SUMMARY

[0020a] In a first aspect, the present invention refers to a PPF polypeptide comprising the amino acid sequence of SEQ ID NO: 438.

[0021] The present disclosure further~~invention~~ relates generally to pancreatic polypeptide family ("PPF") polypeptides having at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 92%, at least 94% or at least 97% sequence identity to PYY(3-36) over the entire length of PYY(3-36), and also comprise at least two PPF motifs including at least the N-terminal polyproline PPF motif and the C-terminal tail PPF motif. Additional PPF motifs disclosed herein~~of the invention~~ may correspond to any motif of any of the PP family polypeptides, including PP, PYY and NPY. In certain example~~embodiments~~, the PPF polypeptides do not include unnatural amino acids. In other example~~embodiments~~, the PPF polypeptides do not include known naturally occurring species variants.

[0022] In one example~~aspect~~, the PPF polypeptides disclosed herein~~of the invention~~ include PYY analog polypeptides. In yet another example~~aspect of the invention~~, the PPF polypeptides of the invention include PPF chimeric polypeptides comprising a fragment of a PP, PYY or NPY polypeptide covalently linked to at least one additional fragment of a PP, PYY or NPY polypeptide, wherein each PP, PYY or NPY fragment includes a PPF motif. Such PPF analog polypeptides and PPF chimeric polypeptides of the invention will exhibit at least 50% sequence identity to a native PYY(3-36) over the entire length of the PYY(3-36). In certain example~~embodiments~~, desirable PPF chimeric polypeptides include an N-terminal PP fragment in combination with a C-terminal PYY fragment. In other example~~embodiments~~, PPF chimeric polypeptides include an N-terminal PP fragment in combination with a C-terminal NPY fragment. In other example~~embodiments~~, PPF chimeric polypeptides include an N-terminal PYY fragment and a C-terminal PP or NPY fragment. In other example~~embodiments~~, PPF chimeric polypeptides include an N-terminal NPY in combination with a C-terminal PYY or PP. In other example~~embodiments~~, PPF chimeric polypeptides may not include an N-terminal PP fragment in combination with a C-terminal NPY fragment. In still other example~~embodiments~~, PPF chimeric polypeptides may not include an N-terminal NPY fragment with a C-terminal PYY fragment.

[0022a] In a second aspect, the present invention refers to the use of a PPF polypeptide of the present invention in the manufacture of a medicament for altering body composition of a subject, wherein said medicament alters the fat to lean ratio, thereby altering body composition, wherein the PPF polypeptide is to be administered peripherally.

[0022b] In a third aspect, the present invention refers to the use of a PPF polypeptide according to the present invention in the manufacture of a medicament for lowering plasma triglyceride levels in a subject, wherein said medicament is to be administered to the subject, wherein cholesterol levels are lowered to a lesser extent, wherein the medicament is to be administered peripherally.

[0022c] In a fourth aspect, the present invention refers to the use as defined herein, wherein triglyceride levels are lowered and cholesterol levels are not lowered.

[0022d] In a fifth aspect, the present invention refers to the use of a PPF polypeptide as defined herein in the manufacture of a medicament for reducing central body fat and for preserving or increasing lean body mass, wherein the medicament is to be administered peripherally.

[0022e] In a sixth aspect, the present invention refers to the use of a PPF polypeptide as defined herein in the manufacture of a medicament for increasing fatty acid β-oxidation while preserving or increasing lean body mass in a subject, wherein the medicament is to be administered peripherally.

[0022f] In a seventh aspect, the present invention refers to the use of a PPF polypeptide as defined herein in the manufacture of a medicament for treating nonalcoholic steatohepatitis or lipodystrophy in a subject, wherein the medicament is to be administered peripherally.

[0023] In another example~~aspect of the invention~~, methods for treating or preventing obesity are provided, wherein the method comprises administering a therapeutically or prophylactically effective amount of a PPF polypeptide disclosed herein~~of the invention~~ to a subject in need thereof. In some example~~embodiments~~, the subject is an obese or overweight subject. While "obesity" is generally defined as a body mass index over 30, for purposes of this disclosure, any subject, including those with a body mass index of less than 30, who needs or wishes to reduce body weight is included in the scope of "obese." Subjects who are insulin resistant, glucose intolerant, or

have any form of diabetes mellitus (*e.g.*, type 1, 2 or gestational diabetes) can benefit from this method.

[0024] In one examplegeneral aspect, methods disclosed hereinof the invention include the use of a PPF polypeptide to modify body composition, for example, reducing body fat, but not lean body mass. The change in body composition can be by, for example, weight (e.g., loss or gain by grams), by percent body fat and percent lean body mass or protein (used interchangeably), or by the ratio of body fat to lean tissue.

[0025] While it has been reported that PYY may be useful in regulating satiety (U.S. 6,558,708) or control of weight (U.S. Patent Application No. 10/016,969, WO2003026591 and WO2003057235), it has now surprisingly been discovered that PPF polypeptides may have a metabolic effect on the body and may be used to affect body composition, leading to the desirable loss of body fat, yet preserving lean body mass or minimizing its loss.

[0026] In certain examplembodiments, methods disclosed hereinof the invention include reducing body fat or reducing or preventing body fat gain, while sparing, minimizing loss, or even increasing lean body mass. Other examplembodiments include controlling body weight and/or sculpting a body's appearance. The subjects to whom these methods may be of interest are those individuals who are overweight or obese, as well as those who are lean. For instance, subjects with lean body composition, e.g., body builders and other athletes, may benefit from the invention as well. It may be desirable for them to reduce or maintain their body weight, e.g., to stay in a certain weight class range, yet preserve or increase their lean body mass for greater strength, stamina, endurance and/or a more muscular appearance. Such methods may also be used on any animal for which a greater lean body mass to fat ratio is desired. Examples of such use include, but are not limited to, creating a superior show dog or creating a superior racehorse or workhorse.

[0027] In one examplegeneral aspect, methods disclosed hereinof the invention include the use of a PPF polypeptide to reduce the fat content in animals for consumption. Methods disclosed hereinof the invention can include producing a leaner meat source. Compositions and methods disclosed hereinof the invention can be used with livestock including, but not limited to, chicken, turkeys, cows, pigs, and sheep.

[0028] It is contemplated that methods disclosed hereinof the invention can be used in combination with other forms of nutritional regimens and weight loss programs, such as those already described above, for example, those that include life-style changes that include monitoring food intake

(quantity and quality) and exercising, as well as surgery. Nutritional regimens include those that are used to increase lean body mass such as those followed by body builders.

[0029] In another examplegeneral aspect, PPF polypeptides reduce the respiratory quotient (RQ) in animals, which is indicative of improved fat utilization for energy at the tissue and cell level (increased fatty acid β-oxidation). Thus, PPF polypeptides may be therapeutically useful in conditions where improved fatty acid β-oxidation in non-adipose tissues is desirable with maintenance, minimization of loss, or an increase in lean body mass. Examples of such conditions include, but are not limited to, nonalcoholic steatohepatitis (NASH) (Grant et al. Nonalcoholic fatty liver disease, Ann Hepatol. 3(3):93-9 Jul-Sep 2004), in which patients display pathologically elevated liver fat content, and lipodystrophy, in which patients lack significant adipose stores, and hence display increased fat build-up in non-adipose tissues such as liver and skeletal muscle (Garg et al. Lipodystrophies: rare disorders causing metabolic syndrome, Endocrinol Metab Clin North Am. 33(2):305-31 Jun 2004).

[0030] In certain examplesembodiments of the invention, a PPF polypeptide may be administered peripherally and not centrally, i.e, not through the central nervous system. In other examplembodiments, a therapeutically or prophylactically effective amount of a PPF polypeptide is administered in a single dose, multiple doses, or continuous administration.

[0031] In yet another exampleaspect of the invention, compounds disclosed hereinof the invention can be used for methods of reducing food intake, reducing nutrient availability, causing weight loss, affecting body composition, altering body energy content or energy expenditure (EE) and improving lipid profile (including reducing LDL cholesterol and/or triglyceride levels and/or changing HDL cholesterol levels). Thus, in certain examplembodiments, the methods disclosed hereinof the invention are useful for treating or preventing conditions or disorders which can be alleviated by reducing nutrient availability in a subject in need thereof, comprising administering to said subject a therapeutically or prophylactically effective amount of a PPF polypeptide disclosed hereinof the invention. Such conditions and disorders include, but are not limited to, hypertension, dyslipidemia, cardiovascular disease, eating disorders, insulin-resistance, obesity, diabetes mellitus of any kind, including Type I, Type II, and gestational diabetes. Compounds disclosed hereinof the invention may also be useful in treating or preventing other conditions associated with obesity including stroke, cancer (*e.g*,. endometrial, breast, prostate, and colon cancer), gallbladder disease, sleep apnea, reduced fertility, and osteoarthritis, (*see* Lyznicki *et al*, *Am. Fam. Phys.* 63:2185, 2001).

[0032] Compounds disclosed hereinof the invention may also be useful for potentiating, inducing, enhancing or restoring glucose responsivity in pancreatic islets or cells. These actions may also be used to treat or prevent conditions associated with metabolic disorders such as those described above and in U.S. patent application no. US20040228846.

[0033] In addition to the amelioration of hypertension in subjects in need thereof, compounds disclosed hereinof the invention may be used to treat or prevent hypotension.

[0034] Compounds disclosed hereinof the invention may also be useful in the treatment or prevention of any number of gastrointestinal disorders that are associated with excess intestinal electrolytes and water secretion as well as decreased absorption, *e.g.*, infectious (*e.g.*, viral or bacterial) diarrhea, inflammatory diarrhea, short bowel syndrome, or the diarrhea which typically occurs following surgical procedure, e.g., ileostomy (*see e.g.*, Harrison's principles of Internal Medicine, McGraw Hill Inc., New York, 12th *ed.*). Examples of infectious diarrhea include, without limitation, acute viral diarrhea, acute bacterial diarrhea (*e.g.*, salmonella, campylobacter, and clostridium) or diarrhea due to protozoal infections, or travellers' diarrhea *(e.g.*, Norwalk virus or rotavirus). Examples of inflammatory diarrhea include, without limitation, malabsorption syndrome, tropical spue, chronic pancreatitis, Crohn's disease, diarrhea, and irritable bowel syndrome. It has also been discovered that the peptides disclosed hereinof the invention can be used to treat or prevent an emergency or life-threatening situation involving a gastrointestinal disorder, e.g., after surgery or due to cholera. Furthermore, the compounds disclosed hereinof the invention can be used to treat intestinal dysfunction in patients with Acquired Immune Deficiency Syndrome (AIDS), especially during cachexia. The compounds disclosed hereinof the invention may also be useful for inhibiting small intestinal fluid and electrolyte secretion, and augmenting nutrient transport, as well as increasing cell proliferation in the gastrointestinal tract, regulating lipolysis in, *e.g.*, adipose tissue and regulating blood flow in a mammal.

[0035] Compounds disclosed hereinof the invention may also be useful for treating or preventing the above conditions by their gastrointestinal protective activity. Accordingly, compounds disclosed hereinof the invention may be used to treat gastrointestinal or muscosal damage. Exemplary types of damage include, but are not limited to, inflammatory bowel disease, bowel atrophy, conditions characterized by loss of bowel mucosa or bowel mucosal function, and other conditions of the gastrointestinal tract, including those which may be brought about by exposure to cytotoxic agents, radiation, toxicity, infection and/or injury. Moreover, these compounds disclosed hereinof the invention may be combined with analgesics, anti-inflammatory agents,

growth hormone, heparin, or any other therapies that may be used to treat inflammatory bowel disease or other conditions listed above.

[0036] Moreover, compounds disclosed hereinof the invention are useful in treating or preventing diseases and disorders that can be alleviated or ameliorated by their anti-secretory properties. Such anti-secretory properties include inhibition of gastric and/or pancreatic secretions and can be useful in the treatment or prevention of diseases and disorders including gastritis, pancreatitis, Barrett's esophagus, and Gastroesophageal Reflux Disease. These diseases may also be treated or prevented by the gastrointestinal protective functions of compounds disclosed hereinof the invention.

[0037] Compounds disclosed hereinof the invention may also be useful for reducing aluminum concentrations in the central nervous system of a subject to treat or prevent a disease or condition associated with abnormal aluminum concentrations (*e.g.*, a patient afflicted with Alzheimer's disease or at risk for developing Alzheimer's disease, dialysis dementia, or increased aluminum levels due to occupational exposure).

[0038] The present disclosureinvention also relates to pharmaceutical compositions comprising a therapeutically or prophylactically effective amount of at least one PPF polypeptide of the invention, or a pharmaceutically acceptable salt thereof, together with pharmaceutically acceptable diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers useful in the delivery of the PPF polypeptides.

[0039] These and other aspects of the invention will be more clearly understood with reference to the following embodiments and detailed description. The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims. All references cited herein are incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0040] **Figure 1** demonstrates the activity of certain PPF polypeptides of the invention in a food intake assay.

[0041] **Figure 2** demonstrates the activity of additional PPF polypeptides of the invention in a food intake assay.

What is claimed is:

1. A PPF polypeptide
comprising the amino acid sequence of SEQ ID NO: 438.

2. The PPF polypeptide according to claim 1, wherein said PPF polypeptide further comprises an N-terminal cap.

3. The PPF polypeptide according to claims 1 or 2, wherein said polypeptide is linked to one or more water-soluble polymers.

4. The PPF polypeptide of claim 3, wherein said polymer is selected from at least one of the group consisting of polyethylene glycol and a fatty acid molecule, wherein said polymer is linked to the N- or C- terminus of the polypeptide, or the side chain of a lysine or serine amino acid residue within the sequence of the polypeptide.

5. Use of a PPF polypeptide of any one of claims 1 through 4 in the manufacture of a medicament for altering body composition of a subject, wherein said medicament alters the fat to lean ratio, thereby altering body composition, wherein the PPF polypeptide is to be administered peripherally.

6. The use of claim 5, wherein body fat is reduced and lean body mass is maintained or increased.

7. The use of claim 6, wherein the body fat and lean body mass are measured as percent body fat and percent lean body mass, respectively.

8. The use of any one of claims 5, 6, and 7, wherein body composition is measured by a method selected from the group consisting of underwater weighing, air displacement plethysmograph, x-ray, MRI, DEXA scan, CT scans, and adiabatic bomb calorimetry.

9. The use of any one of claims 5 through 8, wherein body weight is reduced.

10. The use of any one of claims 5 through 9, wherein said medicament is to be administered with at least one agent selected from the group consisting of an amylin, amylin agonist or amylin analog agonist, salmon calcitonin, a cholecystokinin (CCK) or CCK agonist, a leptin (OB protein) or leptin agonist, an exendin or exendin analog agonist, a GLP-1, GLP-1 agonist or GLP-1 analog agonist, a CCK or CCK agonist, calcitonin, a calcitonin agonist, a small molecule cannabinoid CB1 receptor antagonist, rimonabant, an 11 beta-hydroxysteroid dehydrogenase-1 inhibitor, sibutramine, and phentermine.

11. The use of any one of claims 5 through 10, wherein the subject is overweight or obese.

12. The use of any one of claims 5 through 11, further comprising dieting.

13. The use of any one of claims 5 through 12, wherein the subject is a mammal.

14. The use of claim 13 wherein the mammal is a human.

15. The use of claim 13 wherein the mammal is selected from a group consisting of a primate, a rat, a mouse, a cat, a dog, a pig, a cow, a steer, a horse, a sheep, and a goat.

16. Use of a PPF polypeptide according to any one of claims 1 through 4 in the manufacture of a medicament for lowering plasma triglyceride levels in a subject, wherein said medicament is to be administered to the subject, wherein cholesterol levels are lowered to a lesser extent, wherein the medicament is to be administered peripherally.

17. The use of claim 16, wherein triglyceride levels are lowered and cholesterol levels are not lowered.

18. The use of claim 16, wherein triglyceride levels are lowered and LDL cholesterol levels are not lowered.

19. The use of claim 16, wherein triglyceride levels are lowered and LDL cholesterol levels are lowered to a lesser extent.

20. The use of claim 16, wherein amylase levels are also lowered.

21. Use of a PPF polypeptide according to any one of claims 1 through 4 in the manufacture of a medicament for reducing body fat or body fat gain in a subject while maintaining or increasing lean body mass, wherein the medicament is to be administered peripherally.

22. Use of a PPF polypeptide according to any one of claims 1 through 4 in the manufacture of a medicament for reducing central body fat and for preserving or increasing lean body mass, wherein the medicament is to be administered peripherally.

23. Use of a PPF polypeptide according to any one of claims 1 through 4 in the manufacture of a medicament for increasing fatty acid β-oxidation while preserving or increasing lean body mass in a subject, wherein the medicament is to be administered peripherally.

24. Use of a PPF polypeptide according to any one of claims 1 through 4 in the manufacture of a medicament for treating nonalcoholic steatohepatitis or lipodystrophy in a subject, wherein the medicament is to be administered peripherally.

What is claimed is:

1. A PPF polypeptide
comprising the amino acid sequence of SEQ ID NO: 438.

2. The PPF polypeptide according to claim 1, wherein said PPF polypeptide further comprises an N-terminal cap.

3. The PPF polypeptide according to claims 1 or 2, wherein said polypeptide is linked to one or more water-soluble polymers.

4. The PPF polypeptide of claim 3, wherein said polymer is selected from at least one of the group consisting of polyethylene glycol and a fatty acid molecule, wherein said polymer is linked to the N- or C- terminus of the polypeptide, or the side chain of a lysine or serine amino acid residue within the sequence of the polypeptide.

5. Use of a PPF polypeptide of any one of claims 1 through 4 in the manufacture of a medicament for altering body composition of a subject, wherein said medicament alters the fat to lean ratio, thereby altering body composition, wherein the PPF polypeptide is to be administered peripherally.

6. The use of claim 5, wherein body fat is reduced and lean body mass is maintained or increased.

7. The use of claim 6, wherein the body fat and lean body mass are measured as percent body fat and percent lean body mass, respectively.

8. The use of any one of claims 5, 6, and 7, wherein body composition is measured by a method selected from the group consisting of underwater weighing, air displacement plethysmograph, x-ray, MRI, DEXA scan, CT scans, and adiabatic bomb calorimetry.

9. The use of any one of claims 5 through 8, wherein body weight is reduced.

10. The use of any one of claims 5 through 9, wherein said medicament is to be administered with at least one agent selected from the group consisting of an amylin, amylin agonist or amylin analog agonist, salmon calcitonin, a cholecystokinin (CCK) or CCK agonist, a leptin (OB protein) or leptin agonist, an exendin or exendin analog agonist, a GLP-1, GLP-1 agonist or GLP-1 analog agonist, a CCK or CCK agonist, calcitonin, a calcitonin agonist, a small molecule cannabinoid CB1 receptor antagonist, rimonabant, an 11 beta-hydroxysteroid dehydrogenase-1 inhibitor, sibutramine, and phentermine.

11. The use of any one of claims 5 through 10, wherein the subject is overweight or obese.

12. The use of any one of claims 5 through 11, further comprising dieting.

13. The use of any one of claims 5 through 12, wherein the subject is a mammal.

14. The use of claim 13 wherein the mammal is a human.

15. The use of claim 13 wherein the mammal is selected from a group consisting of a primate, a rat, a mouse, a cat, a dog, a pig, a cow, a steer, a horse, a sheep, and a goat.

16. Use of a PPF polypeptide according to any one of claims 1 through 4 in the manufacture of a medicament for lowering plasma triglyceride levels in a subject, wherein said medicament is to be administered to the subject, wherein cholesterol levels are lowered to a lesser extent, wherein the medicament is to be administered peripherally.

17. The use of claim 16, wherein triglyceride levels are lowered and cholesterol levels are not lowered.

18. The use of claim 16, wherein triglyceride levels are lowered and LDL cholesterol levels are not lowered.

19. The use of claim 16, wherein triglyceride levels are lowered and LDL cholesterol levels are lowered to a lesser extent.

20. The use of claim 16, wherein amylase levels are also lowered.

21. Use of a PPF polypeptide according to any one of claims 1 through 4 in the manufacture of a medicament for reducing body fat or body fat gain in a subject while maintaining or increasing lean body mass, wherein the medicament is to be administered peripherally.

22. Use of a PPF polypeptide according to any one of claims 1 through 4 in the manufacture of a medicament for reducing central body fat and for preserving or increasing lean body mass, wherein the medicament is to be administered peripherally.

23. Use of a PPF polypeptide according to any one of claims 1 through 4 in the manufacture of a medicament for increasing fatty acid β-oxidation while preserving or increasing lean body mass in a subject, wherein the medicament is to be administered peripherally.

24. Use of a PPF polypeptide according to any one of claims 1 through 4 in the manufacture of a medicament for treating nonalcoholic steatohepatitis or lipodystrophy in a subject, wherein the medicament is to be administered peripherally.