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(54) Title: NOVEL METHODS

(57) Abstract: The invention provides methods for screening for compounds that modulate SERCA3 activity, and, in turn, regulate the production of GLP-1, as well as methods of treatment or prophylaxis of metabolic diseases, obesity, diseases and disorders involving abnormal intestinal motility, and other diseases and disorders mediated by GLP-1, and compositions comprising SERCA3 inhibitors. The invention also provides a Serca3 modulator for use as a medicament, a Serca3 modulator for use in the treatment of the aforementioned disease states and, the use of a Serca3 modulator in the manufacture of a medicament for the treatment of the aforementioned disease states.



NOVEL METHODS

TECHNICAL FIELD:

[0001] The field relates to methods of screening, methods of modulating taste, appetite, metabolism and digestion, and methods of treating metabolic diseases. Specifically, the field relates to methods of modulation of GLP-1 activity by inhibiting SERCA3, thereby enhancing GLP-1 release induced by a lipid, sweetener or spice, e.g., a sweetener or spice compound. Furthermore, the field relates to methods of screening designed to identify compounds that modulate SERCA3 activity or production.

BACKGROUND:

[0002] The risk of certain diseases or disorders increases with an individual's increased or excessive weight gain. Such diseases include diabetes mellitus, heart disease, and some forms of cancer. Metabolic disturbances may also be associated with poor digestion and abnormal intestinal motility, with symptoms such as abdominal pain, vomiting, constipation, diarrhoea, and poor absorption of nutrients, as well as serious diseases such as upper gastrointestinal disorders, e.g., gastrointestinal reflux disorder and peptic ulcers; and disorders of the lower bowel such as functional bowel diseases, e.g., irritable bowel disease; colitis, e.g., ulcerative colitis; and diverticulitis or diverticulosis. Unfortunately, despite the numerous concerted efforts to research effective treatments, and the urgent need for such treatments, relatively little is currently known regarding the interactions of the various signalling pathways that are integral in maintaining a normal or healthy human metabolism. Knowledge of these biochemical pathways could be utilized to slow or abate undesirable weight gain and/or to treat or decrease the incidence of diseases or disorders related to abnormal metabolism, weight, digestion or intestinal motility.

[0003] SERCA is an acronym for Sarcoplasmic or Endoplasmic Reticulum Ca^{++} -ATPase (Muscle endoplasmic reticulum is called sarcoplasmic reticulum). The function of SERCA enzymes is to catalyze the hydrolysis of ATP coupled with the translocation of calcium from the cytosol to the endoplasmic reticulum lumen. As well, SERCA enzymes are involved in calcium sequestration associated with muscular excitation and contraction or with secretion. Alternative splicing of the enzyme results in multiple

transcript variants encoding different isoforms of SERCA3. In this regard, there are SERCA3 isoforms labelled a through f.

[0004] SERCA3 is a Ca^{2+} transporting ATPase, which is encoded by ATP2a3. SERCA3 is one of the three intracellular ER Ca^{2+} pumps located in the endoplasmic reticula of muscle cells. The sequence divergences between SERCA3 and the other SERCAs, SERCA1 and SERCA2, are mainly located in the N- and C-terminal domains. SERCA3 is generally non-muscle SERCA isoform and is found in a number of tissues, most abundantly in thymus, trachea, salivary gland, spleen, bone marrow, lymph node, peripheral leukocytes, pancreas and colon. It is found to some extent in cardiomyocytes, although SERCA3 lacks the putative interacting domain for phospholamban, a modulator that regulates the Ca^{2+} pump in cardiac muscle cells, and as a consequence, is not modulated by the presence of phospholamban. SERCA3 is selectively expressed in some endothelial and excretory cells, for example, pancreatic β cells, platelets, and mast cells, Ozog,A., Pouzet,B., Bobe,R. and Lompre,A.M. Characterization of the 3' end of the mouse SERCA 3 gene and tissue distribution of mRNA spliced variants. FEBS Lett. 427 (3), 349-352 (1998); Liu,L.H., Paul,R.J., Sutliff,R.L., Miller,M.L., Lorenz,J.N., Pun,R.Y., Duffy,J.J., Doetschman,T., Kimura,Y., MacLennan,D.H., Hoying,J.B. and Shull,G.E. Defective endothelium-dependent relaxation of vascular smooth muscle and endothelial cell Ca^{2+} signaling in mice lacking sarco(endo)plasmic reticulum Ca^{2+} -ATPase isoform 3. J. Biol. Chem. (1997) 272 (48), 30538-30545; Brini, M. and Carafoli, E. Calcium pumps in health and disease. Physiol. Rev. 89:1341-1378 (2009). Thus, while the general presence and expression of SERCA3, specifically its role in Ca^{2+} pump regulation, is known, less is understood as to what, if any, beneficial application this knowledge may have in the context of regulating the human metabolic processes or gut physiological function.

[0005] Glucagon-like peptide-1 ("GLP-1") is known to be involved in a variety of metabolic functions. GLP-1 belongs to a larger hormone group that is referred to as the glucagon superfamily of peptide hormones. The hormones of this superfamily are categorized in the same family due, in large part, to their close sequence homology with one another. This superfamily includes the following peptides: GLP-1 (1-37), (7-37) and -(7-36) amide, GIP, exendin-3 and -4, secretin, peptide histidine-methionine amide

(PHM), GLP-2, helospectin-1 and-2, helodermin, pituitary adenylyl cyclase-activating polypeptides (PACAP)-38, -27, PACAP-related peptide (PRP), GH- (GRP), and vasoactive intestinal polypeptide (VIP). While these peptide hormones have significantly similar sequence homology they differ in physiological function and role. For example, GLP-1 functions to stimulate insulin releasing factor secretion whereas GLP-2 operates to regulate the distinct function of growth of intestinal epithelial cells. *Id.*

[0006] GLP-1 is a naturally occurring 37 amino acid fragment of a polypeptide hormone proglucagon. The full length GLP-1 is thought to be biologically inactive. The six N-terminal amino acids are cleaved to give the active forms of the peptide, GLP-1(7-37) and GLP-1 (7-36). This cleavage seems to be activated by eating, and the truncated peptide is active in stimulating post—prandial insulin secretion, suppressing glucose-dependent glucagons secretion, delaying gastric emptying, and stimulating beta-cell proliferation. Given its mechanism of action, GLP-1 has not surprisingly received considerable attention as a potential panacea for Type II diabetes. However, GLP-1 is not orally active and has an extremely short plasma half life (about 90 seconds), making it impractical for use as a pharmaceutical.

[0007] There have been several efforts to develop GLP-1 analogs or mimetics with longer half life, for example as described in U.S. Pat. No. 5,545,618. Eli Lilly's exenatide (Byetta®) is a 39 amino acid peptide having a sequence derived from a peptide in Gila monster saliva, having some sequence identity to GLP-1, which binds the GLP-1 receptor. See, e.g., US 5,424,286.

[0008] Originally identified as a β -cell secretagogue, there is widespread evidence that, in humans, GLP-1 operates to regulate gastric motility. Secreted by L cells, in response to nutrients, GLP-1 has already been shown in vivo to have dual roles in decreasing glucagon and stimulating insulin secretion.

[0009] In fact, regarding GLP-1's involvement with insulin secretion, it had long been known that postprandial exposure of glucose to the pancreas could not entirely account for observations of insulin in the bloodstream. Kieffer, TJ., Habener, JF. *Endocrine Reviews*, 20 (6): 876-913. Consequently, before the discovery of the existence of GLP-1, it had been thought that some other incretin type hormone played a role in the

observed secretion of insulin. Over time GLP-1 was eventually discovered and shown to have value as an important intestinal incretin hormone.

[0010] The ability to modulate GLP-1 is potentially critical to human weight control as the slowing or inhibition of gastric emptying functions to prolong the periods where a given individual perceives satiation. Thus, when an individual feels satiated for longer periods this will decrease the perceived need to eat, which, in turn, will reduce an individual's overall intake of calories and promote weight loss or maintain healthy body weight.

[0011] GLP-1 activity can be enhanced by inhibitors of dipeptidyl peptidase 4 (DPP-4), the enzyme that deactivates GLP-1 in plasma. DPP-4 inhibitors or glyptins, are a class of oral hypoglycemics that block DPP-4. They include for example sitagliptin and vildagliptin and are used to treat diabetes mellitus type 2. Their mechanism of action is thought to result from increased incretin levels (GLP-1 and GIP), which inhibit glucagon release, which increases insulin secretion, decreases gastric emptying, and decreases blood glucose levels.

[0012] While the DPP-4 inhibitors show promise, there is still a lack of knowledge regarding the endogenous signal transduction mechanisms capable of modulating GLP-1's mode of action. Better understanding of the signaling pathways that are able to regulate GLP-1 production and activity would allow for greater manipulation of the hormone and, in turn, be of great utility in the addressing increased or excessive weight gain in mammals, as well as metabolic diseases such as diabetes.

Previous research has focused on the role of Serca3 in taste signal transduction in the oral cavity (Iguchi N, Ohkuri T, Slack JP, Zhong P, Huang L (2011) Sarco/Endoplasmic Reticulum Ca^{2+} -ATPases (SERCA) Contribute to GPCR-Mediated Taste Perception.

PLoS ONE volume 6, issue 8, article number e23165.

SUMMARY OF THE INVENTION:

[0013] It is now surprisingly discovered that there is an endogenous cellular signaling connection between SERCA3 and GLP-1. Specifically, it has been observed that inhibition of SERCA3 production enhances the level of GLP-1. This insight provides a method of modulating GLP-1 by administration of a SERCA3 inhibitory

compound without the necessary addition of exogenous GLP-1 or GLP-1 mimetics. Certain sweeteners or spices alone can stimulate GLP-1 release, but at much lower level in the absence of specific SERCA3 blockers. Administration of a SERCA3 inhibitor alone or in conjunction with normal food components such as lipids, sweeteners or spices
5 can thus upregulate local GLP-1 release in the gut, consequently regulating gut motility and helping maintain normal gut physiology. Increase in GLP-1 results in a feeling of satiety, and gastric motility and gastric emptying are both decreased. This results in longer periods of perceived satiation and consequently less overall caloric intake.

[0014] Thus, one aspect of the invention provides a method for preventing or
10 reducing weight gain in mammals, e.g., humans, by administering an effective amount of a SERCA3 inhibitor. Moreover, insulin production is stimulated, which may be desirable in, e.g., people suffering from or at high risk of Type II diabetes.

[0015] In view of the above, one aspect of the invention also relates to a method of screening for potential SERCA3 inhibitors. It is still further contemplated that these
15 same SERCA3 inhibitors are useful to increase GLP-1 production.

[0016] In another aspect, the invention also contemplates a SERCA3 inhibitor that may modulate GLP-1 levels in order to prevent excessive weight gain or to treat excessive weight gain or maintain healthy body weight.

[0017] In another aspect, the invention also contemplates a SERCA3 inhibitor
20 that may modulate GLP-1 levels in order to promote normal intestinal motility and for treatment or prophylaxis of diseases and disorders mediated by abnormal intestinal motility, for example to slow down gut motility and thereby mitigate discomfort caused by abnormal gut tissue contraction; and a method of treatment or prophylaxis of diseases and disorders mediated by abnormal intestinal motility comprising administering an
25 effective amount of a SERCA3 inhibitor to a patient in need thereof.

[0018] In another aspect, the invention also contemplates a SERCA3 inhibitor that may modulate GLP-1 levels in order to treat Type II diabetes, by enhancing insulin secretion mediated by GLP-1, either as sole agent or in combination with one or more anti-diabetic drugs.

[0019] In another aspect, the invention contemplates combining a SERCA3 inhibitor with another modulator of GLP-1 production in order to increase overall production of GLP-1.

5 [0020] It is also contemplated that a SERCA3 inhibitor be taken in conjunction with a GLP-1 mimetic, such as exenatide, or in order increase the production and/or concentration of GLP-1 levels in a given individual.

[0021] In another embodiment, the invention provides a mixture in either food or beverage forms or in capsule, powder, liquid or pill forms that contain at least one SERCA3 inhibitor and at least one lipid, sweetener and/or spice, as well as the use of
10 such mixture to regulate gut motility through GLP-I regulation.

[0022] In another embodiment, the invention provides for administration of a SERCA-3 inhibitor in conjunction with a DPP-4 blocker and optionally a lipid, sweeter or a spice compound, as a single composition or simultaneously or sequentially. Other aspects of the present invention are presented in the accompanying claims and in
15 the following description and drawings. These aspects may be presented under separate section headings. However, it is to be understood that the teachings under each section are not necessarily limited to that particular section heading.

Before describing the present invention it is to be understood that the invention is not
20 limited to particularly exemplified molecules or process parameters as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting. In addition, the practice of the present invention will employ, unless
25 otherwise indicated, conventional methods of virology, microbiology, molecular biology, recombinant DNA techniques and immunology all of which are within the ordinary skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook, *et al.*, *Molecular Cloning: A Laboratory Manual* (2nd Edition, 1989); *DNA Cloning: A Practical Approach*, vol. I & II (D. Glover, ed.); *Oligonucleotide Synthesis* (N. Gait, ed., 1984); *A Practical Guide to Molecular Cloning* (1984); and *Fundamental Virology*, 2nd
30 Edition, vol. I & II (B.N. Fields and D.M. Knipe, eds.).

All publications, patents and patent applications cited herein, whether *supra* or *infra*, are hereby incorporated by reference in their entirety. It must be noted that, as used in this specification and the appended claims, the singular forms “a”, “an” and “the” include
5 plural referents unless the content clearly dictates otherwise. All scientific and technical terms used in this application have meanings commonly used in the art unless otherwise specified. As used in this application, the following words or phrases have the meanings specified.

- 10 The term “comprising” means “including” as well as “consisting” *e.g.* a composition “comprising” X may consist exclusively of X or may include something additional *e.g.* X + Y.

BRIEF DESCRIPTION OF FIGURES:

- 15 **[0023]** Figure 1: The expression of Serca3 in mouse enteroendocrine cells. Mouse small intestine sections are stained with anti-Serca3 antibody. A subset of endocrine cells is immunoreactive to the antibody (green, top and bottom rows). The same sections are also stained with DAPI to visualize nuclei (middle row). Overlapping of the top and middle images shows that most of the SERCA3 staining is concentrated in the cytosol.

- 20 **[0024]** Figure 2: Immunostaining of a human duodenal section with anti-SERCA3 antibody. A subset of human enteroendocrine cells expressed SERCA3 (green). The magnified inset shows that most of the staining are concentrated in the bottom half of the cells.

- [0025]** Figure 3: Co-expression of SERCA3 with TRPM5 in mouse small
25 intestine. Expression of SERCA3 in mouse small intestinal is visualized using anti-SERCA3 antibody (A, red) and anti-TrpM5 (B, green). Superimposition (C) of these two images shows their overlapping pattern. TRPM5 is a taste signalling protein that has been found to be co-expressed in taste receptors and other taste signalling components such as α -gustducin, Gb3, Gg13 and PLC b2(Jang et al, PNAS, 104:105069-74, 2007).

- 30 **[0026]** Figure 4: TrpM5 and SERCA3 are Co-expressed in human duodenal enteroendocrine cells. Double immunostaining of a human duodenal section with anti-

TrpM5 (green) and anti-SERCA3 (red) antibodies indicates that these two proteins are co-expressed in the same set of chemosensory cells in human gut. DAPI was used to stain nuclei (blue).

[0027] Figure 5: Detection of GLP-1 in mouse small intestine. Immunostaining of a mouse small intestinal section with anti-GLP-1 antibody showed that some scattered residual signals (green) were detected all over the section, indicating that low level of secreted GLP-1 are present in the gut. The most concentrated areas are the bottom parts of the enteroendocrine cells (arrows). DAPI was used to visualize the nuclei.

[0028] Figure 6: GLP-1 fold change in response to 10mM saccharin. GLP-1 secretion from mouse small intestine, in response to 10 mM Saccharin, shows an increase in SERCA3 knock-out mice. Secretion of GLP-1 from wild-type and SERCA3-knockout gut tissue was increased in response to 10 mM Saccharin. The increase was about 3-4 fold in the wild-type tissue whereas 9-10 fold increase was observed in the KO tissue. The increased GLP-1 levels are maintained for over 20 min.

[0029] Figure 7: SERCA3 gene knockout further increased the GLP-1 release from mouse intestinal tissue. Thymol and isoeugenol increased the GLP-1 release from the wild-type tissue by 2-3 fold. The increase was 6 fold in the SERCA3-knockout tissue.

[0030] Figure 8: GLP-1 response of gut villi and crypts to lipid administration in SERCA3 wild type and knockout mice.

[0031] Figure 9: Pretreatment of SERCA3-WT gut sample with the SERCA3 Inhibitor BHQ compared to those samples without pretreatment, showing that for maximum effect, the Serca-3 inhibitor should be administered with lipid, sweetener or spice, e.g., a sweetener or spice, molecule at about the same time.

[0032] Figure 10: The combination of BHQ and Saccharin maximized the GLP-1 release from the gut tissue. The control treatments with 0.05% DMSO or a mixture of 0.05% DMSO and 30 mM BHQ slightly augment the GLP-1 level. The mixture of 0.05% DMSO and 5 mM Saccharin increases the GLP-1 level (over 2-fold). The mixture of 5 mM Saccharin, 30 mM BHQ and 0.05% DMSO induced the maximum GLP-1 level (3.5 to 4.5 fold increase).

DETAILED DESCRIPTION OF THE INVENTION:

5 **[0033]** In one embodiment, the invention provides for Method I, wherein Method I is a method of identifying a compound that modulates SERCA3 activity, comprising:

- i.) providing a cell comprising or expressing a SERCA3 polypeptide;
- ii.) contacting said cell with a first compound to be screened;
- 10 iii.) contacting said cell with a second compound wherein the second compound is known to increase GLP-1 levels;
- iv.) measuring the levels of GLP-1 after contacting the cell with the second compound;
- 15 v.) determining whether said first compound functions as a SERCA3 modulator by comparing the levels of GLP-1 in said cell to a control not contacted with said first compound;

wherein steps i and ii are conducted simultaneously or sequentially, e.g. simultaneously.

1.1. The method of Method I wherein said SERCA3 is endogenously expressed within the cell.

20 1.2. The method of Method I wherein nucleic acid encoding for SERCA3, or SERCA3 polypeptide, has been recombinantly introduced into a cell.

1.3. The method of Method I, 1.1, 1.2, wherein said cell is prokaryotic.

1.4. The method of Method I, 1.1, 1.2, 1.3, wherein said cell is eukaryotic.

25 1.5. The method of 1.4 wherein the eukaryotic cell is a yeast, insect, amphibian or mammalian cell.

1.6. The method of 1.4 wherein the eukaryotic cell is a CHO cell, HEK-293 or xenopus oocyte.

1.7. The method of Method I or any of the foregoing methods wherein GLP-1 is endogenously expressed by said cell.

- 1.8. The method of Method I or any of the foregoing methods wherein GLP-1 is recombinantly expressed by said cell.
- 1.9. The method of Method I or any of the foregoing methods wherein determination of GLP-1 levels utilizes a detectable label.
- 5 1.10. Method 1.9 wherein the label is an enzyme substrate.
- 1.11. Method 1.9 wherein the label is a radionuclide.
- 1.12. Method 1.9 wherein the label is a chemiluminescent label.
- 1.13. Method 1.9 wherein the label is a fluorescent label.
- 1.14. The method of Method I or any of the foregoing methods wherein the GLP-1 is
10 linked at the C-terminus to a measurable gene product.
- 1.15. Method 1.14, wherein the determination of GLP-1 levels is done by measurement of said measurable gene product.
- 1.16. The method of Method I or any of the foregoing methods, wherein the determination of GLP-1 levels is done by measurement of GLP-1 mRNA.
- 15 1.17. The method of Method I or any of the foregoing methods, wherein the determination of GLP-1 levels is done by measurement of GLP-1 protein.
- 1.18. The method of Method I or any of the foregoing methods, wherein the determination of GLP-1 levels is done by measurement of the mRNA of a second gene product.
- 20 1.19. The method of Method I or any of the foregoing methods, wherein the determination of GLP-1 levels is done by measurement of insulin.
- 1.20. The method of Method I or any of the foregoing methods, wherein GLP-1 levels are measured using mass spectrometry, e.g., time of flight mass spectrometry, e.g., MALDI-TOF MS.
- 25 1.21. The method of Method I or any of the foregoing methods, wherein GLP-1 levels are measured using specific antibodies to GLP-1, e.g., in an ELISA format.
- 1.22. The method of any of the foregoing methods, wherein the method is performed in a high throughput manner.
- 1.23. The method of Method I or any of the foregoing methods, wherein SERCA3 is
30 overexpressed in a cell.

1.24. The method of Method I or 1.21, wherein said overexpressed SERCA3 is endogenously overexpressed.

1.25. The method of Method I or 1.21, wherein said overexpressed SERCA3 is exogenously introduced.

5 1.26. The method of Method I or any of the foregoing methods, wherein said SERCA3 is overexpressed in a non-recombinant manner.

1.27. The method of Method I or any of the foregoing methods, wherein said SERCA3 is overexpressed in a recombinant manner.

1.28. The method of Method I or any of the foregoing methods, wherein said test
10 compound binds to SERCA3 directly.

1.29. The method of Method I or any of the foregoing methods wherein said test compound binds to a gene or gene product; wherein the binding of said test compound to said gene or gene product modulates SERCA3.

1.30. The method of Method I or any of the foregoing methods, wherein said first
15 compound is administered in conjunction with one or more compounds known to increase levels of GLP-1.

1.31. The method of Method I or 1.30, wherein said one or more compounds known to increase levels of GLP-1 comprises a spice, e.g., thymol and/or isoeugenol.

1.32. The method of Method I or 1.30, wherein said one or more compounds known to
20 increase levels of GLP-1 comprises a sweetener, e.g., saccharin.

1.33. The method of Method I or 1.30, wherein said one or more compounds known to increase levels of GLP-1 comprises a lipid, e.g., a triglyceride and/or a fatty acid.

1.34. The method of Method I or 1.30, wherein said one or more compounds known to increase levels of GLP-1 comprises a DPP-4 inhibitor.

25 1.35. The method of Method I or of any of the foregoing methods, wherein said second compound is a compound known to decrease the levels of GLP-1.

1.36. The method of 1.35 wherein the said compound known to decrease GLP-1 is Dipeptidyl peptidase-4.

30 1.37. The method of Method I or of any of the foregoing methods, wherein the steps of Method I are carried out in silico.

1.38. The method of Method I or of any of the foregoing methods, wherein the steps of Method I are carried out in vitro.

1.39. The method of Method I or any of the foregoing methods, wherein the steps of Method I are carried out in vivo.

5 1.40. The method of Method I or any of the foregoing methods, wherein said first compound is introduced to a SERCA3-knockout animal.

1.41. The method of Method I or any of the foregoing methods, wherein said first compound is introduced to a SERCA3-knock-in animal.

1.42. The method of Method I or any of the foregoing methods, wherein said first
10 compound is administered to a rodent.

1.43. The method of Method I or any of the foregoing methods, wherein said first compound is administered to an animal selected from the group consisting of: *Xenopus laevis* (*X. laevis*), *Mus musculus* (*M. musculus*), or *Rattus norvegicus* (*R. norvegicus*).

1.44. The method of Method I or any of the foregoing methods, wherein said first
15 compound is tested in a patient.

1.45. The method of Method I or any of the foregoing methods, wherein the determination of whether GLP-1 levels are modulated is done by measuring insulin levels in a patient after administration with said first compound.

1.46. The method of Method I or any of the foregoing methods, wherein determination
20 of GLP-1 levels is measured postprandially.

1.47. A SERCA3 modulator identified by the the method of Method I or any one of the foregoing methods (1.1-1.46).

1.48. The SERCA3 modulator identified by the method of Method I, or any one of the foregoing methods, wherein the modulator is selected from the group consisting of: a
25 protein, a peptide, a small molecule, an antihistamine; a cyclosporine; an antibody to SERCA3; an antisense oligonucleotides, triple helix DNA, RNA aptamers, ribozymes or double stranded RNA directed to a nucleic acid sequence of SERCA3, taste receptor ligands such as a bitter receptor ligand, a sweet receptor ligand, an umami receptor ligand, a fat receptor ligand, a sour receptor ligand, 1,4-Dihydroxy-2,5-di-tert-
30 butylbenzene (BHQ); 2,5-di-(tert-butyl)-1,4-hydroquinone; 2,5- di(t-butyl)-1,4-

benzohydroquinone; thapsigargin and structurally similar compounds to any of the foregoing.

[0034] In another embodiment, the invention provides for Method II, wherein Method II is a method of treatment, mitigation or prophylaxis of an abnormal condition mediated by GLP-1 activity, comprising administering an effective amount of a compound that inhibits the activity of SERCA3 to a mammal in need thereof.

2.1. Method II wherein the condition mediated by GLP-1 activity is Type II diabetes.

2.2. Method II wherein the condition mediated by GLP-1 activity is a prediabetic condition.

2.3. Method II wherein the condition mediated by GLP-1 activity is ametabolic disease or disorder.

2.4. Method II wherein the condition mediated by GLP-1 is obesity.

2.5. Method II wherein the condition mediated by GLP-1 is abnormal intestinal motility.

2.6. Method II or any of the previous methods wherein the compound that inhibits the activity of SERCA3 is administered in conjunction with known treatments for obesity.

2.7. Method II or any of the previous methods wherein the compound that inhibits the activity of SERCA3 is administered in conjunction with known treatments for

Type II diabetes.

2.8. Method II or any of the previous methods wherein the compound that inhibits the activity of SERCA3 is administered in conjunction with known treatments for diseases or disorders caused or characterized by abnormal intestinal motility.

2.9. Method II or any of the previous methods wherein the compound that inhibits the activity of SERCA3 is administered in conjunction with known regimens for the prevention of obesity.

2.10. The foregoing method comprising co-administration (concurrently or sequentially) administration of an effective amount of one or more drugs selected from the group consisting of bisguanides, e.g., metformin, a sulfonylurea, thiazolidinediones such as pioglitazone or rosiglitazone, DPP-4 inhibitors, e.g., sitagliptin (Januvia), meglitinides, e.g. repaglinide (Prandin) or nateglinide (Starlix), GLP-1 mimetics, e.g.,

exenatide, and/or insulin or an insulin analog, for example a long acting basal insulin analogue, for example glargine (Lantus).

2.11. Method II, or any of the foregoing methods, wherein the compound that inhibits the activity of SERCA3 is administered in conjunction with a sweetener.

5 2.12. The foregoing method wherein the sweetener is saccharin.

2.13. Method II, or any of the foregoing methods, wherein the compound that inhibits the activity of SERCA3 is administered in conjunction with a spice.

2.14. The foregoing method wherein the spice is thymol, isoeugenol, or a combination thereof.

10 2.15. Method II or any of the foregoing methods wherein the compound that inhibits the activity of SERCA3 is 1,4-Dihydroxy-2,5-di-tert-butylbenzene.

2.16. Method II or any of the foregoing methods wherein the compound that inhibits the activity of SERCA3 is 2,5-di-(tert-butyl)-1,4-hydroquinone.

15 2.17. Method II or any of the foregoing methods wherein the compound that inhibits the activity of SERCA3 is 2,5-di(t-butyl)-1,4-benzohydroquinone

2.18. Method II or any of the foregoing methods wherein the compound that inhibits the activity of SERCA3 is thapsigargin.

2.19. Method II or any of the foregoing methods wherein the compound that inhibits the activity of SERCA3 is an antihistamine [histamine is known to enhance
20 SERCA3 expression; antihistamine is believed to inhibit histamine-mediated SERCA3 expression].

2.20. Method II or any of the foregoing methods wherein the compound that inhibits the activity of SERCA3 is a cyclosporin, e.g., cyclosporine A.

2.21. Method II or any of the foregoing methods wherein the compound that
25 inhibits the activity of SERCA3 is an antibody to SERCA3, e.g., a monoclonal human, humanized or chimeric antibody to SERCA3.

2.22. Method II or any of the foregoing methods wherein the compound that inhibits the activity of SERCA3 is a monoclonal antibody binding to the same epitope as monoclonal antibody PL/IM4302 (See, Chandrasekera, C.P., et al., Jour. Bio. Chem.
30 (2003) 278 (14): 12482–12488, incorporated herein by reference), e.g., wherein the antibody has the complementarity determining regions (CDRs) of monoclonal antibody

PL/IM4302, for example a chimeric or humanized form of monoclonal antibody PL/IM4302.

2.23. Method II or any of the foregoing methods wherein the compound that inhibits the activity of SERCA3 is selected from antisense oligonucleotides, triple helix
5 DNA, RNA aptamers, ribozymes and double stranded RNA directed to a nucleic acid sequence of SERCA3.

2.24. Method II, or any of the foregoing methods, wherein modulation of the mammal's metabolism occurs by inhibition of gastric motility or slowing inhibition of gastric emptying.

10 2.25. Method II, or any of the foregoing methods, wherein the mammal to be treated is a human.

2.26. Method II or any of the foregoing methods wherein the mammal to be treated is a dog or cat.

15 2.27. Method II or any of the foregoing methods wherein the compound that inhibits the activity of SERCA3 is administered within 1-2 hours of a meal.

2.28. Method II or any of the foregoing methods wherein the compound that inhibits the activity of SERCA3 is locally active in the gastrointestinal tract, such that systemic activity is reduced or eliminated, e.g., substantially free of activity other than on GLP-1.

20 **[0034a]** In another embodiment, the invention provides an effective amount of a compound that inhibits the activity of SERCA3 for use as a medicament.

3.0. In another embodiment, the invention provides a further medical use I (FMU I) wherein FMUI is an effective amount of a compound that inhibits the activity of SERCA3 for use in the treatment or prophylaxis of an abnormal condition mediated
25 by GLP-1 activity in a mammal in need thereof.

3.1. The FMU I wherein the condition mediated by GLP-1 activity is Type II diabetes.

3.2. The FMU I wherein the condition mediated by GLP-1 activity is a pre-diabetic condition.
30

- 3.3. The FMU I wherein the condition mediated by GLP-1 activity is a metabolic disease or disorder.
- 3.4. The FMU I wherein the condition mediated by GLP-1 is obesity.
- 3.5. The FMU I wherein the condition mediated by GLP-1 is an abnormal intestinal motility.
- 3.6. The FMU I, or any one of the previous FMU I (3.1 – 3.5), wherein the compound that inhibits the activity of SERCA3 is administered in conjunction with known treatments for obesity.
- 3.7. The FMU I, or any one of the previous FMU I (3.1 – 3.6), wherein the compound that inhibits the activity of SERCA3 is administered in conjunction with known treatments for Type II diabetes.
- 3.8. The FMU I, or any one of the previous FMU I (3.1 – 3.7), wherein the compound that inhibits the activity of SERCA3 is administered in conjunction with known treatments for diseases or disorders caused or characterized by abnormal intestinal motility.
- 3.9. The FMU I, or any one of the previous FMU I (3.1 – 3.8), wherein the compound that inhibits the activity of SERCA3 is administered in conjunction with known regimens for the prevention of obesity.
- 3.10. The foregoing FMU I comprises co-administration (concurrently or sequentially) of an effective amount of one or more drugs selected from the group consisting of biguanides, e.g., metformin, a sulfonylurea, thiazolidinediones such as pioglitazone or rosiglitazone, DPP-4 inhibitors, e.g., sitagliptin (Januvia), meglitinides, e.g. repaglinide (Prandin) or nateglinide (Starlix), GLP-1 mimetics, e.g., exenatide, and/or insulin or an insulin analog, for example a long acting basal insulin analogue, for example glargine (Lantus).
- 3.11. The FMU I, or any one of the previous FMU I (3.1 – 3.10), wherein the compound that inhibits the activity of SERCA3 is administered in conjunction with a sweetener.
- 3.12. The FMU I of 3.11 wherein the sweetener is saccharin.
- 3.13. The FMU I, or any one of the previous FMU I (3.1 – 3.12), wherein the

compound that inhibits the activity of SERCA3 is administered in conjunction with a spice.

3.14. The foregoing FMU I of 3.13 wherein the spice is thymol, isoeugenol, or a combination thereof.

5 3.15. The FMU I, or any one of the previous FMU I (3.1 – 3.14), wherein the compound that inhibits the activity of SERCA3 is 1,4-Dihydroxy-2,5-di-tert-butylbenzene (BHQ).

3.16. The FMU I, or any one of the previous FMU I (3.1 – 3.15), wherein the compound that inhibits the activity of SERCA3 is 2,5-di-(tert-butyl)-1,4-
10 hydroquinone.

3.17. The FMU I, or any one of the previous FMU I (3.1 – 3.16), wherein the compound that inhibits the activity of SERCA3 is 2,5-di(t-butyl)-1,4-benzohydroquinone

3.18. The FMU I, or any one of the previous FMU I (3.1 – 3.17), wherein the
15 compound that inhibits the activity of SERCA3 is thapsigargin.

3.19. The FMU I, or any one of the previous FMU I (3.1 – 3.18), wherein the compound that inhibits the activity of SERCA3 is an antihistamine [histamine is known to enhance SERCA3 expression; antihistamine is believed to inhibit histamine-mediated SERCA3 expression].

20 3.20. The FMU I, or any one of the previous FMU I (3.1 – 3.19), wherein the compound that inhibits the activity of SERCA3 is a cyclosporin, e.g., cyclosporine A.

3.21. The FMU I, or any one of the previous FMU I (3.1 – 3.20), wherein the compound that inhibits the activity of SERCA3 is an antibody to SERCA3, e.g., a
25 monoclonal human, humanized or chimeric antibody to SERCA3.

3.22. The FMU I, or any one of the previous FMU I (3.1 – 3.21), wherein the compound that inhibits the activity of SERCA3 is a monoclonal antibody binding to the same epitope as monoclonal antibody PL/IM4302 (See, Chandrasekera, C.P., et al., Jour. Bio. Chem. (2003) 278 (14): 12482–12488, incorporated herein
30 by reference), e.g., wherein the antibody has the complementarity determining

regions (CDRs) of monoclonal antibody PL/IM4302, for example a chimeric or humanized form of monoclonal antibody PL/IM4302.

- 3.23. The FMU I, or any one of the previous FMU I (3.1 – 3.6), wherein the compound that inhibits the activity of SERCA3 is selected from antisense oligonucleotides, triple helix DNA, RNA aptamers, ribozymes and double stranded RNA directed to a nucleic acid sequence of SERCA3.
- 3.24. The FMU I, or any one of the previous FMU I (3.1 – 3.23), wherein the modulation of the mammal's metabolism occurs by inhibition of gastric motility or slowing inhibition of gastric emptying.
- 3.25. The FMU I of 3.24 wherein the mammal to be treated is a human.
- 3.26. The FMU I of 3.24 wherein the mammal to be treated is a dog or cat.
- 3.27. The FMU I or any one of the previous FMU I (3.1 – 3.26) wherein the compound that inhibits the activity of SERCA3 is administered within 1-2 hours of a meal.
- 3.28. The FMU I, or any one of the previous FMU I (3.1 – 3.27), wherein the compound that inhibits the activity of SERCA3 is locally active in the gastrointestinal tract, such that systemic activity is reduced or eliminated, e.g., substantially free of activity other than on GLP-1.

4.0 In another embodiment, the invention provides for the use of an effective amount of a compound that inhibits the activity of SERCA3 in the manufacture of a medicament for the treatment or prophylaxis of an abnormal condition mediated by GLP-1 activity in a mammal in need thereof.

- 4.1. The foregoing use as defined in 4.0 wherein the condition mediated by GLP-1 activity is Type II diabetes.
- 4.2. The foregoing use as defined in 4.0 wherein the condition mediated by GLP-1 activity is a pre-diabetic condition.
- 4.3. The foregoing use as defined in 4.0 wherein the condition mediated by GLP-1 activity is a metabolic disease or disorder.
- 4.4. The foregoing use as defined in 4.0 wherein the condition mediated by GLP-1 is obesity.
- 4.5. The foregoing use as defined in 4.0 wherein the condition mediated by

GLP-1 is an abnormal intestinal motility.

- 4.6. The foregoing use as defined in any one of 4.0 – 4.5 wherein the compound that inhibits the activity of SERCA3 is used in conjunction with known treatments for obesity.
- 5 4.7. The foregoing use as defined in any one of 4.0 – 4.6 wherein the compound that inhibits the activity of SERCA3 is used in conjunction with known treatments for Type II diabetes.
- 4.8. The foregoing use as defined in any one of 4.0 – 4.7 wherein the compound that inhibits the activity of SERCA3 is used in conjunction with known treatments for diseases or disorders caused or characterized by abnormal intestinal motility.
- 10 4.9. The foregoing use as defined in any one of 4.0 – 4.8 wherein the compound that inhibits the activity of SERCA3 is used in conjunction with known regimens for the prevention of obesity.
- 15 4.10. The foregoing use as defined in any one of 4.0 – 4.9 wherein the medicament is co-administered (concurrently or sequentially) with an effective amount of one or more drugs selected from the group consisting of bisguanides, e.g., metformin, a sulfonylurea, thiazolidinediones such as pioglitazone or rosiglitazone, DPP-4 inhibitors, e.g., sitagliptin (Januvia), meglitinides, e.g., repaglinide (Prandin) or nateglinide (Starlix), GLP-1 mimetics, e.g., exenatide, and/or insulin or an insulin analog, for example a long acting basal insulin analogue, for example glargine (Lantus).
- 20 4.11. The foregoing use as defined in any one of 4.0 – 4.10 wherein the compound that inhibits the activity of SERCA3 is used in conjunction with a sweetener.
- 25 4.12. The foregoing use as defined in 4.11 wherein the sweetener is saccharin.
- 4.13. The foregoing use as defined in any one of 4.0 – 4.12 wherein the compound that inhibits the activity of SERCA3 is used in conjunction with a spice.
- 30 4.14. The foregoing use as defined in 4.13 wherein the spice is thymol, isoeugenol, or a combination thereof.

- 4.15. The foregoing use as defined in any one of 4.0 – 4.14 wherein the compound that inhibits the activity of SERCA3 is 1,4-Dihydroxy-2,5-di-tert-butylbenzene (BHQ).
- 5 4.16. The foregoing use as defined in any one of 4.0 – 4.15 wherein the compound that inhibits the activity of SERCA3 is 2,5-di-(tert-butyl)-1,4-hydroquinone.
- 4.17. The foregoing use as defined in any one of 4.0 – 4.16 wherein the compound that inhibits the activity of SERCA3 is 2,5-di(t-butyl)-1,4-benzohydroquinone
- 10 4.18. The foregoing use as defined in any one of 4.0 – 4.17 wherein the compound that inhibits the activity of SERCA3 is thapsigargin.
- 4.19. The foregoing use as defined in any one of 4.0 – 4.18 wherein the compound that inhibits the activity of SERCA3 is an antihistamine [histamine is known to enhance SERCA3 expression; antihistamine is believed to inhibit
- 15 histamine-mediated SERCA3 expression].
- 4.20. The foregoing use as defined in any one of 4.0 – 4.19 wherein the compound that inhibits the activity of SERCA3 is a cyclosporin, e.g., cyclosporine A.
- 4.21. The foregoing use as defined in any one of 4.0 – 4.20 wherein the
- 20 compound that inhibits the activity of SERCA3 is an antibody to SERCA3, e.g., a monoclonal human, humanized or chimeric antibody to SERCA3.
- 4.22. The foregoing use as defined in any one of 4.0 – 4.21 wherein the compound that inhibits the activity of SERCA3 is a monoclonal antibody binding to the same epitope as monoclonal antibody PL/IM4302 (See, Chandrasekera, C.P., et al., Jour. Bio. Chem. (2003) 278 (14): 12482–12488, incorporated herein
- 25 by reference), e.g., wherein the antibody has the complementarity determining regions (CDRs) of monoclonal antibody PL/IM4302, for example a chimeric or humanized form of monoclonal antibody PL/IM4302.
- 4.23. The foregoing use as defined in any one of 4.0 – 4.22 wherein the

compound that inhibits the activity of SERCA3 is selected from antisense oligonucleotides, triple helix DNA, RNA aptamers, ribozymes and double stranded RNA directed to a nucleic acid sequence of SERCA3.

- 5 4.24. The foregoing use as defined in any one of 4.0 – 4.23 wherein modulation of the mammal's metabolism occurs by inhibition of gastric motility or slowing inhibition of gastric emptying.
- 4.25. The foregoing use as defined in 4.24 wherein the mammal to be treated is a human.
- 10 4.26. The foregoing use as defined in 4.24 wherein the mammal to be treated is a dog or cat.
- 4.27. The foregoing use as defined in any one of 4.0 – 4.26 wherein the medicament is administered within 1-2 hours of a meal.
- 4.28. The foregoing use as defined in any one of 4.0 – 4.27 wherein the compound that inhibits the activity of SERCA3 is locally active in the
- 15 gastrointestinal tract, such that systemic activity is reduced or eliminated, e.g., substantially free of activity other than on GLP-1.

[0035] The invention further provides a compound that inhibits the activity of SERCA3 for use in any of the foregoing methods.

20 **[0036]** The invention further provides the use of a compound that inhibits the activity of SERCA3 in the manufacture of a formulation for use in any of Method II or 2.01-2.28.

[0037] The invention further provides a composition for oral administration, e.g., a food, beverage, capsule, pill, powder, food additive, or beverage additive which contains at least one SERCA3 inhibitor and at least one lipid, sweetener or spice, e.g., a

25 sweetener or spice ingredient, e.g., a non-sugar sweetener selected from stevia, aspartame, sucralose, neotame, acesulfame potassium, saccharin, sugar alcohols, and combinations thereof, or a spice, e.g., thymol and/or eugenol, for use in any of Method II or 2.01-2.24 or for FMUI (3.0) or 3.01 to 3.24 or for use in 4.0 or 4.01 to 4.24. In vivo screening for the effect of a SERCA3 modulator is carried out by treating the animals

30 with regular food plus, for example, a Serca3 inhibitor and then checking the plasma

insulin level or glucose level to assess the effective of the potential inhibitor on plasma insulin or glucose levels.

[0038] In another embodiment, the invention provides for administration of a SERCA-3 inhibitor in conjunction with a DPP-4 blocker and optionally a sweetener or a spice compound, as a single composition or simultaneously or sequentially.

[0039] Lipids for use in the methods and compositions described herein include palatable triglycerides and fatty acids, e.g., fish oil or vegetable oils. Sweeteners include non-sugar sweeteners selected from stevia, aspartame, sucralose, neotame, acesulfame potassium, saccharin, sugar alcohols, and combinations thereof. Spices include edible compounds having a spicy flavor as used to season foods, beverages and compositions for oral administration, e.g., essential oils from plants, as well as dried seed, fruit, root, bark, or vegetative materials, for example thymol and/or eugenol, as well as pepper, cinnamon, and so forth.

[0040] The term "SERCA3" as used herein refers to gene products of naturally occurring variants a-f of the SERCA3 gene or additional functional variants that are expressed in cells in vertebrate species, for example, without limitation, SERCA3 isoforms from mouse, rat, rabbit, ape, monkey, pig, dog, cat, cattle, and frog. These include in particular, without limitation, animals commonly used in laboratories such as *Mus musculus*, *Rattus norvegicus*, and *Xenopus laevis*. Examples of such isoforms are, without limitation, rat SERCA3a (SEQ ID: 1, SEQ ID: 2), mouse SERCA3a (SEQ ID: 3, SEQ ID: 4), mouse SERCA3b (SEQ ID: 5, SEQ ID: 6), and human SERCA3c (SEQ ID: 7 and SEQ ID: 8), Human SERCA3a (SEQ ID: 9, SEQ ID: 10), Human SERCA3b (SEQ ID: 11, SEQ ID: 12), Human SERCA3d (SEQ ID: 13, SEQ ID: 14), Human SERCA3e (SEQ ID: 15, SEQ ID: 16), Human SERCA3f (SEQ ID: 17, SEQ ID: 18), and their SERCA3 gene products.

[0041] The N-terminal part of SERCA3 is highly conserved in pig, cat, rat, mouse and human, whereas the C-termini of SERCA3 are more variable. Vertebrate homologs not yet described can be easily isolated and sequenced employing well-known methods, for example, hybridization with a homolog SERCA3 sequence of another species.

[0042] SERCA3 nucleic acids or polypeptides may be synthesized chemically as is well known in the art based on known sequences. Alternatively they may be amplified

using clones comprising one or more SERCA3 sequences. For transfection and expression, a cDNA clone may be used. Clones that are known to comprise Serca3 may be selected and obtained from commercial sources, or a cDNA library may be screened using all or part of the known Serca3 sequence. Serca3 may be amplified using clones
5 and/or polymerase chain reaction (PCR).

[0043] The synthetic, amplified, or isolated SERCA3 nucleic acid may then be introduced into one of many well known expression systems to produce the SERCA3 gene product/protein. For example, SERCA3 may be heterologously expressed in various cells including, without limitation, vertebrate cells or mammalian cells, as is well known
10 in the art.

[0044] Vertebrates comprise amphibians, reptiles, birds, and mammals (including humans). One or more SERCA homologue genes are known in various vertebrates, including various vertebrates commonly employed in the lab, for example, without limitation, *Xenopus laevis* (*X. laevis*), *Mus musculus* (*M. musculus*), *Rattus norvegicus*
15 (*R. norvegicus*), and other rodents.

[0045] Invertebrates having one or more SERCA homologue may also be used, for example *Caenorhabditis elegans* (*C. elegans*), and the fruit fly *Drosophila melanogaster* (*D. melanogaster*).

[0046] For further methods, the SERCA3 expressing cells may be used, or
20 alternatively, an isolated SERCA3 protein may be used.

[0047] Isolated SERCA3 protein may, for example, be integrated into an artificial lipid bilayer as is well known in the art and the resulting SERCA3 lipid bilayer can be used for further methods as is well known in the art.

[0048] Effects on SERCA3 may be direct or indirect, for example, through an
25 effect on SERCA3 regulation and/or expression. Moreover, the effects on GLP-1 may be direct or indirect.

[0049] The ability of a substance to “modulate” SERCA3 refers to, but is not limited to, the ability of a substance to inhibit the biological activity of SERCA3, or modulate the subcellular localization of the protein, or alter the stability of the protein (e.g., by post-
30 translational modification such as phosphorylation, glycosylation, etc.) and/or inhibit SERCA3 gene expression. Such modulation could also involve affecting the ability of

other proteins to interact with SERCA3.

[0050] Thus a SERCA3 inhibitor may bind to and/or block SERCA3, or may inhibit expression of SERCA3.

The assays described herein may be used to screen libraries for agents that modulate Serca3 activity. The assays may be designed to screen large chemical libraries by automating the assay steps and providing compounds from any convenient source to the assays, which are typically run in parallel (for example in microtiter formats on microtiter plates in robotic assays). Assays may be run in high throughput screening methods (whereby it is possible to screen up to several thousand potential modulators in a single day) that involve providing a combinatorial chemical or peptide library containing a large number of potential enhancers. Such libraries are then screened in one or more assays described herein-above to identify those library agents (particular chemical species or subclasses) that display the activity described herein-above. The enhancers thus identified can be directly used or may serve as leads to identify further modulators by making and testing derivatives.

Synthetic compound libraries are commercially available from a number of companies including Maybridge Chemical Co. (Trevillet, Cornwall, UK), Comgenex (Princeton, N.J.), Brandon Associates (Merrimack, N.H.), and Microsource (New Milford, Conn.).

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A combinatorial chemical library is a collection of diverse chemical compounds generated by either chemical synthesis or biological synthesis, by combining a number of chemical "building blocks" such as reagents. For example, a linear combinatorial chemical library such as a polypeptide library is formed by combining a set of chemical building blocks (amino acids) in every possible way for a given compound length (i.e., the number of amino acids in a polypeptide compound). Millions of chemical compounds can be synthesized through such combinatorial mixing of chemical building blocks. A rare-chemical library is available from Aldrich (Milwaukee, Wis.).

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Libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are commercially available for example from Pan Laboratories (Bothell, Wash.) or

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MycoSearch (NC), or are readily producible by methods well known in the art.

Additionally, natural and synthetically produced libraries and compounds are readily modified through conventional chemical, physical, and biochemical means. Other libraries include protein/expression libraries, cDNA libraries from natural sources, including, for example, foods, plants, animals, bacteria, libraries expressing randomly or systematically mutated variants of one or more polypeptides, and genomic libraries in viral vectors that are used to express the mRNA content of one cell or tissue.

The invention provides a compound that modulates Serca3 activity.

- 10 Non limiting examples of modulators of Serca 3 activity include a protein, a peptide, a small molecule, an antihistamine; a cyclosporine; an antibody to Serca3; an antisense oligonucleotides, triple helix DNA, RNA aptamers, ribozymes or double stranded RNA directed to a nucleic acid sequence of SERCA3, taste receptor ligands such as a bitter receptor ligand, a sweet receptor ligand, an umami receptor ligand, a fat receptor ligand, a
15 sour receptor ligands,.

The term “umami receptor ligands” as used herein also includes compounds that enhance umami taste.

- Non limiting examples of umami receptor ligands include, but are not limited to: 2-(3-phenylpropyl)pyridine, L-Glu (glutamic acid, glutamate, for example in the form of its salts such as monosodium glutamate, monopotassium glutamate, monoammonium glutamate, calcium diglutamate, magnesium diglutamate), L-Asp (L-asparagine,), 5'-ribonucleotides or their salts including, without limitation, calcium 5'-ribonucleotides, disodium 5'-ribonucleotides, and dipotassium 5'-ribonucleotides (e.g. inosinic acid, guanylic acid, adenosinic acid, inosinates, guanylates, and adenylates, including
25 guanosine 5'-monophosphate, inosine 5'-monophosphate, and 5'-adenylate and their salts such as disodium guanylate, disodium inosinate, disodium adenylate; dipotassium guanylate, dipotassium inosinate, dipotassium adenylate, calcium guanylate, calcium inosinate, calcium adenylate), maltol, ethyl maltol, glycine, L-leucine, autolyzed or
30 hydrolyzed proteins (e.g. autolyzed yeast, hydrolyzed yeast, hydrolyzed vegetable

proteins) Many more umami receptor ligands other than those listed herein are known to those of skill in the art and still more can be identified using methods known in the art.

Non-limiting examples of fat receptor ligands include linoleic acids, oleic acids,
 5 palmitates, oleoylethanolamides, omega-3 fatty acids, mixed fatty acid emulsion, and N-acylphosphatidylethanolamine (NAPE), myristoleic acid, palmitoleic acid, alpha-linolenic acid, arachidonic acid, eicosapentaenoic acid, erucic acid, and docosahexaenoic acid.

Many more fat receptor ligands other than those listed herein are known to those of skill in the art and still more can be identified using methods known in the art.

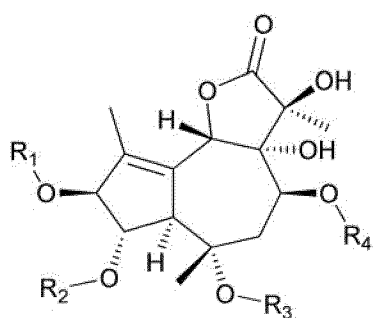
10 .
 Non-limiting examples of sour receptor ligands include citric acid and hydroxycitric acid. Many more sour receptor ligands other than those listed herein are known to those of skill in the art, and still more can be identified using methods known in the art and described herein.

15 Non-limiting bitter receptor ligands include flavanones, flavones, flavonols, flavans, phenolic flavonoids, isoflavones, limonoid aglycones, glucosinolates or hydrolysis product thereof, caffeine, quinine, metformin, metformin hydrochloride, extracts of Momordica charantia (bitter melon), and isothiocyanates. Many more bitter receptor
 20 ligands other than those listed herein are known to those of skill in the art and still more can be identified using methods known in the art.

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 Non-limiting sweet receptor ligands include nutritive and non nutritive sweeteners e.g. sucrose, fructose, glucose, high fructose corn syrup, corn syrup, xylose, arabinose,
 25 rhamnose, erythritol, xylitol, mannitol, sorbitol, inositol, saccharine, swingle extract, rubus extract, rebaudiosides e.g. Rebaudioside A, Rebaudioside B, and Rebaudioside C, methyl chavicol, Theasaponin E1, Acesulfame K, Alitame, Aspartame, CH 401, Dulcin, Neotame, sodium Cyclamate, Sucralose, Superaspartame, Cynarin, Glycyphyllin, Abrusoside A, Abrusoside B, Abrusoside C, Abrusoside D, Abrusoside E,
 30 Apioglycyrrhizin, Araboglycyrrhizin, Baiyunoside, Brazzein, Bryodulcoside, Carnosifloside V, Carnosifloside VI, D. cumminsii, Cyclocarioside A, Cyclocarioside I,

- Dulcoside A, Glycyrrhizic Acid, Hernandulcin, Hernandulcin, 4beta-hydroxy-Hesperitin-7-Glucoside Dihydrochalcone, Huangqioside E, Huangqioside E, 3-Hydroxyphloridzin, 2,3-Dihydro-6-Methoxy 3-O-Acetate, Mabinlin Maltosyl-Alpha-(1,6)-Neohesperidin Dihydrochalcone, Mogroside IIE, Mogroside III, Mogroside IIIE, Mogroside IV,
- 5 Mogroside V, 11-Oxo Mogroside V, Monatin, Monoammonium Glycyrrhizinate (Mag), Mukurozioside Iib, Naringin Dihydrochalcone, Neohesperidin Dihydrochalcone (NHDHC), Neomogroside, Osladin, Periandrin I, Periandrin II, Periandrin III, Periandrin IV, Periandrin V, Phlomisoid I, Phlorizin, Phyllodulcin, Polypodoside A, Potassium magnesium calcium glycyrrhizin, Pterocaryosides A, Pterocaryosides B, Rubusoside,
- 10 Scandenoside R6, Siamenoside I, Sodium glycyrrhizinate, Steviolbioside, Stevioside, alpha-Glycosyl Suavioside A, Suavioside B, Suavioside G, Suavioside H, Suavioside I, Suavioside J, Thaumatin, Triammonium Glycyrrhizinate (TAG), Trilobatin Curculin, Strogen 1, Strogen 2, Strogen 4, Miraculin, Hodulcin, Jujubasaponin II, Jujubasaponin III, Abrusoside E, Periandrinic acid I, monoglucuronide, Periandrinic acid II,
- 15 monoglycuronide, Chlorogenic Acid, beta- (1,3-Hydroxy-4-methoxybenzyl)-Hesperitin Dihydrochalcone, 3'-Carboxy-Hesperitin Dihydrochalcone, 3'- Stevioside analogue. Many more sweet receptor ligands other than those listed herein are known to those of skill in the art, and still more can be identified using methods known in the art.
- 20 Non limiting examples of particular modulators of Serca 3 include 1,4-Dihydroxy-2,5-di-tert-butylbenzene (BHQ); 2,5-di-(tert-butyl)-1,4-hydroquinone; 2,5-di(t-butyl)-1,4-benzohydroquinone; thapsigargin and structurally similar compounds to any of the foregoing.
- 25 Non limiting examples of structurally similar compounds to the foregoing include:
 3,5-Di-tert-butyl-4-hydroxyphenol;
 3,6-Di-tert-butylpyrocatechol;
 1,3-Benzenediol,
 2,5-di-tert-butylbenzene-1,3-diol;
- 30 3,5-Di-tert-butylpyrocatechol;
 1,4-Dihydroxy-2,5-di-tert-butylbenzene;

- 2,5-di-(tert-butyl)-1,4-hydroquinone;
 2,5-di(t-butyl)-1,4-benzohydroquinone;
 2,5-Diethylhydroquinone;
 2-Neopentyl-5-tert-butylhydroquinone;
 5 Hydroquinone;
 2-(tert-butyl)-5-isopropylbenzene-1,4-diol;
 2,5-Di-tert-pentylhydroquinone;
 2-(tert-butyl)-5-(tert-pentyl)benzene-1,4-diol;
 2,5-diisobutylbenzene-1,4-diol;
 10 5-tert-Butyl-2-methyl-hydroquinone;
 Thymoquinol;
 2,5-Dineopentylhydroquinone;
 2,5-Diisopropylhydroquinone;
 3,6-Dihydroxy-2,5-di-tert-butyltoluene;
 15 2-tert-Butyl-5-isopropylphenol;
 3,6-Di-tert-butylphenol;
 2-tert-Butyl-4-methoxy-5-methylphenol;
 2,5-Di-tert-butyl-4-methoxyphenol;
 1,4-Dimethoxy-2,5-di-tert-butylbenzene;
 20 3,5-Di-tert-butyl-4-methoxyphenol;
 A compound of formula (I)



(I)

- Wherein R₁ to R₄ are independently selected from the group consisting of H, and COR₅
 25 and, wherein R₅ is a hydrocarbon residue having 1 to 7 carbon atoms. In particular R₅ is a
 straight or branched C₁-C₇ alkyl, C₂-C₇ alkenyl or C₂-C₇ alkynyl.

In particular R₁ is COC₄H₇, R₃ is COCH₃ and R₂ and R₄ are independently selected from the group consisting of straight or branched C₁-C₇ alkyl, C₂-C₇ alkenyl.

Non limiting examples of compounds of formula (I) are Thapsigargin and

5 Thapsitranstagnin.

[0051] In one aspect, the invention provides a cell line which overexpresses SERCA3 in order to detect a compounds ability to affect GLP-1 production. SERCA3 may be overexpressed by transfection of plasmid DNA that contains a SERCA3 coding sequence, a eukaryotic promoter and a polyadenylation site sequence into a mammalian
10 cell line. Any suitable cells that allow SERCA3 expression and a chosen detection method to measure binding to or interaction with SERCA3 can be used. COS cells derived from kidney cells of the African green monkey are useful mammalian cells. Various eukaryotic promoters may be used, a useful promoter is the CMV (cytomegalovirus) promoter. The SERCA3-transfected cells can be used for detection of
15 GLP-1 levels therein.

[0052] Alternatively, transfection could be performed using any suitable cells or cell lines, for example, any mammalian cells (all mammals have the three SERCA genes including SERCA3), or any vertebrate cells (which have SERCA3 homologous genes). Invertebrate cells may be suitable as well

20 [0053] The term "GLP-1" as used herein refers to gene products of naturally occurring variants GLP-1 (1-37), GLP-1 (7-37) and -(7-36) amide and additional variants that are expressed in gut cells in vertebrate species. These include in particular, without limitation, animals commonly used in laboratories such as *Mus musculus*, *Rattus norvegicus*, and *Xenopus laevis*.

25 [0054] GLP-1 nucleic acids or polypeptides may be synthesized chemically as is well known in the art based on known sequences. Alternatively they may be amplified using clones comprising one or more GLP-1 sequences. For transfection and expression, a cDNA clone may be used. Clones that are known to comprise GLP-1 may be selected and obtained from commercial sources, or a cDNA library may be screened using all or

part of the known GLP-1 sequence. GLP-1 may be amplified using clones and/or polymerase chain reaction (PCR).

[0055] The synthetic, amplified, or isolated GLP-1 nucleic acid may then be introduced into one of many well known expression systems to produce the GLP-1 gene product/protein. For example, GLP-1 may be heterologously expressed in various cells including, without limitation, vertebrate cells or mammalian cells, as is well known in the art.

[0056] For further methods, the GLP-1 expressing cells may be used. The cells may express GLP-1 endogenously or may express GLP-1 after being subject to recombinant techniques used to introduce a nucleic acid encoding for GLP-1 into the cell.

[0057] The term "test compound" as used herein may be used singly or as a mixture of multiple test compounds. Multiple test compounds can be used to test more efficiently and to detect synergistic effects of the test compounds on SERCA3 activity.

[0058] Test compounds may include any compound including, without limitation, bioactive peptides, polypeptides, antibodies. The test compounds can be naturally occurring or synthetically produced, the latter are available as synthetic combinatorial libraries.

[0059] Moreover, GLP-1 is well conserved among mammalian species. Regarding GLP-1, invertebrate cells may be suitable as well.

[0060] If measurement of GLP-1 is used to detect test compound binding to or interaction with SERCA3, the cells chosen for transfection need to be capable of GLP-1 production in response to an exogenous stimulus. For example, without limitation, it is well known in the field that certain enteroendocrine cells are able to produce GLP-1. However, the nucleic acid sequence encoding GLP-1 may be recombinantly introduced into COS and HEK-293 cells, for example, and many other suitable cells that are well known in the art. GLP-1 measurement may then be conducted by any method known in the art or disclosed herein.

[0061] In certain embodiments, the compound that inhibits SERCA3 activity is an antibody. Methods utilized to generate antibodies include hybridoma technology, ribosome display, bacterial and yeast display, and others known in the art. The vast majority of monoclonal antibodies (mAbs) are of rodent origin. When such antibodies are

administered in a different species, patients can mount their own antibody response to such xenogenic antibodies, which may result in the eventual neutralization and elimination of the antibody. This problem for humans is minimized by using antibodies which have fully human sequences, for example made by phage display, or by engineering antibodies so that only the critical binding residues are of rodent origin, while the constant domain and optionally the framework regions of the variable domain are predominantly of human origin. Chimeric antibodies are generally antibodies wherein the constant region is of human origin and the variable region is predominantly murine. Humanized antibodies are human antibodies in which residues from the complementarity determining regions (CDR) are replaced by residues from a CDR of a non-human species such as mouse, having the desired properties such as specificity, affinity, and potency. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. This humanization strategy is generally referred to as "CDR grafting", see, e.g., Winter, U.S. Pat. No. 5,225,539. Back mutation of selected target framework residues to the corresponding donor residues might be required to restore and / or improved affinity. Structure-based methods may also be employed for humanization and affinity maturation, for example as described for humanization in U.S. Patent Appl'n Ser. No. 10/153,159. Comparison of the essential framework residues required in humanization of several antibodies, as well as computer modeling based on antibody crystal structures revealed a set of framework residues termed as "Vernier zone residues" (Foote, *J. Mol. Biol.* 224:487-499 (1992)). In addition, several residues in the VH-VL interface zone have been suggested to be important in maintaining affinity for the antigen (Santos, *Prog Nucleic Acid Res Mol Biol.* 60:169-94 (1998); Kettleborough, et al., *Protein Engin.*, 4:773-783 (1991)). Alternatively, humanized antibodies may contain the CDRs from a non-human sequence grafted into pools (e.g. libraries) of individual human framework regions. This newly engineered antibody is able to bind to the same antigen as the original antibody. The antibody constant region is derived from a human antibody. The methodology for performing this aspect is generally described as framework shuffling (Dall'Acqua, *Methods*, 36:43-60 (2005)). Furthermore, the humanized antibody may contain sequences from two or more framework regions derived from at least two human antibody germline sequences with high homology to the donor species. Antibodies

designed using this method are described as hybrid antibodies (Rother et al., U.S. Pat. No. 7,393,648). Any techniques wherein the affinity and specificity is adequately retained are encompassed herein.

[0062] "Nucleic acid sequence", as used herein, refers to an oligonucleotide, nucleotide or polynucleotide, and fragments or portions thereof, and to DNA or RNA of genomic or synthetic origin that may be single or double stranded, and represent the sense or antisense strand. "Polynucleotide" as used herein refers to DNA or RNA.

[0063] The term "double stranded RNA" as used herein is meant to encompass any and all conventional techniques for RNAi-mediated gene silencing. Such techniques are familiar to one of skill in the art and may include, but are not limited to, use of dsRNA, siRNA and shRNA to knockdown gene expression.

[0064] The term "antisense" as used herein, refers to nucleotide sequences that are complementary to a specific DNA or RNA sequence. The term "antisense strand" is used in reference to a nucleic acid strand that is complementary to the "sense" strand. Antisense molecules may be produced by any method, including synthesis by ligating the gene(s) of interest in a reverse orientation to a viral promoter that permits the synthesis of a complementary strand. Once introduced into a cell, this transcribed strand combines natural sequences produced by the cell to form duplexes. These duplexes then block either the further transcription or translation. The designation "negative" is sometimes used in reference to the antisense strand, and "positive" is sometimes used in reference to the sense strand.

[0065] As contemplated herein, antisense oligonucleotides, triple helix DNA, RNA aptamers, ribozymes and double stranded RNA are "directed to a nucleic acid sequence of SERCA3" such that the nucleotide sequence of SERCA3 chosen will produce gene-specific inhibition of SERCA3 gene expression. For example, knowledge of the SERCA3 nucleotide sequence may be used to design an antisense molecule which gives strongest hybridization to the mRNA. Similarly, ribozymes can be synthesized to recognize specific nucleotide sequences of SERCA3 and cleave it (Cech, J. Amer. Med Assn. 260:3030 (1988). Techniques for the design of such molecules for use in targeted inhibition of gene expression is well known to one of skill in the art.

[0066] In one embodiment, compounds useful in the present invention are locally active in the gastrointestinal tract and have minimal systemic activity. Such compounds may be administered orally or rectally. In other embodiments, the compounds may be administered by any suitable means, depending on the particular properties of the compound, e.g., orally, parenterally (including intravenous, intramuscular, intraperitoneal, intraosseous, or subcutaneous injection), transdermally, by inhalation, rectally, vaginally or topically (including buccal and sublingual administration). The compounds useful in the invention may generally be provided in the form of tablets or capsules, as a powder or granules, or as an aqueous solution or suspension. Tablets for oral use may include the active ingredients mixed with pharmaceutically acceptable excipients such as inert diluents, disintegrating agents, binding agents, lubricating agents, sweetening agents, flavouring agents, colouring agents and preservatives. Suitable inert diluents include sodium and calcium carbonate, sodium and calcium phosphate, and lactose, while corn starch and alginic acid are suitable disintegrating agents. Binding agents may include starch and gelatin, while the lubricating agent, if present, will generally be magnesium stearate, stearic acid or talc. If desired, the tablets may be coated with a material such as glyceryl monostearate or glyceryl distearate, to delay absorption in the gastrointestinal tract.

[0067] Dosages of the compounds of the invention will vary depending upon the condition to be treated or prevented and on the identity of the inhibitor being used. Estimates of effective dosages and in vivo half-lives for the individual compounds encompassed by the invention can be made on the basis of in vivo testing using an animal model, such as the mouse model described herein or an adaptation of such method to larger mammals. Appropriate dosage may range from, e.g., 0.01mg to 5000 mg, and is suitably adjusted to achieve a target blood level roughly corresponding to the effective level observed in vitro.

[0068] In one embodiment, the compounds are administered within one to two hours before a meal.

[0069] Compounds useful according to the invention to inhibit SERCA3 can be administered in combination or in conjunction with other known therapeutic agents useful

for treating Type II diabetes, obesity, pre-diabetic conditions and other metabolic diseases and disorders. In any event, the administering physician can adjust the amount and timing of drug administration on the basis of results observed.

- 5 The following invention will now be further described only by way of example. The following examples are presented only to illustrate the present invention and to assist one of ordinary skill in making and using the same. The examples are not intended in any way to otherwise limit the scope of the invention. Efforts have been made to ensure accuracy with respect to numbers used (*e.g.*, amounts, temperatures, etc.), but some
10 experimental error and deviation should, of course, be allowed for.

Example 1: GLP-1 Release in Wild-Type and SERCA3 Knock-out Mice and in human small intestine tissue

[0070] Immunohistochemistry with the gastrointestinal tissue

- 15 Tissue preparation: C57BL/6J mice are sacrificed, and small intestine excised. The tissue is then rinsed in 1x PBS, and fixed in 4% paraformaldehyde in PBS for one hour on ice, and cryoprotected in 20% sucrose in PBS overnight. Frozen tissues are further sliced into 12- micron thick sections. Duodenum tissue sections of human small intestine are purchased from IMGENEX (San Diego, CA; Catalogue number IMH-1028).

- 20 [0071] Immunostaining: Tissue sections are blocked in 3% BSA, 0.3% Triton X-100, 2% donkey or goat serum and 0.1% sodium azide in PBS for one hour at room temperature and then are incubated overnight in the blocking buffer containing diluted primary antibody: rabbit polyclonal antiserum against SERCA3 or goat polyclonal antiserum against GLP-1. The secondary antibodies were Alexa488-conjugated goat-anti-
25 rabbit antibody, and Alexa488-conjugated donkey anti-goat antibody.

- [0072] Double immunostaining: Tissue sections are blocked first and incubated with rabbit polyclonal anti-TrpM5 antibody, followed by Alexa488-conjugated goat-anti-rabbit antibody containing DAPI to stain nuclei. The rabbit primary antibody against SERCA3 is first labeled with Alexa647 using Zenon Tricolor Rabbit IgG labeling kit
30 (Invitrogen), and the labeled primary antibody is used to incubate the tissue sections for 2

hours at room temperature, followed by post-fixation in 4% paraformaldehyde/PBS for 10 minutes. Images are taken using a confocal microscope.

Results: Determination of whether SERCA3 is also involved in the chemosensory activity in the gastrointestinal tract, an immunostaining is performed with an anti-

5 SERCA3 antibody on mouse and human small intestine tissue sections (FIG. 1 and FIG. 2).

[0073] Results illustrate that a subset of solitary enteroendocrine cells express SERCA3 proteins. Most of the proteins concentrate in the cytosol or in the bottom half of the human cells. Since these cells are known to contain peptide hormone-filled
10 granules, the subcellular localisation of Serca3 suggests that this protein may be directly involved in the regulation of the hormone secretion by controlling the uptake of the free calcium into the endoplasmic reticulum and thus the intracellular concentrations of free calcium ions that triggers the secretion.

[0074] To determine whether these SERCA3-expressing enteroendocrine cells are
15 involved in the chemosensory responses in the gut, a double immunostaining is performed with a taste signaling protein, TrpM5, which has been found to be co-expressed in taste receptors and other taste signaling components such as a-gustducin, Gb3, Ggl3 and PLC b2 (Jang et al, PNAS, 104:15069-74, 2007). Double
20 immunostaining shows that in both mouse and human small intestine, SERCA3 proteins are nearly completely overlapping with TrpM5, suggesting that SERCA3 proteins are part of these chemosensory cells' signaling pathways (Figures 3 and 4). In particular results indicate that some bipolar-shaped cells of human intestine express both Serca3 and TrpM5.

[0075] To determine whether these enteroendocrine cells produce and secrete the
25 gut hormone, immunohistochemical studies on the adjacent mouse small intestinal sections are carried out with an antibody against GLP-1 (Figure 5). The results indicate that although the residual GLP-1 is detected all over the tissue section, the most concentrated areas of GLP-1 are the bottom portions of the enteroendocrine cells.

30 **Example 2: SERCA3 and Tastant-Induced Gut Hormone Secretion.**

[0076] To study the contribution of SERCA3 to tastant-induced gut hormone secretion, wild-type and SERCA3-KO mice are fasted overnight. Enteroendocrine cells-containing villi and crypts are collected from duodenal segment and cultured in 3-ml low-glucose DMEM medium. After cells are settled in the medium for 15 min, 0.2 ml
5 supernatant is taken immediately prior to the application of stimuli, and this sample is referred to as the “time 0” sample. The same amount of supernatant is collected at specified time points subsequent to the stimulus application. The quantity of GLP-1 in these samples is determined using an ELISA kit. The data are normalized by dividing GLP-1 amount at later time points by that at time 0, and expressed as RFU (relative fold
10 unit) (Figure 6).

Enzyme-linked immunoabsorbent assays (ELISA) or radioimmunoassays (RIA) suitable for measuring concentrations of the hormones e.g. GLP-1 mentioned herein are commercially available. One example of an ELISA kit for determining concentrations of GLP-1 is commercially available from Merck Millipore catalogue EGLP-35K.

[0077] A series of experiments is performed to determine GLP-1 production in response to different stimuli. Wild-type and SERCA3-knockout mice are fasted overnight, and then sacrificed. Median laparotomy is performed, and an incision on the antimesenteric side of the duodenum is made near the gastroduodenal junction. The duodenum is flushed with 20 ml of calcium-/magnesium-free HBSS, and the proximal 5
20 cm of duodenum is dissected out. The luminal tissues including villi and crypts are scrapped off using a glass slide, minced and washed three times in the regular HBSS. Tissue is then cultured in low-glucose DMEM with antibiotics and 5%CO₂ at 37 °C. The cultured tissue is challenged with stimulus including saccharin, thymol and isoeugenol, linolenic acid, and the culture supernatant is collected at different post-stimulation time
25 points. Dipeptidyl peptidase IV inhibitor is added to the supernatants, and GLP-1 levels in these samples is then determined using an ELISA (Millipore).

[0078] The secretion of GLP-1 from both the wild-type and Serca3-knockout gut tissue is boosted in response to 10 mM Saccharin. But the increase is about 3-4 fold in the wild-type tissue whereas 9-10 fold increase occurs in the KO tissue. And the relatively
30 high level of GLP-1 is maintained for over 20 min in the KO sample. This result indicates that the null-mutation of SERCA3 gene results in a more robust GLP-1

response to 10 mM Saccharin and the response is maintained at a relatively high level for a longer time period in comparison with that in the wild-type intestinal tissue.

Example 3: Use of SERCA3 in Conjunction with Thymol and Isoeugenol and Effects on GLP-1 Release.

[0079] Previous studies have reported that spices such as thymol and isoeugenol can also stimulate the gut hormone serotonin release (Braun et al, *Gastroenterology* 2007, 132:1890-1901). To investigate whether these spices can increase GLP-1 release from intestinal cells, and how SERCA3 contributes to the spice-evoked GLP-1 release, GLP-1 levels are assessed from the intestinal villi and crypts of both the wild-type and SERCA3-knockout mice upon stimulation of 0.1 mM of thymol and 0.5 mM of isoeugenol (Figure 7). The result illustrates a 2-3-fold increase in the release of GLP-1 from the wild-type tissue. However, a 6-fold increase is observed in the SERCA3-KO tissue sample.

Example 4: SERCA3 inhibitor and GLP-1 release.

[0080] The effect of a pharmacological reagent, the SERCA3 specific inhibitor BHQ (1,4-Dihydroxy-2,5-di-tert-butylbenzene), is tested on the appropriate gut samples (Figure 9). Prior treatment of the tissue with 30 mM BHQ for 20 minutes before the application of 5 mM Saccharin (red trace) results in a smaller GLP-1 release response in comparison to those samples without prior treatment with 30mM BHQ. The GLP-1 level was stable for the duration of the test, for example 20 minutes, during which period the GLP-1 in the BHQ-free control started to decline.

[0081] Smaller increases in GLP-1 release from the BHQ-pretreated samples are attributable to the reduced SERCA3 function by BHQ inhibition and hence a partially depleted endoplasmic reticulum of calcium content. To minimize any pre-depletion, a tastant stimulus, for example Saccharin, may be added simultaneously with BHQ to the test tissue (Figure 10). In this experiment, 0.05% DMSO is included as a control because the stock solution of BHQ is prepared in DMSO. The results illustrate that 0.05% DMSO alone or 30 mM BHQ in 0.05% DMSO cause a slight increase in GLP-1 release.

Saccharin (5 mM) in combination with 0.05% DMSO causes over a 2-fold increase. The

mixture of Saccharin and BHQ increase the GLP-1 level by 3-4.5 fold; this effect lasts for at least 20 minutes.

Immunohistochemistry has shown that Serca3 is expressed in a subset of mouse
5 enteroendocrine cells, and human enteroendocrine cells. Double immunostaining indicates that in both mouse and human small intestinal tissue sections, a group of TRPM5-expressing enteroendocrine cells also express Serca3. As in taste bud cells, TRPM5 is also involved in tastant-elicited cellular responses in the gastrointestinal tract, regulating the release of gut hormones such as glucagon-like peptide 1 (GLP-1). GLP-1
10 assays showed that knocking out Serca3 boosted the amount of GLP-1 released from the endocrine cells stimulated by such tastants as saccharin, which is apparently due to the impairment of the intracellular clearance in these cells, thus prolonging the duration of GLP-1 release. Interestingly, knocking out Serca3 also significantly augmented the spicy compound (thymol and isoeugenol) but not the fatty acid (linolenic acid) -induced GLP-1
15 release from mouse small intestine.

Pre-treatment of the tissue samples with the Serca3 inhibitor BHQ (1,4-Dihydroxy-2,5-di-tert-butylbenzene) somewhat reduced the GLP-1 release which is probably attributable to the partial depletion of calcium stores caused by Serca3 inhibition.

Simultaneous application of the tastant saccharin and the inhibitor BHQ, however, more
20 robustly induced the release of GLP-1 from mouse enteroendocrine cells than did saccharin alone. These results strongly suggest that Serca3 can be used as a target for regulating tastant-induced GLP-1 release.

**Example 5: Characterisation of the effect of Serca3 inhibition by BHQ on the
25 calcium response of human duodenal cells upon stimulation by tastants and spices.**

Biopsies of human duodenal tissue are briefly treated with calcium/magnesium- free medium and dissociated into individual cells which are then cultured in complete DMEM medium (Dulbecco's Modified Eagle medium) containing 10% fetal bovine serum. Once attached to the coverslip these cells are loaded with a calcium sensitive dye such as Fura-
30 2 AM, and stimulated with tastants and spices: saccharin, thymol and isoeugenol. The calcium responses of these cells to these stimuli are recorded in the presence and absence

of the SERCA3 inhibitor BHQ. The effect of BHQ on the calcium response amplitude and duration is determined.

- After the calcium imaging has finished the cells are fixed and immunohistochemistry is carried out to determine the expression of SERCA3, taste receptors and signalling molecules such as TRPM5 in the responsive cells.

Example 6: Characterisation of the effect of Serca3 inhibition by BHQ on the GLP-1 release from human duodenal cells upon stimulation by tastants and spices.

- Human duodenal biopsies are cultured in HBSS (Hank's balanced salt solution) medium. Saccharin, thymol and isoeugenol in the absence or presence of the SERCA3 inhibitor BHQ is added to the medium. The GLP-1 level in the medium before and after addition of these compounds is quantitatively determined using ELISA essays.
- After the ELISA assays is finished, the tissue is fixed and immunohistochemistry is carried out to determine the co-expression of SERCA3, taste receptors and signalling molecules such as TRPM5 in the responsive cells.

Example 7: Characterisation of the effect of Serca3 modulators on the calcium response of the GLP-1 release from human duodenal cells upon stimulation by tastants and spices.

- Food components such as sugars activate sweet taste receptors on some intestinal sensory cells, triggering intracellular signaling cascades that eventually elevate intracellular calcium concentrations. The increase in cytosolic calcium results in the secretion of gut hormones such as GLP-1. The hormone release is terminated when the concentration of the cytosolic calcium is restored to the pre-stimulation basal level, largely due to the sequestration of ions back into the intracellular stores by calcium pumps on the endoplasmic reticulum. By temporarily inhibiting these calcium pumps' activity, the cytosolic calcium concentration can remain at an elevated level for a longer time period, allowing more GLP-1 release from these cells.

Human gut cells or biopsies are cultured in Dulbecco's Modified Eagle Medium (DMEM). Artificial sweeteners such as saccharin that are pre-dissolved in DMEM are applied to the tissue culture. An aliquot of the medium is taken at time 0, followed by additional aliquots taken every 5 minutes. The concentration of GLP-1 in each aliquot is
5 determined using an ELISA assays. The effect of the artificial sweetener on GLP-1 release is determined by fold change; that is, the GLP-1 concentration in the later aliquot is divided by that in the aliquot at time 0.

To determine the effect of a potential SERCA3 modulator, a parallel experiment with another portion of human gut cells or biopsies is carried out in the same way as described
10 above, except that in addition to saccharin, the modulator is premixed with saccharin, and the two chemicals are simultaneously applied to the human tissue.

Negative control experiments are also performed in the same manner as described above, except that the modulator alone, or neither the modulator nor the saccharin, are applied. The GLP-1 fold changes from these four experiments are compared to determine the
15 effect of the potential modulator.

Example 8: Characterisation of Serca3's role in gut chemosensory cells' response to sweet compounds

Previous data indicated that the Serca3 knock out (KO) mice seem to have less intake of
20 sucrose, suggesting that the knockout of this gene may render the animal with a stronger anorexigenic response to caloric taste stimuli in the gut lumen, negatively regulating appetite and consequently reducing food intake. To determine whether the Serca3 knockout affects the sweet compound-mediated gut hormone release, wild type (WT) and Serca3 knockout (KO) mice are gavaged-administrated with glucose, and plasma
25 concentrations of the hormones GLP-I, insulin like growth factor 1(IGF-I), insulin like growth factor 2 (GF-II), Serotonin, and cholecystokinin (CCK) are determined using enzyme-linked immunoabsorbent assays (ELISA) or radioimmunoassay (RIA). The data is comparatively analysed between the WT and KO samples.

Enzyme-linked immunoabsorbent assays (ELISA) or radioimmunoassay (RIA) suitable
30 for measuring concentrations of the aforementioned hormones are commercially available

Example 9: Characterisation of Serca3's role in the response of gut chemosensory cells to dietary fat

In addition to the sweet taste receptors, fat receptors are also expressed in the gut
5 chemosensory cells. Dietary fat or metabolites produced by gut microbiota can regulate
gut hormone release. To determine whether the Serca3 knockout affects dietary fat-
mediated gut hormone release, WT and KO mice are fasted and then fed with a mixture
of short-, medium- and long chain fatty acids (capric, lauric, stearic, and linolenic acids),
and plasma concentrations of the hormones GLP-I, IGF-I, IGF-II, Serotonin, and CCK
10 are determined using ELISA or RIA assays. The data is comparatively analysed between
the WT and KO samples.

**Example 10: Characterisation of Serca3's role in the response of gut chemosensory
15 cells to spice**

Spice is an important flavour component. Spicy compounds such as thymol, eugenol and
isoeugenol are known to stimulate enterocytes, triggering the release of gut hormones
such as serotonin. To determine whether the Serca3 knockout affects spice-mediated gut
hormone release, WT and KO mice are fed with a mixture of thymol, eugenol and
20 isoeugenol, and plasma concentrations of the hormones GLP-I, IGF-I, IGF-II, Serotonin,
and CCK are determined using ELISA or RIA assays. The data is comparatively analysed
between the WT and KO samples.

Example 11: Administration of a Serca3 modulator in Diabetic mammals

Numerous established and accepted diabetic rat models exist for the assessment of
therapies for the treatment of diabetes. One example of a suitable diabetic animal model
is a hypoinsulinemic animal model. This model is useful because it provides for a low
insulin level in the blood and so it facilitates the assessment of any therapies on the
30 release of insulin, and in particular, the effect of any therapies on an increase in the
release of insulin in the blood stream"

Diabetic rat models are commercially available from the The Jackson Laboratory. More information can be found on the Jackson Laboratory website www.jax.org/jaxmice

- 5 A single Serca3 modulator (e.g. BHQ) can be assayed in the diabetic rat model detailed herein below.

Diabetic rats and Wistar rats are selected for administration of the Serca3 modulator (e.g. BHQ) for the treatment of diabetes. Animals are grouped according to dosage, and
10 increasing dosages (range of 0.01-100mg/kg) are utilised. The Serca3 modulator is instilled into the animal via silastic tubing inserted into the duodenum through the mouths of the lightly anesthetised animals.

Blood samples are collected via cannulation of the tail vein, and samples are withdrawn
15 at baseline, 15, 30, 60 and 120 minutes post-instillation. Blood samples are collected in collection tubes containing standard cocktails of peptidase inhibitors and preservatives, and samples are stored at -25.degree. C. until assayed. Blood samples are assayed for the presence of the hormone GLP-1. Assays for the hormone are performed using standard ELISA methodologies. Results are analyzed for efficacy of Serca3 modulators
20 administration for the treatment of diabetic rats.

Metabolites and other analyte concentrations, including glucose, free fatty acids, triglycerides, calcium, potassium, sodium, magnesium, phosphate, are also assessed. Circulating concentrations of GLP-1 are expected to increase.

25 **Example 12: Administration of a Serca3 modulator in Obese mammals**

Obesity rat models are commercially available from the The Jackson Laboratory. More information can be found on the Jackson Laboratory website www.jax.org/jaxmice

- 30 A single Serca3 modulator (e.g. BHQ) can be assayed in the obesity rat model detailed herein below.

- Industry standard diet induced obesity rats and applicable controls (healthy rats) are administered with a Serca3 modulator (e.g. BHQ) for the treatment of obesity following the procedure described above in example 11. As in example 11, animals are grouped according to dosage, and increasing dosages (range of 0.01-100mg/kg) are utilised.

Samples are collected and GLP-1 hormone assays are performed as detailed above in example 11. Circulating concentrations of GLP-1 are expected to increase.

10 SEQUENCE LISTING

[0082]

SEQ ID NO:1&2	Rat Serca3a cDNA nucleotide (SEQ ID NO:1) and amino acid sequence (999 amino acid residues) (SEQ ID NO:2) This is the only isoform of Serca3 expressed in rat taste papillae as determined by gene specific PCR with rat taste tissue cDNA.	GenBank Accession number: NM_012914.1
SEQ ID NO:3&4	Mouse Serca3a nucleotide (SEQ ID NO:3) and amino acid sequence (999 amino acid residues) (SEQ ID NO:4). Mouse taste tissues express isoforms a and b.	GenBank Accession number: NM_001163336.1
SEQ ID NO: 5&6	Mouse Serca3b nucleotide (SEQ ID NO:5) and amino acid sequence (1038 amino acid residues) (SEQ ID NO:6). Mouse taste tissues express isoforms a and b.	GenBank Accession number: NM_016745.3
SEQ ID	Human Serca3c cDNA nucleotide (SEQ ID NO:7) and amino acid	GenBank Accession number:

NO:7&8	sequence(1029 amino acid residues) (SEQ ID NO:8). This is the only isoform of Serca3 expressed in human taste papillae as determined by gene specific PCR.	NM_174958.2 NM_174956
SEQ ID NO:9&10	Human Serca3a cDNA nucleotide (SEQ ID NO:9) and amino acid sequence (SEQ ID NO:10).	GenBank Accession number: NM_005173
SEQ ID NO:11&12	Human Serca3b cDNA nucleotide (SEQ ID NO:11) and amino acid sequence (SEQ ID NO:12).	GenBank Accession number: NM_174955
SEQ ID NO:13&14	Human Serca3d cDNA nucleotide (SEQ ID NO:13) and amino acid sequence (SEQ ID NO:14).	GenBank Accession number: NM_174954
SEQ ID NO:15&16	Human Serca3e cDNA nucleotide (SEQ ID NO:5) and amino acid sequence (SEQ ID NO:16).	GenBank Accession number: NM_174953
SEQ ID NO:17&18	Human Serca3f cDNA nucleotide (SEQ ID NO:17) and amino acid sequence (SEQ ID NO:18).	GenBank Accession number: NM_174957

Rat Serca3a

- One example of a Serca3 protein is set forth as SEQ ID NO: 2 (GenBank Accession number: NM_012914.1). Preferred Serca3 proteins for use with the invention comprise
- 5 an amino acid sequence: (a) having 50% or more identity (e. g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 2; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 2, wherein n is 7 or more (e. g. 8,10, 12,14, 16,18, 20,25, 30,35, 40,50,

60,70, 80,90, 100,150, 200,250 or more). These SERCA3 proteins include variants (e. g. allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 2.

Fragments may lack one or more amino acids (e. g. 1,2, 3, 4,5, 6,7, 8,9, 10,15, 20,25 or more) from the C-terminus and/or one or more amino acids (e. g. 1,2, 3,4, 5,6, 7, 8, 9,10,

5 15,20, 25 or more) from the N-terminus of SEQ ID NO: 2. Other fragments may omit one or more domains of the protein (eg. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

One example of a nucleotide sequence encoding a Serca3 protein is set forth as SEQ ID

10 NO: 1 (GenBank Accession number: NM_012914.1). Preferred Serca3 nucleotide sequences for use with the invention comprise a nucleotide sequence: (a) having 50% or more identity (e. g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 1; and/or (b) which is a fragment of at least n consecutive nucleotides of SEQ ID NO: 1, wherein n is 7 or more (e. g. 8,10, 15 12,14, 16,18, 20,25, 30,35, 40,50, 60,70, 80,90, 100,150, 200,250 or more). These Serca3 nucleotide sequences include variants (e. g. allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 1.

SEQ ID No 2

20 MEEAHLLSAADVLRFRSVTAEGGLTLEQVTDARERYGPNELPTEEGKSLWELVVEQFEDLLVRIL
LLAALVSFVLAWFEEGEETTTAFVEPLVIMLILVANAIVGVWQERNAESAIEALKEYEPEMGKVir
SDRKGvQRIrARDIVPGDIVEVAVGDKVPADLRLEIKSTTLRVDQSILTGESVSVTKHTDAIPDPRA
VNQDKKNMLFSGTNIASGKALGVAVATGLHTELKGIRSQMAAVEPERTPLQRKLDEFGRQLSHAI
SVICVAVWVINIGHFADPAHGGSWLRGAVYYFKIAVALAVAAIPEGLPAVITTCLALGTRRMARK
25 NAIVRSLPSVETLGCTSVICSDKTGTLTTNQMSVCRMVVAEAEAGACRLHEFTISGTTYTPEGEV
RQGEQLVRCGQFDGLVELATICALCNDsALDYNEAKGVYEKVGEEATETALTCLVEKMNVFDTDL
KGLSRVERAGACNSVIKQLMQKEFTLEFSRDRKSMSVYCTPTRADPKAQGSKMFVKGAPESVIER
CSSVRVGSRTVPLSATSREHILAKIRDWGSgSHTLRCLALATRDTPPRKEDMQLDDCSQFVQYETG
LTFVGCvGMLDPPRPEVAACITRCSRAGIRVVMITGDNKGTAVAICRRLGIFGDTEdVLGKAYTGR
30 EFDDLSPeQQRQACRTARCFARVEPAHKSrIVENLQSFNEITAMTGDGVNDAPALKKAeIGIAMGS
GTAVAKSAAEMVLSDDNFASIVAAVEEGRAIYNNMKQFIRYLISSNVGEVVCIFLTAILGLPEALIP
VQLLWVNLVTDGLPATALGFNPPDLIMEKLPRNPREALISGWLFFRYLAIGVYVGLATVAAATW
WFLYDAEGPQVTFHQLRNFLKCEDNPLFAGIDCEVFESRFPTTMALSVLVTIEMCNALNSVSENQ

SLLRMPPWLNPNWLLGAVVMSMALHFLILLVPPLPLIFQVTPLSGRQWGVVLQMSLPVILLDEALK
YLSRHHVDEKKDLK

Mouse Serca3a

- 5 One example of a Serca3 protein is set forth as SEQ ID NO: 4 (GenBank Accession number: NM_001163336.1). Preferred Serca3 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e. g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 4; and/or (b) which is a fragment of at least n consecutive amino
10 acids of SEQ ID NO: 4, wherein n is 7 or more (e.g. 8,10, 12,14, 16,18, 20,25, 30,35, 40,50, 60,70, 80,90, 100,150, 200,250 or more). These Serca3 proteins include variants (e. g. allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 4. Fragments may lack one or more amino acids (e. g. 1,2, 3, 4,5, 6,7, 8,9, 10,15, 20,25 or more) from the C-terminus and/or one or more amino acids (e. g. 1,2, 3,4, 5,6, 7, 8, 9,10,
15 15,20, 25 or more) from the N-terminus of SEQ ID NO: 4. Other fragments may omit one or more domains of the protein (eg. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

- One example of a nucleotide sequence encoding a Serca3 protein is set forth as SEQ ID
20 NO: 3 (GenBank Accession number: NM_001163336.1). Preferred Serca3 nucleotide sequences for use with the invention comprise a nucleotide sequence: (a) having 50% or more identity (e. g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 3; and/or (b) which is a fragment of at least n consecutive nucleotides of SEQ ID NO: 3, wherein n is 7 or more (e. g. 8,10,
25 12,14, 16,18, 20,25, 30,35, 40,50, 60,70, 80,90, 100,150, 200,250 or more). These Serca3 nucleotide sequences include variants (e. g. allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 3.

SEQ ID No 4

- 30 MEEAHLISAADVLRFRFSVTAEGGLSLEQVTDARERYGPNELPTEEGKSLWELVVEQFEDLLVRILL
LAALVSFVLAWFEEGEETTTAFVEPLVIMLILVANAIVGVWQERNAESAIEALKEYEPENGKVIRES
DRKGVQRIRARDIVPGDIVEVAVGDKVPADLRLLIEIKSTTLRVDQSILTGESVSVTKHTDAIPDPRA

VNQDKKNMLFSGTNIASGKALGVAVATGLQTELGKIRSQMAAVEPERTPLQRKLDEFGRQLSHAI
 SVICVAVVWINIGHFADPAHGGSWLRGAVYYFKIAVALAVAAIPEGLPAVITTCLALGTRRMARK
 NAIVRSLPSVETLGCTSVICSDKTGTLTTNQMSVCRMVVAEAEAGTCRLHEFTISGTTYTPEGEVR
 QGEQPVRCGQFDGLVELATICALCNDSSALDYNEAKGVYEKVGEATETALTCLVEKMNVFDTDLK
 5 GLSRVERAGACNSVIKQLMRKEFTLEFSRDRKSMSVYCTPTRADPKVQGSKMFVKGAPESVIERC
 SSVRVGSRTAPLSTTSREHILAKIRDWGSGLTLRCLALATRDTPPRKEDMHLDDCSRFBVQYETDL
 TFVGCVGMLDPPRPEVAACITRCSRAGIRVVMITGDNKGTAVAICRRLGIFGDTEDVLGKAYTGRE
 FDDLSPQQRQACRTARCFARVEPAHKSRIVENLQSFNEITAMTGDGVNDAPALKKAEIGIAMGSG
 TAVAKSAAEMVLSDDNFASIVAAVEEGRAIYNNMKQFIRYLISSNVGEVVCIFLTAILGLPEALIPV
 10 QLLWVNLVTDGLPATALGFNPPDLIMEKPPRNPREALISGWLFFRYLAIGVYVGLATVAAATW
 WFLYDTEGPQVTFYQLRNFLKCEDNPLFAGIDCKVFESRFPPTMALSVLVTIEMCNALNSVSENQ
 SLLRMPPWLNPNWLLGAVVMSMALHFLILLVPLPLIFQVTPLSGRQWGVVLQMSLPVILLDEALK
 YLSRNHMDEKKDLK

15 Mouse Serca3b

- One example of a Serca3 protein is set forth as SEQ ID NO: 6 (GenBank Accession
 number: NM_016745.3). Preferred Serca3 proteins for use with the invention comprise
 an amino acid sequence: (a) having 50% or more identity (e. g. 60%, 65%, 70%, 75%,
 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to
 20 SEQ ID NO: 6; and/or (b) which is a fragment of at least n consecutive amino acids of
 SEQ ID NO: 6, wherein n is 7 or more (e. g. 8,10, 12,14, 16,18, 20,25, 30,35, 40,50,
 60,70, 80,90, 100,150, 200,250 or more). These Serca3 proteins include variants (e. g.
 allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 6.
 Fragments may lack one or more amino acids (e. g. 1,2, 3, 4,5, 6,7, 8,9, 10,15, 20,25 or
 25 more) from the C-terminus and/or one or more amino acids (e. g. 1,2, 3,4, 5,6, 7, 8, 9,10,
 15,20, 25 or more) from the N-terminus of SEQ ID NO: 6. Other fragments may omit one
 or more domains of the protein (eg. omission of a signal peptide, of a cytoplasmic
 domain, of a transmembrane domain, or of an extracellular domain).
- 30 One example of a nucleotide sequence encoding a Serca3 protein is set forth as SEQ ID
 NO: 5 (GenBank Accession number: NM_016745.3). Preferred Serca3 nucleotide
 sequences for use with the invention comprise a nucleotide sequence: (a) having 50% or
 more identity (e. g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%,

96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 5; and/or (b) which is a fragment of at least n consecutive nucleotides of SEQ ID NO: 5, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Serca3 nucleotide sequences include variants (e.g. allelic variants, homologs, orthologs, 5 paralog, mutants, etc.) of SEQ ID NO: 5.

SEQ ID No 6

MEEAHLLSAADVLRFRSVTAEGGLSLEQVTDARERYGNELPTEEGKSLWELVVEQFEDLLVRILL
 LAALVSFVLAWFEEGEETTTAFVEPLVIMLILVANAIVGWQERNAESAIEALKEYEPENMGKVIRS
 10 DRKGVQRIRARDIVPGDIVEVAVGDKVPADLRLEIKSTTLRVDQSILTGESVSVTKHTDAIPDPRA
 VNQDKKNMLFSGTNIASGKALGVAVATGLQTELGKIRSQMAAVEPERTPLQRKLDEFGRQLSHAI
 SVICVAVWVINIGHFADPAHGGSWLRGAVYYFKIAVALAVAAIPEGLPAVITTCALGTRRMARK
 NAIVRSLPSVETLGCTSVICSDKTGTLTTNQMSVCRMVVAEAEAGTCRLHEFTISGTTYTPEGEVR
 QGEQPVRCGQFDGLVELATICALCNDSDLDYNEAKGVYEVGEATETALTCLVEKMNVDFTDLK
 15 GLSRVERAGACNSVIKQLMRKEFTLEFSRDRKSMSVYCTPTRADPKVQGSKMFVKGAPESVIERC
 SSVRVGSRTAPLSTTSREHILAKIRDWGSGLTLRCLALATRDTPPRKEDMHLDDCSRFBVQYETDL
 TFVGCVGMLDPPRPEVAACITRCSRAGIRVVMITGDNKGTAVAICRRLGIFGDTEDVLGKAYTGRE
 FDDLSPQQRQACRTARCFARVEPAHKSRIVENLQSFNEITAMTGDGVNDAPALKKAIEIGIAMGSG
 TAVAKSAAEMVLSDDNFASIVAAVEEGRAIYNNMKQFIRYLISSNVGEVVCIFLTAILGLPEALIPV
 20 QLLWVNLVTDGLPATALGFNPPDLIDIMEKPPRNPREALISGWLFRLAIGVYVGLATVAAATW
 WFLYDTEGPQVTFYQLRNFLKCEDNPLFAGIDCKVFESRFPPTMALSVLVTIEMCNALNSVSENQ
 SLLRMPPWLNPNWLLGAVVMSMALHFLILLVPLPLIFQVTPLSGRQWGVVLQMSLPVILLDEALK
 YLSRNHMDGVLGTFMQARSRLPTTSRTPYHTGKKGPVENPGSRGESPVWPSD

Human Serca3c

One example of a Serca3 protein is set forth as SEQ ID NO: 8 (GenBank Accession number: NM_174958.2). Preferred Serca3 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to 30 SEQ ID NO: 8; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 8, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Serca3 proteins include variants (e.g. allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 8. Fragments may lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or

more) from the C-terminus and/or one or more amino acids (e.g. 1,2, 3,4, 5,6, 7, 8, 9,10, 15,20, 25 or more) from the N-terminus of SEQ ID NO: 8. Other fragments may omit one or more domains of the protein (eg. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

5

One example of a nucleotide sequence encoding a Serca3 protein is set forth as SEQ ID NO: 7 (GenBank Accession number: NM_174958.2). Preferred Serca3 nucleotide sequences for use with the invention comprise a nucleotide sequence: (a) having 50% or more identity (e. g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 10 96%, 97%, 98%, 99%, 99. 5% or more) to SEQ ID NO: 7; and/or (b) which is a fragment of at least n consecutive nucleotides of SEQ ID NO: 7, wherein n is 7 or more (e. g. 8,10, 12,14, 16,18, 20,25, 30,35, 40,50, 60,70, 80,90, 100,150, 200,250 or more). These Serca3 nucleotide sequences include variants (e. g. allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 7.

15

SEQ ID No 8

MEAAHLLPAADVLRHFSVTAEGGLSPAQVTGARERYGPNELPSEEGKSLWELVLEQFEDLLVRIL
LLAALVSFVLAWFEEGEETTTAFVEPLVIMLILVANAIVGVWQERNAESAIEALKEYEPEMGKVIR
SDRKGVQIRIRARDIVPGDIVEVAVGDKVPADRLRIEIKSTTLRVDQSILTGESVSVTKHTEAIPDPRA
20 VNQDKKNMLFSGTNITSGKAVGVAVATGLHTELGKIRSQMAAVEPERTPLQRKLDEFGRQLSHAI
SVICVAVVWINIGHFADPAHGGSWLRGAVYYFKIAVALAVAAIPEGLPAVITTCLALGTRRMARK
NAIVRSLPSVETLGCTSVICSDKTGTLTNQMSVCRMVVAEADAGSCLLHEFTISGTTYTPEGEVR
QGDQPVRRCGQFDGLVELATICALCNDSDLDYNEAKGVYEKVGEATETALTCLVEKMNVFDTDQL
ALS RVERAGACNTVIKQLMRKEFTLEFSRDRKSMSVYCTPTRPHPTGQGSKMFVKGAPESVIERCS
25 SVRVGSRTAPLTPTSREQILAKIRDWGS GSDTLRCLALATRDAPPRKEDMELDDCSKFVQYETDLT
FVGCVGMLDPPRPEVAACITRCYQAGIRVVMITGDNKGTAVAICRRLGIFGDTEDEVAGKAYTGRE
FDDLSPEQQRQACRTARCFARVEPAHKSRIVENLQSFNEITAMTGDGVNDAPALKKAEIGIAMGSG
TAVAKSAAEMVLSDDNFASIVA AVEEGRAIYSNMKQFIRYLISNVGEVVCIFLTAILGLPEALIPV
QLLWVNLVTDGLPATALGFNPPDL DIMEKLPRSPREALISGWLFFRYLAIGVYVGLATVAAATWW
30 FVYDAEGPHINFYQLRNFLKCEDNPLFAGIDCEVFESRFPTTMALSVLVTIEMCNALNSVSENQSL
LRMPPWMNPWLLVAVAMSMALHFLILLVPPLPLIFQVTPLSGRQWVVVLQISLPVILLDEALKYLS
RNHMHACLYPGLLRTVSQAWSRQPLTTSWTPDHTGLASLKK

Human Serca3a

One example of a Serca3 protein is set forth as SEQ ID NO: 10 (GenBank Accession number: NM_005173). Preferred Serca3 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e. g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 10; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 10, wherein n is 7 or more (e. g. 8,10, 12,14, 16,18, 20,25, 30,35, 40,50, 60,70, 80,90, 100,150, 200,250 or more). These Serca3 proteins include variants (e. g. allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 10. Fragments may lack one or more amino acids (e. g. 1,2, 3, 4,5, 6,7, 8,9, 10,15, 20,25 or more) from the C-terminus and/or one or more amino acids (e. g. 1,2, 3,4, 5,6, 7, 8, 9,10, 15,20, 25 or more) from the N-terminus of SEQ ID NO: 10. Other fragments may omit one or more domains of the protein (eg. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

One example of a nucleotide sequence encoding a Serca3 protein is set forth as SEQ ID NO: 9 (GenBank Accession number: NM_005173). Preferred Serca3 nucleotide sequences for use with the invention comprise a nucleotide sequence: (a) having 50% or more identity (e. g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 9; and/or (b) which is a fragment of at least n consecutive nucleotides of SEQ ID NO: 9, wherein n is 7 or more (e. g. 8,10, 12,14, 16,18, 20,25, 30,35, 40,50, 60,70, 80,90, 100,150, 200,250 or more). These Serca3 nucleotide sequences include variants (e. g. allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 9.

SEQ ID No 10

MEAAHLLPAADVLRHFSVTAEGGLSPAQVTGARERYGPNELPSEEGKSLWELVLEQFEDLLVRIL
LLAALVSFVLAWFEEGEETTTAFVEPLVIMLILVANAIVGWQERNAESAIEALKEYEPEMGKVIR
SDRKGVQIRIRARDIVPGDIVEVAVGDKVPADLRLEIKSTTLRVDQSILTGESVSVTKHTEAIPDPRA
VNQDKKNMLFSGTNITSGKAVGVAVATGLHTELGKIRSQMAAVEPERTPLQRKLDEFGRQLSHAI
SVICVAVWVINIGHFADPAHGGSWLRGAVYYFKIAVALAVAAIPEGLPAVITTCLALGTRRMARK
NAIVRSLPSVETLGCTSVICSDKTGTLTTNQMSVCRMVVAEADAGSCLLHEFTISGTTYTPEGEVR

QGDQPVRCGQFDGLVELATICALCNDSDALDYNEAKGVYEKVGAEATETALTCLVEKMNVFDTDLQ
 ALSRVERAGACNTVIKQLMRKEFTLEFSRDRKSMSVYCTPTRPHPTGQGSKMFMVKGAPESVIERCS
 SVRVGSRAPLTPTSREQILAKIRDWGSQSDTLRCLALATRDAPPRKEDMELDDCSKFVQYETDLT
 FVGCVGMLDPPRPEVAACITRCYQAGIRVVMITGDNKGTAVAICRRLGIFGDTEDVAGKAYTGRE
 5 FDDLSPQQRQACRTARCFARVEPAHKSRIVENLQSFNEITAMTGDGVNDAPALKKAEIGIAMGSG
 TAVAKSAAEMVLSDDNF
 ASIVAAVEEGRAIYSNMKQFIRYLISSNVGEVVCIFLTAILGLPEALIPVQLLWVNLVTDGLPATAL
 GENPPDLIMEKLPRSPREALISGWLFFRYLAIGVYVGLATVAAATWWFVYDAEGPHINFYQLRN
 FLKCEDNPLFAGIDCEVFESRFPPTMALSVLVTIEMCNALNSVSENQSLLRMPWMPWNPWLLVAV
 10 AMSMALHFLILLVPPLPLIFQVTPLSGRQWVVVLQISLPVILLDEALKYLSRNMHEEMSQK

Human Serca3b

One example of a Serca3 protein is set forth as SEQ ID NO: 12 (GenBank Accession
 number: NM_174955). Preferred Serca3 proteins for use with the invention comprise an
 15 amino acid sequence: (a) having 50% or more identity (e. g. 60%, 65%, 70%, 75%, 80%,
 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ
 ID NO: 12; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ
 ID NO: 12, wherein n is 7 or more (e. g. 8,10, 12,14, 16,18, 20,25, 30,35, 40,50, 60,70,
 80,90, 100,150, 200,250 or more). These Serca3 proteins include variants (e. g. allelic
 20 variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 12. Fragments
 may lack one or more amino acids (e. g. 1,2, 3, 4,5, 6,7, 8,9, 10,15, 20,25 or more) from
 the C-terminus and/or one or more amino acids (e. g. 1,2, 3,4, 5,6, 7, 8, 9,10, 15,20, 25 or
 more) from the N-terminus of SEQ ID NO: 12. Other fragments may omit one or more
 domains of the protein (eg. omission of a signal peptide, of a cytoplasmic domain, of a
 25 transmembrane domain, or of an extracellular domain).

One example of a nucleotide sequence encoding a Serca3 protein is set forth as SEQ ID
 NO: 11 (GenBank Accession number: NM_174955). Preferred Serca3 nucleotide
 sequences for use with the invention comprise a nucleotide sequence: (a) having 50% or
 30 more identity (e. g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%,
 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 11; and/or (b) which is a
 fragment of at least n consecutive nucleotides of SEQ ID NO: 11, wherein n is 7 or more
 (e. g. 8,10, 12,14, 16,18, 20,25, 30,35, 40,50, 60,70, 80,90, 100,150, 200,250 or more).

These Serca3 nucleotide sequences include variants (e. g. allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 11..

SEQ ID No 12

5 MEAAHLLPAADVLRHFSVTAEGGLSPAQVTGARERYGPNELPSEEGKSLWELVLEQFEDLLVRIL
LLAALVSFVLAWFEEGEETTTAFVEPLVIMLILVANAIVGVWQERNAESAIEALKEYEPEMGKVIR
SDRKGVQRIRARDIVPGDIVEVAVGDKVPADRLRLIEIKSTTLRVDQSILTGESVSVTKHTEAIPDPRA
VNQDKKNMLFSGTNITSGKAVGVAVATGLHTEL GKIRSQMAAAVEPERTPLQRKLDEFGRQLSHAI
SVICVAVWVINIGHFADPAHGGSWLRGAVYYFKIAVALAVAAIPEGLPAVITTCALGTRRMARK
10 NAIVRSLPSVETLGCTSVICSDKTGTLTTNQMSVCRMVVAEADAGSCLLHEFTISGTTYTPEGEVR
QGDQPVRCGQFDGLVELATICALCNDSDLDYNEAKGVYEKVGATETALTCLVEKMNVFDTDLQ
ALS RVERAGACNTVIKQLMRKEFTLEFSRDRKSMSVYCTPTRPHPTGQGSKMFVKGAPESVIERCS
SVRVGSR TAPLTPTSREQILAKIRDWGSQSDTLRCLALATRDAPPRKEDMELDDCSKFVQYETDLT
FVGCVGMLDPPRPEVAACITRCYQAGIRVVMITGDNKGTAVAICRRLGIFGDTEDVAGKAYTGRE
15 FDDLSPQQRQACRTARCFARVEPAHKS RIVENLQSFNEITAMTGDGVNDAPALKKAEIGIAMGSG
TAVAKSAAEMVLSDDNF
ASIVA AVEEGRAIYSNMKQFIRYLISSNVGEVVCIFLTAILGLPEALIPVQLLWVNLVTDGLPATAL
GFNPPDL DIMEKLPRSPREALISGWLFFRYLAIGVYVGLATVAAATWWFVYDAEGPHINFYQLRN
FLKCEDNPLFAGIDCEVFESRFPTTMALSVLVTIEMCNALNSVSENQSLLRMPWPWNPWLLVAV
20 AMSMALHFLILLVPPLPLIFQVTPLSGRQWVVVLQISLPVILLDEALKYLSRNHMHACLYPGLLRT
VSQAWSRQPLTTSWTPDHTGRNEPEVSAGNRVESPVCTSD

Human Serca3d

One example of a Serca3 protein is set forth as SEQ ID NO: 14 (GenBank Accession
25 number: NM_174954). Preferred Serca3 proteins for use with the invention comprise an
amino acid sequence: (a) having 50% or more identity (e. g. 60%, 65%, 70%, 75%, 80%,
85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ
ID NO: 14; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ
ID NO: 14, wherein n is 7 or more (e. g. 8,10, 12,14, 16,18, 20,25, 30,35, 40,50, 60,70,
30 80,90, 100,150, 200,250 or more). These Serca3 proteins include variants (e. g. allelic
variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 14. Fragments
may lack one or more amino acids (e. g. 1,2, 3, 4,5, 6,7, 8,9, 10,15, 20,25 or more) from
the C-terminus and/or one or more amino acids (e. g. 1,2, 3,4, 5,6, 7, 8, 9,10, 15,20, 25 or
more) from the N-terminus of SEQ ID NO: 14. Other fragments may omit one or more

domains of the protein (eg. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

- One example of a nucleotide sequence encoding a Serca3 protein is set forth as SEQ ID NO: 13 (GenBank Accession number: NM_174954). Preferred Serca3 nucleotide sequences for use with the invention comprise a nucleotide sequence: (a) having 50% or more identity (e. g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 13; and/or (b) which is a fragment of at least n consecutive nucleotides of SEQ ID NO: 13, wherein n is 7 or more (e. g. 8,10, 12,14, 16,18, 20,25, 30,35, 40,50, 60,70, 80,90, 100,150, 200,250 or more). These SERCA3 nucleotide sequences include variants (e. g. allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 13.

SEQ ID No 14

- 15 MEAAHLLPAADVLRHFSVTAEGGLSPAQVTGARERYGPNELPSEEGKSLWELVLEQFEDLLVRIL
LLAALVSFVLAWFEEGEETTTAFVEPLVIMLILVANAIVGWVQERNAESAIEALKEYEPEMGKVir
SDRKGvQRIRARDIVPGDIVEVAVGDKVPADLRLEIKSTTLRVDQSILTGESVSVTKHTEAIPDPRA
VNQDKKNMLFSGTNITSGKAVGVAVATGLHTELGKIRSQMAAVEPERTPLQRKLDEFGRQLSHAI
SVICVAVWVINIGHFADPAHGGSWLRGAVYYFKIAVALAVAAIPEGLPAVITTCLALGTRRMARK
20 NAIVRSLPSVETLGCTSVICSDKTGTLTTNQMSVCRMVVAEADAGSCLLHEFTISGTTYTPEGEVR
QGDQPVRRCGQFDGLVELATICALCNDSDLDYNEAKGVYEKVGATETALTCLVEKMNVFDTDLQ
ALSRVERAGACNTVIKQLMRKEFTLEFSRDRKSMSVYCTPTRPHPTGQGSKMFVKGAPESVIERCS
SVRVGSRTAPLTPTSREQILAKIRDWGSGLTLRCLALATRDAPPRKEDMELDDCSKFVQYETDLT
FVGCVGMLDPPRPEVAACITRCYQAGIRVVMITGDNKGTAVAICRRLGIFGDTEDVAGKAYTGRE
25 FDDLSPQQRQACRTARCFARVEPAHKSRIVENLQSFNEITAMTGDGVNDAPALKKAIEIGIAMGSG
TAVAKSAAEMVLSDDNFASIVAAVEEGRAIYSNMKQFIRYLISSNVGEVVCIFLTAILGLPEALIPV
QLLWVNLVTDGLPATALGFNPPDLIMEKLPRSPREALISGWLFFRYLAIGVYVGLATVAAATWW
FVYDAEGPHINFYQLRNFLKCEDNPLFAGIDCEVFESRFPPTMALSVLVTIEMCNALNSVSENQSL
LRMPWPWNPWLLVAVAMSMALHFLILLVPPLPLIFQVTPLSGRQWVVVLQISLPVILLDEALKYLS
30 RNHMHACLYPGLLRTVSQAWSRQPLTTSWTPDHTGARDTASSRCQSCSREEAGKK

Human Serca3e

One example of a Serca3 protein is set forth as SEQ ID NO: 16 (GenBank Accession number: NM_174953). Preferred Serca3 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e. g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 16; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 16, wherein n is 7 or more (e. g. 8,10, 12,14, 16,18, 20,25, 30,35, 40,50, 60,70, 80,90, 100,150, 200,250 or more). These Serca3 proteins include variants (e. g. allelic variants, homologs, orthologs, paralog, mutants, etc.) of SEQ ID NO: 16. Fragments may lack one or more amino acids (e. g. 1,2, 3, 4,5, 6,7, 8,9, 10,15, 20,25 or more) from the C-terminus and/or one or more amino acids (e. g. 1,2, 3,4, 5,6, 7, 8, 9,10, 15,20, 25 or more) from the N-terminus of SEQ ID NO: 16. Other fragments may omit one or more domains of the protein (eg. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

One example of a nucleotide sequence encoding a Serca3 protein is set forth as SEQ ID NO: 15 (GenBank Accession number: NM_174953). Preferred Serca3 nucleotide sequences for use with the invention comprise a nucleotide sequence: (a) having 50% or more identity (e. g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 15; and/or (b) which is a fragment of at least n consecutive nucleotides of SEQ ID NO: 15, wherein n is 7 or more (e. g. 8,10, 12,14, 16,18, 20,25, 30,35, 40,50, 60,70, 80,90, 100,150, 200,250 or more). These Serca3 nucleotide sequences include variants (e. g. allelic variants, homologs, orthologs, paralog, mutants, etc.) of SEQ ID NO: 15.).

SEQ ID No 16

MEAAHLLPAADVLRHFSVTAEGGLSPAQVTGARERYGPNELPSEEGKSLWELVLEQFEDLLVRIL
LLAALVSFVLAWFEEGEETTTAFVEPLVIMLILVANAIVGVWQERNAESAIEALKEYEPEMGKVir

SDRKGVQRIRARDIVPGDIVEVAVGDKVPADLRLEIKSTTLRVDQSILTGESVSVTKHTEAIPDPRA
 VNQDKKNMLFSGTNITSGKAVGVAVATGLHTELGKIRSQMAAVEPERTPLQRKLDEFGRQLSHAI
 SVICVAVWVINIGHFADPAHGGSWLRGAVYYFKIAVALAVAAIPEGLPAVITTCALGTRRMARK
 NAIVRSLPSVETLGCTSVICSDKTGTLTTNQMSVCRMVVAEADAGSCLLHEFTISGTTYTPEGEVR
 5 QGDQPVRRCGQFDGLVELATICALCNDSDLDYNEAKGVYEKVGAEATETALTCLVEKMNVFDTDLQ
 ALSRVERAGACNTVIKQLMRKEFTLEFSRDRKSMSVYCTPTRPHPTGQGSKMFVKGAPESVIERCS
 SVRVGSRTAPLTPTSREQILAKIRDWGSGLDLRCLALATRDAPPRKEDMELDDCSKFVQYETDLT
 FVGCVGMLDPPRPEVAACITRCYQAGIRVVMITGDNKGTAVAICRRLGIFGDTEDVAGKAYTGRE
 FDDLSPQQRQACRTARCFARVEPAHKSRIVENLQSFNEITAMTGDGVNDAPALKKAEIGIAMGSG
 10 TAVAKSAAEMVLSDDNFASIVAAVEEGRAIYSNMKFIRYLISNVGEVVCIFLTAILGLPEALIPV
 QLLWVNLVTDGLPATALGFNPPDLIMEKLPRSPREALISGWLFFRYLAIGVYVGLATVAAATWW
 FVYDAEGPHINFYQLRNFLKCEDNPLFAGIDCEVFESRFPTTMALSVLVTIEMCNALNSVSENQSL
 LRMPPWMNPWLLVAVAMSMALHFLILLVPLPLIFQVTPLSGRQWVVVLQISLPVILLDEALKYLS
 RNHMHACLYPGLLRTVSQAWSRQPLTTSWTPDHTGLASLGQGHISIVLSSELLREGGSREEMSQK

15

Human Serca3f

One example of a Serca3 protein is set forth as SEQ ID NO: 18 (GenBank Accession number: NM_174957). Preferred Serca3 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e. g. 60%, 65%, 70%, 75%, 80%,
 20 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 18; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 18, wherein n is 7 or more (e. g. 8,10, 12,14, 16,18, 20,25, 30,35, 40,50, 60,70, 80,90, 100,150, 200,250 or more). These Serca3 proteins include variants (e. g. allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 18. Fragments
 25 may lack one or more amino acids (e. g. 1,2, 3, 4,5, 6,7, 8,9, 10,15, 20,25 or more) from the C-terminus and/or one or more amino acids (e. g. 1,2, 3,4, 5,6, 7, 8, 9,10, 15,20, 25 or more) from the N-terminus of SEQ ID NO: 18. Other fragments may omit one or more domains of the protein (eg. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

30

One example of a nucleotide sequence encoding a Serca3 protein is set forth as SEQ ID NO: 17 (GenBank Accession number: NM_174957). Preferred Serca3 nucleotide sequences for use with the invention comprise a nucleotide sequence: (a) having 50% or

more identity (e. g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 17; and/or (b) which is a fragment of at least n consecutive nucleotides of SEQ ID NO: 17, wherein n is 7 or more (e. g. 8,10, 12,14, 16,18, 20,25, 30,35, 40,50, 60,70, 80,90, 100,150, 200,250 or more).

- 5 These Serca3 nucleotide sequences include variants (e. g. allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 17..

SEQ ID No 18

MEAAHLLPAADVLRHFSVTAEGGLSPAQVTGARERYGPNELPSEEGKSLWELVLEQFEDLLVRIL
 10 LLAALVSFVLAWFEEGEETTTAFVEPLVIMLILVANAIVGVWQERNAESAIEALKEYEPEMGKVIR
 SDRKGVQRIRARDIVPGDIVEVAVGDKVPADLRLEIKSTTLRVDQSILTGESVSVTKHTEAIPDPRA
 VNQDKKNMLFSGTNITSGKAVGVAVATGLHTELGKIRSQMAAVEPERTPLQRKLDEFGRQLSHAI
 SVICVAVVWINIGHFADPAHGGSWLRGAVYYFKIAVALAVAAIPEGLPAVITTCLALGTRRMARK
 NAIVRSLPSVETLGCTSVICSDKTGTLTTNQMSVCRMVVAEADAGSCLLHEFTISGTTYTPEGEVR
 15 QGDQPVRCGQFDGLVELATICALCNDSDLDYNEAKGVYEKVGATETALTCLVEKMNVFDTDLQ
 ALSRVERAGACNTVIKQLMRKEFTLEFSRDRKSMSVYCTPTRPHPTGQGSKMFVKGAPESVIERCS
 SVRVGSRTAPLTPTSREQILAKIRDWGSGLTLRCLALATRDAPPRKEDMELDDCSKFVQYETDLT
 FVGCVGMLDPPRPEVAACITRCYQAGIRVVMITGDNKGTAVAICRRLGIFGDTEDVAGKAYTGRE
 FDDLSPQQRQACRTARCFARVEPAHKSRIVENLQSFNEITAMTGDGVNDAPALKKAEIGIAMGSG
 20 TAVAKSAAEMVLSDDNFASIVAAVEEGRAIYSNMKQFIRYLISSNVGEVVCIFLTAILGLPEALIPV
 QLLWVNLVTDGLPATALGFNPPDLIMEKLPRSPREALISGWLFFRYLAIGVYVGLATVAAATWW
 FVYDAEGPHINFYQLRNFLKCEDNPLFAGIDCEVFESRFPPTMALSVLVTIEMCNALNSVSENQSL
 LRMPWPWNPLLVAVAMSMALHFLILLVPPLPLIFQVTPLSGRQWVVVLQISLPVILLDEALKYLS
 RNHMHEMSQK

25

It will be understood by the skilled person that as a result of the degeneracy of the genetic code numerous different nucleotide sequences can encode the same Serca3 protein of the present invention. In addition, it is to be understood that skilled persons may, using routine techniques, make nucleotide substitutions that do not affect the Serca3 protein
 30 encoded by the nucleotide sequence of the present invention to reflect the codon usage of any particular host organism in which the Serca3 protein of the present invention is to be expressed.

Examples of nucleotide sequences encoding Serca3 protein are set forth as SEQ ID NO: 1 (GenBank Accession number: NM_012914.1), SEQ ID NO: 3 (GenBank Accession number: NM_001163336.1), SEQ ID NO: 5 (GenBank Accession number: NM_016745.3), SEQ ID NO: 7 (GenBank Accession number: NM_174958.2), SEQ ID NO: 9 (GenBank Accession number: NM_005173), SEQ ID NO: 11 (GenBank Accession number: NM_174955), SEQ ID NO: 13 (GenBank Accession number: NM_174954), SEQ ID NO: 15 (GenBank Accession number: NM_174953), SEQ ID NO: 17 (GenBank Accession number: NM_174957). Preferred nucleotide sequences for use with the invention comprise a nucleotide sequence that is substantially homologous to SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, or SEQ ID NO: 17.

A "substantially homologous" nucleotide sequence of the present invention has 50% or more identity (e. g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, or SEQ ID NO: 17.

In general, "identity" refers to an exact nucleotide-to-nucleotide or amino acid-to- amino acid correspondence of two polynucleotides or polypeptide sequences, respectively. Two or more sequences (polynucleotide or amino acid) can be compared by determining their "percent identity". The percent identity of two sequences, whether nucleic acid or amino acid sequences, is the number of exact matches between two aligned sequences divided by the length of the shorter sequences and multiplied by 100. An approximate alignment for nucleic acid sequences is provided by the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics* 2: 482-489 (1981). This algorithm can be applied to amino acid sequences by using the scoring matrix developed by Dayhoff, *Atlas of Protein Sequences and Structure*, M. O. Dayhoff ed. , 5 suppl. 3: 353-358, National Biomedical Research Foundation, Washington, D. C. , USA, and normalized by Gribskov, *Nucl. Acids Res.* 14 (6): 6745- 6763 (1986). An exemplary implementation of this algorithm to determine percent identity of a sequence is provided by the Genetics

- Computer Group (Madison, WI) in the "BestFit" utility application. The default parameters for this method are described in the Wisconsin Sequence Analysis Package Program Manual, Version 8 (1995) (available from Genetics Computer Group, Madison, WI). A preferred method of establishing percent identity in the context of the present
- 5 invention is to use the MPSRCH package of programs copyrighted by the University of Edinburgh, developed by John F. Collins and Shane S. Sturrock, and distributed by IntelliGenetics, Inc. (Mountain View, CA). From this suite of packages the Smith-Waterman algorithm can be employed where default parameters are used for the scoring table (for example, gap open penalty of 12, gap extension penalty of one, and a gap of
- 10 six). From the data generated the "Match" value reflects "sequence identity." Other suitable programs for calculating the percent identity or similarity between sequences are generally known in the art, for example, another alignment program is BLAST, used with default parameters. For example, BLASTN and BLASTP can be used using the following default parameters: genetic code = standard; filter = none; strand = both; cutoff = 60 ;
- 15 expect = 10 ; Matrix = BLOSUM62; Descriptions = 50 sequences; sort by = HIGH SCORE; Databases = non-redundant, GenBank + EMBL + DDBJ + PDB + GenBank CDS translations + Swiss protein + Spupdate + PIR. Details of these programs can be found at the following internet address: <http://www.ncbi.nlm.gov/cgi-bin/BLAST>.
- 20 Alternatively, a nucleotide sequence is considered "substantially homologous" nucleotide if it is capable of selectively hybridizing to the nucleotide sequences disclosed herein under, for example, stringent conditions, as defined for that particular system. For example, stringent hybridization conditions can include 50% formamide, 5x Denhardt's Solution, 5x SSC, 0.1% SDS and 100 pg/ml denatured salmon sperm DNA and the
- 25 washing conditions can include 2x SSC, 0.1% SDS at 37 C followed by 1x SSC, 0.1% SDS at 68 C.
- Defining appropriate hybridization conditions is well within the purview of the person skilled in the art.
- 30 The identity between SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16 and, SEQ ID NO: 18

is 90% or more (e. g. 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more).

CLAIMS:

1. A method of identifying a compound that modulates SERCA3 activity, comprising:
 - a. providing a cell comprising or expressing a SERCA3 polypeptide;
 - 5 b. contacting said cell with a first compound to be screened;
 - c. contacting said cell with a second compound wherein the second compound is known to affect GLP-1 levels;
 - d. measuring the levels of GLP-1 after contacting the cell with the second compound;
 - 10 e. determining whether said first compound functions as a SERCA3 modulator by comparing the levels of GLP-1 in said cell to a control not contacted with said first compound;

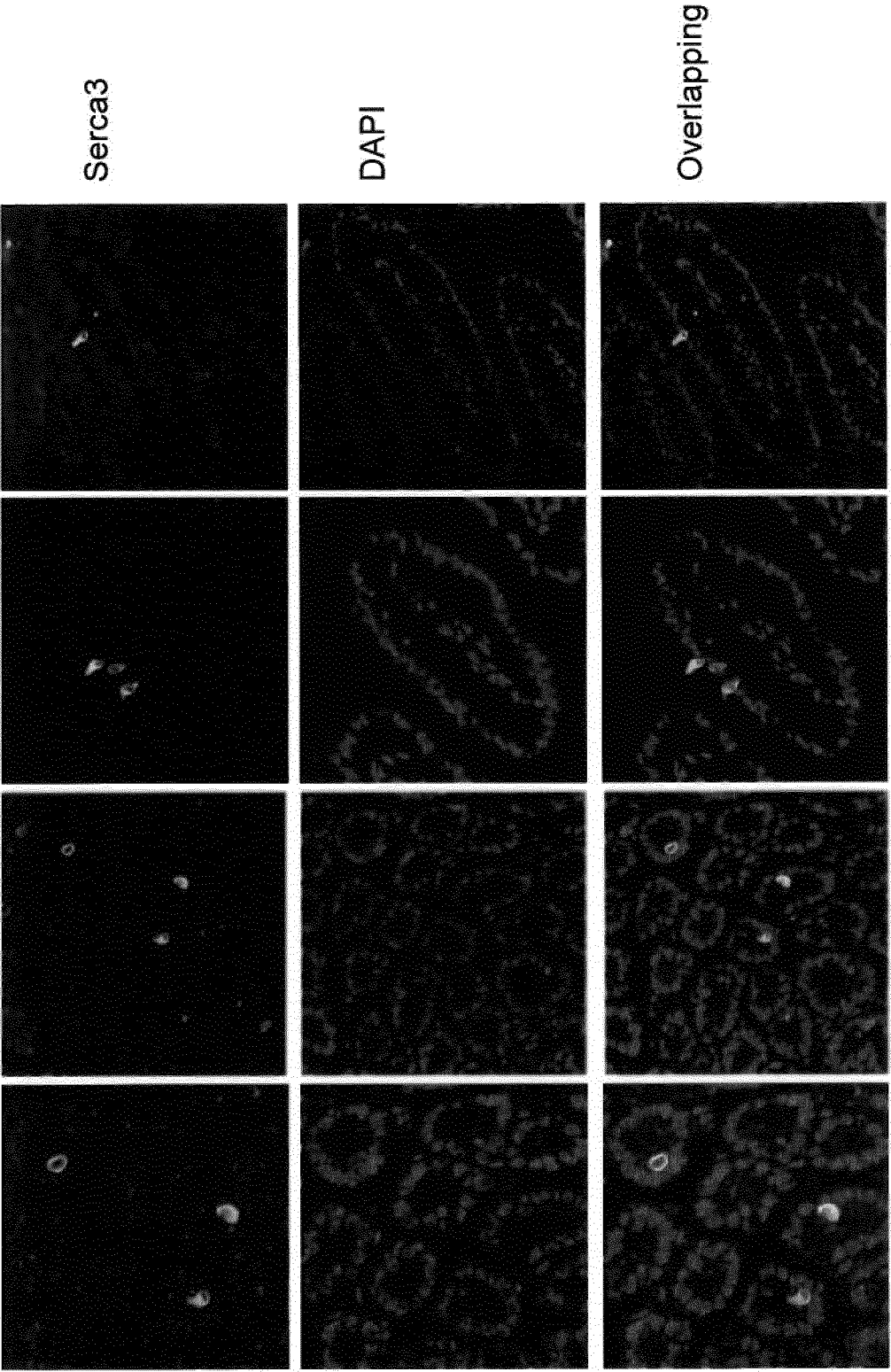
wherein steps b and c are performed together or sequentially.
2. The method of claim 1 wherein said second compound is thymol, isoeugenol,
- 15 saccharin, a DPP-4 inhibitor, or combinations thereof.
3. The method of claim 1 wherein the second compound is dipeptidyl peptidase-4.
4. A method of treatment or prophylaxis for a condition mediated by GLP-1 activity in a mammal in need thereof, comprising administering an effective amount of a compound that inhibits the activity of SERCA3 to the mammal.
- 20 5. The method of claim 4 wherein the condition is type II diabetes, a pre-diabetic condition, a metabolic disease, obesity, or a disease or disorder caused or characterized by abnormal intestinal motility.
6. The method of claim 4 or 5 wherein the compound is administered in conjunction with a compound capable of enhancing GLP-1 levels, e.g., a sweetener, a spice, a DPP-4
- 25 inhibitor, or a combination thereof.
7. The method of claim 4, 5, or 6 wherein the compound is 1,4-Dihydroxy-2,5-di-tert-butylbenzene; 2,5-di-(tert-butyl)-1,4-hydroquinone; 2,5-di(t-butyl)-1,4-benzohydroquinone; thapsigargin; an antihistamine; a cyclosporine; an antibody to

SERCA3; or antisense oligonucleotides, triple helix DNA, RNA aptamers, ribozymes or double stranded RNA directed to a nucleic acid sequence of SERCA3.

8. The method of any of claims 4-7 wherein the mammal to be treated is a human, dog or cat.
- 5 9. The method of any of claims 4-8 wherein the compound is administered within 1-2 hours before a meal.
10. The method of any of claims 4-9 wherein the compound is locally active in the gastrointestinal tract and substantially free of activity other than GLP-1 effects.
11. An effective amount of a compound that inhibits the activity of SERCA3 for use as a
10 medicament
12. An effective amount of a compound that inhibits the activity of SERCA3 for use in the treatment or prophylaxis of a condition mediated by GLP-1 activity in a mammal in need thereof.
13. The compound of claim 12 wherein the condition is type II diabetes, a pre-diabetic
15 condition, a metabolic disease, obesity, or a disease or disorder caused or characterized by abnormal intestinal motility.
14. The compound of any one of claims 11 to 13 wherein the compound is administered in conjunction with a compound capable of enhancing GLP-1 levels, e.g., a sweetener, a spice, a DPP-4 inhibitor, or a combination thereof.
- 20 15. The compound of any one of claims 11 to 14 wherein the compound is 1,4-Dihydroxy-2,5-di-tert-butylbenzene (BHQ); 2,5-di-(tert-butyl)-1,4-hydroquinone; 2,5-di(t-butyl)-1,4-benzohydroquinone; thapsigargin; an antihistamine; a cyclosporine; an antibody to SERCA3; or antisense oligonucleotides, triple helix DNA, RNA aptamers, ribozymes or double stranded RNA directed to a nucleic acid sequence of SERCA3.
- 25 16. The compound of any one of claims 11 to 15 wherein the mammal to be treated is a human, dog or cat.
17. The compound of any one of claims 11-16 wherein the compound is administered within 1-2 hours before a meal.
18. The compound of any one of claims 11-17 wherein the compound is locally active in
30 the gastrointestinal tract and substantially free of activity other than GLP-1 effects.

19. The use of an effective amount of a compound that inhibits the activity of SERCA3 in the manufacture of a medicament for the treatment or prophylaxis of an abnormal condition mediated by GLP-1 activity in a mammal in need thereof.
20. The use as defined in claim 19 wherein the condition is type II diabetes, a pre-diabetic condition, a metabolic disease, obesity, or a disease or disorder caused or characterized by abnormal intestinal motility.
21. The use as defined in claim 19 or 20 wherein the compound is administered in conjunction with a compound capable of enhancing GLP-1 levels, e.g., a sweetener, a spice, a DPP-4 inhibitor, or a combination thereof.
22. The use as defined in any one of claims 19 to 21 wherein the compound is 1,4-Dihydroxy-2,5-di-tert-butylbenzene; 2,5-di-(tert-butyl)-1,4-hydroquinone; 2,5-di(t-butyl)-1,4-benzohydroquinone; thapsigargin; an antihistamine; a cyclosporine; an antibody to SERCA3; or antisense oligonucleotides, triple helix DNA, RNA aptamers, ribozymes or double stranded RNA directed to a nucleic acid sequence of SERCA3.
23. The use as defined in any one of claims 19 to 22 wherein the mammal to be treated is a human, dog or cat.
24. The use as defined in any one of claims 19 to 23 wherein the compound is administered within 1-2 hours before a meal.
25. The use as defined in any one of claims 19 to 24 wherein the compound is locally active in the gastrointestinal tract and substantially free of activity other than GLP-1 effects.

Figure 1



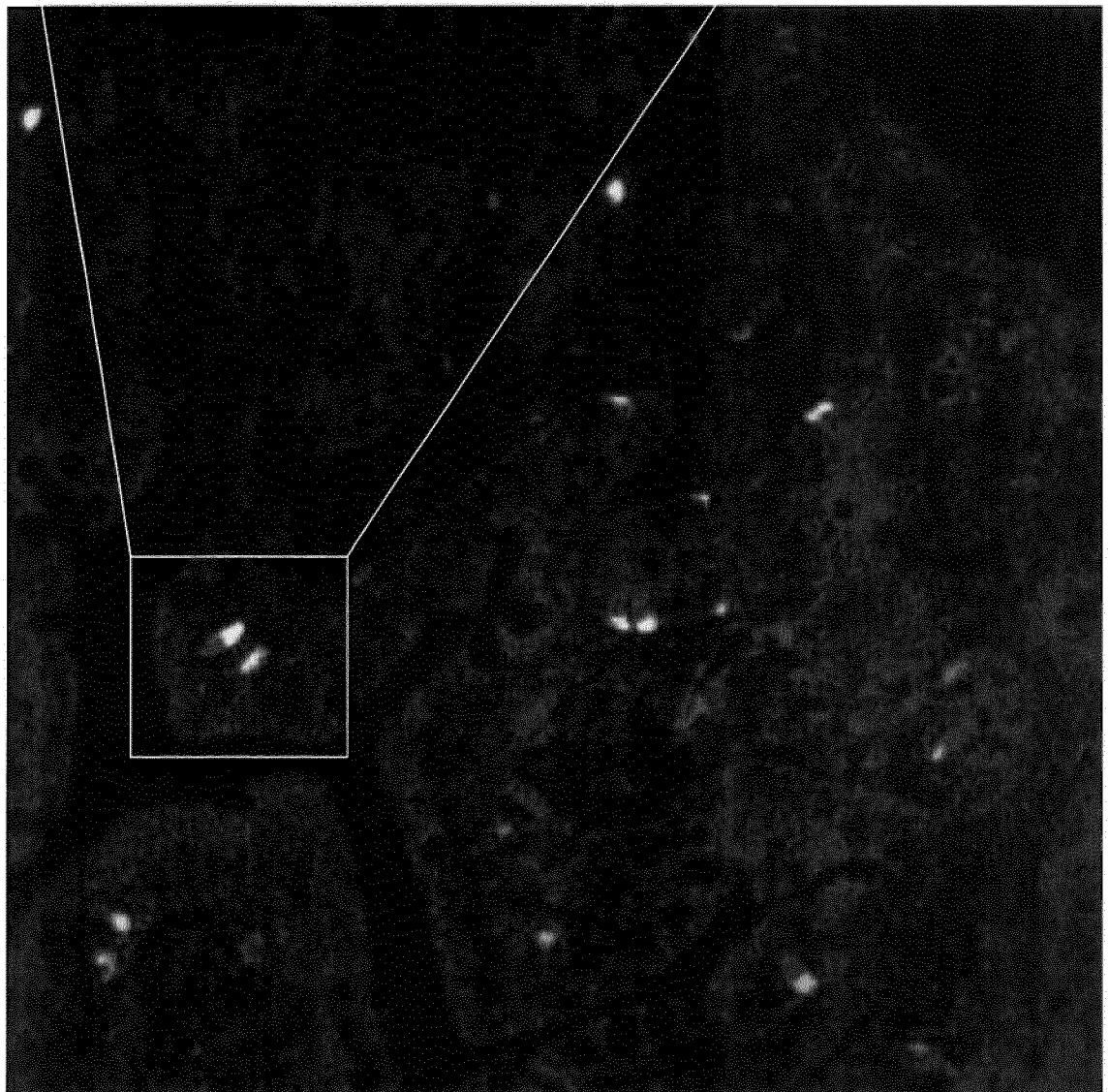
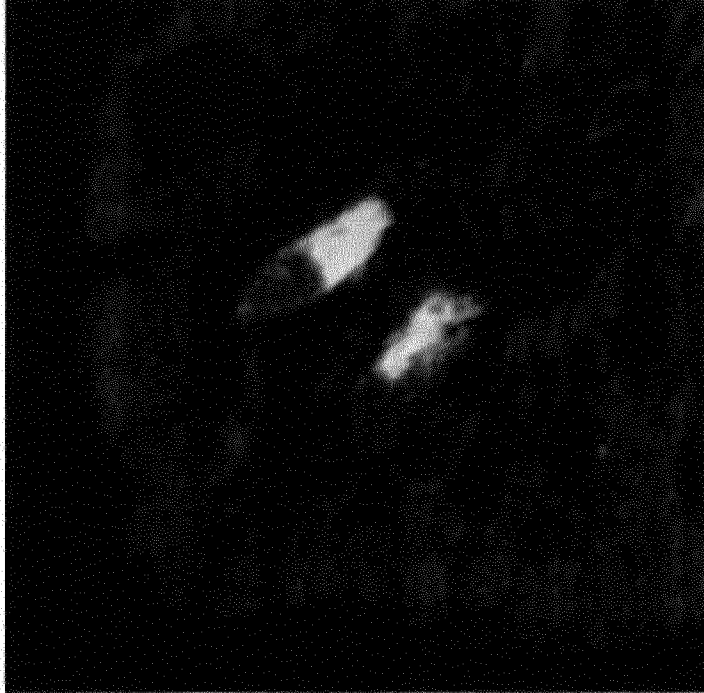


Figure 2

Figure 3

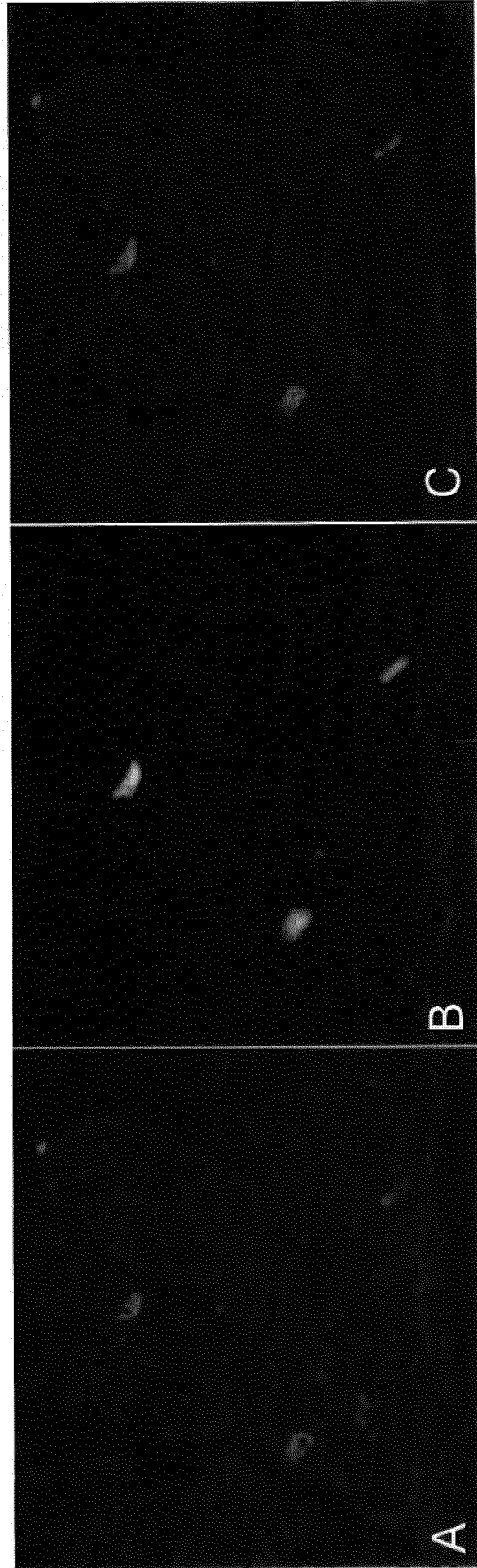
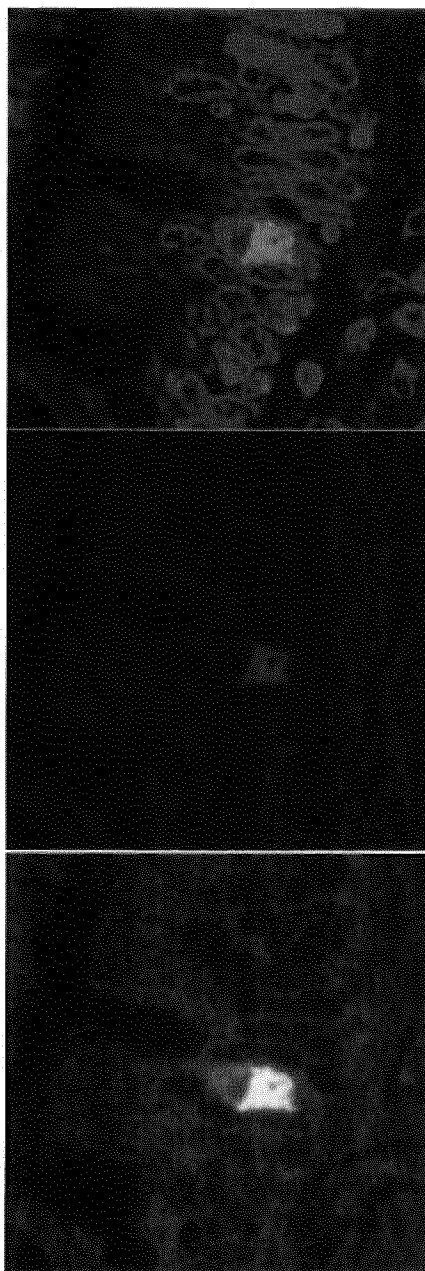


Figure 4



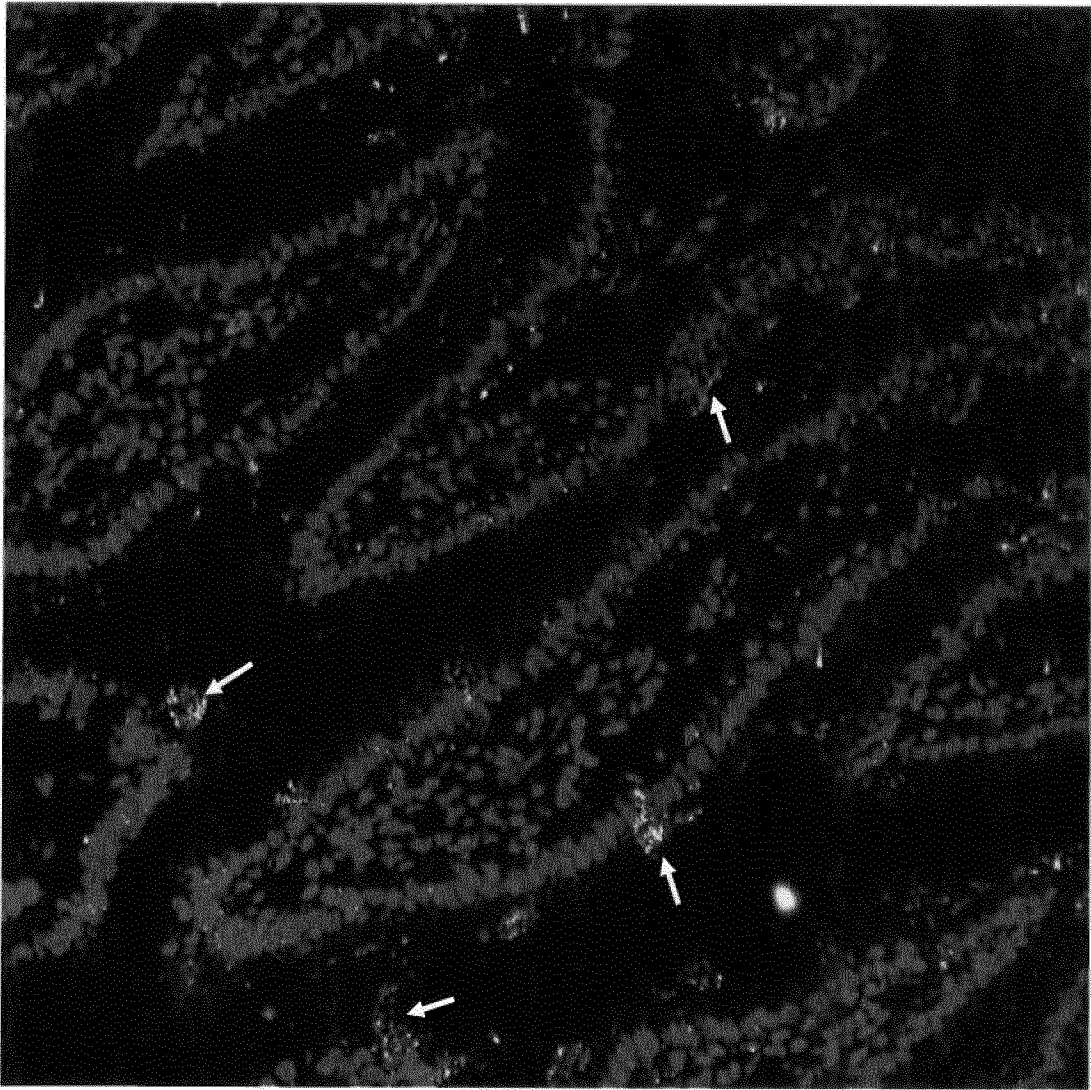


Figure 5

Figure 6

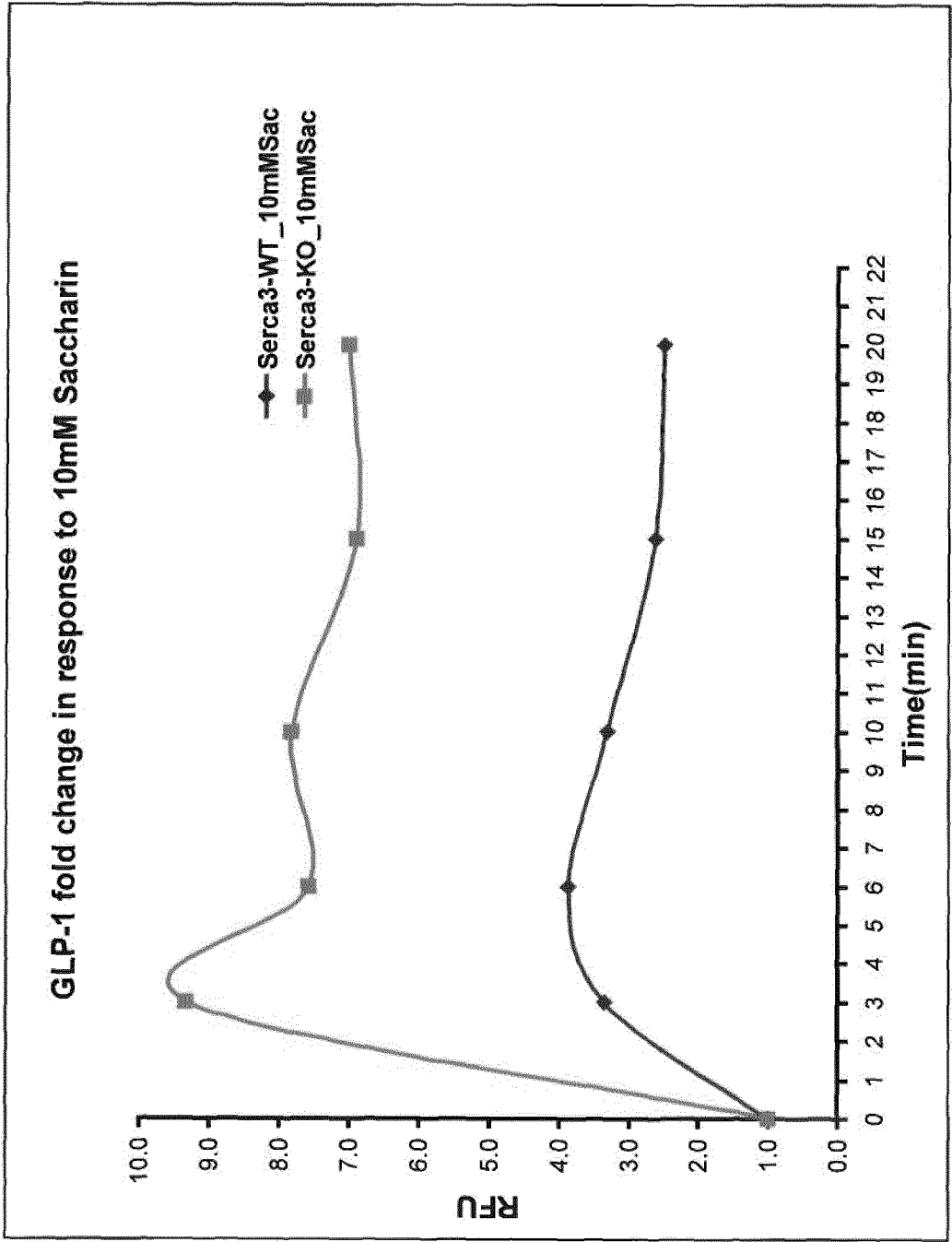


Figure 7

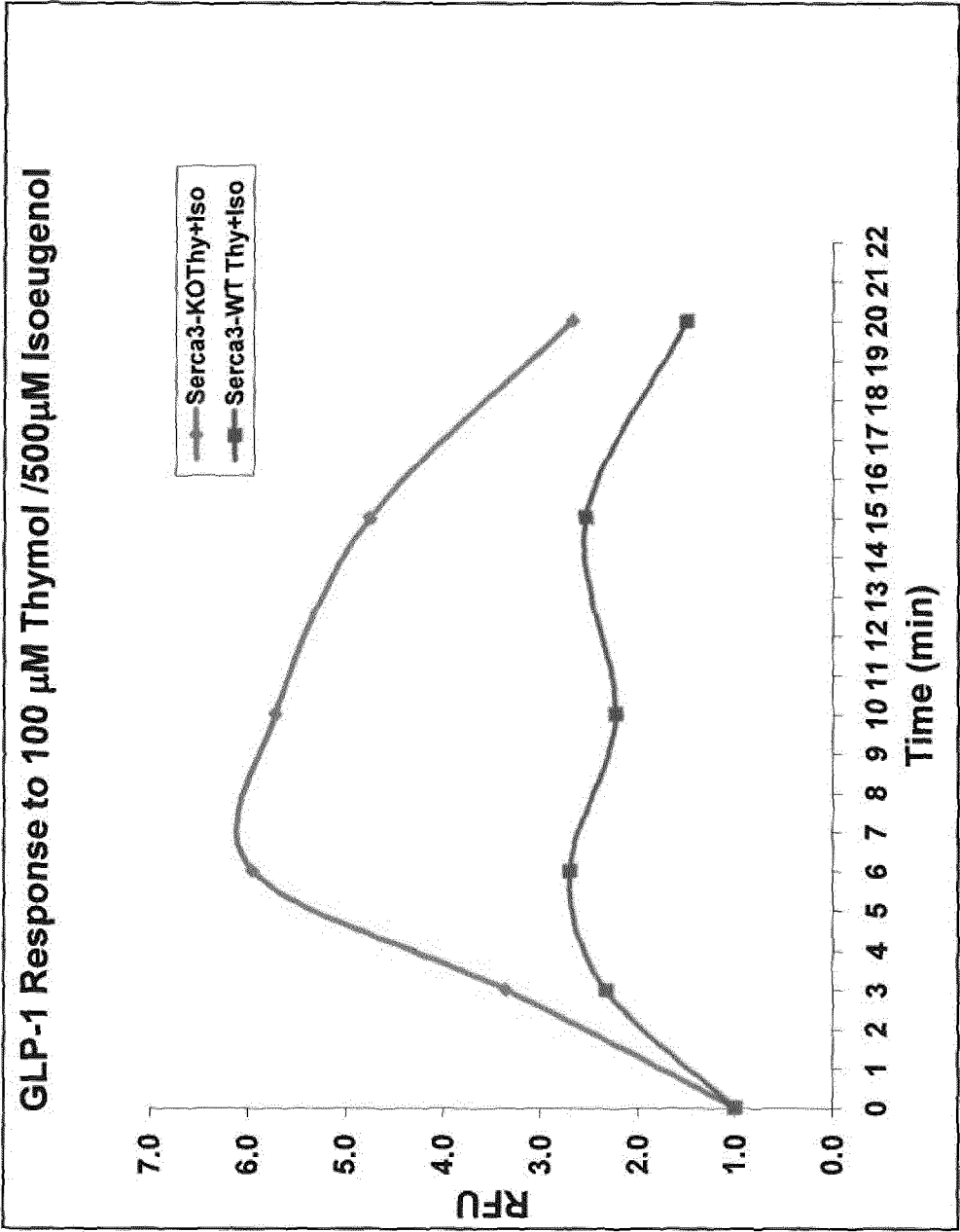


Figure 8:

GLP-1 Response of Gut Villi and Crypts to Lipid: 100 μ M Linolenic Acid

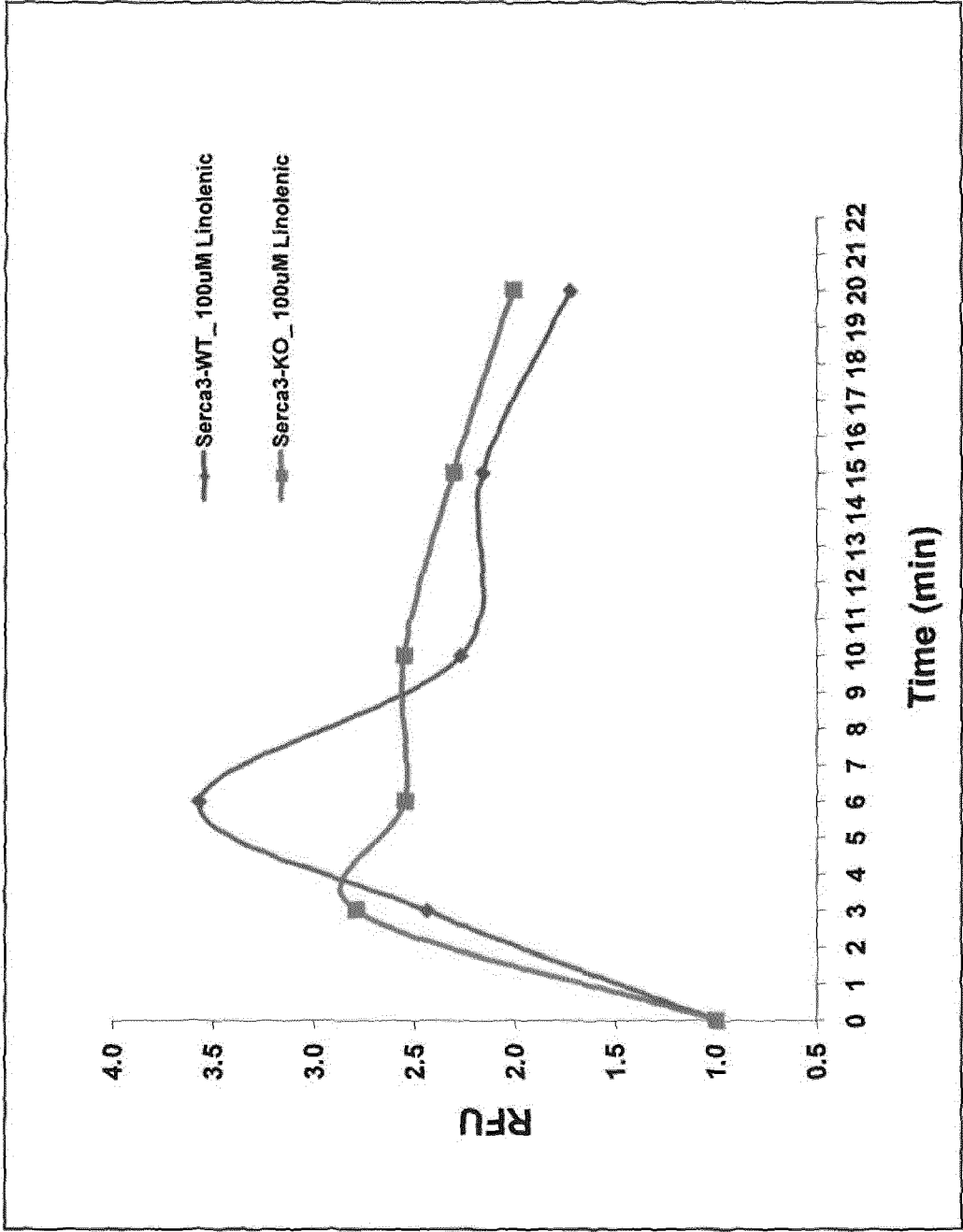
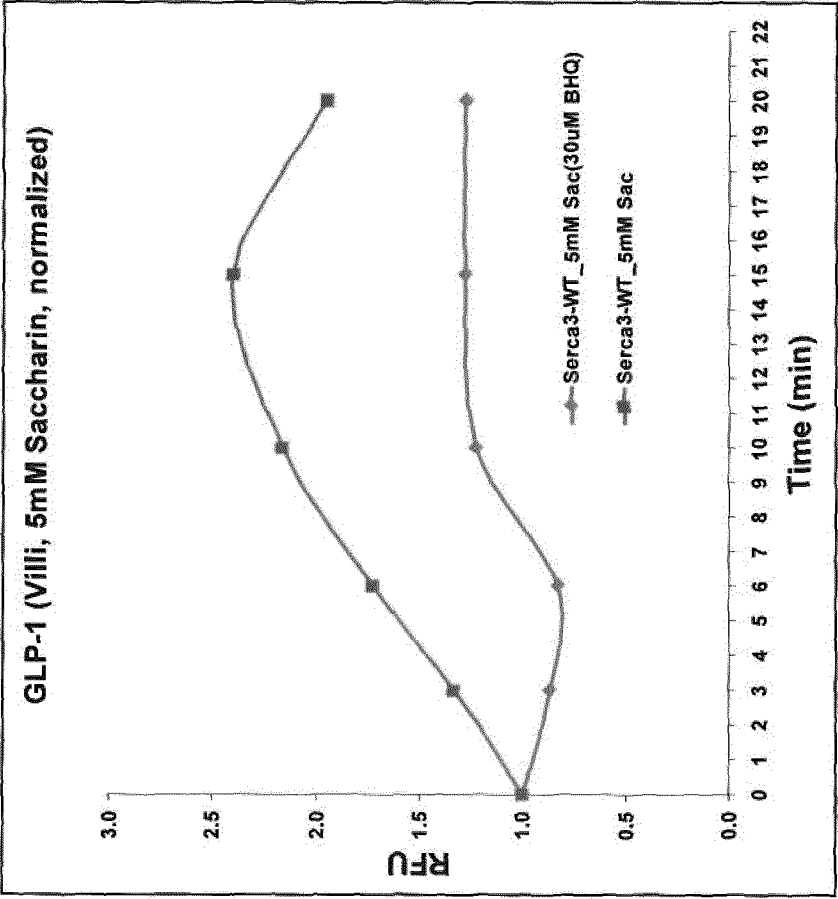


Figure 9



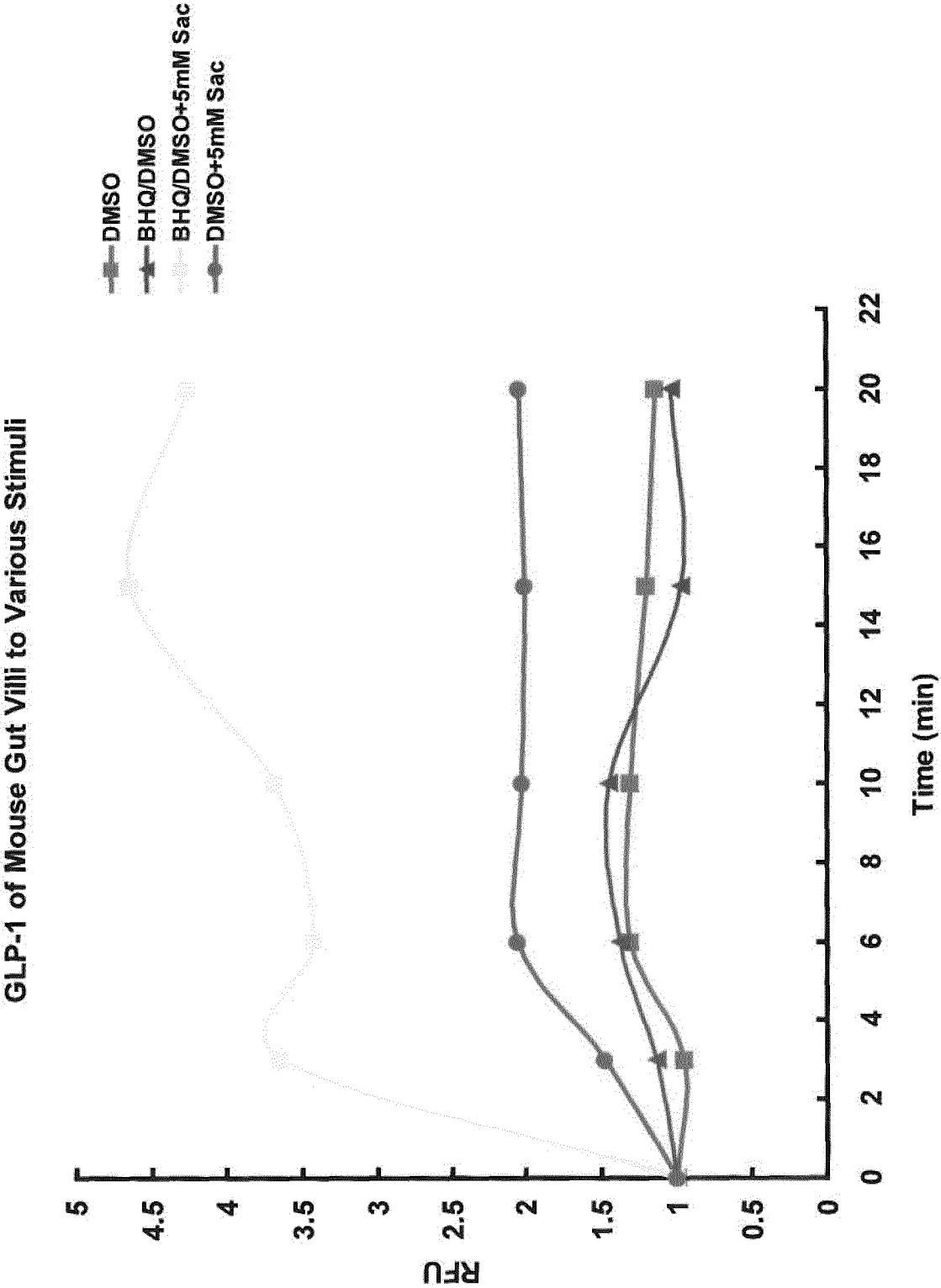


Figure 10

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/061023

A. CLASSIFICATION OF SUBJECT MATTER

INV. G01N33/50 A61K31/00 A61K31/05 A61K31/55 A61P3/00
A61P1/00

ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, PAJ, EMBASE, BIOSIS, COMPENDEX, INSPEC, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2002/132770 A1 (CAPLAN MICHAEL T [US] ET AL CAPLAN MICHAEL S [US] ET AL) 19 September 2002 (2002-09-19) page 7, paragraph 0123; claims 1,3,37,38,56	11
X	US 5 545 618 A (BUCKLEY DOUGLAS I [US] ET AL) 13 August 1996 (1996-08-13) cited in the application claims 1-14	4-25
A	WO 00/63426 A2 (DEVGEN NV [BE]; ZWAAL RICHARD [BE]; GROENEN JOSE [BE]; BOGAERT THIERRY) 26 October 2000 (2000-10-26) claims 1-3,7-10	1-3
	----- -/-	



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

27 September 2012

Date of mailing of the international search report

10/10/2012

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
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Fax: (+31-70) 340-3016

Authorized officer

van der Kooij, M

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2012/061023

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	P. D. BORGE ET AL: "Insulin Receptor Substrate 1 Regulation of Sarco-endoplasmic Reticulum Calcium ATPase 3 in Insulin-secreting beta -Cells", JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 278, no. 13, 21 March 2003 (2003-03-21), pages 11359-11368, XP55038891, ISSN: 0021-9258, DOI: 10.1074/jbc.M209521200 abstract page 11367, column 1, paragraph 2; figure 9 -----	4-25
A	AISHA AL-JARALLAH ET AL: "Mechanism of reduced colonic contractility in experimental colitis: role of sarcoplasmic reticulum pump isoform-2", MOLECULAR AND CELLULAR BIOCHEMISTRY, KLUWER ACADEMIC PUBLISHERS, BO, vol. 298, no. 1-2, 25 November 2006 (2006-11-25), pages 169-178, XP019506115, ISSN: 1573-4919 abstract -----	4-25
A	ARREDOUANI ABDELILAH ET AL: "SERCA3 ablation does not impair insulin secretion but suggests distinct roles of different sarcoendoplasmic reticulum Ca²⁺ pumps for Ca²⁺ homeostasis in pancreatic beta-cells.", DIABETES, vol. 51, no. 11, November 2002 (2002-11), pages 3245-3253, XP002684209, ISSN: 0012-1797 abstract page 3250, column 1, paragraph 2 - column 2, paragraph 2 -----	4-25
A	US 2006/063187 A1 (HOTAMISLIGIL GOKHAN S [US] ET AL) 23 March 2006 (2006-03-23) page 3, paragraph 0022; figure 8 page 32, paragraphs 0262,0263; example 2 -----	4-25

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2012/061023

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2002132770	A1	19-09-2002	NONE
US 5545618	A	13-08-1996	NONE
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		JP 2002541859 A	10-12-2002
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US 2006063187	A1	23-03-2006	NONE