

DESCRIPTION**ACTIVATING ADIPONECTIN BY CASEIN HYDROLYSATE****TECHNICAL FIELD**

[0001] The present disclosure relates to a method of stimulating adiponectin using a casein hydrolysate.

BACKGROUND ART

[0002] Adiponectin (also referred to as GBP-28, apM1, AdipoQ and 30-kDa adipocyte complement-related protein (Acrp30)) is a protein which in humans is encoded by the *ADIPOQ* gene and is secreted by adipocytes (fat cells). It is involved in regulating glucose levels as well as fatty acid breakdown.

[0003] Adiponectin is the second best known adipokine, but in contrast to leptin, has several beneficial and protective effects. These effects include anti-inflammatory, vasculoprotective and anti-diabetic effects. Adiponectin is a 247 amino-acid protein monomer which forms trimers which further polymerize into larger polymeric complexes varying in size between 180kDa (hexameres; LMW) or 400-600kDa (16-meres; HMW).

[0004] Levels of adiponectin in human blood are between 5-15 µg/ml and are decreased in subjects with insulin resistance and type 2 diabetes. In general women have a higher adiponectin plasma concentration (10-12 µg/ml) than men (7-8 µg/ml). It was also shown that adiponectin-deficient mice display diabetes. Moreover, adiponectin has been shown to promote insulin sensitivity in experimental models. Administration of adiponectin causes glucose-lowering effects and ameliorates insulin resistance. It is therefore beneficial to increase the level of adiponectin in human blood.

[0005] It has surprisingly been found that peptides selected from a casein hydrolysate activates adiponectin.

DISCLOSURE OF THE INVENTION

[0006] In a first aspect, the present disclosure is directed to a method for activating adiponectin by administering a composition comprising peptides selected from a casein hydrolysate.

[0007] In a second aspect, the present disclosure is directed to a method to reduce risk of heart attack by administering a composition comprising peptides selected from a casein hydrolysate.

[0008] In a further aspect, the present disclosure is directed to a method to maintain healthy weight by administering a composition comprising peptides selected from a casein hydrolysate.

[0009] In a preferred embodiment of the disclosure and/or embodiments thereof in the method of the disclosure the plasma concentration of adiponectin is increased.

[0010] In a preferred embodiment of the disclosure and/or embodiments thereof in the method of the disclosure the plasma concentration of adiponectin is adjusted to between 5-15 $\mu\text{g/ml}$. Woman have generally a higher adiponectin plasma concentration than men, and thus in a preferred embodiment the plasma concentration of adiponectin in woman is adjusted to 10-15 $\mu\text{g/ml}$, more preferably from 10-12 $\mu\text{g/ml}$, while for men the plasma concentration is preferably adjusted to 5-10 $\mu\text{g/ml}$, more preferably to 7-9 $\mu\text{g/ml}$.

[0011] In a preferred embodiment of the disclosure and/or embodiments thereof the composition comprising peptides selected from a casein hydrolysate is a nutritional composition.

[0012] In a preferred embodiment of the disclosure and/or embodiments thereof the casein hydrolysate is a cow's milk hydrolysate.

[0013] In a preferred embodiment of the disclosure and/or embodiments thereof the hydrolysate is an extensively hydrolyzed cow's milk peptide-containing hydrolysate.

[0014] In a preferred embodiment of the disclosure and/or embodiments thereof the hydrolysate consists of peptides with a molecular weight of more than 500 Da.

[0015] In a preferred embodiment of the disclosure and/or embodiments thereof the hydrolysate comprises at least one peptide selected from the group consisting of SEQ ID NO: 1-68, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides..

[0016] In a preferred embodiment of the disclosure and/or embodiments thereof the hydrolysate comprises at least one peptide selected from the group consisting of SEQ ID NO: 1-24, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0017] In a preferred embodiment of the disclosure and/or embodiments thereof the hydrolysate comprises at least one peptide selected from the group consisting of SEQ ID NO: 1-15, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0018] In a preferred embodiment of the disclosure and/or embodiments thereof the hydrolysate comprises at least one peptide selected from the group consisting of SEQ ID NO: 1-12, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0019] In a preferred embodiment of the disclosure and/or embodiments thereof the hydrolysate is administered in a nutritional composition, comprising a lipid or a fat phase, and a protein source.

[0020] In a preferred embodiment of the disclosure and/or embodiments thereof the nutritional composition comprises about 0.1 to about 1 g/100 kcal of a prebiotic composition, wherein the prebiotic composition comprises at least 20% of an oligosaccharide.

[0021] In a preferred embodiment of the disclosure and/or embodiments thereof the nutritional composition further comprises about 5 to about 100 mg/100 kcal of a source of long chain polyunsaturated fatty acids which comprises docosahexanoic acid.

[0022] In a preferred embodiment of the disclosure and/or embodiments thereof the nutritional composition further comprises arachidonic acid.

[0023] In a preferred embodiment of the disclosure and/or embodiments thereof hydrolysate is administered to human, preferably a child or juvenile.

[0024] In a preferred embodiment of the disclosure and/or embodiments thereof hydrolysate is administered to an adult.

[0026] In a preferred embodiment of the disclosure and/or embodiments thereof the human has a cow's milk allergy.

BRIEF DESCRIPTION OF THE DRAWINGS

[0027] Figure 1: Schematic representation of in vitro human adiponectin culture assay.

[0028] Figure 2: Results of adiponectin secretion of adipocytes upon stimulation of hydrolysate (APZ1) or single acid amino acid mixture (APAA6).

[0029] Figure 3: Graph depicting the effect of various long chain polyunsaturated fatty acids on adiponectin secretion by human primary adipocytes in the presence or absence of APZ-1.

[0030] Figure 4: Graph depicting the effect of various long chain polyunsaturated fatty acids on adiponectin intracellular expression by human primary adipocytes in the presence or absence of APZ-1.

[0031] Figure 5: Graph depicting serum adiponectin levels in mice fed a low fat diet, a high fat diet, a high fat diet with a phosphate buffered saline gavage and high fat diet supplemented with a casein hydrolysate and DHA/ARA with LGG gavage (NHLL).

BEST MODE FOR CARRYING OUT THE INVENTION

[0032] The term "nutritional composition" as used herein describes a solid or liquid formulation which can therefore be eaten or drunk by a human subject for nutrition. The nutritional composition of the disclosure preferably has a nutritional value of at least 1, more preferred at least 10 and even more preferred 50 kcal(kilo calorie)/100ml for liquid formulations and preferably at least 1, more preferred at least 10, even more preferred at least 50, such as at least 100, and most preferred at least 300 kcal/100g for dry food formulations. In a preferred embodiment of the disclosure the nutritional formulation of the

disclosure has a nutritional value of at least 50-200 kcal/100ml for liquid formulations and at least 300-600 kcal/100g for dry food formulations. A nutritional composition is distinguished from a vaccine. In contrast to a vaccine, a nutritional composition does not comprise any of adjuvants (unless as contaminations), activated or inactivated viral compounds (unless as contaminations), activated or inactivated bacterial compounds (unless as contaminations), and pathogenic compounds (unless as contaminations). The term "supplement" as used herein relates to a nutritional supplement which is a concentrated source of nutrient or alternatively other substances with a nutritional or physiological effect whose purpose is to supplement the normal diet.

[0033] In addition to the above recited ingredients further ingredients may be selected from lipids, minerals, carbohydrates, amino acids, amino acid chelates, anabolic nutrients, vitamins, antioxidants, probiotic bacterial strain and lipotropic agents in order to provide an optimal sustained energy and anabolic nutritional formulation. The nutritional composition may be a nutritional supplement or may provide complete nutrition. Preferably the nutritional composition is in the form of a dry food concentrate. The nutritional composition of the disclosure provides a human subject with increasing preference with at least 5%, at least 10%, at least 25%, at least 50%, at least 75% or at least 90% of the daily calorie requirement of a human subject. The person skilled in the art is well aware that the daily calorie requirement is dependent on the gender, height and age of a human subject. For example, a 30 year old male of 80kg body weight and 180cm height has a daily calorie requirement of around 2900 cal (calories) to maintain his body weight whereas a 30 year old female of 55kg body weight and 165cm height has a daily calorie requirement of around 2100 cal to maintain her body weight. In a preferred embodiment, the nutritional formulation of the present disclosure is an infant or a nutritional product for infants or juvenile.

[0034] The term "peptide" as used herein describes linear molecular chains of amino acids, including single chain molecules or their fragments. A peptide in accordance with the disclosure contains with increasing preference about 2 to 100 amino acids, about 5 to 50 amino acids, or about 5 to 40 amino acids. Peptides may further form oligomers consisting of at least two identical or different molecules. The corresponding higher order structures of such multimers are, correspondingly, termed homo- or heterodimers, homo- or heterotrimers etc. Furthermore, peptidomimetics of such peptides where amino acid(s) and/or peptide bond(s) have been replaced by functional analogs are also encompassed by the term "peptide". Such functional analogues include all known amino acids other than the 20 gene-encoded amino acids, such as selenocysteine. The term "peptide" also refers to naturally modified peptides where the modification is effected e.g. by glycosylation, acetylation, phosphorylation and similar modifications which are well known in the art. A peptide has to

be distinguished from a protein in the present disclosure. A protein in accordance with the present disclosure describes an organic compound made of amino acids arranged in a linear chain and folded into a globular form. Furthermore, a protein in accordance with the present disclosure describes a chain of more than 100 amino acids. Peptides may, e.g., be produced recombinantly, (semi-) synthetically, or obtained from natural sources such as after hydrolysis of proteins, all according to methods known in the art.

[0035] The term "casein hydrolysate" as used herein defines a formula which comprises peptides derived from hydrolyzed cow's casein milk proteins. In this regard, a hydrolyzed protein is a protein that has been broken down into peptides and/or component amino acids. While there are many means of achieving protein hydrolysis, two of the most common means are prolonged boiling in a strong acid or strong base or using an enzyme such as the pancreatic protease enzyme to stimulate the naturally-occurring hydrolytic process. Hydrolysis of proteins derived from milk is preferably achieved using an enzyme or a mixture of enzyme. A casein cow milk hydrolysate can comprise peptides derived from milk, wherein the proteins of said milk have been hydrolyzed to various degrees. Accordingly, one can distinguish between a partially hydrolyzed cow's milk peptide-containing hydrolysate and an extensively hydrolyzed cow's milk peptide-containing hydrolysate. In this regard, a partially hydrolyzed cow's milk peptide-containing hydrolysate comprises more than 20% of intact cow's milk protein whereas an extensively hydrolyzed cow's milk peptide-containing hydrolysate comprises less than 1% of peptides having a size of greater than 1.5kD. Furthermore, an extensively hydrolyzed cow's milk peptide-containing hydrolysate is preferably hypoallergenic.

[0036] A nonlimiting example of a method of hydrolysis is disclosed herein. In some embodiments, this method may be used to obtain the protein hydrolysate and peptides of the present disclosure. The proteins are hydrolyzed using a proteolytic enzyme, Protease N. Protease N "Amano" is commercially available from Amano Enzyme U.S.A. Co., Ltd., Elgin, Ill. Protease N is a proteolytic enzyme preparation that is derived from the bacterial species *Bacillus subtilis*. The protease powder is specified as "not less than 150,000 units/g", meaning that one unit of Protease N is the amount of enzyme which produces an amino acid equivalent to 100 micrograms of tyrosine for 60 minutes at a pH of 7.0. To produce the infant formula of the present invention, Protease N can be used at levels of about 0.5% to about 1.0% by weight of the total protein being hydrolyzed.

[0037] The protein hydrolysis by Protease N is typically conducted at a temperature of about 50° C. to about 60° C. The hydrolysis occurs for a period of time so as to obtain a degree of hydrolysis between about 4% and 10%. In a particular embodiment, hydrolysis occurs for a period of time so as to obtain a degree of hydrolysis between about 6% and 9%.

In another embodiment, hydrolysis occurs for a period of time so as to obtain a degree of hydrolysis of about 7.5%. This level of hydrolysis may take between about one half hour to about 3 hours.

[0038] A constant pH should be maintained during hydrolysis. In the method of the present invention, the pH is adjusted to and maintained between about 6.5 and 8. In a particular embodiment, the pH is maintained at about 7.0.

[0039] In order to maintain the optimal pH of the solution of whey protein, casein, water and Protease N, a caustic solution of sodium hydroxide and/or potassium hydroxide can be used to adjust the pH during hydrolysis. If sodium hydroxide is used to adjust the pH, the amount of sodium hydroxide added to the solution should be controlled to the level that it comprises less than about 0.3% of the total solid in the finished protein hydrolysate. A 10% potassium hydroxide solution can also be used to adjust the pH of the solution to the desired value, either before the enzyme is added or during the hydrolysis process in order to maintain the optimal pH.

[0040] The amount of caustic solution added to the solution during the protein hydrolysis can be controlled by a pH-stat or by adding the caustic solution continuously and proportionally. The hydrolysate can be manufactured by standard batch processes or by continuous processes.

[0041] To better ensure the consistent quality of the protein partial hydrolysate, the hydrolysate is subjected to enzyme deactivation to end the hydrolysis process. The enzyme deactivation step may consist include at heat treatment at a temperature of about 82° C. for about 10 minutes. Alternatively, the enzyme can be deactivated by heating the solution to a temperature of about 92° C. for about 5 seconds. After enzyme deactivation is complete, the hydrolysate can be stored in a liquid state at a temperature lower than 10° C.

[0042] A method for producing a protein partial hydrolysate includes the following procedure. Initially, 60.3 kg non-milk solids (milk powder) and 37.4 kg whey protein concentrate (60%) were intermixed in a tank containing water at 54° C. The slurry had a total solids content of between 20% and 23%. The pH of the slurry was then measured. Sodium and potassium hydroxide were added to the slurry to adjust the pH of the slurry to 7.0. After adjusting the pH, 0.5 kg of Amano N enzyme was added to the slurry. Following the addition of Amano N to the slurry, the pH was continuously adjusted to a pH of 7.0 using sodium hydroxide and potassium hydroxide. The total amount of sodium hydroxide added to the slurry was 0.3 kg. The total amount of potassium hydroxide added to the slurry was 1.5 kg.

[0043] The hydrolysis was permitted to occur for 90 minutes, the time starting with the addition of Amano N enzyme to the slurry. At the end of 90 minutes, the slurry was heat treated to inactivate the enzyme. The heat treatment consisted of raising the temperature of

the slurry to 82° C. for 10 minutes. The degree of hydrolysis obtained in this example was between 6% and 9%. The slurry was then cooled and spray dried to obtain a powdered hydrolysate.

[0044] A non-limiting method of determining the molecular weight distribution of the hydrolysate peptides includes. Size exclusion chromatography (SEC) to determine the molecular weight distribution of the hydrolysate peptides created by the presently-described hydrolysis process. Specifically, a sufficient amount of the powdered infant formula was weighed out to provide 0.5 grams of protein into a 50 ml conical centrifuge tube. Water was added to bring the tube to a volume of 45 ml. The mixture was placed in a Sarstedt D-5223 Mixer and mixed for one hour. After mixing, a 1% protein solution was created by adding another 5 ml of water to the tube. A stock standard was prepared and mixed for one hour as well.

[0045] Separately, 14.91 grams potassium chloride (KCl) was added to a 1000 ml beaker. The KCl was dissolved by adding 700 ml of water to the beaker. 250 ml acetonitrile and 1.0 ml trifluoroacetic acid were then added to the KCl solution (eluent). The pH was adjusted to 3.0 using a 0.2M K₂HPO₄ solution.

[0046] An HPCL reagent bottle was filled and the bottle was washed with eluent, reserving about 50 ml for dilution of samples and standards. The Hitachi L-6200 A Intelligent Pump lines were purged with eluent and the columns were equilibrated with eluent for one hour.

[0047] After the samples were mixed for one hour, 5.0 ml of each sample was pipetted into glass screw-cap tubes. 5.0 ml Dichloromethane was also pipetted into each tube. The tubes were capped and mixed by inversion four times. The samples were then centrifuged for five minutes at 200×g.

[0048] While the samples were in the centrifuge, the stock standards 1-5 were diluted with eluent (800 ul+3200 ul). Approximately 1 ml of each standard was pipetted into each of two autosampler vials and capped.

[0049] The upper (aqueous) layer of the centrifuged samples 1-10 were diluted with eluent (100 ul+900 ul). The vials were loaded into the autosampler tray as follows: blank, standard, samples and second standard. The tray was placed in the Hitachi autosampler. The total number of vials to be run were entered into the autosampler program using the keys on the front of the autosampler and the samples were run. The results indicated the molecular weight profile of the protein.

[0050] The term "peptide derived from cow's milk" as used herein defines a peptide which has an amino acid sequence which is a partial amino acid sequence of a cow's milk protein. Such peptides may be obtained as outlined above by hydrolysis or may be

synthesized in vitro by methods known to the skilled person and described in the examples of the disclosure.

[0051] The term "peptide-containing fraction of the hydrolysate" refers to a mixture of peptides comprising at least 2, preferably at least 5, more preferably at least 10 and most preferably at least 20 which have been isolated from the hydrolysate of the disclosure by filtration techniques which are known to the skilled person. Furthermore, techniques for the isolation of peptides from the hydrolysate of the disclosure are described herein below.

[0052] The term "child" or the term "juvenile" is used herein in accordance with the definitions provided in the art. Thus, the term "child" means a human subject between the stages of birth and the age of about 10 and the term "juvenile" means a human subject between the age of about 10 and puberty (before sexual maturity).

[0053] The term "adult" is used herein in accordance with the definitions provided in the art. Thus, this term means a human subject after puberty (after sexual maturity). A further preferred embodiment of the disclosure relates to the nutritional formulation of the disclosure, wherein the human subject has a cow's milk allergy.

[0054] The term "cow's milk allergy" describes a food allergy, i.e. an immune adverse reaction to one or more of the proteins contained in cow's milk in a human subject. The principal symptoms are gastrointestinal, dermatological and respiratory symptoms. These can translate into skin rashes, hives, vomiting, diarrhea, constipation and distress. The clinical spectrum extends to diverse disorders: anaphylactic reactions, atopic dermatitis, wheeze, infantile colic, gastro esophageal reflux disease (GERD), esophagitis, colitis gastroenteritis, headache/migraine and constipation.

[0055] The present inventors have surprisingly found that a casein hydrolysate has a beneficial effect on plasma concentration of adiponectin. Low adiponectin plasma levels are associated with type 1 diabetes, and increased weight.

[0056] It was also found that an extensively hydrolyzed cow's milk peptide-containing hydrolysate had positive effects on the plasma concentration of adiponectin. Suitable hydrolysates casein hydrolysates include casein hydrolysates. It was furthermore found that dialysis of the hydrolysate with a cut-off of 500 Da so as to include peptide sequences 500 Da and larger renders a hydrolysate fraction that has even better effect on the adiponectin plasma concentration. Accordingly, in particular embodiments, the hydrolysate comprises peptides with a molecular weight of more than 500 Da, and in further embodiments, the hydrolysate comprises peptides with a molecular weight in a range of 500 to 2000 Da. In other embodiments, the hydrolysate consists of peptides with a molecular weight of more than 500 Da, and in further embodiments, the hydrolysate consists of peptides with a molecular weight in a range of 500 to 2000 Da.

[0057] The following peptides have been identified as possibly contributing to the beneficial effect on adiponectin levels:

Table 1: identified peptide in the hydrolysate:

SEQ ID NO: 1*	IPNPIG
SEQ ID NO: 2	IGSESTEDQ
SEQ ID NO 3:	DKTEIPT
SEQ ID NO: 4	IVPN
SEQ ID NO: 5	LEDSP
SEQ ID NO: 6	NQEQPI
SEQ ID NO: 7	NVPGE
SEQ ID NO: 8	PFP GPI
SEQ ID NO: 9	TEDEL
SEQ ID NO: 10	VPSE
SEQ ID NO: 11	YFP GP
SEQ ID NO: 12	YPSGA
SEQ ID NO 13	FPGPI
SEQ ID NO: 14	MHQPHQPLPPT
SEQ ID NO: 15	YFP GPIN
SEQ ID NO: 16	DMEST
SEQ ID NO: 17	FPGPIN
SEQ ID NO: 18	IPNPI
SEQ ID NO: 19	MESTEV
SEQ ID NO: 20	PGPIN
SEQ ID NO: 21	PHQPLPPT
SEQ ID NO: 22	PNPI
SEQ ID NO: 23	SKDIGSE
SEQ ID NO: 24	YFP GPI
SEQ ID NO: 25	AINPSKEN
SEQ ID NO: 26	APFPE
SEQ ID NO: 27	DIGSES
SEQ ID NO: 28	DMPI
SEQ ID NO: 29	DVPS
SEQ ID NO: 30	EDI
SEQ ID NO: 31	ELF
SEQ ID NO: 32	EMP

SEQ ID NO: 33	ETAPVPL
SEQ ID NO: 34	GPFP
SEQ ID NO: 35	GPIV
SEQ ID NO: 36	IGSSSEES
SEQ ID NO: 37	IGSSSEESA
SEQ ID NO: 38	INPSKE
SEQ ID NO: 39	IPPLTQTPV
SEQ ID NO: 40	ITAP
SEQ ID NO: 41	KHQGLPQ
SEQ ID NO: 42	LDVTP
SEQ ID NO: 43	LPLPL
SEQ ID NO: 44	NAVPI
SEQ ID NO: 45	NEVEA
SEQ ID NO: 46	NLL
SEQ ID NO: 47	PITPT
SEQ ID NO: 48	PNSLPQ
SEQ ID NO: 49	PQLEIVPN
SEQ ID NO: 50	PQNIPPL
SEQ ID NO: 51	PVLGPV
SEQ ID NO: 52	PVPQ
SEQ ID NO: 53	PVVVP
SEQ ID NO: 54	PVVVPP
SEQ ID NO: 55	SIGSSSEESAE
SEQ ID NO: 56	SISSEE
SEQ ID NO: 57	SISSEEIVPN
SEQ ID NO: 58	SPPEIN
SEQ ID NO: 59	SPPEINT
SEQ ID NO: 60	TDAPSFS
SEQ ID NO: 61	VATEEV
SEQ ID NO: 62	VLPVP
SEQ ID NO: 63	VPGE
SEQ ID NO: 64	VPGEIV
SEQ ID NO: 65	VPITPT
SEQ ID NO: 66	VVPPFLQPE
SEQ ID NO: 67	VVPP

SEQ ID NO: 68

YPVEP

[0058] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 1 and at least one peptide selected from the group consisting of SEQ ID NO: 2-68, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0059] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 2 and at least one peptide selected from the group consisting of SEQ ID NO:1, and SEQ ID NO: 3-68, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0060] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 3 and at least one peptide selected from the group consisting of SEQ ID NO:1-2, and SEQ ID NO: 4-68, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0061] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 4 and at least one peptide selected from the group consisting of SEQ ID NO: 1-3, and SEQ ID NO: 5-68, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0062] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 5 and at least one peptide selected from the group consisting of SEQ ID NO: 1-4, and SEQ ID NO: 6-68, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0063] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 6 and at least one peptide selected from the group consisting of SEQ ID NO:1-5, and SEQ ID NO: 7-68, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0064] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 7 and at least one peptide selected from the group consisting of SEQ ID NO:1-6, and SEQ ID NO: 8-68, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0065] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 8 and at least one peptide selected from the group consisting of SEQ ID NO: 1-7, and SEQ ID NO: 9-68, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0066] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 9 and at least one peptide selected from the group consisting of SEQ ID NO: 1-8, and SEQ ID NO: 10-68, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0067] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 10 and at least one peptide selected from the group consisting of SEQ ID NO: 1-9, and SEQ ID NO: 11-68, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0068] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 11 and at least one peptide selected from the group consisting of SEQ ID NO: 1-10, and SEQ ID NO: 12-68, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0069] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 12 and at least one peptide selected from the group consisting of SEQ ID NO: 1-11, and SEQ ID NO: 13-68, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0070] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 13 and at least one peptide selected from the group consisting of SEQ ID NO: 1-12, and SEQ ID NO: 14-68, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0071] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 14 and at least one peptide selected from the group consisting of SEQ ID NO: 1-13, and SEQ ID NO: 15-68, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0072] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 15 and at least one peptide selected from the group consisting of SEQ ID NO: 1-14, and SEQ ID NO: 16-68, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0073] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 16 and at least one peptide selected from the group consisting of SEQ ID NO: 1-15, and SEQ ID NO: 17-68, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0074] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 17 and at least one peptide selected from the group consisting of SEQ ID NO: 1-16, and SEQ ID NO: 18-68, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0075] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 18 and at least one peptide selected from the group consisting of SEQ ID NO: 1-17, and SEQ ID NO: 19-68, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0076] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 19 and at least one peptide selected from the group consisting of SEQ ID NO: 1-18, and SEQ ID NO: 20-68, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0077] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 20 and at least one peptide selected from the group consisting of SEQ ID NO: 1-19, and SEQ ID NO: 21-68, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0078] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 21 and at least one peptide selected from the group consisting of SEQ ID NO: 1-20, and SEQ ID NO: 22-68, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0079] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 22 and at least one peptide selected from the group consisting of SEQ ID NO: 1-21, and SEQ ID NO: 23-68, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0080] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 23 and at least one peptide selected from the group consisting of SEQ ID NO: 1-22, and SEQ ID NO: 24-68, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0081] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 24 and at least one peptide selected from the group consisting of SEQ ID NO: 1-23, and SEQ ID NO: 25-68, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0082] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 1 and at least one peptide selected from the group consisting of SEQ ID NO: 2- 24, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0083] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 2 and at least one peptide selected from the group consisting of SEQ ID NO: 1, and SEQ ID NO: 3- 24, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0084] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 3 and at least one peptide selected from the group consisting of SEQ ID NO: 1-2, and SEQ ID NO: 4- 24, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0085] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 4 and at least one peptide selected from the group consisting of SEQ ID NO: 1-3, and SEQ ID NO: 5- 24, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0086] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 5 and at least one peptide

selected from the group consisting of SEQ ID NO: 1-4, and SEQ ID NO: 6- 24, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0087] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 6 and at least one peptide selected from the group consisting of SEQ ID NO: 1-5, and SEQ ID NO: 7- 24, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0088] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 7 and at least one peptide selected from the group consisting of SEQ ID NO: 1-6, and SEQ ID NO: 8- 24, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0089] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 8 and at least one peptide selected from the group consisting of SEQ ID NO: 1-7, and SEQ ID NO: 9- 24, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0090] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 9 and at least one peptide selected from the group consisting of SEQ ID NO: 1-8, and SEQ ID NO: 10- 24, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0091] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 10 and at least one peptide selected from the group consisting of SEQ ID NO: 1-9, and SEQ ID NO: 11- 24, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0092] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 11 and at least one peptide selected from the group consisting of SEQ ID NO: 1-10, and SEQ ID NO: 12- 24, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0093] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 12 and at least one peptide selected from the group consisting of SEQ ID NO: 1-11, and SEQ ID NO: 13- 24, preferably

at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0094] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 13 and at least one peptide selected from the group consisting of SEQ ID NO: 1-12, and SEQ ID NO: 14- 24, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0095] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 14 and at least one peptide selected from the group consisting of SEQ ID NO: 1-13, and SEQ ID NO: 15- 24, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0096] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 15 and at least one peptide selected from the group consisting of SEQ ID NO: 1-14, and SEQ ID NO: 16- 24, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0097] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 16 and at least one peptide selected from the group consisting of SEQ ID NO: 1-15, and SEQ ID NO: 17- 24, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0098] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 17 and at least one peptide selected from the group consisting of SEQ ID NO: 1-16, and SEQ ID NO: 18- 24, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0099] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 18 and at least one peptide selected from the group consisting of SEQ ID NO: 1-17, and SEQ ID NO: 19- 24, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0100] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 19 and at least one peptide selected from the group consisting of SEQ ID NO: 1-18, and SEQ ID NO: 20- 24, preferably

at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0101] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 20 and at least one peptide selected from the group consisting of SEQ ID NO: 1-19, and SEQ ID NO: 21- 24, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0102] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 21 and at least one peptide selected from the group consisting of SEQ ID NO: 1-20, and SEQ ID NO: 22- 24, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0103] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 22 and at least one peptide selected from the group consisting of SEQ ID NO: 1-21, and SEQ ID NO: 23- 24, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0104] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 23 and at least one peptide selected from the group consisting of SEQ ID NO: 1-22, and SEQ ID NO: 24, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0105] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 24 and at least one peptide selected from the group consisting of SEQ ID NO: 1-23, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0106] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 1 and at least one peptide selected from the group consisting of SEQ ID NO: 2-15, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0107] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 2 and at least one peptide selected from the group consisting of SEQ ID NO: 1, and SEQ ID NO: 3- 15, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0108] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 3 and at least one peptide selected from the group consisting of SEQ ID NO: 1-2, and SEQ ID NO: 4-15, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0109] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 4 and at least one peptide selected from the group consisting of SEQ ID NO: 1-3, and SEQ ID NO: 5-15, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0110] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 5 and at least one peptide selected from the group consisting of SEQ ID NO: 1-4, and SEQ ID NO: 6-15, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0111] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 6 and at least one peptide selected from the group consisting of SEQ ID NO: 1-5, and SEQ ID NO: 7-15, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0112] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 7 and at least one peptide selected from the group consisting of SEQ ID NO: 1-6, and SEQ ID NO: 8- 15, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0113] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 8 and at least one peptide selected from the group consisting of SEQ ID NO: 1-7, and SEQ ID NO: 9-15, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0114] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 9 and at least one peptide selected from the group consisting of SEQ ID NO: 1-8, and SEQ ID NO: 10-15, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0115] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 10 and at least one peptide selected from the group consisting of SEQ ID NO: 1-9, and SEQ ID NO: 11-15, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0116] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 11 and at least one peptide selected from the group consisting of SEQ ID NO: 1-10, and SEQ ID NO: 12-15, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0117] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 12 and at least one peptide selected from the group consisting of SEQ ID NO: 1-11, and SEQ ID NO: 13-15, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0118] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 13 and at least one peptide selected from the group consisting of SEQ ID NO: 1-12, and SEQ ID NO: 14-15, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0119] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 14 and at least one peptide selected from the group consisting of SEQ ID NO: 1-13, and SEQ ID NO: 15, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0120] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 15 and at least one peptide selected from the group consisting of SEQ ID NO: 1-14, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0121] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 16 and at least one peptide selected from the group consisting of SEQ ID NO: 1-15, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0122] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 17 and at least one peptide

selected from the group consisting of SEQ ID NO: 1-15, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0123] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 18 and at least one peptide selected from the group consisting of SEQ ID NO: 1-15, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0124] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 19 and at least one peptide selected from the group consisting of SEQ ID NO: 1-15, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0125] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 20 and at least one peptide selected from the group consisting of SEQ ID NO: 1-15, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0126] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 21 and at least one peptide selected from the group consisting of SEQ ID NO: 1-15, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0127] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 22 and at least one peptide selected from the group consisting of SEQ ID NO: 1-15, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0128] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 23 and at least one peptide selected from the group consisting of SEQ ID NO: 1-15, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0129] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 24 and at least one peptide selected from the group consisting of SEQ ID NO: 1-15, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0130] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 1 and at least one peptide selected from the group consisting of SEQ ID NO: 2-12, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0131] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 2 and at least one peptide

selected from the group consisting of SEQ ID NO: 1, and SEQ ID NO: 3-12, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0132] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 3 and at least one peptide selected from the group consisting of SEQ ID NO: 1-2, and SEQ ID NO: 4-12, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0133] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 4 and at least one peptide selected from the group consisting of SEQ ID NO: 1-3, and SEQ ID NO: 5-12, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0134] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 5 and at least one peptide selected from the group consisting of SEQ ID NO: 1-4, and SEQ ID NO: 6-12, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0135] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 6 and at least one peptide selected from the group consisting of SEQ ID NO: 1-5, and SEQ ID NO: 7-12, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0136] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 7 and at least one peptide selected from the group consisting of SEQ ID NO: 1-6, and SEQ ID NO: 8-12, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0137] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 8 and at least one peptide selected from the group consisting of SEQ ID NO: 1-7, and SEQ ID NO: 9-12, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0138] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 9 and at least one peptide selected from the group consisting of SEQ ID NO: 1-8, and SEQ ID NO: 10-12, preferably at

least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0139] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 10 and at least one peptide selected from the group consisting of SEQ ID NO: 1-9, and SEQ ID NO: 11-12, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0140] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 11 and at least one peptide selected from the group consisting of SEQ ID NO: 1-10, and SEQ ID NO: 12, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0141] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 12 and at least one peptide selected from the group consisting of SEQ ID NO: 1-11, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0142] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 13 and at least one peptide selected from the group consisting of SEQ ID NO: 1-12, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0143] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 14 and at least one peptide selected from the group consisting of SEQ ID NO: 1-12, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0144] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 15 and at least one peptide selected from the group consisting of SEQ ID NO: 1-12, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0145] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 16 and at least one peptide selected from the group consisting of SEQ ID NO: 1-12, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0146] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 17 and at least one peptide selected from the group consisting of SEQ ID NO: 1-12, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0147] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 18 and at least one peptide selected from the group consisting of SEQ ID NO: 1-12, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0148] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 19 and at least one peptide selected from the group consisting of SEQ ID NO: 1-12, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0149] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 20 and at least one peptide selected from the group consisting of SEQ ID NO: 1-12, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0150] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 21 and at least one peptide selected from the group consisting of SEQ ID NO: 1-12, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0151] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 22 and at least one peptide selected from the group consisting of SEQ ID NO: 1-12, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0152] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 23 and at least one peptide selected from the group consisting of SEQ ID NO: 1-12, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0153] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate comprises a peptide with SEQ ID NO: 24 and at least one peptide selected from the group consisting of SEQ ID NO: 1-12, preferably at least 2 peptides, preferably at least 3 peptides, preferably at least 4 peptides, preferably at least 5 peptides.

[0154] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate is a hydrolysate comprising combinations of SEQ ID NO: 1, and SEQ ID NO: 2.

[0155] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate is a hydrolysate comprising combinations of SEQ ID NO: 1, and SEQ ID NO: 3.

[0156] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate is a hydrolysate comprising combinations of SEQ ID NO: 1, and SEQ ID NO: 4.

[0157] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate is a hydrolysate comprising combinations of SEQ ID NO: 1, and SEQ ID NO: 5.

[0158] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate is a hydrolysate comprising combinations of SEQ ID NO: 1, and SEQ ID NO: 6.

[0159] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate is a hydrolysate comprising combinations of SEQ ID NO: 1, and SEQ ID NO: 7.

[0160] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate is a hydrolysate comprising combinations of SEQ ID NO: 1, and SEQ ID NO: 8.

[0161] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate is a hydrolysate comprising combinations of SEQ ID NO: 1, and SEQ ID NO: 9.

[0162] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate is a hydrolysate comprising combinations of SEQ ID NO: 1, and SEQ ID NO: 10.

[0163] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate is a hydrolysate comprising combinations of SEQ ID NO: 1, and SEQ ID NO: 11.

[0164] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate is a hydrolysate comprising combinations of SEQ ID NO: 1, and SEQ ID NO: 12.

[0165] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate is a hydrolysate comprising combinations of SEQ ID NO: 1, and SEQ ID NO: 13.

[0166] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate is a hydrolysate comprising combinations of SEQ ID NO: 1, and SEQ ID NO: 14.

[0167] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate is a hydrolysate comprising combinations of SEQ ID NO: 1, and SEQ ID NO: 15.

[0168] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate is a hydrolysate comprising combinations of SEQ ID NO: 1, and SEQ ID NO: 16.

[0169] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate is a hydrolysate comprising combinations of SEQ ID NO: 1, and SEQ ID NO: 17.

[0170] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate is a hydrolysate comprising combinations of SEQ ID NO: 1, and SEQ ID NO: 18.

[0171] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate is a hydrolysate comprising combinations of SEQ ID NO: 1, and SEQ ID NO: 19.

[0172] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate is a hydrolysate comprising combinations of SEQ ID NO: 1, and SEQ ID NO: 20.

[0173] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate is a hydrolysate comprising combinations of SEQ ID NO: 1, and SEQ ID NO: 21.

[0174] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate is a hydrolysate comprising combinations of SEQ ID NO: 1, and SEQ ID NO: 22.

[0175] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate is a hydrolysate comprising combinations of SEQ ID NO: 1, and SEQ ID NO: 23.

[0176] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate is a hydrolysate comprising combinations of SEQ ID NO: 1, and SEQ ID NO: 24.

[0177] In a preferred embodiment of the disclosure and/or embodiments thereof the nutritional formulation additionally comprises one or more of carbohydrates, nucleic acids, lipids, minerals, anabolic nutrients, vitamins, antioxidants, probiotic bacterial strains and lipotropic agents.

[0178] In a preferred embodiment of the present disclosure and/or embodiments thereof the nutritional composition comprises a fat phase wherein the lipid or fat is present at a level of up to about 7 g/100 kcal.

[0179] In a preferred embodiment of the present disclosure and/or embodiments thereof the nutritional composition wherein the protein source is present at a level of up to about 5 g/100 kcal.

[0180] In a preferred embodiment of the present disclosure and/or embodiments thereof the nutritional composition comprises an oligosaccharide wherein the oligosaccharide comprises galacto-oligosaccharide.

[0181] In a preferred embodiment of the present disclosure and/or embodiments thereof the nutritional composition further comprises polydextrose.

[0182] The present disclosure is also directed to a peptide-containing fraction of a casein hydrolysate for use in activating adiponectin.

[0183] The present is also directed to peptides selected from a casein hydrolysate for use in maintaining healthy weight, by administering a composition comprising peptides selected from a casein hydrolysate.

[0184] The present is also directed to peptides selected from a casein hydrolysate for use in reducing the risk of heart attack by administering a composition comprising peptides selected from a casein hydrolysate.

[0185] In a preferred embodiment of the present disclosure and/or embodiments thereof the plasma concentration of adiponectin is increased. Preferably the plasma concentration of adiponectin is adjusted to between 5-10 mg/ml.

[0186] In a preferred embodiment of the present disclosure and/or embodiments thereof the composition comprising peptides selected from a casein hydrolysate is a nutritional composition.

[0187] In a preferred embodiment of the present disclosure and/or embodiments thereof the casein hydrolysate is a cow's milk hydrolysate.

[0188] In a preferred embodiment of the present disclosure and/or embodiments thereof the hydrolysate is an extensively hydrolyzed cow's milk peptide-containing hydrolysate.

[0189] The preferred embodiments of the method of the disclosure and/or embodiments thereof are also preferred embodiments of the casein hydrolysate for use in activating adiponectin, for use in maintaining healthy weight, and/or for use in reducing the risk of heart attack.

[0190] The disclosure is now exemplified by the following non limiting examples.

EXAMPLES

Example 1:

Adipocyte isolation and culture

[0191] Subcutaneous adipose tissue was obtained from healthy lean or moderately overweight women undergoing plastic surgery. The procedure was approved by the ethical committee of the Heinrich-Heine-University (Düsseldorf, Germany). Preadipocytes were isolated by collagenase digestion. Isolated cell pellets were resuspended in DMEM/F12 medium supplemented with 10% FCS, seeded in six-well or 12-well culture dishes, respectively, and maintained at 37°C with 5% CO₂. After reaching confluence (day 0 of differentiation), cell cultures were incubated in an adipocyte differentiation medium (DMEM/F12, 33 mM biotin, 17 mmol/l d-pantothenic-acid, 66 nM insulin, 1 nM triiodo-L-thyronine, 100 nM cortisol, 10 mg/ml apo-transferrin, 50 mg/ml gentamycin, 0.25 mg/ml amphotericin B, 15 mM HEPES, 14 nM NaHCO₃, pH 7.4) with troglitazone (5 µM) for 3 days. Once differentiation was started the cells were further incubated in adipocyte differentiation medium with medium changes every 2-3 days for a total differentiation period of 14 days.

[0192] After the differentiation period (14 days), the adipocytes were challenged with extensive casein hydrolysate at different concentrations (0.01%, 0.1% and 1%, respectively) for 24 h. See figure 1 for a schematic overview of the in vitro human primary adipocyte cultures assay.

Adiponectin release upon casein hydrolysate stimulation

[0193] The isolated human preadipocytes were carefully counted and the same cell number per well was plated. After the differentiation period, the cells are treated with casein hydrolysate at 0.01%, 0.1% and 1%. After 24 h, the supernatants were collected and stored at -20°C for analysis of adipokine content with an ELISA kit.

[0194] The ELISA kits includes a plate with wells that are coated with a primary antibody against human adiponectin. The supernatants are added and after the appropriate incubation time, the sample is washed so that only the adipokine bound to the antibody is left. Another buffer containing the secondary antibody conjugated with HRP is added to the wells. After the indicated incubation time, the excess of secondary antibody is removed by washing and the remaining HRP bound to the adipokine-antibody complex reacts when adding the TMB buffer. The reaction is stopped by adding an acidic solution and the reacting yellow colour is measured. The absorbance is proportional to the yellow colour which indicates the presence of the adipokine of interest. A standard curve is obtained by plotting the concentration of the standards versus their absorbances, interpolating from the standard curve the concentration of adipokine in the sample is calculated.

[0195] The kits included a standard of human recombinant adiponectin used to calculate the adiponectin concentration. Moreover, the kits include a Quality control high and low standards with known concentrations. The kit for adiponectin ELISA recognizes natural and

recombinant human adiponectin (full length, mutation-modified trimer only forming and globular domain).

[0196] Once the incubation conditions were validated, and unspecific effects of the milk fractions alone were discarded, we assessed the adiponectin secretion in the supernatants of the adipocytes previously stimulated with casein hydrolysate.

[0197] A casein hydrolysate of the present disclosure (APZ1) triggered a significant upregulation of adiponectin secretion at 1%. ($290.6 \pm 56.6\%$ vs. control, see figure), following a dose-dependent trend. The effect of the APZ1 fraction is independent of an single amino acid mixture (APAA6), the latter did not exert any significant effect on adiponectin secretion. See figure 2 showing the results of adiponectin secretion of adipocytes upon stimulation of hydrolysate (APZ1) or single acid amino acid mixture (APAA6).

Example 2

[0198] The effect of casein hydrolysate (APZ-1) on adiponectin secretion and intracellular expression was evaluated in primary human adipocytes was investigated. As depicted in Figure 3, APZ-1 (1%) combined with ARA:DHA (100 μ M:50 μ M) does not appear to enhance adiponectin secretion by primary human adipocytes when compared to LCPUFA's in the absence of APZ-1 when measured at 24 hours. However, Figure 4 shows that APZ-1 combined with ARA:DHA (100 μ M:50 μ M) does synergistically increase adiponectin intracellular expression by human primary adipocytes compared to the effect of LC-PUFAs in the absence of APZ-1 at 24 hours. Since adiponectin expression is seen at 24 hours, it is therefore possible that adiponectin secretion by the adipocytes would be increased at a later time point.

[0199] The effect of a high fat diet (HFD) supplemented with a casein hydrolysate and DHA/ARA, along with oral gavage administration of LGG on the development of risk factors for CVD and type 2 diabetes and the inflammatory state of adipose tissue was evaluated. Low fat diet (LFD) and high fat diet (HFD) fed mice were used as references. The effect of the above formulation was compared to a HFD + phosphate buffered saline (PBS) gavage control group to control for the repeated oral gavage treatment with LGG.

[0200] The treatment of mice with a high fat diet supplemented with a casein hydrolysate and DHA/ARA with LGG gavage (NHLL) showed beneficial effects compared to the HFD with PBS gavage control mice. As seen in Figure 5, mice given a high fat diet supplemented with NHLL demonstrated an increase in serum adiponectin levels. Additional specific beneficial effects included lower body weight gain despite an increased food intake, Lower body weight gain, despite an increased food intake, body adiposity was strongly reduced, lower fasting insulin, lower plasma cholesterol, lower plasma triglycerides, lower Serum Amyloid A (SAA; systemic inflammation marker), lower microalbuminurea (suggesting improved kidney

function), lower mesenteric and inguinal fat mass, strongly diminished epididymal fat inflammation, lower liver mass and circulating alanine aminotransferase (ALT) levels, improved histopathology, and reduced inflammation.

CLAIMS

What is claimed is:

1. A method for promoting healthy weight in a subject by administering a composition comprising a casein hydrolysate to the subject, wherein the hydrolysate consists of peptides with a molecular weight of more than 500 Da, wherein the peptides comprise at least 10 peptides selected from the following: SEQ ID NO: 1-3, SEQ ID NO: 5-12, SEQ ID NO: 14-16, SEQ ID NO: 18-20, SEQ ID NO: 22-23, SEQ ID NO: 25-29, SEQ ID NO: 33-45, SEQ ID NO: 47-56, SEQ ID NO: 58-59, SEQ ID NO: 61-62 and SEQ ID NO: 64-68, further wherein healthy weight is promoted by an increase in the concentration of adiponectin in the subject.
2. The method according to claim 1, wherein the plasma concentration of adiponectin is adjusted to between 5-15 $\mu\text{g/ml}$
3. The method according to claim 1, wherein the hydrolysate comprises at least three additional peptides selected from the group consisting of SEQ ID NO: 1-29, SEQ ID NO: 33-45 and SEQ ID NO: 47-68.
4. The method according to claim 3, wherein the hydrolysate comprises at least three additional peptides selected from the group consisting of SEQ ID NO: 1-24.
5. The method according to claim 4, wherein the hydrolysate comprises at least three additional peptides selected from the group consisting of SEQ ID NO: 1-15.
6. The method according to claim 3, wherein the hydrolysate comprises a peptide with SEQ ID NO: 1 and at least three additional peptides selected from the group consisting of SEQ ID NO: 2-29, SEQ ID NO: 33-45 and SEQ ID NO: 47-68.
7. The method according to claim 1, wherein the hydrolysate is administered in a nutritional composition, comprising a lipid or a fat phase, and a protein source.
8. The method according to claim 7, wherein the nutritional composition comprises about 0.1 to about 1 g/100 kcal of a prebiotic composition, wherein the prebiotic composition comprises at least 20% of an oligosaccharide.
9. The method according to claim 7, wherein the nutritional composition further comprises about 5 to about 100 mg/100 kcal of a source of long chain polyunsaturated fatty acids which comprises docosahexaenoic acid.
10. The method according to claim 9, wherein the nutritional composition further comprises arachidonic acid.
11. The method according to claim 1, wherein the subject is a human child or juvenile.
12. The method according to claim 1, wherein the composition additionally comprises one or more of carbohydrates, nucleic acids, lipids, minerals, anabolic nutrients, vitamins, antioxidants, probiotic bacterial strains and lipotropic agents.