 12 h (during dark cycle) and then intermittently up to 30 h.

Figure 20. Swiss Webster male (25-30 g) or female (20-25 g) mice were acclimatized to 12 h dark/light cycle and placed in groups of 6-8 mice per cage. Vehicle (0.5 ml of PBS w 1% of BSA) alone or vehicle + DC7-2 was administered three times per day (08:00; 16:00; 24:00), and feed intake was monitored daily for a week. Data were fitted with linear regression (R2>0.99) and 95 % confidence intervals (grey lines).

DETAILED DISCLOSURE OF THE INVENTION

The inventors of the present invention have found novel polypeptides that may be used to induce signalling in intestinal cells and may consequently induce satiety. Although a specific peptide has been identified from a proteolytic digest of muscle-specific alpha-actinin-2 protein, it is envisioned that similar polypeptides will bind the same receptors in the intestine and provide the same biological activity, i.e. signal to induce satiation and satiety. Similar peptides may contain e.g. conservative substitutions or be truncated. The rationale for using the polypeptides of the invention is that the energy content due to the relatively small length of the peptide is low as compared to the effect on satiety.

Definitions

When terms such as "one", "a" or "an" are used in this disclosure they mean "at least one", or "one or more" unless otherwise indicated. Further, the term "comprising" is intended to

mean "including" and thus allows for the presence of other constituents, features, conditions, or steps than those explicitly recited.

In some specific embodiments, the first 1, 2, or 3 amino acids in the N-terminal of the amino acid sequences according to the invention are in the D-form. It is assumed that the N-terminal trimming and thereby degradation of the peptides are somewhat delayed by having amino acids of the D-form in the N-terminal of these polypeptides. Alternatively and in some embodiments, the first 1, 2, or 3 amino acids in the N-terminal of the amino acid sequences according to the invention are amino acids in beta or gamma forms. Beta amino acids have their amino group bonded to the beta carbon rather than the alpha carbon as in the 20 standard natural amino acids. A capital D-letter subscript after the letter representing the amino acid residue designate herein amino acids specified to be in D-form, such as W_D referring to a tryptophan in D-form. A capital L-letter subscript after the letter representing the amino acid residue designate herein amino acids specified to be in L-form, such as W_L referring to a tryptophan in L-form. If not otherwise indicated, an amino acid is in its natural L-form.

Alternatively, the first 1, 2, or 3 amino acids in the N-terminal of the amino acid sequences according to the invention may be modified by incorporation of protective groups, e.g. fluorine, or alternatively cyclic amino acids or other suitable non-natural amino acids are used.

A "variant" or "analogue" of a peptide refers to a peptide having an amino acid sequence that is substantially identical to a reference peptide, typically a native or "parent" polypeptide, or a polypeptide of formula I or II. The peptide variant may possess one or more amino acid substitutions, deletions, and/or insertions at certain positions within the native amino acid sequence. The "variant" within this definition still has functional activity. In some embodiment a variant has at least 80 % sequence identity with the reference polypeptide. In some embodiments a variant has at least 85 % sequence identity with the reference polypeptide. In other embodiments a variant has at least 90 % sequence identity with the reference polypeptide. In a further embodiment a variant has at least 95 % sequence identity with the reference polypeptide.

"Conservative" amino acid substitutions are those in which an amino acid residue is replaced with an amino acid residue having a side chain with similar physicochemical properties. Families of amino acid residues having similar side chains are known in the art, and include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (e.g.,

alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). A particular form of conservative amino acid substitutions include those with amino acids, which are not among the normal 20 amino acids encoded by the genetic code. Since preferred embodiments of the present invention entail use of synthetic peptides, it is unproblematic to provide such "non-naturally occurring" amino acid residues in the peptides disclosed herein, and thereby it is possible to exchange the natural saturated carbon chains in the side chains of amino acid residues with shorter or longer saturated carbon chains – for instance, lysine may be substituted with an amino acid having a side chain -(CH2)nNH3, where n is different from 4, and arginine may be substituted with an amino acid having the side chain (CH2)nNHC(=NH2)NH2, where n is different from 3, etc. Similarly, the acidic amino acids aspartic acid and glutamic acid may be substituted with amino acid residues having the side chains –(CH2)nCOOH, where n>2.

The polypeptides of this invention may in some embodiments benefit from having higher stability than polypeptides containing only naturally occurring amino acids, and its modification enables to have much higher stability, such as a modification in the N-terminal of the polypeptide.

Accordingly and in some embodiments, the polypeptides of this invention have at their N-terminal a protection group, such as a protection group selected from the group consisting of acetyl group, fluorenyl methoxy carbonyl group, formyl group, palmitoyl group, myristyl group, stearyl group and polyethylene glycol (PEG).

The active peptide may also be di- or multimerized, e.g. through cross-linking with suitable di- or multivalent chemical cross-linkers, e.g. disuccinimidyl suberate, containing spacers of different length, e.g. 10-100 Å, and different functionality, e.g. homo- or heterofunctional, for coupling through non-critical amino or other reactive groups. Alternatively, photoactivation or enzymatic cross-linking may be used to increase stability and potency in vivo.

The modifications of peptides described above greatly increase the stability of the peptides of this invention. The term used herein "stability" refers to in vivo stability, such as the stability in the gut of a subject receiving such polypeptide. The protection group described above protects the peptides from the attack of protease in vivo.

The polypeptides according to the invention may be derived from a proteolytic digests of meat and be resistant to pepsin degradation. Accordingly, in some embodiments a polypeptide according to the invention may only contain naturally occurring amino acids.

In other embodiments, a polypeptide according to the invention is more stable towards degradation in the gastrointestinal tract, e.g. as measured in a stability assay described in the examples of the present invention, as compared to a control peptide. In some embodiments, a polypeptide according to the invention is more stable towards degradation in the gastrointestinal tract, e.g. measured in a stability assay described in the examples of the present invention as compared to a control peptide with the sequence RRPYIL, (SEQ ID NO:39).

In some embodiments, a polypeptide according to the invention has an half-life (T½) of degradation in vivo in the gut or in vitro, e.g. measured in a stability assay described in the example 2 of the present invention, which is higher than 2 min, such as higher than 4 min, such as higher than 6 min, such as higher than 8 min, such as higher than 10 min, such as higher than 15 min, such as higher than 20 min, such as higher than 25 min, such as higher than 30 min, such as higher than 35 min, such as higher than 40 min, such as higher than 45 min, such as higher than 50 min, such as higher than 55 min, such as higher than 60 min.

The term "substantially identical" in the context of two amino acid sequences means that the sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least about 50, at least about 60, at least about 70, at least about 80, at least about 90, at least about 95, at least about 98, or at least about 99 percent sequence identity. In some embodiments, when measuring the sequence identity between two different peptide sequences, a gap of one or two amino acids is allowed when the two peptide sequences are aligned without having any influence on the value of sequence identity. In some embodiments, a residue position that is not identical differ by only a conservative amino acid substitution. Sequence identity is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, the publicly available GCG software contains programs such as "Gap" and "BestFit" which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild-type protein and a mutein thereof. See, e.g., GCG Version 6.1. Polypeptide sequences can also be compared using FASTA or ClustalW, applying default or recommended parameters. A program in GCG Version 6.1., FASTA (e.g., FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson, Methods Enzymol. 1990; 183:63-98; Pearson, Methods Mol. Biol. 2000;132:185-219). Another preferred algorithm when comparing a sequence to a database containing a large number of sequences from various organisms is the computer program BLAST, especially blastp, using default parameters. See, e.g., Altschul et al., J. Mol. Biol.

1990;215:403-410; Altschul et al., Nucleic Acids Res. 1997;25:3389-402 (1997); each herein incorporated by reference. "Corresponding" amino acid positions in two substantially identical amino acid sequences are those aligned by any of the protein analysis software mentioned herein, typically using default parameters.

The term "functional activity" as used herein refers to a polypeptide that stimulates cell signalling measured as fluorescence by elevated intracellular calcium or cellular release of gut hormones, such as measured in the signalling assays described in the examples. The functional activity of a variant may exhibit at least about 25%, such as at least about 50%, such as at least about 75%, such as at least about 90% of the specific activity of a reference polypeptide, such as the octapeptide ASDKPYIL, when tested in the assays as described herein. Alternatively, the functional activity of a variant may exhibit higher activity than a reference polypeptide, such as the octapeptide ASDKPYIL, when tested in the assays as described herein.

An "isolated" molecule is a molecule that is the predominant species in the composition wherein it is found with respect to the class of molecules to which it belongs (i.e., it makes up at least about 5% of the type of molecule in the composition and typically will make up at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or more of the species of molecules, e.g., peptides, in the composition). Commonly, a composition of a specific peptide sequence may exhibit 90% - 99% homogeneity for peptides in the context of all present peptide species in the composition or at least with respect to substantially active peptide species in the context of proposed use. If produced synthetically, a composition of a specific peptide sequence will exhibit 98% - 99%, or even higher and close to 100 % homogeneity for peptides in the context of all present peptide species in the composition or at least with respect to substantially active peptide species in the context of proposed use.

Unless otherwise indicated the polypeptides within the present invention is a linear sequence of amino acids. The term "linear sequence" as used herein refers to the specific sequence of amino acids connected by standard peptide bonds in standard N- to C-terminal direction. The peptide may contain only peptide bonds. In some embodiments however, a second part of a peptide sequence may be bound to and continue from the side chain of a terminal amino acid in a first part of an amino acid sequence. Also the term does not exclude that an amino acid within a sequence, such as within AA1-AA8, may be connected, such as through the side chains, with another amino acid at a distant location within the peptide sequence, such as a distant location within AA1-AA8.

In the context of the present invention, "treatment" or "treating" refers to preventing, alleviating, managing, curing or reducing one or more symptoms or clinically relevant manifestations of a disease or disorder, unless contradicted by context. For example, "treatment" of a patient in whom no symptoms or clinically relevant manifestations of a disease or disorder have been identified is preventive or prophylactic therapy, whereas "treatment" of a patient in whom symptoms or clinically relevant manifestations of a disease or disorder have been identified generally does not constitute preventive or prophylactic therapy.

The terms "patient" and "subject" refer to any human or animal that may be treated using the methods of the present invention.

Many aspect of the present invention relates to the use of polypeptides or compositions to promote satiety in a subject. The underlying cause of a metabolic syndrome or disorder that may treated by the polypeptides or compositions according to the invention, is an overconsumption of calories, while still not feeling satiety. By inducing or promoting satiety in a subject, such total amounts of calories, including calories derived from fat and carbohydrates are reduced in the subject. Accordingly, the polypeptides and compositions of the invention may be used in preventing or reducing a metabolic syndrome or disorder, such as obesity, insulin-deficiency or insulin-resistance related disorders, Diabetes Mellitus (such as, for example, Type 2 Diabetes), glucose intolerance, abnormal lipid metabolism, atherosclerosis, hypertension, cardiac pathology, stroke, non-alcoholic fatty liver disease, hyperglycemia, hepatic steatosis, dyslipidemia, dysfunction of the immune system associated with overweight and obesity, cardiovascular diseases, high cholesterol, elevated triglycerides, asthma, sleep apnoea, osteoarthritis, neuro- degeneration, gallbladder disease, syndrome X, inflammatory and immune disorders, atherogenic dyslipidemia and cancer.

Preparation of polypeptides of the invention

The invention also relates to a method of preparing polypeptides of the invention as mentioned above. The method of synthesis or preparation thereof includes, but is not limited to recombinant (whether produced from cDNA, genomic DNA, synthetic DNA or other form of nucleic acid), synthetic, and transgenic means.

The polypeptides of the invention described herein may be produced by means of recombinant nucleic acid techniques. In general, a nucleic acid sequence encoding the desired polypeptide is then inserted into an expression vector, which is in turn transformed or transfected into host cells.

As an alternative and also the preferred option, the polypeptides of the invention are produced by synthetic means, i.e. by polypeptide synthesis. In some embodiments, the invention relates to a method of manufacturing an analogue comprising non-natural amino acids from about 5 total residues to about 20 total residues. In some embodiments, an analogue comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 non-natural amino acids, such as any one of the following non-naturally occurring amino acid residues.

The polypeptides of the present invention can also comprise non-naturally occurring amino acid residues. Non-naturally occurring amino acids include, without limitation, beta-alanine, desaminohistidine, trans-3-methylproline, 2,4-methanoproline, cis-4-hydroxyproline, trans-4-hydroxyproline, N-methylglycine, allo-threonine, methylthreonine, hydroxyethylcys-teine, hydroxyethylhomocysteine, nitroglutamine, homoglutamine, pipecolic acid, thiazolidine carboxylic acid, dehydroproline, 3- and 4-methylproline, 3,3-dimethylproline, tert-leucine, nor-valine, 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, and 4-fluorophenylalanine. Several methods are known in the art for incorporating non-naturally occurring amino acid residues into polypeptides. For example, an in vitro system can be employed wherein nonsense mutations are suppressed using chemically aminoacylated suppressor tRNAs. Methods for synthesizing amino acids and aminoacylating tRNA are known in the art. Transcription and translation of plasmids containing nonsense mutations is carried out in a cell-free system comprising an E. coli S30 extract and commercially available enzymes and other reagents. Polypeptides are purified by chromatography. See, for example, Robertson et al., J. Am. Chem. Soc. 113:2722, 1991; Ellman et al., Methods Enzymol. 202:301, 1991; Chung et al., Science 259:806-9, 1993; and Chung et al., Proc. Natl. Acad. Sci. USA 90:10145-9, 1993). In a second method, translation is carried out in Xenopus oocytes by microinjection of mutated mRNA and chemically aminoacylated suppressor tRNAs (Turcatti et al., J. Biol. Chem. 271:19991-8, 1996). Within a third method, E. coli cells are cul-tured in the absence of a natural amino acid that is to be replaced (e.g., phenylalanine) and in the presence of the desired non-naturally occurring amino acid(s) (e.g., 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, or 4-fluorophenylalanine). The non-naturally occurring amino acid is incorporated into the polypeptide in place of its natural counterpart. See, Koide et al., Biochem. 33:7470-6, 1994. Naturally occurring amino acid residues can be converted to non-naturally occurring species by in vitro chemical modification. Chemical modification can be combined with site-directed mutagenesis to further expand the range of substitutions (Wynn and Richards, Protein Sci. 2:395-403, 1993).

As another alternative to synthetic preparation, the polypeptides of the invention may be purified from any natural source containing such polypeptide, such as from the proteolytic

hydrolysate of muscle tissue, such as any source containing alpha-actinin-2 protein, such as by the methods described in the example section.

Accordingly, in some embodiments the sequence of the polypeptides of the invention is derived from a sequence found in nature, such as a fragment of alpha-actinin-2 protein.

The polypeptides of the present invention may be purified by a variety of procedures known in the art including, but not limited to, chromatography (e.g., ion exchange, affinity, hydrophobic, chromatofocusing, and size exclusion), electrophoretic procedures (e.g., preparative isoelectric focusing (IEF), differential solubility (e.g., ammonium sulfate precipitation), or extraction (see, e.g., Protein Purification, J.-C. Janson and Lars Ryden, editors, VCH Publishers, New York, 1989). They may be purified by affinity chromatography on an antibody column. Additional purification may be achieved by conventional chemical purification means, such as high performance liquid chromatography. Other methods of purification, including barium citrate precipitation, are known in the art, and may be applied to the purification - see, for example, Scopes, R., Protein Purification, Springer-Verlag, N.Y., 1982.

For the methods of the invention including the therapeutic purposes it is not critical to have a high purity of a specific peptide of the invention. However, the higher the concentration of a specific peptide of the invention the higher is the effect in terms of inducing satiation and satiety relative to amount of total protein and total amount of calories consumed by the subject receiving the composition of polypeptides. It is to be understood that the idea of the invention is to administer polypeptides that induce satiation or satiety without administering a lot of calories to the subject.

In some embodiments the compositions of polypeptides of the invention are substantially pure. Thus, in an embodiment of the invention the polypeptides of the invention are purified to at least about 90 to 95% homogeneity, preferably to at least about 98% homogeneity. Purity may be assessed by e.g. HPLC and amino-terminal amino acid sequencing.

Administration and pharmaceutical compositions

Administration of the polypeptides according to the invention may be through several routes of administration, for example, lingual, sublingual, buccal, in the mouth, oral, in the stomach and intestine, nasal, pulmonary, for example, through the bronchioles and alveoli or a combination thereof, epidermal, dermal, transdermal, vaginal, rectal, ocular, for examples through the conjunctiva, uretal, and parenteral to patients in need of such a treatment.

Some kind of oral administration is preferred since these types of polypeptides are derived from a source that naturally has to pass through the mouth and to the intestinal mucosa.

Compositions of the current invention may be administered in several dosage forms, for example, as solutions, suspensions, emulsions, microemulsions, multiple emulsion, foams, salves, pastes, plasters, ointments, tablets, coated tablets, rinses, capsules, for example, hard gelatine capsules and soft gelatine capsules, suppositories, rectal capsules, drops, gels, sprays, powder, aerosols, inhalants, eye drops, ophthalmic ointments, ophthalmic rinses, vaginal pessaries, vaginal rings, vaginal ointments, injection solution, in situ transforming solutions, for example in situ gelling, in situ setting, in situ precipitating, in situ crystallization, infusion solution, and implants.

One of skill in the art will recognize that the appropriate dosage of the compositions and pharmaceutical compositions may vary depending on the individual being treated and the purpose. For example, the age, body weight, and medical history of the individual patient may affect the therapeutic efficacy of the therapy. Further, a lower dosage of the composition may be needed to produce a transient cessation of symptoms, while a larger dose may be needed to produce a complete cessation of symptoms associated with the disease, disorder, or indication. A competent physician can consider these factors and adjust the dosing regimen to ensure the dose is achieving the desired therapeutic outcome without undue experimentation. It is also noted that the clinician and/or treating physician will know how and when to interrupt, adjust, and/or terminate therapy in conjunction with individual patient response. Dosages may also depend on the strength of the particular polypeptide of the invention chosen for the pharmaceutical composition.

The dose of the composition or pharmaceutical compositions may vary. The dose of the composition may be once per day. In some embodiments, multiple doses may be administered to the subject per day. In some embodiments, the total dosage is administered in at least two application periods, In some embodiments, the period can be an hour, a day, a month, a year, a week, or a two-week period. In an additional embodiment of the invention, the total dosage is administered in two or more separate application periods, or separate doses.

In some embodiments, subjects can be administered the composition in which the composition is provided in a daily dose range of about 0.0001 mg/kg to about 5000 mg/kg of the weight of the subject. The dose administered to the subject can also be measured in terms of total amount of polypeptide of the invention administered per day. In some embodiments, a subject is administered from about 0.001 to about 3000 milligrams of polypeptide of the invention per day. In some embodiments a subject is administered up to

about 2000 milligrams of polypeptide of the invention per day. In some embodiments, a subject is administered up to about 1800 milligrams of polypeptide of the invention per day. In some embodiments, a subject is administered up to about 1600 milligrams of polypeptide of the invention per day. In some embodiments, a subject is administered up to about 1400 milligrams of polypeptide of the invention per day. In some embodiments, a subject is administered up to about 1200 milligrams of polypeptide of the invention per day. In some embodiments, a subject is administered up to about 1000 milligrams of polypeptide of the invention per day. In some embodiments, a subject is administered up to about 800 milligrams of polypeptide of the invention per day. In some embodiments, a subject is administered from about 0.001 milligrams to about 700 milligrams of polypeptide of the invention per dose. In some embodiments, a subject is administered up to about 700 milligrams of polypeptide of the invention per dose. In some embodiments, a subject is administered up to about 600 milligrams of polypeptide of the invention per dose. In some embodiments, a subject is administered up to about 500 milligrams of polypeptide of the invention per dose. In some embodiments, a subject is administered up to about 400 milligrams of polypeptide of the invention per dose. In some embodiments, a subject is administered up to about 300 milligrams of polypeptide of the invention per dose. In some embodiments, a subject is administered up to about 200 milligrams of polypeptide of the invention per dose. In some embodiments, a subject is administered up to about 100 milligrams of polypeptide of the invention per dose. In some embodiments, a subject is administered up to about 50 milligrams of polypeptide of the invention per dose.

A composition, wherein a polypeptide of the invention is added may be any food composition, food product, or food ingredient. Here, the term "food" is used in a broad sense – and covers food for humans as well as food for animals (i.e. a feed). In a preferred aspect, the food is for human consumption. The food may be in the form of a solution or as a solid – depending on the use and/or the mode of application and/or the mode of administration.

When used as – or in the preparation of - a food – such as functional food - the composition of the present invention may be used in conjunction with one or more of: a nutritionally acceptable carrier, a nutritionally acceptable diluent, a nutritionally acceptable excipient, a nutritionally acceptable adjuvant, a nutritionally active ingredient.

The composition of the present invention may be used as a food ingredient.

As used herein the term "food ingredient" includes a formulation which is or can be added to functional foods or foodstuffs as a nutritional supplement. The term food ingredient as used here also refers to formulations which can be used at low levels in a wide variety of products

that require gelling, texturising, stabilising, suspending, film-forming and structuring, retention of juiciness and improved mouthfeel, without adding viscosity.

The food ingredient may be in the form of a solution or as a solid – depending on the use and/or the mode of application and/or the mode of administration.

The composition of the present invention may be – or may be added to - food supplements.

The composition of the present invention may be – or may be added to - functional foods.

As used herein, the term "functional food" means food which is capable of providing not only a nutritional effect and/or a taste satisfaction, but is also capable of delivering a further beneficial effect to consumer.

Accordingly, functional foods are ordinary foods that have components or ingredients (such as those described herein) incorporated into them that impart to the food a specific functional – e.g. medical or physiological benefit - other than a purely nutritional effect.

Although there is no legal definition of a functional food, most of the parties with an interest in this area agree that they are foods marketed as having specific health effects.

Some functional foods are nutraceuticals. Here, the term "nutraceutical" means a food which is capable of providing not only a nutritional effect and/or a taste satisfaction, but is also capable of delivering a therapeutic (or other beneficial) effect to the consumer. Nutraceuticals cross the traditional dividing lines between foods and medicine.

Surveys have suggested that consumers place the most emphasis on functional food claims relating to heart disease. Preventing cancer is another aspect of nutrition which interests consumers a great deal, but interestingly this is the area that consumers feel they can exert least control over. In fact, according to the World Health Organization, at least 35% of cancer cases are diet-related. Furthermore claims relating to osteoporosis, gut health and obesity effects are also key factors that are likely to incite functional food purchase and drive market development.

The composition of the present invention can be used in the preparation of or added to food products such as one or more of: jams, marmalades, jellies, dairy products (such as milk or cheese), meat products, poultry products, fish products, vegetable-based soups, and bakery products.

By way of example, the composition of the present invention can be used as ingredients to soft drinks, a fruit juice or a beverage comprising whey protein, health teas, cocoa drinks, milk drinks and lactic acid bacteria drinks, yoghurt and drinking yoghurt, cheese, ice cream, water ices and desserts, confectionery, biscuits cakes and cake mixes, snack foods, breakfast cereals, instant noodles and cup noodles, instant soups and cup soups, balanced foods and drinks, sweeteners, texture improved snack bars, fibre bars, bake stable fruit fillings, care glaze, chocolate bakery filling, cheese cake flavoured filling, fruit flavoured cake filling, cake and doughnut icing, heat stable bakery filling, instant bakery filling creams, filing for cookies, ready-to-use bakery filling, reduced calorie filling, adult nutritional beverage, acidified soy/juice beverage, aseptic/retorted chocolate drink, bar mixes, beverage powders, calcium fortified soy/plaim and chocolate milk, calcium fortified coffee beverage.

A composition according to the present invention can further be used as an ingredient in food products such as American cheese sauce, anti-caking agent for grated & shredded cheese, chip dip, cream cheese, dry blended whip topping fat free sour cream, freeze/thaw dairy whipping cream, freeze/thaw stable whipped topping, low fat & lite natural cheddar cheese, low fat Swiss style yoghurt, aerated frozen desserts, and novelty bars, hard pack ice cream, label friendly, improved economics & indulgence of hard pack ice cream, low fat ice cream: soft serve, barbecue sauce, cheese dip sauce, cottage cheese dressing, dry mix Alfredo sauce, mix cheese sauce, dry mix tomato sauce and others.

For certain aspects, preferably the foodstuff is a beverage.

For certain aspects, preferably the foodstuff is a bakery product - such as bread, Danish pastry, biscuits or cookies.

The present invention also provides a method of preparing a food or a food ingredient, the method comprising mixing a polypeptide according to the present invention or the composition according to the present invention with another food ingredient.

Specific embodiments of the invention

One aspect of the invention related to an isolated polypeptide comprising the amino acid sequence

AA1-AA2-AA3-K-AA5-AA6-AA7-AA8 (formula I, SEQ ID NO:1),

wherein AA1 is an optional amino acid selected from A, L, I, and V; AA2 is an optional amino acid selected from S, T, G, A, N, E and D; AA3 is an optional amino acid selected from D, E, and G; AA5 is selected from P, N, S, D, A, T, K, and G; AA6 is selected from Y, N, I, W, and F; AA7 is selected from I, L, R, and V; AA8 is selected from L, I, V, S, M, and T; which polypeptide is not more than 50 amino acids in length; or a variant thereof with a sequence identity of at least 80%.

Another aspect of the invention related to a method of promoting satiety in a subject or to a method of preventing or reducing the incidence of obesity in a subject comprising enteral administering to a subject in need thereof a polypeptide comprising or consisting of the amino acid sequence

AA1-AA2-AA3-AA4-AA5-AA6-AA7-AA8 (formula III, SEQ ID NO:3),

wherein AA1 is an optional amino acid selected from A, L, I, and V; AA2 is an optional amino acid selected from S, T, G, A, N, E and D; AA3 is an optional amino acid selected from D, R, K, E, and G; AA4 is an amino acid selected from K and R; AA5 is selected from P, N, S, D, A, T, K, and G; AA6 is selected from Y, N, I, W, and F; AA7 is selected from I, L, R, and V; AA8 is selected from L, I, V, S, M, and T; which polypeptide is not more than 50 amino acids in length; or a variant thereof with a sequence identity of at least 80%.

In the following AA1-AA8 may refer to the amino acids of either formula I, II, or III.

In some embodiments AA1 is absent. In some embodiments AA1 is any one natural amino acid selected from Y, W, V, T, S, R, Q, P, N, M, L, K, I, H, G, F, E, D, C, and A. In some embodiments AA2 is absent. In some embodiments AA2 is any one natural amino acid selected from Y, W, V, T, S, R, Q, P, N, M, L, K, I, H, G, F, E, D, C, and A. In some embodiments AA3 is absent. In some embodiments AA1 is present. In some embodiments AA2 is present. In some embodiments AA3 is present. In some embodiments AA1 is A. In some embodiments AA2 is S. In some embodiments AA3 is D. In some embodiments AA3 is selected from any one amino acid C,D,E,N,P, and Q. In some embodiments AA3 is selected from E and G. In some embodiments AA3 is P. In some embodiments AA3 is C. In some embodiments AA4 is K. In some embodiments AA6 is Y. In some embodiments AA7 is I. In some embodiments AA8 is L. In some embodiments the amino acid sequence is not found in nature. In some embodiments AA8 is the C-terminal amino acid. In some embodiments AA5 is P. In some embodiments AA6 is selected from Y and W. In some embodiments AA7 is selected from I and L.

In some embodiments AA2 is an optional amino acid selected from S, T, A, N, E and D. In some embodiments AA5 is selected from P, S, D, A, T, K, and G. In some embodiments AA6 is selected from Y, N, I, and W. In some embodiments AA8 is selected from L, I, V, S, and M.

In some embodiments the polypeptide does not comprise any one of the sequences AVTEKKYILYDFSVTS (SEQ ID NO:5), PRRPYIL (SEQ ID NO:38), RRPYIL (SEQ ID NO:39), RPYIL (SEQ ID NO:40), RRPWIL (SEQ ID NO:41), KRPYIL (SEQ ID NO:42), KKPYIL (SEQ ID NO:43), Adamantoyl-KPYIL (SEQ ID NO:9), H-Lys-psi(CH_2NH)Lys-Pro-Tyr-Ile-Leu-OH (SEQ ID NO:44). In some embodiments the polypeptide does not comprise derivatives of Lys.

In some embodiments the polypeptide does not consists of any one of the sequences AVTEKKYILYDFSVTS, PRRPYIL, RRPYIL, RPYIL, RRPWIL, KRPYIL, KKPYIL, Adamantoyl-KPYIL, H-Lys-psi(CH_2NH)Lys-Pro-Tyr-Ile-Leu-OH. In some embodiments the polypeptide is not a derivative of KPYIL.

In some embodiments the amino acid sequence only contains natural amino acids.

In some embodiments the polypeptide of the invention is 5-50, such as 5-50, 5-49, 5-48, 5-47, 5-46, 5-45, 5-44, 5-43, 5-42, 5-41, 5-40, 5-39, 5-38, 5-37, 5-36, 5-35, 5-34, 5-33, 5-32, 5-31, 5-30, 5-29, 5-28, 5-27, 5-26, 5-25, 5-24, 5-23, 5-22, 5-21, 5-20, 5-19, such as 5-18, such as 5-17, such as 5-16, such as 5-15, such as 5-14, such as 5-13, such as 5-12, such as 5-11, such as 5-10, such as 5-9, such as 5-8, such as 5-7, such as 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 amino acids in length.

In some embodiments the polypeptide of the invention is less than 50, 49, 48, 47, 46, 45, 44, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, such as 18, such as 17, such as 16, such as 15, such as 14, such as 13, such as 12, such as 11, such as 10, such as 9, such as 8, such as 7 amino acids in length.

In some embodiments the polypeptide of the invention is 5-50, such as 6-50, such as 7-50, such as 8-50, such as 9-50, such as 10-50, such as 11-50, such as 12-50, such as 13-50, such as 14-50, such as 15-50, such as 16-50, such as 17-50, such as 18-50, such as 19-50, such as 20-50, such as 21-50, such as 22-50, such as 23-50, such as 24-50, such as 25-50, such as 26-50, such as 27-50, such as 28-50, such as 29-50, such as 30-50, such as 31-50, such as 32-50, such as 33-50, such as 34-50, such as 35-50, such as 36-50, such as 37-50, such as 38-50, such as 39-50, such as 40-50, such as 41-50, such as 42-50, such as 43-50,

such as 44-50, such as 45-50, such as 46-50, such as 47-50, such as 48-50, such as 49-50 amino acids in length.

In some embodiments the polypeptide of the invention is more than 5, such as 6, such as 7, such as 8, such as 9, such as 10, such as 11, such as 12, such as 13, such as 14, such as 15, such as 16, such as 17, such as 18, such as 19, such as 20, such as 21, such as 22, such as 23, such as 24, such as 25, such as 26, such as 27, such as 28, such as 29, such as 30, such as 31, such as 32, such as 33, such as 34, such as 35, such as 36, such as 37, such as 38, such as 39, such as 40, such as 41, such as 42, such as 43, such as 44, such as 45, such as 46, such as 47, such as 48, such as more than 49 amino acids in length.

In some embodiments the polypeptide of the invention is an octapeptide or a heptapeptide

In some embodiments the polypeptide of the invention has or comprises a sequence selected from ASDKPYIL, SDKPYIL, DKPYIL, and KPYIL.

In some embodiments the polypeptide of the invention consist of or comprises a sequence selected from ASDKPYIL, AGDKNYIL, AGDKNYIT, AGDKSYIT, ADGKPYIV, AEDKDFIT, AADKPYIL, ATDKPYIL, AGDKPYIT, ASEKPYIL, ADGKPYVT, AGDKPYIL, ASDKPNIL, ASDKPYIT, AADKPFIL, ASDKAYIT, AGDKAYIT, ANGKPFIT, AGDKNFIT, ASDKSYIT, ASDKTYIT, ASDKNYIT, AGDKKYIT, AGDKNYIS, AADKNYIT, AGDKNYIM, AADKNFIM, AADKNFIT, and AGDKGIRS.

In some embodiments the polypeptide of the invention is an isolated polypeptide.

In some embodiments the polypeptide of the invention is synthetically made.

In some embodiments the polypeptide of the invention is a purified fragment.

In some embodiments the polypeptide of the invention is purified from animal sources.

In some embodiments the polypeptide of the invention is generated by enzymatic treatment of proteins from animal sources.

In some embodiments the polypeptide of the invention has been modified by N terminal acylation or other chemical modifications to introduce protection groups.

In some specific embodiments, the polypeptide of the invention consists of or comprises an amino acid sequence selected from the group consisting of KPYIL, KPYII (SEQ ID NO:45),

KPYIV (SEQ ID NO:46), KPYLL (SEQ ID NO:47), KPYLI (SEQ ID NO:48), KPYLV (SEQ ID NO:49), KPYVL (SEQ ID NO:50), KPYVI (SEQ ID NO:51), KPYVV (SEQ ID NO:52), KPWIL (SEQ ID NO:53), KPWII (SEQ ID NO:54), KPWIV (SEQ ID NO:55), KPWLL (SEQ ID NO:56), KPWLI (SEQ ID NO:57), KPWLV (SEQ ID NO:58), KPWVL (SEQ ID NO:59), KPWVI (SEQ ID NO:60), KPWVV (SEQ ID NO:61), RPYIL (SEQ ID NO:40), RPYII (SEQ ID NO:62), RPYIV (SEQ ID NO:63), RPYLL (SEQ ID NO:64), RPYLI (SEQ ID NO:65), RPYLV (SEQ ID NO:66), RPYVL (SEQ ID NO:67), RPYVI (SEQ ID NO:68), RPYVV (SEQ ID NO:69), RPWIL (SEQ ID NO:70), RPWII (SEQ ID NO:71), RPWIV (SEQ ID NO:72), RPWLL (SEQ ID NO:73), RPWLI (SEQ ID NO:74), RPWLV (SEQ ID NO:75), RPWVL (SEQ ID NO:76), RPWVI (SEQ ID NO:77), and RPWVV (SEQ ID NO:78).

In some specific embodiments, the polypeptide of the invention consist of or comprises an amino acid sequence selected from the group consisting of DKPYIL (SEQ ID NO:8), DKPYII (SEQ ID NO:79), DKPYIV (SEQ ID NO:80), DKPYLL (SEQ ID NO:81), DKPYLI (SEQ ID NO:82), DKPYLV (SEQ ID NO:83), DKPYVL (SEQ ID NO:84), DKPYVI (SEQ ID NO:85), DKPYVV (SEQ ID NO:86), DKPWIL (SEQ ID NO:87), DKPWII (SEQ ID NO:88), DKPWIV (SEQ ID NO:89), DKPWLL (SEQ ID NO:90), DKPWLI (SEQ ID NO:91), DKPWLV (SEQ ID NO:92), DKPWVL (SEQ ID NO:93), DKPWVI (SEQ ID NO:94), DKPWVV (SEQ ID NO:95), DRPYIL (SEQ ID NO:96), DRPYII (SEQ ID NO:97), DRPYIV (SEQ ID NO:98), DRPYLL (SEQ ID NO:99), DRPYLI (SEQ ID NO:100), DRPYLV (SEQ ID NO:101), DRPYVL (SEQ ID NO:102), DRPYVI (SEQ ID NO:103), DRPYVV (SEQ ID NO:104), DRPWIL (SEQ ID NO:105), DRPWII (SEQ ID NO:106), DRPWIV (SEQ ID NO:107), DRPWLL (SEQ ID NO:108), DRPWLI (SEQ ID NO:109), DRPWLV (SEQ ID NO:110), DRPWVL (SEQ ID NO:111), DRPWVI (SEQ ID NO:112), DRPWVV (SEQ ID NO:113), EKPYIL (SEQ ID NO:114), EKPYII (SEQ ID NO:115), EKPYIV (SEQ ID NO:116), EKPYLL (SEQ ID NO:117), EKPYLI (SEQ ID NO:118), EKPYLV (SEQ ID NO:119), EKPYVL (SEQ ID NO:120), EKPYVI (SEQ ID NO:121), EKPYVV (SEQ ID NO:122), EKPWIL (SEQ ID NO:123), EKPWII (SEQ ID NO:124), EKPWIV (SEQ ID NO:125), EKPWLL (SEQ ID NO:126), EKPWLI (SEQ ID NO:127), EKPWLV (SEQ ID NO:128), EKPWVL (SEQ ID NO:129), EKPWVI (SEQ ID NO:130), EKPWVV (SEQ ID NO:131), ERPYIL (SEQ ID NO:132), ERPYII (SEQ ID NO:133), ERPYIV (SEQ ID NO:134), ERPYLL (SEQ ID NO:135), ERPYLI (SEQ ID NO:136), ERPYLV (SEQ ID NO:137), ERPYVL (SEQ ID NO:138), ERPYVI (SEQ ID NO:139), ERPYVV (SEQ ID NO:140), ERPWIL (SEQ ID NO:141), ERPWII (SEQ ID NO:142), ERPWIV (SEQ ID NO:143), ERPWLL (SEQ ID NO:144), ERPWLI (SEQ ID NO:145), ERPWLV (SEQ ID NO:146), ERPWVL (SEQ ID NO:147), ERPWVI (SEQ ID NO:148), ERPWVV (SEQ ID NO:149), RKPYIL (SEQ ID NO:150), RKPYII (SEQ ID NO:151), RKPYIV (SEQ ID NO:152), RKPYLL (SEQ ID NO:153), RKPYLI (SEQ ID NO:154), RKPYLV (SEQ ID NO:155), RKPYVL (SEQ ID NO:156), RKPYVI (SEQ ID NO:157), RKPYVV (SEQ ID NO:158), RKPWIL (SEQ ID NO:159), RKPWII (SEQ ID NO:160), RKPWIV (SEQ ID NO:161), RKPWLL (SEQ ID NO:162), RKPWLI (SEQ ID NO:163), RKPWLV (SEQ ID

NO:164), RKPWVL (SEQ ID NO:165), RKPWVI (SEQ ID NO:166), RKPWVV (SEQ ID NO:167), RRPYIL (SEQ ID NO:39), RRPYII (SEQ ID NO:168), RRPYIV (SEQ ID NO:169), RRPYLL (SEQ ID NO:170), RRPYLI (SEQ ID NO:171), RRPYLV (SEQ ID NO:172), RRPYVL (SEQ ID NO:173), RRPYVI (SEQ ID NO:174), RRPYVV (SEQ ID NO:175), RRPWIL (SEQ ID NO:41), RRPWII (SEQ ID NO:176), RRPWIV (SEQ ID NO:177), RRPWLL (SEQ ID NO:178), RRPWLI (SEQ ID NO:179), RRPWLV (SEQ ID NO:180), RRPWVL (SEQ ID NO:181), RRPWVI (SEQ ID NO:182), RRPWVV (SEQ ID NO:183), GKPYIL (SEQ ID NO:184), GKPYII (SEQ ID NO:185), GKPYIV (SEQ ID NO:186), GKPYLL (SEQ ID NO:187), GKPYLI (SEQ ID NO:188), GKPYLV (SEQ ID NO:189), GKPYVL (SEQ ID NO:190), GKPYVI (SEQ ID NO:191), GKPYVV (SEQ ID NO:192), GKPWIL (SEQ ID NO:193), GKPWII (SEQ ID NO:194), GKPWIV (SEQ ID NO:195), GKPWLL (SEQ ID NO:196), GKPWLI (SEQ ID NO:197), GKPWLV (SEQ ID NO:198), GKPWVL (SEQ ID NO:199), GKPWVI (SEQ ID NO:200), GKPWVV (SEQ ID NO:201), GRPYIL (SEQ ID NO:202), GRPYII (SEQ ID NO:203), GRPYIV (SEQ ID NO:204), GRPYLL (SEQ ID NO:205), GRPYLI (SEQ ID NO:206), GRPYLV (SEQ ID NO:207), GRPYVL (SEQ ID NO:208), GRPYVI (SEQ ID NO:209), GRPYVV (SEQ ID NO:210), GRPWIL (SEQ ID NO:211), GRPWII (SEQ ID NO:212), GRPWIV (SEQ ID NO:213), GRPWLL (SEQ ID NO:214), GRPWLI (SEQ ID NO:215), GRPWLV (SEQ ID NO:216), GRPWVL (SEQ ID NO:217), GRPWVI (SEQ ID NO:218), and GRPWVV (SEQ ID NO:219).

In some specific embodiments, the polypeptide of the invention consists of or comprises an amino acid sequence selected from the group consisting of SDKPYIL (SEQ ID NO:220), SDKPYII (SEQ ID NO:221), SDKPYIV (SEQ ID NO:222), SDKPYLL (SEQ ID NO:223), SDKPYLI (SEQ ID NO:224), SDKPYLV (SEQ ID NO:225), SDKPYVL (SEQ ID NO:226), SDKPYVI (SEQ ID NO:227), SDKPYVV (SEQ ID NO:228), SDKPWIL (SEQ ID NO:229), SDKPWII (SEQ ID NO:230), SDKPWIV (SEQ ID NO:231), SDKPWLL (SEQ ID NO:232), SDKPWLI (SEQ ID NO:233), SDKPWLV (SEQ ID NO:234), SDKPWVL (SEQ ID NO:235), SDKPWVI (SEQ ID NO:236), SDKPWVV (SEQ ID NO:237), SDRPYIL (SEQ ID NO:238), SDRPYII (SEQ ID NO:239), SDRPYIV (SEQ ID NO:240), SDRPYLL (SEQ ID NO:241), SDRPYLI (SEQ ID NO:242), SDRPYLV (SEQ ID NO:243), SDRPYVL (SEQ ID NO:244), SDRPYVI (SEQ ID NO:245), SDRPYVV (SEQ ID NO:246), SDRPWIL (SEQ ID NO:247), SDRPWII (SEQ ID NO:248), SDRPWIV (SEQ ID NO:249), SDRPWLL (SEQ ID NO:250), SDRPWLI (SEQ ID NO:251), SDRPWLV (SEQ ID NO:252), SDRPWVL (SEQ ID NO:253), SDRPWVI (SEQ ID NO:254), SDRPWVV (SEQ ID NO:255), SEKPYIL (SEQ ID NO:256), SEKPYII (SEQ ID NO:257), SEKPYIV (SEQ ID NO:258), SEKPYLL (SEQ ID NO:259), SEKPYLI (SEQ ID NO:260), SEKPYLV (SEQ ID NO:261), SEKPYVL (SEQ ID NO:262), SEKPYVI (SEQ ID NO:263), SEKPYVV (SEQ ID NO:264), SEKPWIL (SEQ ID NO:265), SEKPWII (SEQ ID NO:266), SEKPWIV (SEQ ID NO:267), SEKPWLL (SEQ ID NO:268), SEKPWLI (SEQ ID NO:269), SEKPWLV (SEQ ID NO:270), SEKPWVL (SEQ ID NO:271), SEKPWVI (SEQ ID NO:272), SEKPWVV (SEQ ID NO:273), SERPYIL (SEQ ID NO:274), SERPYII (SEQ ID NO:275), SERPYIV

(SEQ ID NO:276), SERPYLL (SEQ ID NO:277), SERPYLI (SEQ ID NO:278), SERPYLV (SEQ ID NO:279), SERPYVL (SEQ ID NO:280), SERPYVI (SEQ ID NO:281), SERPYVV (SEQ ID NO:282), SERPWIL (SEQ ID NO:283), SERPWII (SEQ ID NO:284), SERPWIV (SEQ ID NO:285), SERPWLL (SEQ ID NO:286), SERPWLI (SEQ ID NO:287), SERPWLV (SEQ ID NO:288), SERPWVL (SEQ ID NO:289), SERPWVI (SEQ ID NO:290), SERPWVV (SEQ ID NO:291), TDKPYIL (SEQ ID NO:292), TDKPYII (SEQ ID NO:293), TDKPYIV (SEQ ID NO:294), TDKPYLL (SEQ ID NO:295), TDKPYLI (SEQ ID NO:296), TDKPYLV (SEQ ID NO:297), TDKPYVL (SEQ ID NO:298), TDKPYVI (SEQ ID NO:299), TDKPYVV (SEQ ID NO:300), TDKPWIL (SEQ ID NO:301), TDKPWII (SEQ ID NO:302), TDKPWIV (SEQ ID NO:303), TDKPWLL (SEQ ID NO:304), TDKPWLI (SEQ ID NO:305), TDKPWLV (SEQ ID NO:306), TDKPWVL (SEQ ID NO:307), TDKPWVI (SEQ ID NO:308), TDKPWVV (SEQ ID NO:309), TDRPYIL (SEQ ID NO:310), TDRPYII (SEQ ID NO:311), TDRPYIV (SEQ ID NO:312), TDRPYLL (SEQ ID NO:313), TDRPYLI (SEQ ID NO:314), TDRPYLV (SEQ ID NO:315), TDRPYVL (SEQ ID NO:316), TDRPYVI (SEQ ID NO:317), TDRPYVV (SEQ ID NO:318), TDRPWIL (SEQ ID NO:319), TDRPWII (SEQ ID NO:320), TDRPWIV (SEQ ID NO:321), TDRPWLL (SEQ ID NO:322), TDRPWLI (SEQ ID NO:323), TDRPWLV (SEQ ID NO:324), TDRPWVL (SEQ ID NO:325), TDRPWVI (SEQ ID NO:326), TDRPWVV (SEQ ID NO:327), TEKPYIL (SEQ ID NO:328), TEKPYII (SEQ ID NO:329), TEKPYIV (SEQ ID NO:330), TEKPYLL (SEQ ID NO:331), TEKPYLI (SEQ ID NO:332), TEKPYLV (SEQ ID NO:333), TEKPYVL (SEQ ID NO:334), TEKPYVI (SEQ ID NO:335), TEKPYVV (SEQ ID NO:336), TEKPWIL (SEQ ID NO:337), TEKPWII (SEQ ID NO:338), TEKPWIV (SEQ ID NO:339), TEKPWLL (SEQ ID NO:340), TEKPWLI (SEQ ID NO:341), TEKPWLV (SEQ ID NO:342), TEKPWVL (SEQ ID NO:343), TEKPWVI (SEQ ID NO:344), TEKPWVV (SEQ ID NO:345), TERPYIL (SEQ ID NO:346), TERPYII (SEQ ID NO:347), TERPYIV (SEQ ID NO:348), TERPYLL (SEQ ID NO:349), TERPYLI (SEQ ID NO:350), TERPYLV (SEQ ID NO:351), TERPYVL (SEQ ID NO:352), TERPYVI (SEQ ID NO:353), TERPYVV (SEQ ID NO:354), TERPWIL (SEQ ID NO:355), TERPWII (SEQ ID NO:356), TERPWIV (SEQ ID NO:357), TERPWLL (SEQ ID NO:358), TERPWLI (SEQ ID NO:359), TERPWLV (SEQ ID NO:360), TERPWVL (SEQ ID NO:361), TERPWVI (SEQ ID NO:362), and TERPWVV (SEQ ID NO:363).

In some specific embodiments, the polypeptide of the invention consists of or comprises an amino acid sequence selected from the group consisting of ASDKPYII (SEQ ID NO:364), ASDKPYIV (SEQ ID NO:365), ASDKPYLL (SEQ ID NO:366), ASDKPYLI (SEQ ID NO:367), ASDKPYLV (SEQ ID NO:368), ASDKPYVL (SEQ ID NO:369), ASDKPYVI (SEQ ID NO:370), ASDKPYVV (SEQ ID NO:371), ASDKPWIL (SEQ ID NO:372), ASDKPWII (SEQ ID NO:373), ASDKPWIV (SEQ ID NO:374), ASDKPWLL (SEQ ID NO:375), ASDKPWLI (SEQ ID NO:376), ASDKPWLV (SEQ ID NO:377), ASDKPWVL (SEQ ID NO:378), ASDKPWVI (SEQ ID NO:379), ASDKPWVV (SEQ ID NO:380), ASDRPYIL (SEQ ID NO:381), ASDRPYII (SEQ ID NO:382), ASDRPYIV (SEQ ID NO:383), ASDRPYLL (SEQ ID NO:384), ASDRPYLI (SEQ ID NO:385), ASDRPYLV (SEQ ID NO:386), ASDRPYVL (SEQ ID NO:387), ASDRPYVI (SEQ ID NO:388),

ASDRPYVV (SEQ ID NO:389), ASDRPWIL (SEQ ID NO:390), ASDRPWII (SEQ ID NO:391), ASDRPWIV (SEQ ID NO:392), ASDRPWLL (SEQ ID NO:393), ASDRPWLI (SEQ ID NO:394), ASDRPWLV (SEQ ID NO:395), ASDRPWVL (SEQ ID NO:396), ASDRPWVI (SEQ ID NO:397), ASDRPWVV (SEQ ID NO:398), ASEKPYIL (SEQ ID NO:399), ASEKPYII (SEQ ID NO:400), ASEKPYIV (SEQ ID NO:401), ASEKPYLL (SEQ ID NO:402), ASEKPYLI (SEQ ID NO:403), ASEKPYLV (SEQ ID NO:404), ASEKPYVL (SEQ ID NO:405), ASEKPYVI (SEQ ID NO:406), ASEKPYVV (SEQ ID NO:407), ASEKPWIL (SEQ ID NO:408), ASEKPWII (SEQ ID NO:409), ASEKPWIV (SEQ ID NO:410), ASEKPWLL (SEQ ID NO:411), ASEKPWLI (SEQ ID NO:412), ASEKPWLV (SEQ ID NO:413), ASEKPWVL (SEQ ID NO:414), ASEKPWVI (SEQ ID NO:415), ASEKPWVV (SEQ ID NO:416), ASERPYIL (SEQ ID NO:417), ASERPYII (SEQ ID NO:418), ASERPYIV (SEQ ID NO:419), ASERPYLL (SEQ ID NO:420), ASERPYLI (SEQ ID NO:421), ASERPYLV (SEQ ID NO:422), ASERPYVL (SEQ ID NO:423), ASERPYVI (SEQ ID NO:424), ASERPYVV (SEQ ID NO:425), ASERPWIL (SEQ ID NO:426), ASERPWII (SEQ ID NO:427), ASERPWIV (SEQ ID NO:428), ASERPWLL (SEQ ID NO:429), ASERPWLI (SEQ ID NO:430), ASERPWLV (SEQ ID NO:431), ASERPWVL (SEQ ID NO:432), ASERPWVI (SEQ ID NO:433), ASERPWVV (SEQ ID NO:434), ATDKPYIL (SEQ ID NO:435), ATDKPYII (SEQ ID NO:436), ATDKPYIV (SEQ ID NO:437), ATDKPYLL (SEQ ID NO:438), ATDKPYLI (SEQ ID NO:439), ATDKPYLV (SEQ ID NO:440), ATDKPYVL (SEQ ID NO:441), ATDKPYVI (SEQ ID NO:442), ATDKPYVV (SEQ ID NO:443), ATDKPWIL (SEQ ID NO:444), ATDKPWII (SEQ ID NO:445), ATDKPWIV (SEQ ID NO:446), ATDKPWLL (SEQ ID NO:447), ATDKPWLI (SEQ ID NO:448), ATDKPWLV (SEQ ID NO:449), ATDKPWVL (SEQ ID NO:450), ATDKPWVI (SEQ ID NO:451), ATDKPWVV (SEQ ID NO:452), ATDRPYIL (SEQ ID NO:453), ATDRPYII (SEQ ID NO:454), ATDRPYIV (SEQ ID NO:455), ATDRPYLL (SEQ ID NO:456), ATDRPYLI (SEQ ID NO:457), ATDRPYLV (SEQ ID NO:458), ATDRPYVL (SEQ ID NO:459), ATDRPYVI (SEQ ID NO:460), ATDRPYVV (SEQ ID NO:461), ATDRPWIL (SEQ ID NO:462), ATDRPWII (SEQ ID NO:463), ATDRPWIV (SEQ ID NO:464), ATDRPWLL (SEQ ID NO:465), ATDRPWLI (SEQ ID NO:466), ATDRPWLV (SEQ ID NO:467), ATDRPWVL (SEQ ID NO:468), ATDRPWVI (SEQ ID NO:469), ATDRPWVV (SEQ ID NO:470), ATEKPYIL (SEQ ID NO:471), ATEKPYII (SEQ ID NO:472), ATEKPYIV (SEQ ID NO:473), ATEKPYLL (SEQ ID NO:474), ATEKPYLI (SEQ ID NO:475), ATEKPYLV (SEQ ID NO:476), ATEKPYVL (SEQ ID NO:477), ATEKPYVI (SEQ ID NO:478), ATEKPYVV (SEQ ID NO:479), ATEKPWIL (SEQ ID NO:480), ATEKPWII (SEQ ID NO:481), ATEKPWIV (SEQ ID NO:482), ATEKPWLL (SEQ ID NO:483), ATEKPWLI (SEQ ID NO:484), ATEKPWLV (SEQ ID NO:485), ATEKPWVL (SEQ ID NO:486), ATEKPWVI (SEQ ID NO:487), ATEKPWVV (SEQ ID NO:488), ATERPYIL (SEQ ID NO:489), ATERPYII (SEQ ID NO:490), ATERPYIV (SEQ ID NO:491), ATERPYLL (SEQ ID NO:492), ATERPYLI (SEQ ID NO:493), ATERPYLV (SEQ ID NO:494), ATERPYVL (SEQ ID NO:495), ATERPYVI (SEQ ID NO:496), ATERPYVV (SEQ ID NO:497), ATERPWIL (SEQ ID NO:498), ATERPWII (SEQ ID NO:499), ATERPWIV (SEQ ID NO:500), ATERPWLL (SEQ ID NO:501), ATERPWLI (SEQ ID NO:502), ATERPWLV (SEQ ID NO:503), ATERPWVL (SEQ ID NO:504), ATERPWVI (SEQ ID NO:505),

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LTEKPYVV (SEQ ID NO:623), LTEKPWIL (SEQ ID NO:624), LTEKPWII (SEQ ID NO:625), LTEKPWIV (SEQ ID NO:626), LTEKPWLL (SEQ ID NO:627), LTEKPWLI (SEQ ID NO:628), LTEKPWLV (SEQ ID NO:629), LTEKPWVL (SEQ ID NO:630), LTEKPWVI (SEQ ID NO:631), LTEKPWVV (SEQ ID NO:632), LTERPYIL (SEQ ID NO:633), LTERPYII (SEQ ID NO:634), LTERPYIV (SEQ ID NO:635), LTERPYLL (SEQ ID NO:636), LTERPYLI (SEQ ID NO:637), LTERPYLV (SEQ ID NO:638), LTERPYVL (SEQ ID NO:639), LTERPYVI (SEQ ID NO:640), LTERPYVV (SEQ ID NO:641), LTERPWIL (SEQ ID NO:642), LTERPWII (SEQ ID NO:643), LTERPWIV (SEQ ID NO:644), LTERPWLL (SEQ ID NO:645), LTERPWLI (SEQ ID NO:646), LTERPWLV (SEQ ID NO:647), LTERPWVL (SEQ ID NO:648), LTERPWVI (SEQ ID NO:649), LTERPWVV (SEQ ID NO:650), ISDKPYIL (SEQ ID NO:651), ISDKPYII (SEQ ID NO:652), ISDKPYIV (SEQ ID NO:653), ISDKPYLL (SEQ ID NO:654), ISDKPYLI (SEQ ID NO:655), ISDKPYLV (SEQ ID NO:656), ISDKPYVL (SEQ ID NO:657), ISDKPYVI (SEQ ID NO:658), ISDKPYVV (SEQ ID NO:659), ISDKPWIL (SEQ ID NO:660), ISDKPWII (SEQ ID NO:661), ISDKPWIV (SEQ ID NO:662), ISDKPWLL (SEQ ID NO:663), ISDKPWLI (SEQ ID NO:664), ISDKPWLV (SEQ ID NO:665), ISDKPWVL (SEQ ID NO:666), ISDKPWVI (SEQ ID NO:667), ISDKPWVV (SEQ ID NO:668), ISDRPYIL (SEQ ID NO:669), ISDRPYII (SEQ ID NO:670), ISDRPYIV (SEQ ID NO:671), ISDRPYLL (SEQ ID NO:672), ISDRPYLI (SEQ ID NO:673), ISDRPYLV (SEQ ID NO:674), ISDRPYVL (SEQ ID NO:675), ISDRPYVI (SEQ ID NO:676), ISDRPYVV (SEQ ID NO:677), ISDRPWIL (SEQ ID NO:678), ISDRPWII (SEQ ID NO:679), ISDRPWIV (SEQ ID NO:680), ISDRPWLL (SEQ ID NO:681), ISDRPWLI (SEQ ID NO:682), ISDRPWLV (SEQ ID NO:683), ISDRPWVL (SEQ ID NO:684), ISDRPWVI (SEQ ID NO:685), ISDRPWVV (SEQ ID NO:686), ISEKPYIL (SEQ ID NO:687), ISEKPYII (SEQ ID NO:688), ISEKPYIV (SEQ ID NO:689), ISEKPYLL (SEQ ID NO:690), ISEKPYLI (SEQ ID NO:691), ISEKPYLV (SEQ ID NO:692), ISEKPYVL (SEQ ID NO:693), ISEKPYVI (SEQ ID NO:694), ISEKPYVV (SEQ ID NO:695), ISEKPWIL (SEQ ID NO:696), ISEKPWII (SEQ ID NO:697), ISEKPWIV (SEQ ID NO:698), ISEKPWLL (SEQ ID NO:699), ISEKPWLI (SEQ ID NO:700), ISEKPWLV (SEQ ID NO:701), ISEKPWVL (SEQ ID NO:702), ISEKPWVI (SEQ ID NO:703), ISEKPWVV (SEQ ID NO:704), ISERPYIL (SEQ ID NO:705), ISERPYII (SEQ ID NO:706), ISERPYIV (SEQ ID NO:707), ISERPYLL (SEQ ID NO:708), ISERPYLI (SEQ ID NO:709), ISERPYLV (SEQ ID NO:710), ISERPYVL (SEQ ID NO:711), ISERPYVI (SEQ ID NO:712), ISERPYVV (SEQ ID NO:713), ISERPWIL (SEQ ID NO:714), ISERPWII (SEQ ID NO:715), ISERPWIV (SEQ ID NO:716), ISERPWLL (SEQ ID NO:717), ISERPWLI (SEQ ID NO:718), ISERPWLV (SEQ ID NO:719), ISERPWVL (SEQ ID NO:720), ISERPWVI (SEQ ID NO:721), ISERPWVV (SEQ ID NO:722), ITDKPYIL (SEQ ID NO:723), ITDKPYII (SEQ ID NO:724), ITDKPYIV (SEQ ID NO:725), ITDKPYLL (SEQ ID NO:726), ITDKPYLI (SEQ ID NO:727), ITDKPYLV (SEQ ID NO:728), ITDKPYVL (SEQ ID NO:729), ITDKPYVI (SEQ ID NO:730), ITDKPYVV (SEQ ID NO:731), ITDKPWIL (SEQ ID NO:732), ITDKPWII (SEQ ID NO:733), ITDKPWIV (SEQ ID NO:734), ITDKPWLL (SEQ ID NO:735), ITDKPWLI (SEQ ID NO:736), ITDKPWLV (SEQ ID NO:737), ITDKPWVL (SEQ ID NO:738), ITDKPWVI (SEQ ID NO:739),

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In some specific embodiments, the polypeptide of the invention consists of or comprises an amino acid sequence derived from Alpha-actinin-1, such as a sequence selected from ASDKPYIL, AGDKNYIL, AGDKNYIT, AGDKSYIT, ADGKPYIV, and AEDKDFIT.

In some specific embodiments, the polypeptide of the invention consists of or comprises an amino acid sequence derived from Alpha-actinin-2, such as a sequence selected from ASDKPYIL, AADKPYIL, AGDKNYIT, ATDKPYIL, AGDKPYIT, ASEKPYIL, ADGKPYVT, AGDKPYIL, ASDKPNIL, ASDKPYIT, AADKPFIL, ASDKAYIT, AGDKAYIT, ANGKPFIT, and AGDKNFIT.

In some specific embodiments, the polypeptide of the invention consists of or comprises an amino acid sequence derived from Alpha-actinin-3, such as a sequence selected from ASDKPYIL, AADKPYIL, ASDKAYIT, ASDKSYIT, ASDKTYIT, ASDKNYIT, AGDKNYIL, AGDKSYIT, AGDKNYIT, AGDKKYIT, and AGDKNYIS.

In some specific embodiments, the polypeptide of the invention consists of or comprises an amino acid sequence derived from Alpha-actinin-4, such as a sequence selected from ASDKPYIL, AGDKPYIL, AADKNYIT, AGDKNYIM, AGDKNYIT, AADKNFIM, AADKNFIT, AGDKGIRS, and AGDKNFIT.

The present invention further relates to compositions comprising the polypeptides of the invention. In some embodiments the compositions of the invention is capable of promoting satiety in a subject upon consumption.

In some embodiments in the compositions of the invention the amount of said polypeptide in the composition is less than about 10 g, such as less than 9 g, 8 g, 7 g, 6 g, 5 g, 4 g, 3 g, 2 g, 1 g, 900 mg, 800 mg, 700 mg, 600 mg, 500 mg, 400 mg, 300 mg, 200 mg, 150 mg, 100 mg, 90 mg, 80 mg, 70 mg, 60 mg, 50 mg, 40 mg, 30 mg, 25 mg, 20 mg, 15 mg, 10 mg, or 5 mg.

In some embodiments in the compositions of the invention the amount of said polypeptide in the composition is at least about 5 mg, such as at least about 10 mg, 15 mg, 20 mg, 25 mg, 30 mg, 40 mg, 50 mg, 60 mg, 70 mg, 80 mg, 90 mg, 100 mg, 150 mg, 200 mg, 300 mg, 400 mg, 500 mg, 600 mg, 700 mg, 800 mg, 900 mg, 1 g, 2 g, 3 g, 4 g, 5 g, 6 g, 7 g, 8 g, 9 g, or 10 g.

In some embodiments in the compositions of the invention the energy content derived through the process of cellular respiration is less than 50 kilojoules (kJ), such as less than 40 kJ, such as less than 30 kJ, such as less than 20 kJ, such as less than 10 kJ, such as less than 5000 Joules (J), such as less than 1000 J, such as less than 900 J, such as less than 800 J, such as less than 700 J, such as less than 600 J, such as less than 500 J, such as less than 400 J, such as less than 300 J, such as less than 200 J, such as less than 100 J, such as less than 50 J.

In some embodiments the compositions of the invention is a food composition.

In some embodiments the compositions of the invention is a fermented composition.

In some embodiments the compositions of the invention is a dairy product.

In some embodiments the compositions of the invention is a pharmaceutical composition.

In some embodiments the compositions of the invention is a nutritional composition.

In some embodiments the compositions of the invention is an oral dosage form. In some embodiments the oral dosage form is selected from the group comprising tablets, capsules, caplets, slurries, sachets, suspensions, chewing gum, and powder formulation that may be dissolved in a liquid. In some embodiments the oral dosage form is a suspension. In some embodiments the oral dosage form is a powder formulation that may be dissolved in a liquid. In some embodiments the liquid is water, milk, juice, or yogurt.

EXAMPLE 1

Assays:

Ca^{2+} Flux assay:

Elevation of intracellular calcium level was measured using the fluorescent calcium chelating dye Fluo-4 AM (ThermoFischer Scientific, Denmark). Briefly, cells were grown as a monolayer in 96-well tissue culture plates (Sarstedt, Germany) to near confluence in appropriate growth medium as described in the cell culture section. Prior to the start of the assay, the cells were incubated with 1.5 µM Fluo-4 AM in complete culture media mixed 1:1 with Hank's balanced salt solution (HBSS, ThermoFischer Scientific, Denmark) containing 25 mM HEPES (pH 7.4), 1% BSA (Sigma-Aldrich, Denmark), 2% ink (Soluro GMBH, Germany), 0.01% Pluronic F-127 (Sigma-Aldrich, Denmark) and 1 mM Probenecide (Sigma-Aldrich) for 60 minutes at 37°C.

All test compounds were dissolved in water, and then diluted in 1xHBSS containing 25 mM HEPES (pH 7.4), 1% BSA and 2% ink. Without any removal of excess Fluo-4 AM, test compounds were added directly into the wells and fluorescence were measured using instrument settings for excitation at 488 nm and emission at 525 nm in a microtiter plate reader (SpectraMax M5, Molecular Devices, USA).

Cell culture:

Cell culture media, Dulbecco's phosphate-buffered saline, pH 7.4 (DPBS), glutamine, trypsin-EDTA and antibiotics were obtained from ThermoFischer Scientific (Denmark). Fetal bovine

serum and all other chemicals were purchased from Sigma-Aldrich (Denmark), unless otherwise stated.

Murine intestinal enteroendocrine L-cell lines that expresses the proglucagon gene and secretes GLP-1 in vitro were used. Cells were grown in DMEM containing 1 g/L D-glucose, 10% fetal bovine serum, 2 mM glutamine, 1% penicillin/streptomycin/neomycin and cultured in a humidified incubator in 95% air and 5% CO2 at 37°C.

Other murine intestinal cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 2 mM glutamine, 2.5 g/L glucose, 20 mM HEPES, 60 nM sodium selenite, 5 µg/ml transferrin, 5 µg/ml insulin, 50 nM dexamethasone, 10 nM EGF, 1 nM triiodothyronine, 2% fetal bovine serum and 1% penicillin/streptomycin/neomycin at 37°C in 5% CO2-95% air atmosphere.

Human intestinal cell lines were cultured in McCoy's modified 5A medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin/neomycin at 37°C and 5% CO2 in a humidified incubator.

Cells were routinely sub-cultivated 1:3 and given new media every second day.

Determination of GLP-1 levels:

GLP-1 levels were determined using a sandwich enzyme-linked immunoabsorbant assay (ELISA). The primary antibody to GLP-1 [2.5 µg/ml mouse monoclonal (HYB 147-06) in 0.05M bicarbonate/carbonate buffer; BioPorto Diagnostics A/S, Gentofte, Denmark) was coated on a flat-bottom 96-well plate (Sarstedt, Nümbrecht, Germany) for at least 24 hours at 4°C. This primary antibody is specific for the amidated C-terminus of the peptide and reacts with GLP-1 (7-36), GLP-1 (9-36) and GLP-1 (1-36), but not with GLP-1 (7-37). After blocking the plate using a PBS buffer containing 4% w/v BSA (Sigma-Aldrich, Denmark) and 0.1% v/v Tween 20 (Sigma-Aldrich) for 1 hour at room temperature, the plate was washed four times with PBS buffer containing 0.1% v/v Tween 20. A standard curve with GLP-1 peptide [human GLP-1 (7-36), Sigma-Aldrich, Denmark) concentrations ranging from 0 pg/ml to 1000 pg/ml was prepared in PBS buffer containing 0.5% BSA and 0.05% Tween 20, and samples were diluted if necessary. Samples and standards were added to the microtiter plate and incubated with the primary antibody for two hours at room temperature. Subsequently, the plate was washed four times, and the wells were incubated with a secondary biotinylated antibody to GLP-1 [1 µg/ml; mouse monoclonal (ABS 033-01), BioPorto Diagnostics A/S, Gentofte, Denmark) for two hours at room temperature. After another washing step, samples were incubated with streptavidin-horseradish peroxidase (1:200, Dako A/S, Denmark) for 45

minutes followed by an incubation with TMB solution (containing 3,3',5,'5-tetramethylbenzidine and H2O2, SMS-gruppen, Denmark). The reaction was stopped by adding H2SO4 (0.2M), and the absorbance of the yellow end product was measured at 450 nm on a microtiter plate spectrophotometer (SpectraMax M5, Molecular Devices, USA). The concentrations of the samples were determined by interpolation to the concentrations of the standard solutions.

Cells (~5x10^5 per sample) were incubated for up to 90 min in Dulbeccos Modified Eagle Medium (DMEM) containing 5,56 mM glucose in absence or presence of different amounts (weight/volume) of protein hydrolysate (pig heart). Supernatant was filtered through 0,45 micron filters and assayed for content of GLP-1 as described in ELISA protocol. Data are mean + SEM from quadruplicate samples.

Preparation of bioactive peptides by enzymatic digestion of meat

Minced meat is diluted 1-10 times with distilled water, adjusted to pH 1-3 with hydrochloric acid, and incubated with 0,01-10% pepsin (w/w) at 4-40 oC for ½-12 h with adequate mixing. Insoluble material is removed by centrifugation at 100-1000 xg for 3-30 min, and supernatant is neutralized with NaOH. Using sterile conditions, low molecular weight peptides in supernatant are recovered by tangential ultrafiltration at 4-40 °C for ½-12 h, and excess water is evaporated at 25-50 °C for up to 12 h. The concentrated dialysate is tested for bioactivity with cells and used for further purification by HPLC.

Purification and identification of bioactive peptide ASDKPYIL

Upconcentrated dialysates were fractionated on preparative C18 columns using buffer B: 20 mM phosphate buffer pH 8.25/10% ACN and a gradient of 0-40% in buffer A: 60% ACN in same buffer. Fractions were tested for bioactivity and further purified by isocratic elution using EVO C18 columns with 4.5% ACN in 0.1%FA isocratic for 30 min. Fractions were subject to MS characterization, where a dominating peak with m/z 453.75 (+2) was observed. Extracted ions chromatograms show this peak to be present in all active fractions. De novo sequencing of 453.75 peak gives [A]SDKPY[I,L][I,L]. N-terminal A is calculated from parent ion –A7. I and L are not resolved by MS because of equal molecular weights. Search of protein sequences gives only ASDKPYIL as match. ASDKPYIL is only found in alpha-actinin-2, a major muscle protein.

Stability of peptides ex vivo.

Peptides are degraded by proteases in the gastrointestinal tract. However the speed of this degradation depends on the sequence of the peptide. In order to measure stability of the ASDKPYIL peptide series and to compare with e.g. RRKPYIL, 10 or 50 mg (wet weight) of mouse or rat intestinal tissue (distal ileum) was equilibrated in V-bottom 24 well plates in 800 µl HBSS, 25 mM HEPES, pH 7,4 at 37 °C with shaking at 350 rpm. Identical amounts of different peptides (final concentration of 1 µg/ml) were added to the intestinal pieces and incubation continued. At various time points, 100 µl aliquots were removed and diluted into whey protein hydrolysate (final concentration of 10 mg/ml) to non-specifically compete protease activity. Peptide solutions were then diluted and tested for bioactivity (fig 8). Peptides incubated under same conditions but in absence of intestine served as controls (no degradation). Determination of EC50 for stimulation of cells allowed calculation of recovered peptide (fig 9), assuming simple inactivation by the tissue.

EXAMPLE 2

1) Structure-activity relationship and stability (SAR)

a. Extended versions
b. Substituted versions

2) In vivo studies in mice

c. Acute effects on feed intake (satiety)
d. Long-term effects on weight may be determined

Based on structural modelling studies of DC7-2 and NTR-1 interactions, peptides being octapeptides, heptapeptides, hexapeptides, or pentapeptides to exhibit increased potency due to increased binding may be predicted.

Comparison with SAR studies using synthetic peptides, peptides with increased potency and stability may be both predicted and observed.

Assays:

Synthetic peptides:

Based on the sequence of the natural hormone Neurotensin (QLYENKPRRPYIL), the bioactive Neurotensin fragment NT(8-13)(RRPYIL) and the identified bioactive octapeptide DC7-2 (ASDKPYIL), synthetic peptides with systematic substitutions of N-terminal amino acids of the octapeptide (X-SDKPYIL), the heptapeptide (X-DKPYIL), the hexapeptide (X-KPYIL) and the pentapeptide (X-PYIL) were synthesised using standard techniques (Schafer-N, Denmark). All peptides were dissolved in pure HPLC-grade water and stored at -20°C.

Stability of peptides:

Concentration determination:

Protein concentration of synthetic peptides (Schafer-N, Denmark), NT (Sigma-Aldrich, Denmark) and NT (8-13)(Sigma-Aldrich, Denmark) were determined by measuring absorbance at 280 nm in Costar ® 96-well UV-transparent plates (Corning, Sigma-Aldrich, Denmark). Each peptide was measured in 4 different concentrations by dilution in Hank's balanced salt solution (HBSS, ThermoFischer Scientific, Denmark) containing 25 mM HEPES (pH 7.4) (Sigma-Aldrich, Denmark). For stability assays, all peptides were diluted to $3x10^{-5}$ M in HBSS; 25 mM HEPES (Ph 7.4) and stored at +4°C.

Intestine homogenate:

Small intestines from 20 Swiss-Webster males were homogenized in 350 ml Dulbecco's phosphate-buffered saline (PBS) (pH 7.4) (ThermoFischer Scientific, Denmark) with a IKA® basic 18 Ultra-Turrax tissue homogenizer set a speed 5 followed by filtration using 100 µm nylon mesh filter. Protein concentration was 6 mg/ml using the bicinconinic acid assay (ThermoFischer Scientific, Denmark) and bovine serum albumin as standard. The intestine homogenate was diluted 10 times in HBSS containing 25 mM HEPES (pH 7.4), and further diluted 30X, 90X, 270X, 810X or 2430X before incubation with peptides. All solutions were prewarmed to 37°C before mixing with peptide solutions.

Peptides were incubated at 10^{-5} M with dilutions of small intestine homogenate at 37°C for 90 minutes with shaking. Reactions were stopped by addition of 1 M phosphoric acid (final 0.4 M, pH ~1.2). Each peptide incubation mix was then neutralized with NaOH to pH 7.2-7.4 and immediately tested for activity in intestinal cells. Control for zero degradation, i.e. addition of 1 M phosphoric acid prior to addition of intestine homogenate, was included for each peptide.

Fetal Bovine Serum:

All peptides were incubated at 10^{-5} M with Fetal Bovine Serum (FBS; final concentration of 66.7%) (Sigma Aldrich, Denmark) at 37°C for 3 hours. The peptide degradation was terminated using 1 M phosphoric acid (final 0.4 M, pH ~1.2) and neutralized to pH 7.2-7.4 with NaOH before testing activity in intestinal cells. As for small intestine homogenates, a zero degradation control was included for each peptide.

Kinetic studies of selected peptides:

DC7-2, NT, DKPYIL and NT-(8-13) (final concentration of 10^{-6} M) were incubated either with FBS or with 270X diluted intestinal homogenate at 37°C for various time points with shaking. Degradation was stopped with 1 M phosphoric acid and the samples were subsequently neutralized and immediately tested with intestinal cells as described above. Control for zero degradation was included for each peptide as above.

Study of hexapeptides:

The 20 hexapeptides with systematic N-terminal substitutions (X-KPYIL) (Schafer-N, Denmark) and NT (8-13) was incubated at 10^{-6} M in either FBS for 10 minutes or with 270X diluted intestinal homogenate for 30 minutes at 37°C with shaking. The degradation was stopped with 1 M phosphoric acid. Peptide solutions were neutralized with NaOH (pH 7.2-7.4), diluted and immediately tested for bioactivity using murine intestinal cells. Determination of EC50 for stimulation of cells allowed calculation of recovered peptide.

Systematic substitutions of N-terminal amino acids in octapeptide ASDKPYIL and their importance for activity and stability. Sequence, activity and stability of DC7-2 is indicated in grey.

Peptide ID	Sequence	Cell signaling activity (EC50, nM)		Stability in serum (activity remaining)[1)]	Stability in intestine (activity remaining)[2)]
		Mean	SEM		
36055	Y SDKPYIL (SEQ ID NO:939)	5,92E-09	5,53E-10	0,0491	3,0
36054	W SDKPYIL (SEQ ID NO:940)	8,87E-09	7,94E-10	0,0426	3,6
36053	V SDKPYIL (SEQ ID NO: 795)	5,15E-09	5,32E-10	0,0488	5,6
36052	T SDKPYIL (SEQ ID NO:941)	5,63E-09	5,67E-10	0,0535	8,4
36051	S SDKPYIL (SEQ ID NO:942)	4,27E-09	3,92E-10	0,0609	19,5
36050	R SDKPYIL (SEQ ID NO:943)	4,01E-09	4,43E-10	0,0735	2,6
36049	Q SDKPYIL (SEQ ID NO:944)	4,01E-09	4,51E-10	0,0742	8,8
36048	P SDKPYIL (SEQ ID NO:945)	4,14E-09	4,54E-10	0,0772	4,6
36047	N SDKPYIL (SEQ ID NO:946)	4,41E-09	4,28E-10	0,0872	3,9
36046	M SDKPYIL (SEQ ID NO:947)	4,88E-09	5,01E-10	0,0762	3,8
36045	L SDKPYIL (SEQ ID NO:507)	8,06E-09	8,25E-10	0,0917	7,9
36044	K SDKPYIL (SEQ ID NO:948)	1,07E-08	1,05E-09	0,0527	6,6
36043	I SDKPYIL (SEQ ID NO:651)	6,87E-09	7,41E-10	0,0956	6,0
36042	H SDKPYIL (SEQ ID NO:949)	3,63E-09	3,24E-10	0,0980	6,4
36041	G SDKPYIL (SEQ ID NO:950)	4,07E-09	5,07E-10	0,0912	10,3
36040	F SDKPYIL (SEQ ID NO:951)	3,85E-09	4,95E-10	0,0944	3,4
36039	E SDKPYIL (SEQ ID NO:952)	4,82E-09	5,76E-10	0,0964	23,0
36038	D SDKPYIL (SEQ ID NO:953)	6,15E-09	6,85E-10	0,0947	29,9
36037	C SDKPYIL (SEQ ID NO:954)	5,44E-09	6,25E-10	0,0963	14,0
36036	A SDKPYIL (SEQ ID NO:6)	3,51E-09	4,75E-10	0,0988	6,0

Notes for Tables 1) Stability in serum is expressed as fraction of peptide activity left after 10 min of incubation in serum at 37 °C compared with undigested sample as described in Examples. 2) Stability in intestine is expressed as % activity left after 30 min incubation in intestine homogenate at 37 oC as described in Examples.

Systematic substitutions of N-terminal amino acid in heptapeptide SDKPYIL and their importance for activity and stability. Sequence, activity and stability of peptide contained in DC7-2 is indicated in grey.

Peptide ID	Sequence	Cell signaling activity (EC50, nM)		Stability in serum (t½, min)	Stability in intestine (t½, min)
		Mean	STD		
36035	Y DKPYIL (SEQ ID NO:955)	6,83E-09	5,97E-10	0,0531	3,2
36034	W DKPYIL (SEQ ID NO:956)	1,41E-08	1,18E-09	0,0427	6,3
36033	V DKPYIL (SEQ ID NO:957)	4,94E-09	4,75E-10	0,0528	4,4
36032	T DKPYIL (SEQ ID NO:292)	5,61E-09	5,33E-10	0,0593	13,3
36031	S DKPYIL (SEQ ID NO:7)	5,00E-09	4,88E-10	0,0587	10,6
36030	R DKPYIL (SEQ ID NO:958)	4,68E-09	4,77E-10	0,0597	6,9
36029	Q DKPYIL (SEQ ID NO:959)	4,97E-09	4,86E-10	0,0620	11,5
36028	P DKPYIL (SEQ ID NO:960)	4,67E-09	4,64E-10	0,0558	10,9
36027	N DKPYIL (SEQ ID NO:961)	5,92E-09	5,21E-10	0,0580	40,5
36026	M DKPYIL (SEQ ID NO:962)	6,08E-09	5,69E-10	0,0601	7,1
36025	L DKPYIL (SEQ ID NO:963)	6,41E-09	9,98E-10	0,0969	3,9
36024	K DKPYIL (SEQ ID NO:964)	1,12E-08	1,61E-09	0,0910	6,9
36023	I DKPYIL (SEQ ID NO:965)	4,77E-09	8,27E-10	0,0928	2,8
36022	H DKPYIL (SEQ ID NO:966)	2,65E-09	3,53E-10	0,0932	4,7
36021	G DKPYIL (SEQ ID NO:967)	2,91E-09	3,86E-10	0,0920	4,5
36020	F DKPYIL (SEQ ID NO:968)	9,52E-09	1,38E-09	0,0901	2,4
36019	E DKPYIL (SEQ ID NO:969)	3,96E-09	6,89E-10	0,0846	17,2
36018	D DKPYIL (SEQ ID NO:970)	1,05E-08	1,47E-09	0,0419	44,3
36017	C DKPYIL (SEQ ID NO:971)	7,72E-09	1,17E-09	0,0407	9,4
36016	A DKPYIL (SEQ ID NO:972)	3,52E-09	6,43E-10	0,0952	2,5

Systematic substitutions of N-terminal amino acid in hexapeptide DKPYIL and their importance for activity and stability. Sequence, activity and stability of peptide contained in DC7-2 is indicated in grey.

Peptide ID	Sequence	Cell signaling activity (EC50, nM)		Stability in serum (t½, min)	Stability in intestine (t½, min)
		Mean	STD		
35995	Y KPYIL (SEQ ID NO:973)	3,34E-09	5,33E-10	0,0346	0,1
35994	W KPYIL (SEQ ID NO:974)	5,58E-09	8,57E-10	0,0334	0,2
35993	V KPYIL (SEQ ID NO:975)	1,17E-09	1,73E-10	0,0375	0,2
35992	T KPYIL (SEQ ID NO:976)	1,16E-09	1,72E-10	0,0424	0,3
35991	S KPYIL (SEQ ID NO:977)	1,15E-09	1,70E-10	0,0477	1,3
35990	R KPYIL (SEQ ID NO:150)	4,40E-10	6,59E-11	0,0480	0,1
35989	Q KPYIL (SEQ ID NO:978)	3,78E-10	5,67E-11	0,0495	0,8
35988	P KPYIL (SEQ ID NO:979)	2,32E-10	3,53E-11	0,0550	0,2
35987	N KPYIL (SEQ ID NO:980)	5,00E-10	7,42E-11	0,0709	1,0
35986	M KPYIL (SEQ ID NO:981)	3,88E-10	5,82E-11	0,0504	0,1
35985	L KPYIL (SEQ ID NO:982)	3,30E-10	4,60E-11	0,0310	0,1
35984	K KPYIL (SEQ ID NO:43)	2,64E-10	3,72E-11	0,0403	0,1
35983	I KPYIL (SEQ ID NO:983)	2,40E-10	3,37E-11	0,0315	0,0
35982	H KPYIL (SEQ ID NO:984)	2,71E-10	3,82E-11	0,0363	0,1
35981	G KPYIL (SEQ ID NO:184)	3,64E-10	5,05E-11	0,0353	0,1
35980	F KPYIL (SEQ ID NO:985)	3,15E-10	4,39E-11	0,0365	0,1
35979	E KPYIL (SEQ ID NO:114)	5,65E-10	7,80E-11	0,0478	0,6
35978	D KPYIL (SEQ ID NO:8)	8,03E-10	1,11E-10	0,0623	2,2
35977	C KPYIL (SEQ ID NO:986)	1,11E-09	1,53E-10	0,0477	2,6
35976	A KPYIL (SEQ ID NO:987)	2,51E-10	3,50E-11	0,0454	0,1

Systematic substitutions of N-terminal amino acid in pentapeptide KPYIL and their importance for activity and stability. Sequence, activity and stability of peptide contained in DC7-2 is indicated in grey.

Peptide ID	Sequence	Cell signaling activity (EC50, nM)		Stability in serum (t½, min)	Stability in intestine (t½, min)
		Mean	STD		
36015	Y PYIL (SEQ ID NO:988)	1,40E-07	1,01E-08	0,0091	2,0
36014	W PYIL (SEQ ID NO:989)	1,33E-07	1,32E-08	0,0066	0,2
36013	V PYIL (SEQ ID NO:990)	3,78E-08	2,71E-09	0,0094	0,3
36012	T PYIL (SEQ ID NO:991)	4,36E-08	3,13E-09	0,0163	2,9
36011	S PYIL (SEQ ID NO:992)	2,25E-08	1,62E-09	0,0241	0,0
36010	R PYIL (SEQ ID NO:40)	1,18E-09	1,00E-10	0,0176	1,1
36009	Q PYIL (SEQ ID NO:993)	2,05E-08	1,47E-09	0,0372	0,2
36008	P PYIL (SEQ ID NO:994)	9,61E-09	6,98E-10	0,0197	2,6
36007	N PYIL (SEQ ID NO:995)	3,93E-08	2,81E-09	0,0148	0,4
36006	M PYIL (SEQ ID NO:996)	1,62E-08	1,17E-09	0,0034	0,2
36005	L PYIL (SEQ ID NO:997)	4,10E-08	4,52E-09	0,0373	0,1
36004	K PYIL (SEQ ID NO:9)	7,71E-09	8,77E-10	0,0124	0,2
36003	I PYIL (SEQ ID NO:998)	2,38E-08	2,63E-09	0,0132	0,2
36002	H PYIL (SEQ ID NO:999)	3,56E-08	3,94E-09	0,0366	0,1
36001	G PYIL (SEQ ID NO:1000)	1,74E-08	1,94E-09	0,0125	0,3
36000	F PYIL (SEQ ID NO:1001)	1,95E-08	2,17E-09	0,0343	2,4
35999	E PYIL (SEQ ID NO:1002)	1,02E-07	1,12E-08	0,0405	3,6
35998	D PYIL (SEQ ID NO:1003)	1,58E-07	1,74E-08	0,0352	12,5
35997	C PYIL (SEQ ID NO:1004)	6,99E-08	7,73E-09	0,0349	0,2
35996	A PYIL (SEQ ID NO:1005)	1,18E-08	1,31E-09	0,0036	0,4

In conclusion, the results demonstrates that octa- and heptapeptides are more stable, and that the N-terminal aa in the hexapeptide has a significant implication on the stability.

As compared to the hexapeptide of a natural homone, neurotensin (8-13) (NT with the sequence RRPYIL), one specific peptide of the present invention DKPYIL is nearly 100 times more stable in serum and around 100-1000x more stable in intestine homogenate.

In vivo studies

Acute effects of DC7-2 on satiety is shown in figure 12, 17-20.

5

CLAIMS

1. An isolated polypeptide consisting of the amino acid sequence

R1-AA1-AA2-AA3-K-P-Y-I-L-R2 (formula II, SEQ ID NO:2),

wherein AA1 is an optional amino acid selected from A, L, I, and V; AA2 is an optional amino acid selected from S, T, G, A, N, E and D; AA3 is an amino acid selected from D, E, and G; R1 defines the N-term (-NH2) or a protection group; R2 defines the C-term (-COOH).

2. The polypeptide according to claim 1, wherein AA3 is an amino acid selected from D and E.

3. The polypeptide according to any one of the previous claims, wherein AA1 is A and/or wherein AA2 is S, and/or wherein AA3 is D.

4. The polypeptide according to any one of the previous claims, which amino acid sequence only contains natural amino acids.

5. The polypeptide according to any one of the previous claims having a sequence selected from ASDKPYIL, SDKPYIL, DKPYIL, AADKPYIL, ATDKPYIL, ASEKPYIL, and AGDKPYIL.

6. An isolated polypeptide of 17-50 amino acids comprising the amino acid sequence AA1-AA2-AA3-K-P-Y-I-L; wherein AA1 is an optional amino acid selected from A, L, I, and V; AA2 is an optional amino acid selected from S, T, G, A, N, E and D; and AA3 is an amino acid selected from D and E.

7. An isolated polypeptide of 17-50 amino acids comprising a sequence selected from ASDKPYIL, SDKPYIL, DKPYIL, AADKPYIL, ATDKPYIL, and AGDKPYIL.

8. A composition comprising a polypeptide as defined in any one of claims 1-7.

9. A polypeptide as defined in any one of claims 1-7 for use in promoting satiety in a subject.

10. A polypeptide as defined in any one of claims 1-7 for use in weight management, and/or for preventing or reducing the incidence of overweight and/or obesity in a subject, and/or for use in preventing or reducing cardiovascular diseases, atherosclerosis, hypertension, hepatosteatosis, cancer and/or diabetes.

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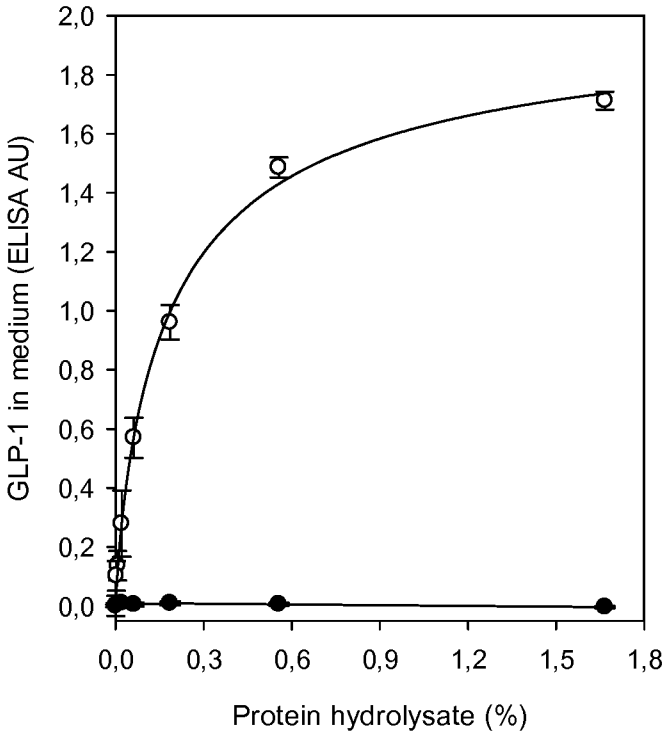
11. A method of promoting satiety or for reducing feed intake in a subject, comprising administering to a subject in need thereof a polypeptide, wherein said polypeptide is as defined in any one of claims 1-7.

12. A method of preventing or reducing the incidence of obesity in a subject, comprising administering to a subject in need thereof a polypeptide, wherein said polypeptide is as defined in any one of claims 1-7.

13. A method to prevent or reduce metabolic syndrome or disorder comprising administering to a subject in need thereof a polypeptide, wherein said polypeptide is as defined in any one of claims 1-7.

Figure 1


GLP-1 in medium (ELISA AU)
2,0
1,8
1,6
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0,0
0,0
0,3
0,6
0,9
1,2
1,5
1,8
Protein hydrolysate (%)

5

Figure 2

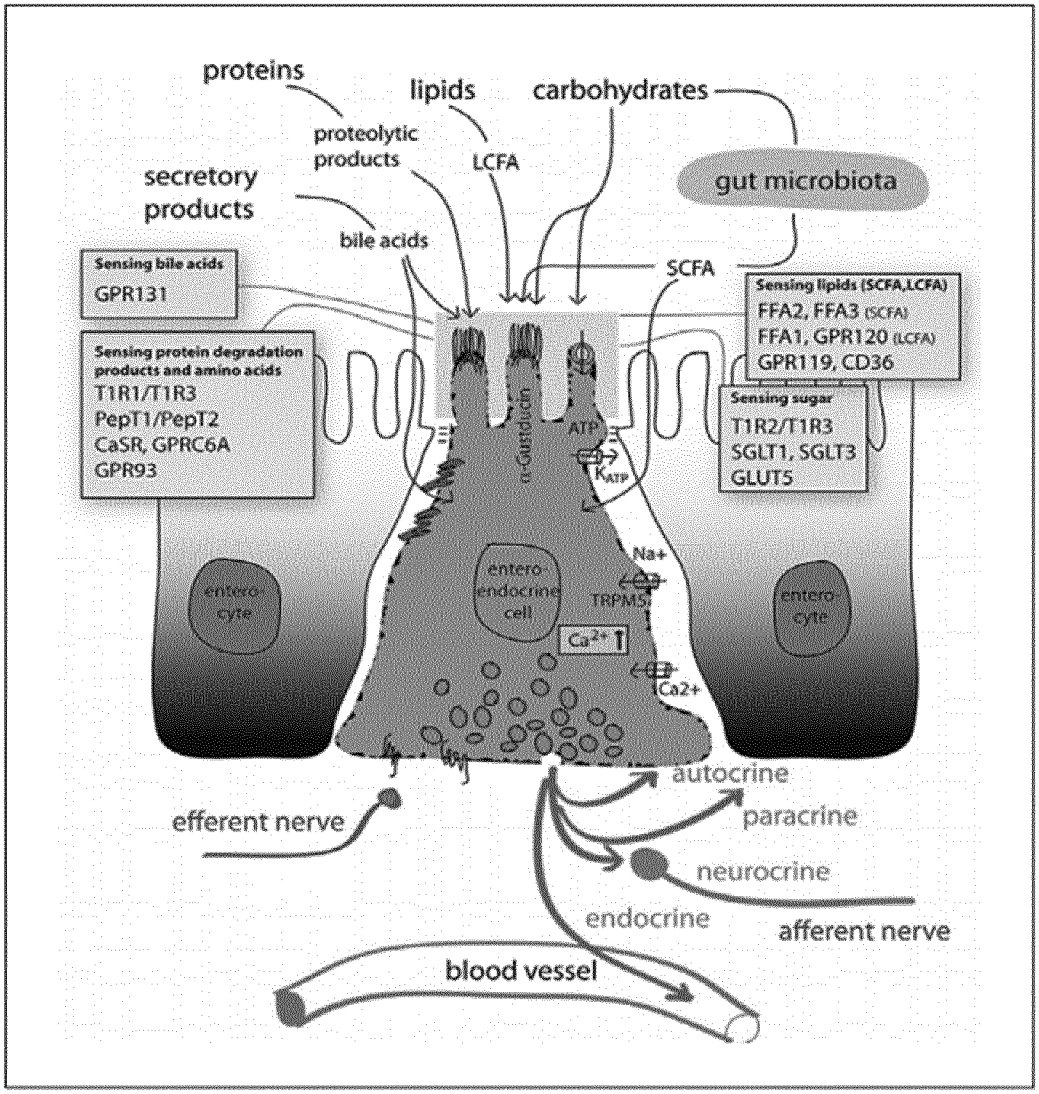

proteins
lipids
carbohydrates
proteolytic products
LCFA
secretory products
bile acids
gut microbiota
SCFA
Sensing bile acids
GPR131
Sensing protein degradation products and amino acids
T1R1/T1R3
PepT1/PepT2
CaSR, GPRC6A
GPR93
Sensing lipids (SCFA,LCFA)
FFA2, FFA3 (SCFA)
FFA1, GPR120 (LCFA)
GPR119, CD36
Sensing sugar
T1R2/T1R3
SGLT1, SGLT3
GLUT5
α-Gustducin
ATP
K_{ATP}
Na+
TRPM5
entero-endocrine cell
entero-cyte
entero-cyte
Ca^{2+}
Ca2+
autocrine
paracrine
neurocrine
endocrine
afferent nerve
efferent nerve
blood vessel

Figure 3

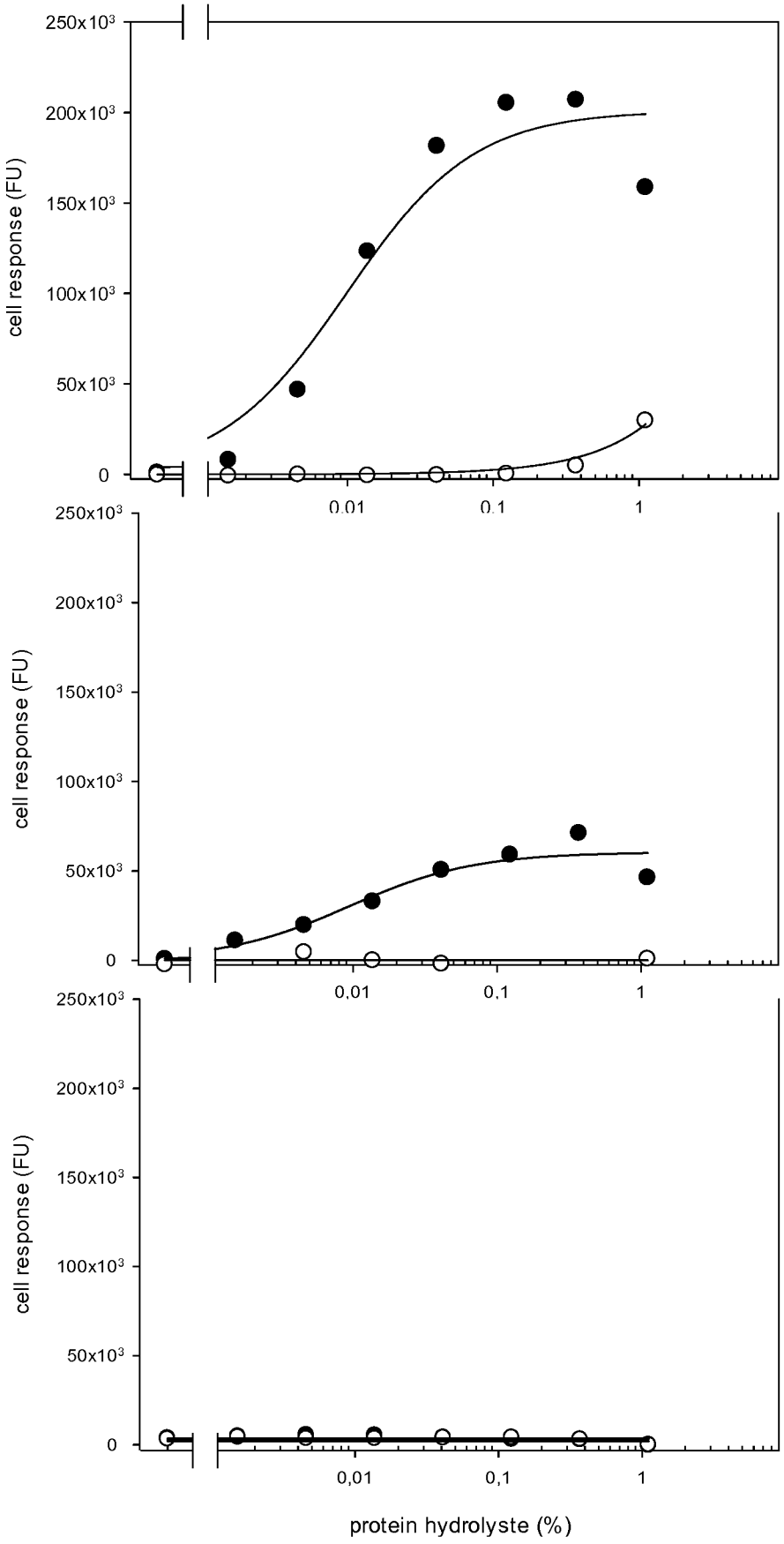

250x10³
200x10³
150x10³
100x10³
50x10³
0
cell response (FU)
0,01
0,1
1
protein hydrolyste (%)

Figure 4

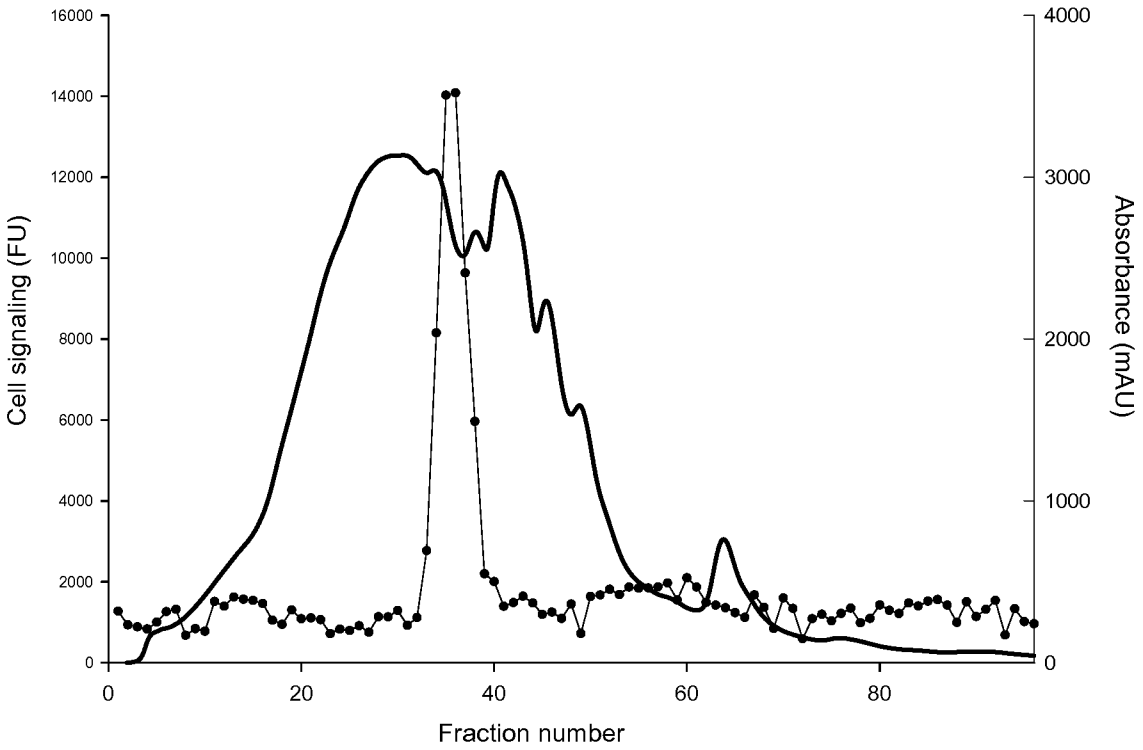

16000
14000
12000
10000
8000
6000
4000
2000
0
Cell signaling (FU)
4000
3000
2000
1000
0
Absorbance (mAU)
0
20
40
60
80
Fraction number

Figure 5

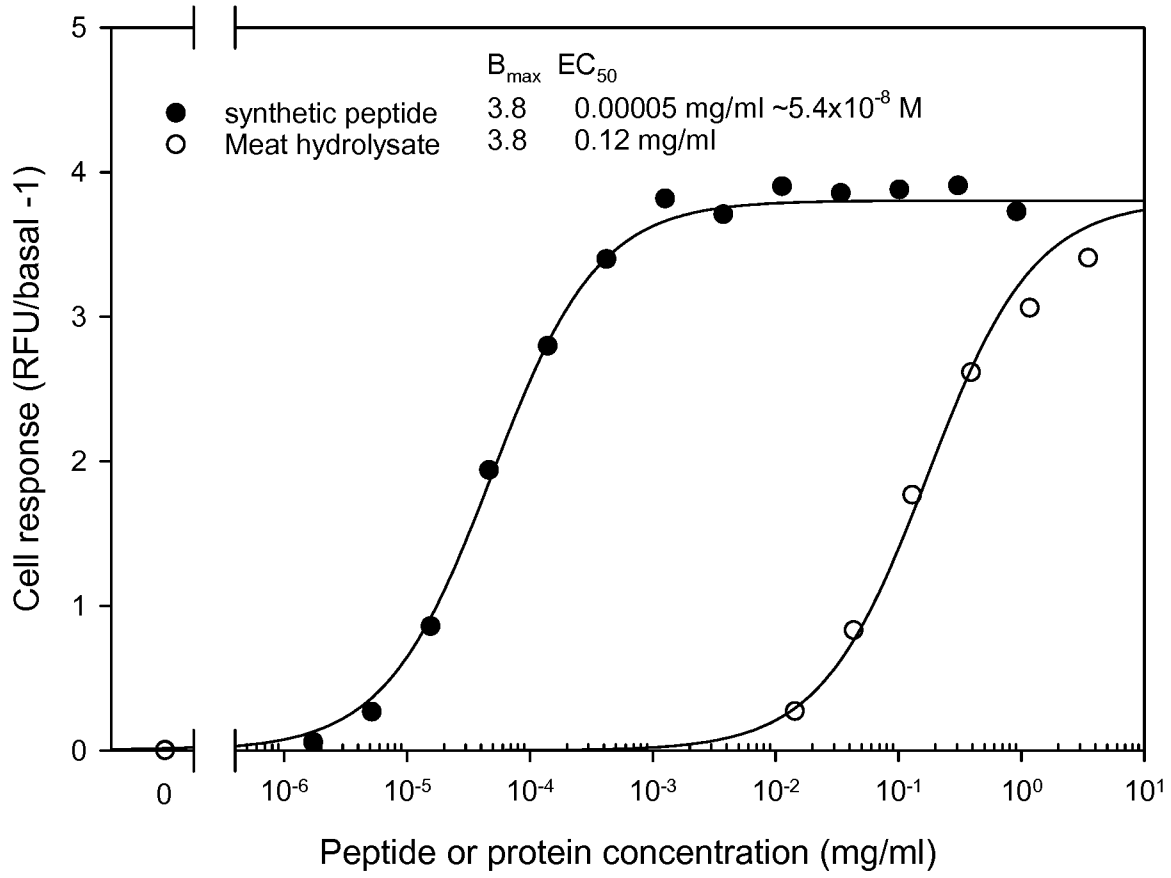

B_max EC_50
synthetic peptide 3.8 0.00005 mg/ml ~5.4x10^-8 M
Meat hydrolysate 3.8 0.12 mg/ml
Cell response (RFU/basal -1)
Peptide or protein concentration (mg/ml)
0
1
2
3
4
5
10^-6
10^-5
10^-4
10^-3
10^-2
10^-1
10^0
10^1

Figure 6

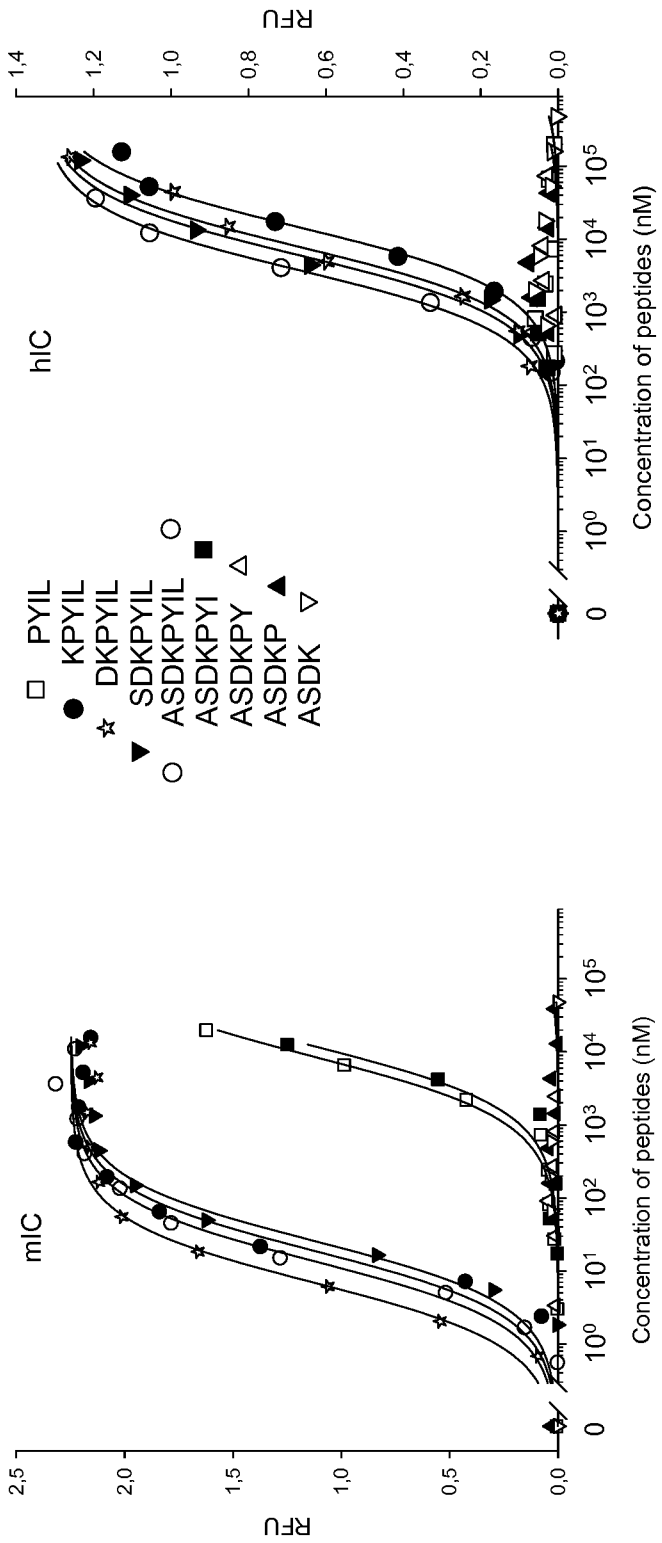

mIC
hIC
RFU
Concentration of peptides (nM)
PYIL
KPYIL
DKPYIL
SDKPYIL
ASDKPYIL
ASDKPYI
ASDKPY
ASDKP
ASDK

Figure 7

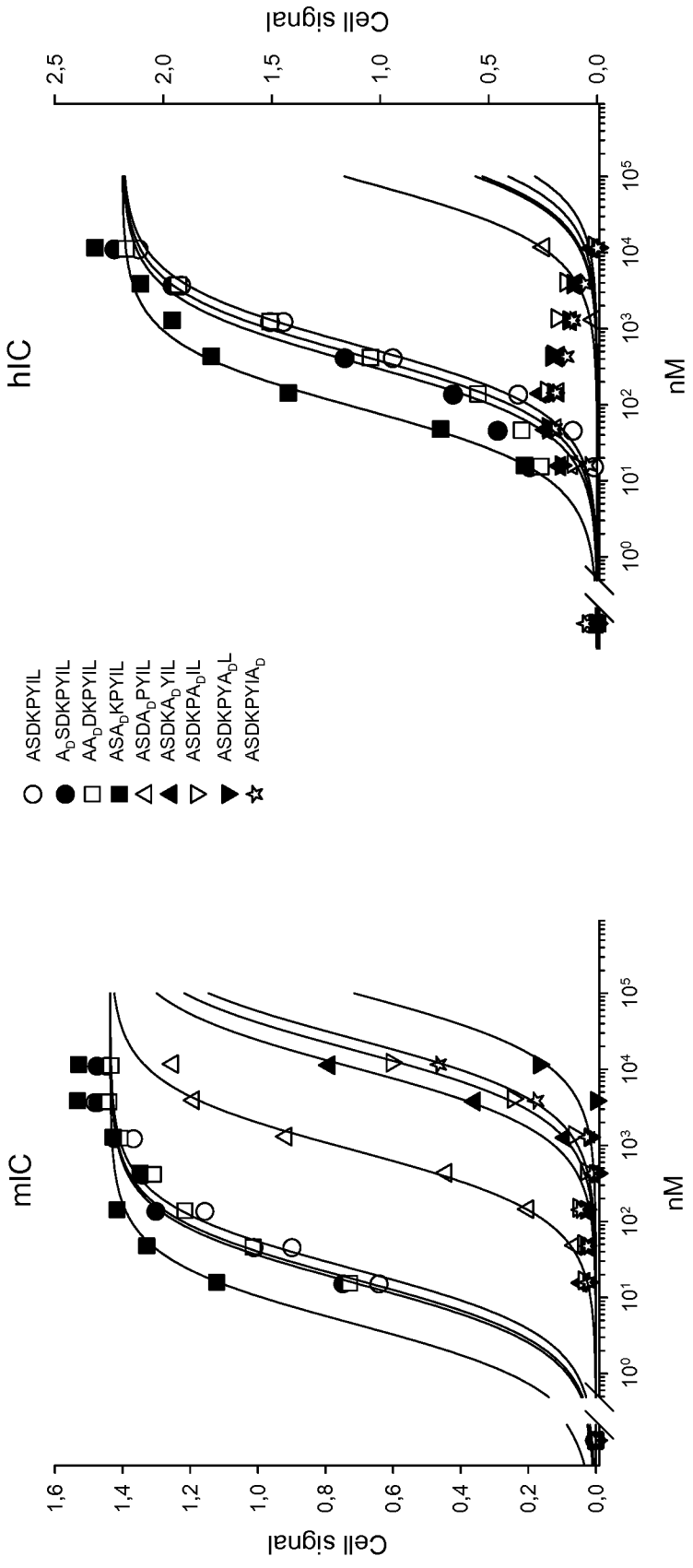

hIC
mIC
Cell signal
nM
ASDKPYIL
A$_D$SDKPYIL
AA$_D$DKPYIL
ASA$_D$KPYIL
ASDA$_D$PYIL
ASDKA$_D$YIL
ASDKPA$_D$IL
ASDKPYA$_D$L
ASDKPYIA$_D$

Figure 8

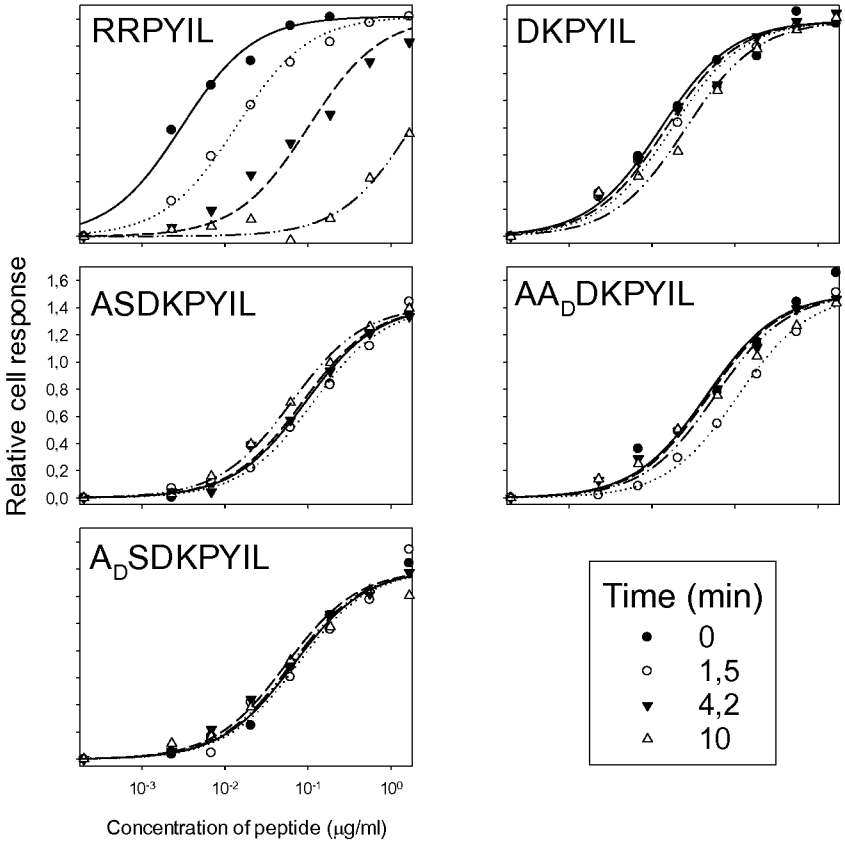
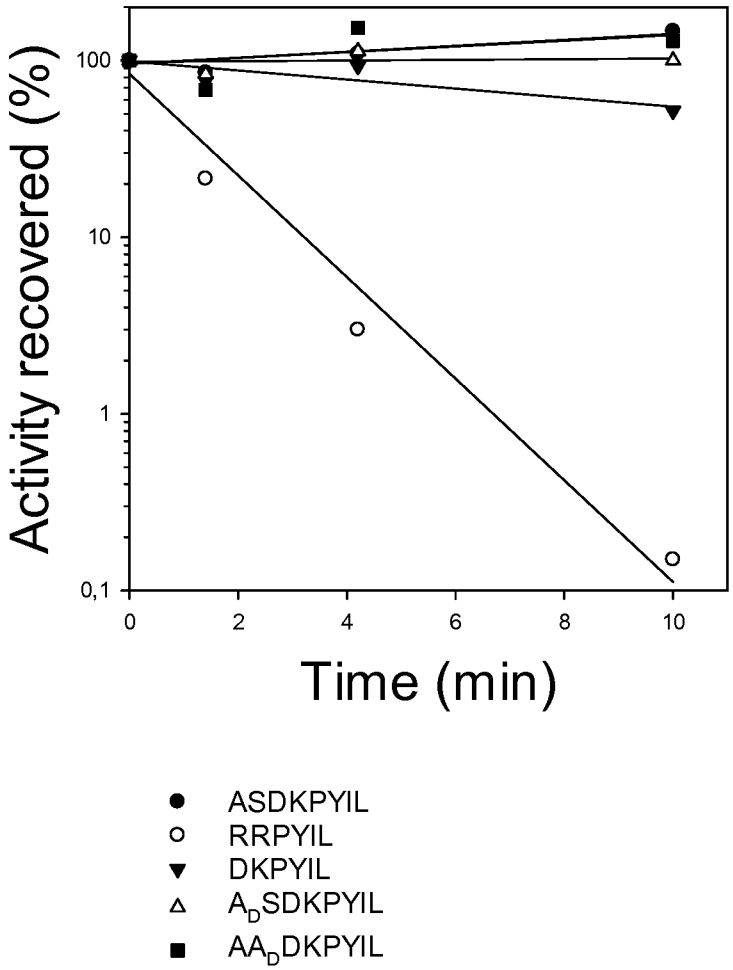

RRPYIL
DKPYIL
ASDKPYIL
AA$_D$DKPYIL
A$_D$SDKPYIL
Relative cell response
0,0
0,2
0,4
0,6
0,8
1,0
1,2
1,4
1,6
10^{-3}
10^{-2}
10^{-1}
10^{0}
Concentration of peptide (µg/ml)
Time (min)
0
1,5
4,2
10

Figure 9


Activity recovered (%)
100
10
1
0,1
0
2
4
6
8
10
Time (min)
ASDKPYIL
RRPYIL
DKPYIL
A$_D$SDKPYIL
AA$_D$DKPYIL

5

Figure 10

5

Neurotensin (NT) /Neuromedin N (NN) precursor

MRGMNLQLVCLTLLAFSSWSLCSDSEEDVRALEADLLTNMHTSKISKASPPSWKMTLLNVCSLINNVNSPAEEAGDMHDDDLVGK

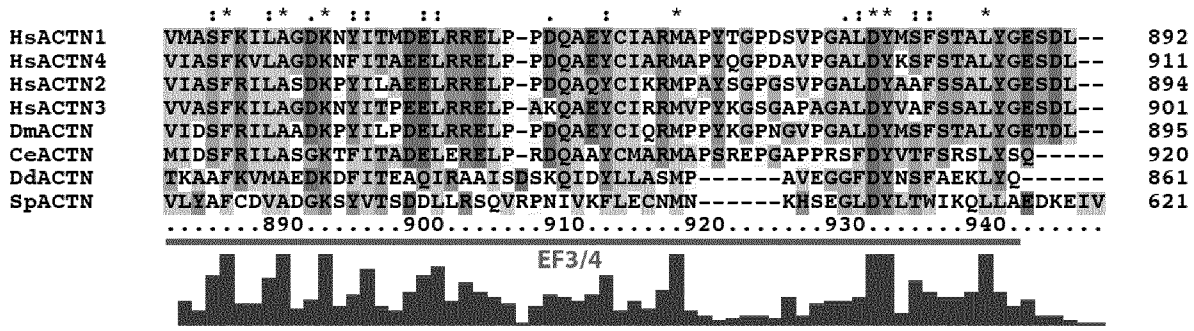
RKLPLVLDGFSLEAMLTIFQLQKICRSRAFQHWEIIQEDILDNVNDKNEKEEVIKRKIPYILKRQLYENKPRRPYILKRGSYYY

ASDKPYIL

ASDKPYIL DC7-2

MLTKFETKSARVKGLSFHPKRPWIL Xenin

Figure 11



HsACTN1 **AGDKNYIT**
HsACTN4 **AGDKNFIT**
HsACTN2 **ASDKPYIL**
HsACTN3 **AGDKNYIT**
DmACTN **AADKPYIL**
CeACTN **ASGKTFIT**
DdACTN **AEDKDFIT**
SpACTN **ADGKSYVT**
DrACTN **AADKPYIL**

Figure 12

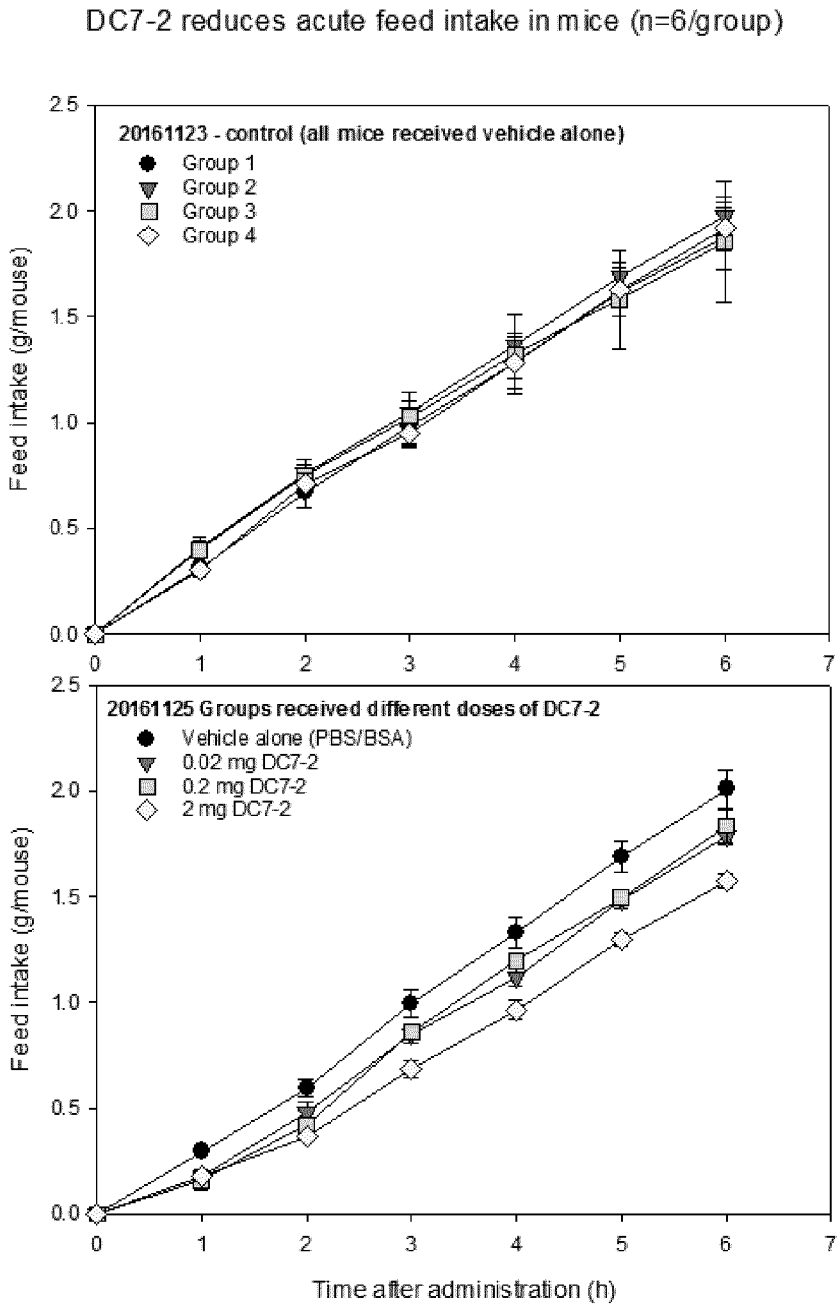

DC7-2 reduces acute feed intake in mice (n=6/group)
20161123 - control (all mice received vehicle alone)
Group 1
Group 2
Group 3
Group 4
Feed intake (g/mouse)
20161125 Groups received different doses of DC7-2
Vehicle alone (PBS/BSA)
0.02 mg DC7-2
0.2 mg DC7-2
2 mg DC7-2
Feed intake (g/mouse)
Time after administration (h)

Figure 13

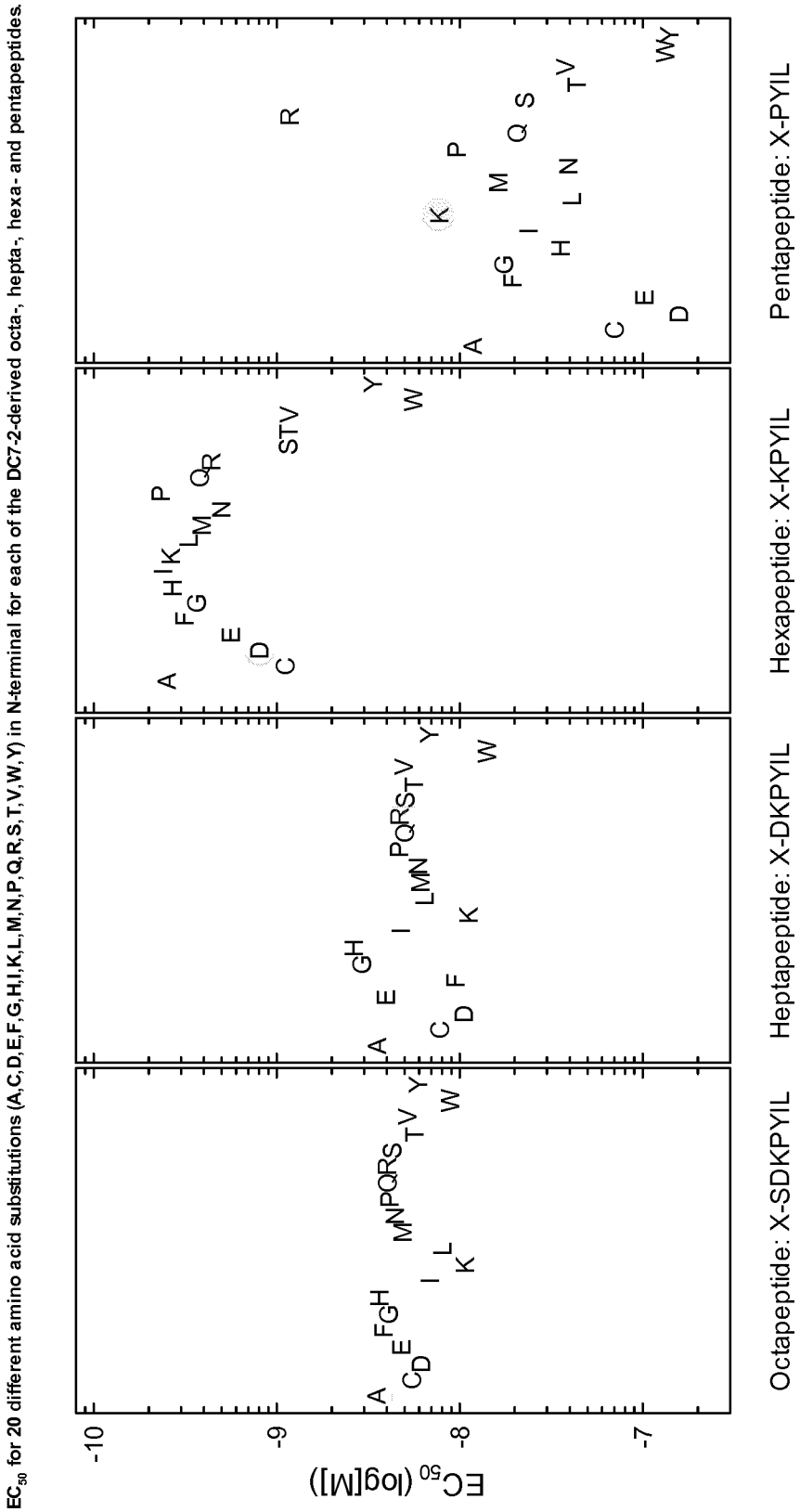

EC50 for 20 different amino acid substitutions (A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y) in N-terminal for each of the DC7-2-derived octa-, hepta-, hexa- and pentapeptides.
EC50 (log[M])
-10
-9
-8
-7
Octapeptide: X-SDKPYIL
Heptapeptide: X-DKPYIL
Hexapeptide: X-KPYIL
Pentapeptide: X-PYIL

Figure 14

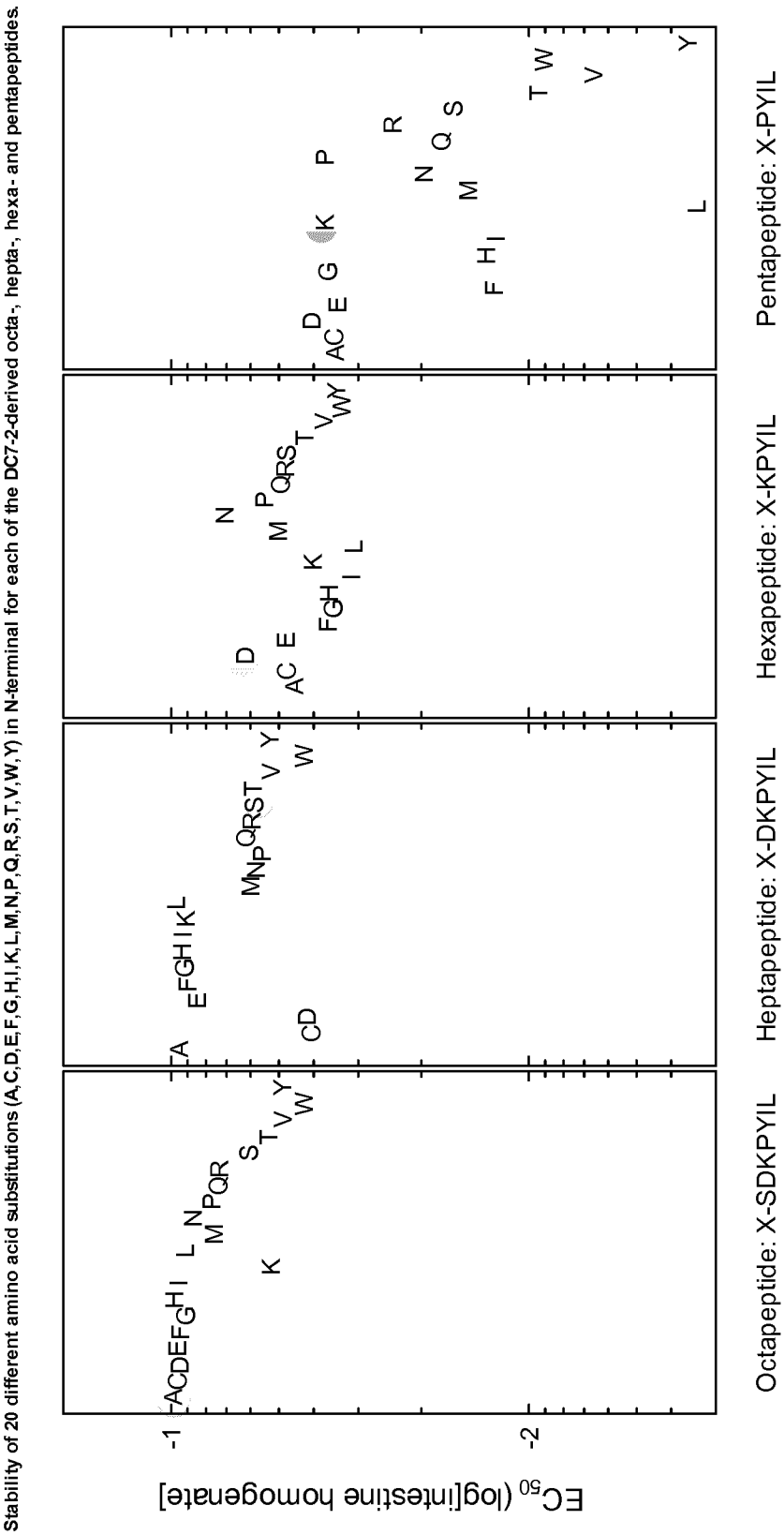

Stability of 20 different amino acid substitutions (A,C,D,E,F,G,H,I,,K,L,M,N,P,Q,R,S,T,V,W,Y) in N-terminal for each of the DC7-2-derived octa-, hepta-, hexa- and pentapeptides.
EC50 (log[intestine homogenate]
-1
-2
Octapeptide: X-SDKPYIL
Heptapeptide: X-DKPYIL
Hexapeptide: X-KPYIL
Pentapeptide: X-PYIL

Figure 15

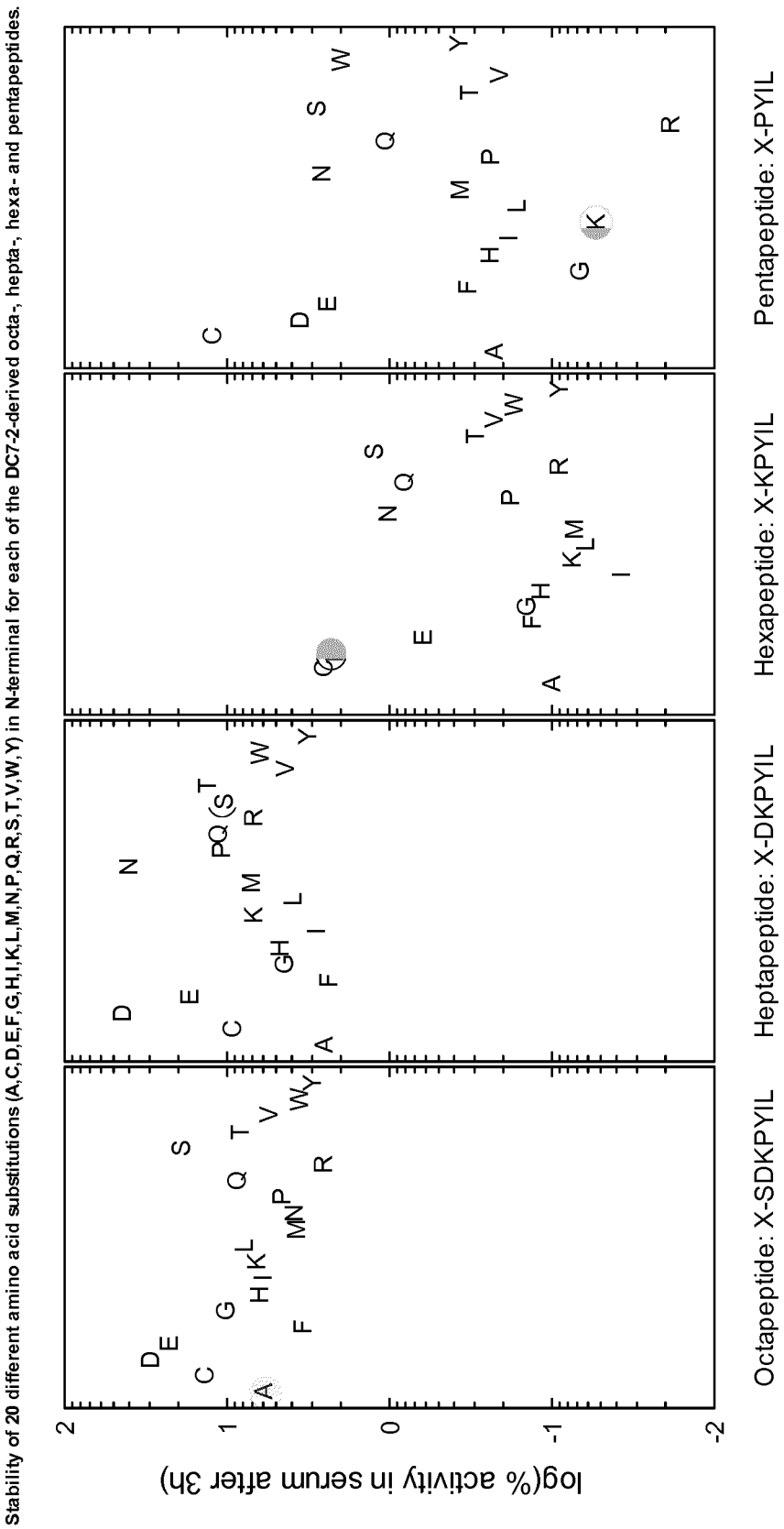

Stability of 20 different amino acid substitutions (A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y) in N-terminal for each of the DC7-2-derived octa-, hepta-, hexa- and pentapeptides.
log(% activity in serum after 3h)
2
1
0
-1
-2
Octapeptide: X-SDKPYIL
Heptapeptide: X-DKPYIL
Hexapeptide: X-KPYIL
Pentapeptide: X-PYIL

Figure 16

Stability of 20 different amino acid substitutions (A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y) in N-terminal of the DC7-2-derived hexapeptide.

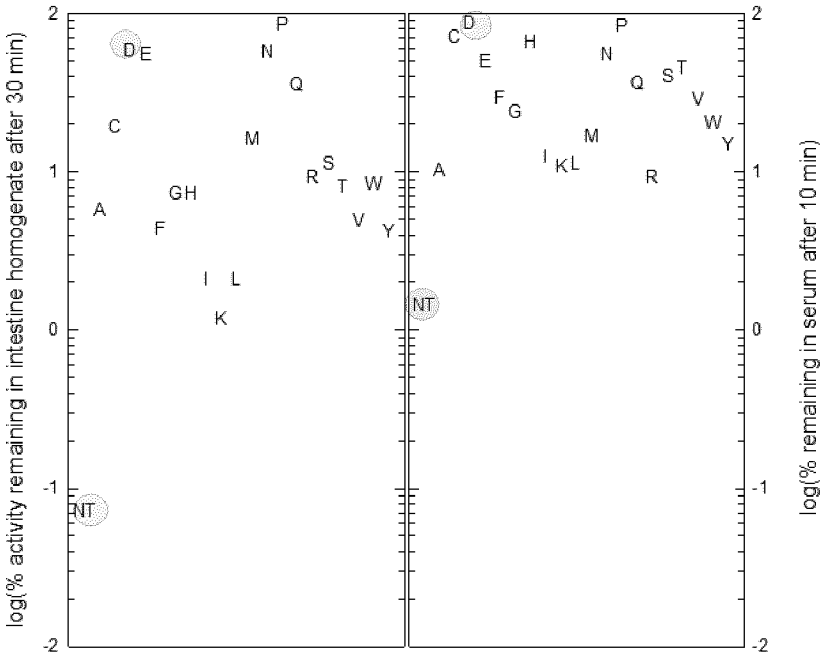


Figure 17

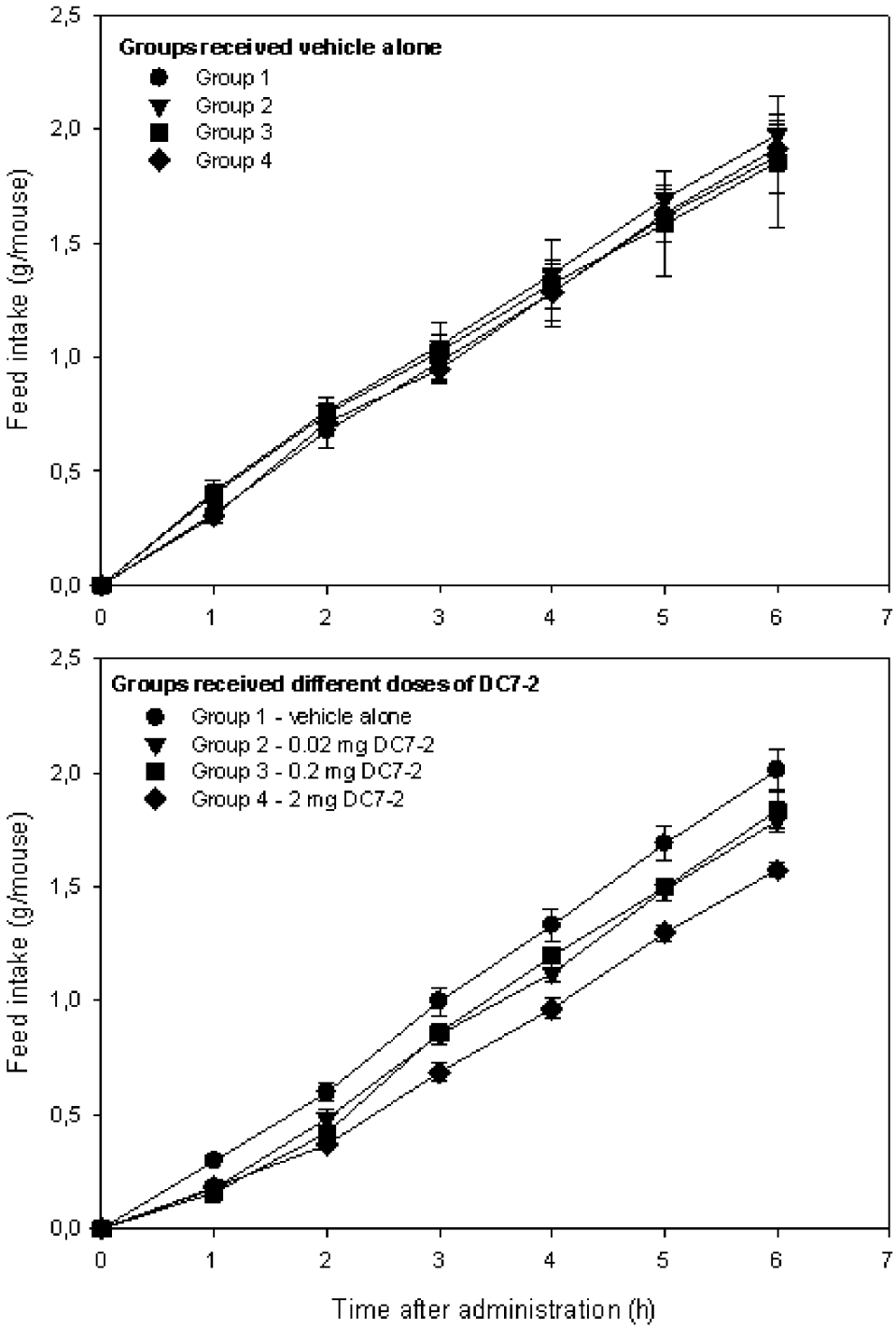
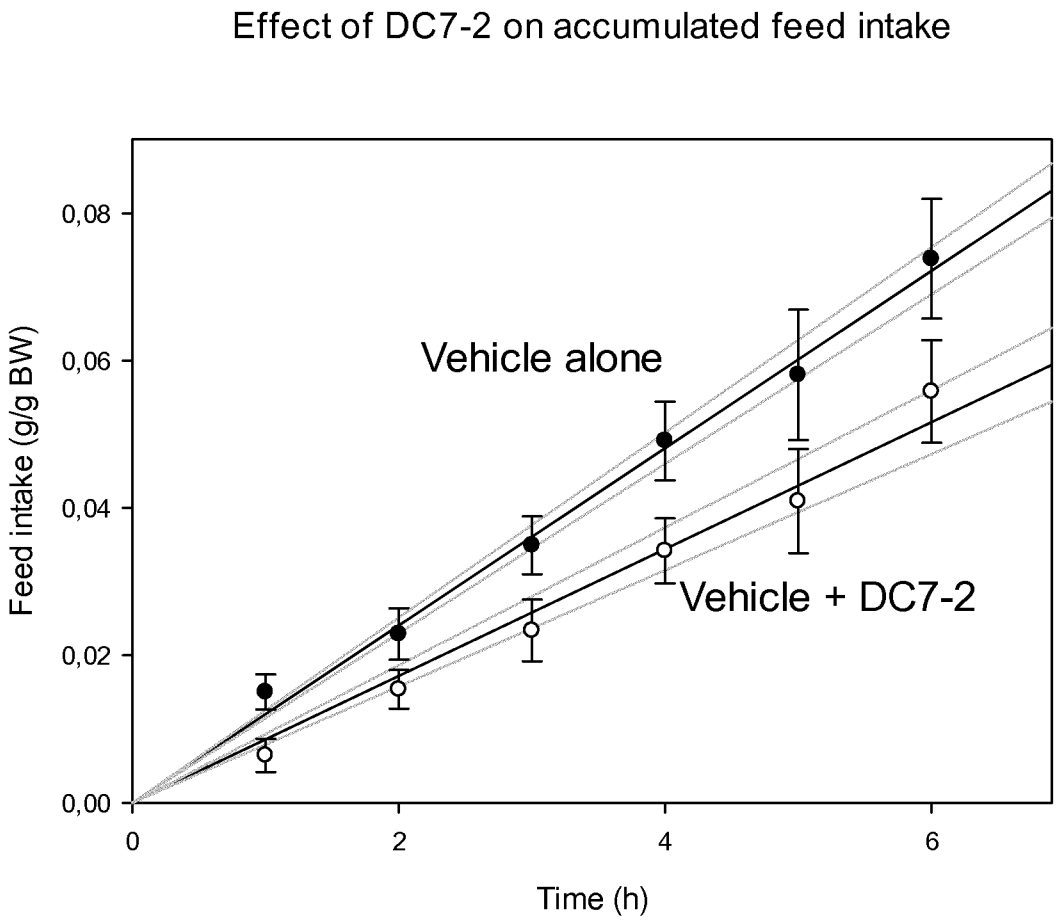
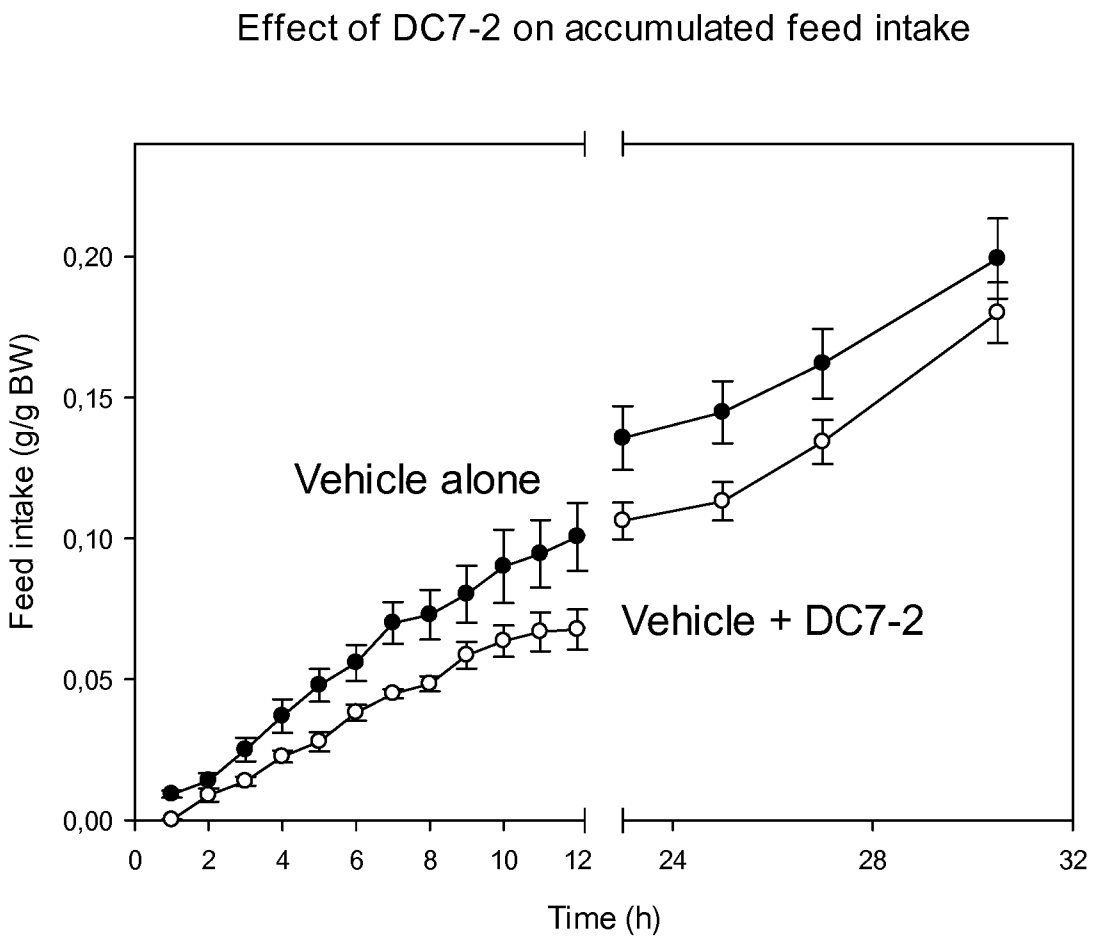

Groups received vehicle alone
Group 1
Group 2
Group 3
Group 4
Feed intake (g/mouse)
2,5
2,0
1,5
1,0
0,5
0,0
0
1
2
3
4
5
6
7
Groups received different doses of DC7-2
Group 1 - vehicle alone
Group 2 - 0.02 mg DC7-2
Group 3 - 0.2 mg DC7-2
Group 4 - 2 mg DC7-2
Feed intake (g/mouse)
Time after administration (h)

Figure 18


Effect of DC7-2 on accumulated feed intake
Feed intake (g/g BW)
0,08
0,06
0,04
0,02
0,00
Vehicle alone
Vehicle + DC7-2
0
2
4
6
Time (h)

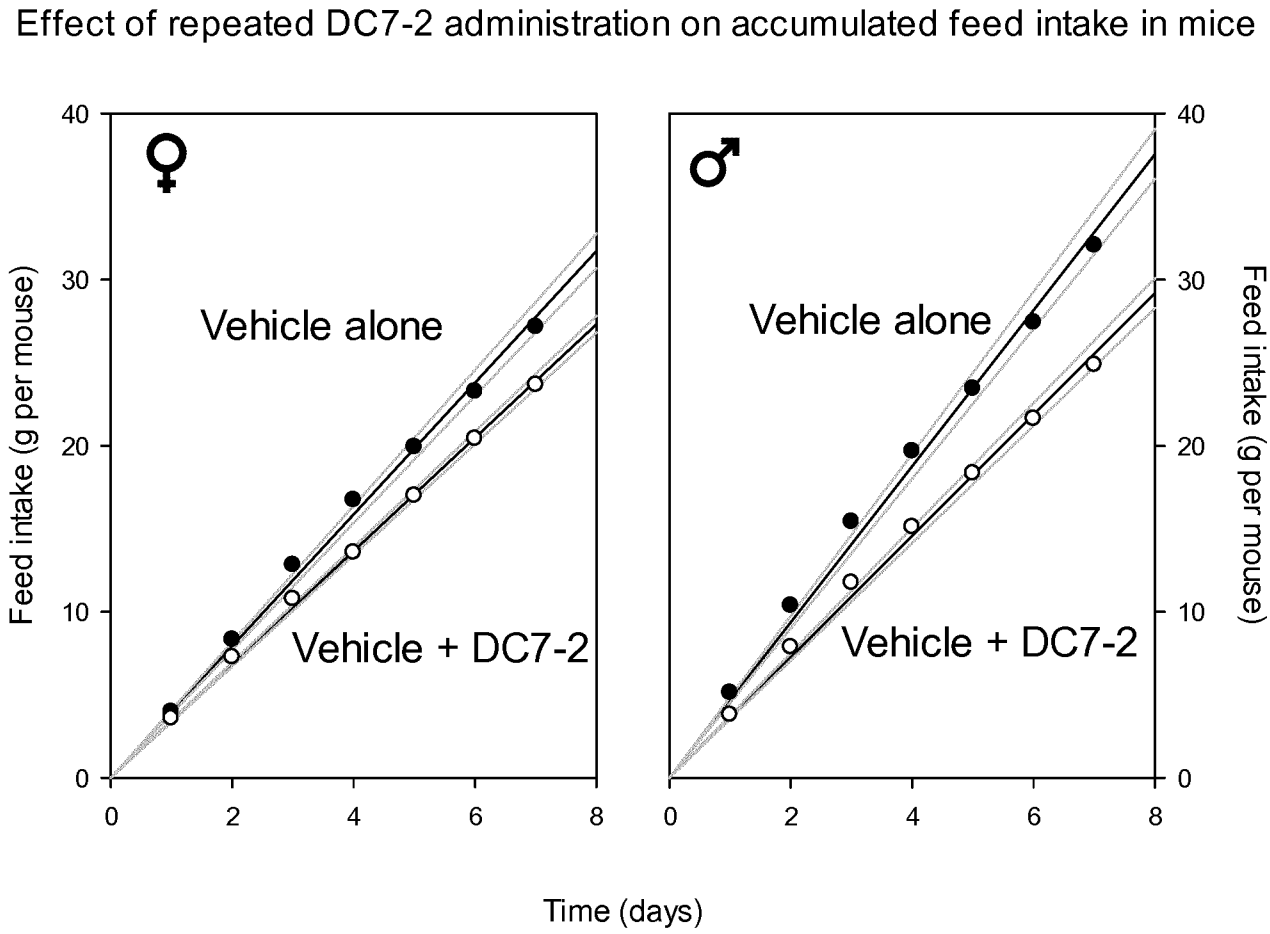
5

Figure 19


Effect of DC7-2 on accumulated feed intake
Feed intake (g/g BW)
0,00
0,05
0,10
0,15
0,20
Vehicle alone
Vehicle + DC7-2
0
2
4
6
8
10
12
24
28
32
Time (h)

5

Figure 20


Effect of repeated DC7-2 administration on accumulated feed intake in mice
♀
♂
Vehicle alone
Vehicle + DC7-2
Vehicle alone
Vehicle + DC7-2
Feed intake (g per mouse)
Feed intake (g per mouse)
Time (days)
0
10
20
30
40
0
2
4
6
8

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