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(54) Title: OBESITY-RELATED GENES AND THEIR PROTEINS AND USES THEREOF

(57) Abstract: The invention provides for methods of treating obesity or an obesity-associated disorder.

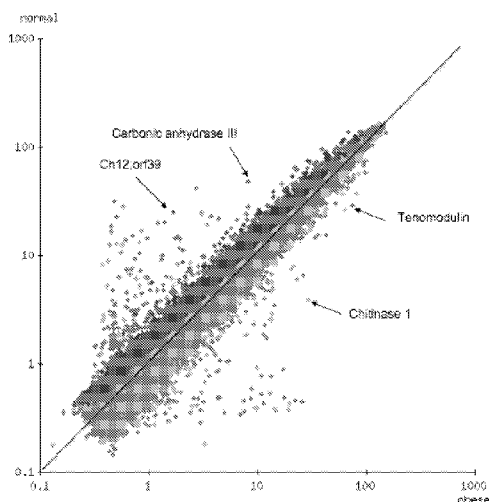


FIG. 1

OBESITY-RELATED GENES AND THEIR PROTEINS AND USES THEREOF

[0001] All patents, patent applications and publications cited herein are hereby incorporated by reference in their entirety. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art as known to those skilled therein as of the date of the invention described and claimed herein.

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BACKGROUND OF THE INVENTION

[0004] Obesity is a chronic disease manifested by an excess of fat mass in proportion to body size. Obesity is, *de facto*, the increased deposition of long chain fatty acids (LCFA), principally in the form of triglycerides (TG), in adipose and other tissues. Obese individuals have an excess of body fat relative to lean body mass that can contribute to other diseases.

[0005] Obesity is one of the most serious public health problems in the 21st century. According to the World Health Organization (WHO), obesity has reached epidemic proportions globally. More than 1 billion adults are overweight and at least 300 million of them are classified as clinically obese. Today, every third American is considered overweight (Body Mass Index (BMI) $>25 \text{ kg/m}^2$). 60% of adults in the US are obese or overweight, and obesity is widely recognized as the largest pharmaceutical market in the world, with few approved drugs that do not work well, leading to, at most, a 5 – 10% weight loss, that is not permanent. It is believed that genetic predisposition significantly increases the risk of becoming obese but specific details of the pathways involved have not been identified.

SUMMARY OF THE INVENTI

[0006] An aspect of the invention provides methods for treating obesity or an obesity-associated disorder in a subject. The methods comprise administering to a subject an effective amount of Spexin, thereby treating obesity or an obesity-associated disorder in the subject. In one embodiment, the subject is a human or non-human animal. In another embodiment, the non-human animal is a mouse, rat, dog, or cat. In a further embodiment, the obesity-associated disorder comprises a metabolic disorder, hypertension, congestive heart failure, a lipid related disorder, type II diabetes mellitus, gallbladder disease, osteoarthritis, sleep apnea, cancer, polycystic ovary syndrome (PCOS), or a combination thereof. In some embodiments, the metabolic disorder comprises hyperglycemia, insulin resistance, hyperinsulinemia, metabolic syndrome, or a combination of such disorders. In other embodiments, the lipid related disorder comprises atherosclerosis, HIV lipodystrophy, coronary heart disease, dyslipidemia, or a combination of a disorder listed herein. In further embodiments, the cancer comprises endometrial cancer, breast cancer, colon cancer, or a combination of such a cancer. In some embodiments, the subject has a Body Mass Index (BMI) greater than about 25 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 30 kg/m². In further embodiments, the subject has a Body Mass Index (BMI) greater than about 35 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 40 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 45 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 50 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 55 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 60 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 65 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 70 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 75 kg/m². In one embodiment, the subject displays a decrease in adipose tissue mass after treatment with Spexin. In another embodiment, the Spexin amount administered results in at least about 1 ng/ml in the serum. In another embodiment, the Spexin amount administered results in at least about 3 ng/ml in the serum. In a further embodiment, the Spexin amount administered results in at least about 10 ng/ml in the serum. In some embodiments, the Spexin amount administered results in at least about 30 ng/ml in the serum. In other embodiments, the Spexin amount administered results in at least about 100 ng/ml in the serum. In further

embodiments, the Spexin amount administered results in at least about 500 ng/ml in the serum. In some embodiments, the Spexin amount administered results in at least about 500 ng/ml in the serum. In one embodiment, Spexin is administered at least once daily or at least twice daily. In another embodiment, Spexin is administered for at least 1 week, for at least 2 weeks, for at least 3 weeks, for at least 4 weeks, for at least 5 weeks, for at least 6 weeks, for at least 8 weeks, for at least 10 weeks, for at least 12 weeks, for at least 24 weeks, or for at least 48 weeks. In a further embodiment, Spexin is administered for at least 1 year, for at least 1.5 years, for at least 2 years, for at least 2.5 years, for at least 5 years, for at least 7.5 years, for at least 10 years, or for at least 15 years.

[0007] The invention provides methods for treating obesity or an obesity-associated disorder in a subject. The methods comprise administering to a subject in need thereof a polypeptide comprising SEQ ID NO: 1, or a pharmaceutically acceptable salt thereof, thereby treating obesity or an obesity-associated disorder in the subject. In one embodiment, the subject is a human or non-human animal. In another embodiment, the non-human animal is a mouse, rat, dog, or cat. In a further embodiment, the obesity-associated disorder comprises a metabolic disorder, hypertension, congestive heart failure, a lipid related disorder, type II diabetes mellitus, gallbladder disease, osteoarthritis, sleep apnea, cancer, polycystic ovary syndrome (PCOS), or a combination thereof. In some embodiments, the metabolic disorder comprises hyperglycemia, insulin resistance, hyperinsulinemia, metabolic syndrome, or a combination of such disorders. In other embodiments, the lipid related disorder comprises atherosclerosis, HIV lipodystrophy, coronary heart disease, dyslipidemia, or a combination of a disorder listed herein. In further embodiments, the cancer comprises endometrial cancer, breast cancer, colon cancer, or a combination of such a cancer. In some embodiments, the subject has a Body Mass Index (BMI) greater than about 25 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 30 kg/m². In further embodiments, the subject has a Body Mass Index (BMI) greater than about 35 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 40 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 45 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 50 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 55 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 60 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 65 kg/m².

In other embodiments, the subject has a Body Mass Index (BMI)

In other embodiments, the subject has a Body Mass Index (BMI) greater than about 75 kg/m².

[0008] An aspect of the invention provides for methods for promoting satiety in an obese subject or a subject afflicted with an obesity-associated disorder, the method comprising administering to a subject an effective amount of Spexin, thereby promoting satiety in an obese subject. In one embodiment, the subject is a human or non-human animal. In another embodiment, the non-human animal is a mouse, rat, dog, or cat. In a further embodiment, the obesity-associated disorder comprises a metabolic disorder, hypertension, congestive heart failure, a lipid related disorder, type II diabetes mellitus, gallbladder disease, osteoarthritis, sleep apnea, cancer, polycystic ovary syndrome (PCOS), or a combination thereof. In some embodiments, the metabolic disorder comprises hyperglycemia, insulin resistance, hyperinsulinemia, metabolic syndrome, or a combination of such disorders. In other embodiments, the lipid related disorder comprises atherosclerosis, HIV lipodystrophy, coronary heart disease, dyslipidemia, or a combination of a disorder listed herein. In further embodiments, the cancer comprises endometrial cancer, breast cancer, colon cancer, or a combination of such a cancer. In some embodiments, the subject has a Body Mass Index (BMI) greater than about 25 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 30 kg/m². In further embodiments, the subject has a Body Mass Index (BMI) greater than about 35 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 40 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 45 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 50 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 55 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 60 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 65 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 70 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 75 kg/m². In one embodiment, the subject displays a decrease in adipose tissue mass after treatment with Spexin. In another embodiment, the Spexin amount administered results in at least about 1 ng/ml in the serum. In another embodiment, the Spexin amount administered results in at least about 3 ng/ml in the serum. In a further embodiment, the Spexin amount administered results in at least about 10 ng/ml in the serum. In some embodiments, the Spexin amount administered results in at least about 30 ng/ml in the serum. In other

embodiments, the Spexin amount administered results in at least about 250 ng/ml in the serum. In some embodiments, the Spexin amount administered results in at least about 500 ng/ml in the serum. In one embodiment, Spexin is administered at least once daily or at least twice daily. In another embodiment, Spexin is administered for at least 1 week, for at least 2 weeks, for at least 3 weeks, for at least 4 weeks, for at least 5 weeks, for at least 6 weeks, for at least 8 weeks, for at least 10 weeks, for at least 12 weeks, for at least 24 weeks, or for at least 48 weeks. In a further embodiment, Spexin is administered for at least 1 year, for at least 1.5 years, for at least 2 years, for at least 2.5 years, for at least 5 years, for at least 7.5 years, for at least 10 years, or for at least 15 years.

[0009] The invention further provides for methods for promoting satiety in an obese subject or a subject afflicted with an obesity-associated disorder, the method comprising administering to a subject in need thereof a polypeptide comprising SEQ ID NO: 1, or a pharmaceutically acceptable salt thereof, thereby promoting satiety in an obese subject. In one embodiment, the subject is a human or non-human animal. In another embodiment, the non-human animal is a mouse, rat, dog, or cat. In a further embodiment, the obesity-associated disorder comprises a metabolic disorder, hypertension, congestive heart failure, a lipid related disorder, type II diabetes mellitus, gallbladder disease, osteoarthritis, sleep apnea, cancer, polycystic ovary syndrome (PCOS), or a combination thereof. In some embodiments, the metabolic disorder comprises hyperglycemia, insulin resistance, hyperinsulinemia, metabolic syndrome, or a combination of such disorders. In other embodiments, the lipid related disorder comprises atherosclerosis, HIV lipodystrophy, coronary heart disease, dyslipidemia, or a combination of a disorder listed herein. In further embodiments, the cancer comprises endometrial cancer, breast cancer, colon cancer, or a combination of such a cancer. In some embodiments, the subject has a Body Mass Index (BMI) greater than about 25 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 30 kg/m². In further embodiments, the subject has a Body Mass Index (BMI) greater than about 35 kg/m².

[0010] An aspect of the invention also provides for a method of promoting weight loss in an obese subject or a subject afflicted with an obesity-associated disorder. The method comprises: a) administering to a subject an effective amount of Spexin; and b) determining whether Spexin decreased the subject's body mass as compared to the subject's body mass

prior to treatment with Spexin, thereby promoting weight loss subject afflicted with an obesity-associated disorder. In one embodiment, the subject is a human or non-human animal. In another embodiment, the non-human animal is a mouse, rat, dog, or cat. In a further embodiment, the obesity-associated disorder comprises a metabolic disorder, hypertension, congestive heart failure, a lipid related disorder, type II diabetes mellitus, gallbladder disease, osteoarthritis, sleep apnea, cancer, polycystic ovary syndrome (PCOS), or a combination thereof. In some embodiments, the metabolic disorder comprises hyperglycemia, insulin resistance, hyperinsulinemia, metabolic syndrome, or a combination of such disorders. In other embodiments, the lipid related disorder comprises atherosclerosis, HIV lipodystrophy, coronary heart disease, dyslipidemia, or a combination of a disorder listed herein. In further embodiments, the cancer comprises endometrial cancer, breast cancer, colon cancer, or a combination of such a cancer. In some embodiments, the subject has a Body Mass Index (BMI) greater than about 25 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 30 kg/m². In further embodiments, the subject has a Body Mass Index (BMI) greater than about 35 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 40 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 45 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 50 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 55 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 60 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 65 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 70 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 75 kg/m². In one embodiment, the subject displays a decrease in adipose tissue mass after treatment with Spexin. In another embodiment, the Spexin amount administered results in at least about 1 ng/ml in the serum. In another embodiment, the Spexin amount administered results in at least about 3 ng/ml in the serum. In a further embodiment, the Spexin amount administered results in at least about 10 ng/ml in the serum. In some embodiments, the Spexin amount administered results in at least about 30 ng/ml in the serum. In other embodiments, the Spexin amount administered results in at least about 100 ng/ml in the serum. In further embodiments, the Spexin amount administered results in at least about 250 ng/ml in the serum. In some embodiments, the Spexin amount administered results in at least about 500 ng/ml in the serum. In one embodiment, Spexin is administered at least once daily or at least twice daily. In another embodiment, Spexin is administered for at least 1 week, for

at least 2 weeks, for at least 3 weeks, for at least 4 weeks, for a weeks, for at least 8 weeks, for at least 10 weeks, for at least 12 weeks, for at least 24 weeks, or for at least 48 weeks. In a further embodiment, Spexin is administered for at least 1 year, for at least 1.5 years, for at least 2 years, for at least 2.5 years, for at least 5 years, for at least 7.5 years, for at least 10 years, or for at least 15 years.

[0011] An aspect of the invention provides for methods of promoting weight loss in an obese subject or a subject afflicted with an obesity-associated disorder. The methods comprise: a) administering to a subject in need thereof a therapeutic amount of a polypeptide comprising SEQ ID NO: 1 or a pharmaceutically acceptable salt thereof; and b) determining whether the polypeptide comprising SEQ ID NO: 1 decreased the subject's body mass as compared to the subject's body mass prior to treatment with polypeptide comprising SEQ ID NO: 1, thereby promoting weight loss in the obese subject or the subject afflicted with an obesity-associated disorder. In one embodiment, the subject is a human or non-human animal. In another embodiment, the non-human animal is a mouse, rat, dog, or cat. In a further embodiment, the obesity-associated disorder comprises a metabolic disorder, hypertension, congestive heart failure, a lipid related disorder, type II diabetes mellitus, gallbladder disease, osteoarthritis, sleep apnea, cancer, polycystic ovary syndrome (PCOS), or a combination thereof. In some embodiments, the metabolic disorder comprises hyperglycemia, insulin resistance, hyperinsulinemia, metabolic syndrome, or a combination of such disorders. In other embodiments, the lipid related disorder comprises atherosclerosis, HIV lipodystrophy, coronary heart disease, dyslipidemia, or a combination of a disorder listed herein. In further embodiments, the cancer comprises endometrial cancer, breast cancer, colon cancer, or a combination of such a cancer. In some embodiments, the subject has a Body Mass Index (BMI) greater than about 25 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 30 kg/m². In further embodiments, the subject has a Body Mass Index (BMI) greater than about 35 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 40 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 45 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 50 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 55 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 60 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 65 kg/m².

In other embodiments, the subject has a Body Mass Index (BMI)

In other embodiments, the subject has a Body Mass Index (BMI) greater than about 75 kg/m².

[0012] An aspect of the invention provides for methods of decreasing serum Leptin levels in an obese subject or a subject afflicted with an obesity-associated disorder. The methods comprise: a) administering to a subject an effective amount of Spexin; and b) determining whether Spexin decreased the subject's serum Leptin levels as compared to the subject's serum Leptin levels prior to treatment with Spexin, thereby decreasing serum Leptin levels in the obese subject or the subject afflicted with an obesity-associated disorder. In one embodiment, the subject is a human or non-human animal. In another embodiment, the non-human animal is a mouse, rat, dog, or cat. In a further embodiment, the obesity-associated disorder comprises a metabolic disorder, hypertension, congestive heart failure, a lipid related disorder, type II diabetes mellitus, gallbladder disease, osteoarthritis, sleep apnea, cancer, polycystic ovary syndrome (PCOS), or a combination thereof. In some embodiments, the metabolic disorder comprises hyperglycemia, insulin resistance, hyperinsulinemia, metabolic syndrome, or a combination of such disorders. In other embodiments, the lipid related disorder comprises atherosclerosis, HIV lipodystrophy, coronary heart disease, dyslipidemia, or a combination of a disorder listed herein. In further embodiments, the cancer comprises endometrial cancer, breast cancer, colon cancer, or a combination of such a cancer. In some embodiments, the subject has a Body Mass Index (BMI) greater than about 25 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 30 kg/m². In further embodiments, the subject has a Body Mass Index (BMI) greater than about 35 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 40 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 45 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 50 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 55 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 60 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 65 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 70 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 75 kg/m². In one embodiment, the subject displays a decrease in adipose tissue mass after treatment with Spexin. In another embodiment, the Spexin amount administered results in at least about 1 ng/ml in the serum. In another embodiment, the Spexin amount administered results in at least about 3 ng/ml in the serum.

In a further embodiment, the Spexin amount administered results in at least about 30 ng/ml in the serum. In some embodiments, the Spexin amount administered results in at least about 100 ng/ml in the serum. In other embodiments, the Spexin amount administered results in at least about 250 ng/ml in the serum. In some embodiments, the Spexin amount administered results in at least about 500 ng/ml in the serum. In one embodiment, Spexin is administered at least once daily or at least twice daily. In another embodiment, Spexin is administered for at least 1 week, for at least 2 weeks, for at least 3 weeks, for at least 4 weeks, for at least 5 weeks, for at least 6 weeks, for at least 8 weeks, for at least 10 weeks, for at least 12 weeks, for at least 24 weeks, or for at least 48 weeks. In a further embodiment, Spexin is administered for at least 1 year, for at least 1.5 years, for at least 2 years, for at least 2.5 years, for at least 5 years, for at least 7.5 years, for at least 10 years, or for at least 15 years.

[0013] The invention provides for a method of decreasing serum Leptin levels in an obese subject or a subject afflicted with an obesity-associated disorder. The method comprises: a) administering to a subject in need thereof a therapeutic amount of a polypeptide comprising SEQ ID NO: 1 or a pharmaceutically acceptable salt thereof; and b) determining whether the polypeptide comprising SEQ ID NO: 1 decreased the subject's serum Leptin levels as compared to the subject's serum Leptin levels prior to treatment with polypeptide comprising SEQ ID NO: 1, thereby decreasing serum Leptin levels in the obese subject or the subject afflicted with an obesity-associated disorder. In one embodiment, the subject is a human or non-human animal. In another embodiment, the non-human animal is a mouse, rat, dog, or cat. In a further embodiment, the obesity-associated disorder comprises a metabolic disorder, hypertension, congestive heart failure, a lipid related disorder, type II diabetes mellitus, gallbladder disease, osteoarthritis, sleep apnea, cancer, polycystic ovary syndrome (PCOS), or a combination thereof. In some embodiments, the metabolic disorder comprises hyperglycemia, insulin resistance, hyperinsulinemia, metabolic syndrome, or a combination of such disorders. In other embodiments, the lipid related disorder comprises atherosclerosis, HIV lipodystrophy, coronary heart disease, dyslipidemia, or a combination of a disorder listed herein. In further embodiments, the cancer comprises endometrial cancer, breast cancer, colon cancer, or a combination of such a cancer. In some embodiments, the subject has a Body Mass Index (BMI) greater than about 25 kg/m². In other embodiments, the subject has a Body Mass Index (BMI) greater than about 30 kg/m². In further embodiments,

the subject has a Body Mass Index (BMI) greater than about 3:

embodiments, the subject has a Body Mass Index (BMI) greater than about 40 kg/m². In

other embodiments, the subject has a Body Mass Index (BMI) greater than about 45 kg/m². In

other embodiments, the subject has a Body Mass Index (BMI) greater than about 50 kg/m².

In other embodiments, the subject has a Body Mass Index (BMI) greater than about 55 kg/m².

In other embodiments, the subject has a Body Mass Index (BMI) greater than about 60 kg/m².

In other embodiments, the subject has a Body Mass Index (BMI) greater than about 65 kg/m².

In other embodiments, the subject has a Body Mass Index (BMI) greater than about 70 kg/m².

In other embodiments, the subject has a Body Mass Index (BMI) greater than about 75 kg/m².

[0014] In one embodiment, the subject displays a decrease in adipose tissue mass after treatment with Spexin. In another embodiment, the Spexin amount administered results in at least about 1 ng/ml in the serum. In another embodiment, the Spexin amount administered results in at least about 3 ng/ml in the serum. In a further embodiment, the Spexin amount administered results in at least about 10 ng/ml in the serum. In some embodiments, the Spexin amount administered results in at least about 30 ng/ml in the serum. In other embodiments, the Spexin amount administered results in at least about 100 ng/ml in the serum. In further embodiments, the Spexin amount administered results in at least about 250 ng/ml in the serum. In some embodiments, the Spexin amount administered results in at least about 500 ng/ml in the serum. In one embodiment, Spexin is administered at least once daily or at least twice daily. In another embodiment, Spexin is administered for at least 1 week, for at least 2 weeks, for at least 3 weeks, for at least 4 weeks, for at least 5 weeks, for at least 6 weeks, for at least 8 weeks, for at least 10 weeks, for at least 12 weeks, for at least 24 weeks, or for at least 48 weeks. In a further embodiment, Spexin is administered for at least 1 year, for at least 1.5 years, for at least 2 years, for at least 2.5 years, for at least 5 years, for at least 7.5 years, for at least 10 years, or for at least 15 years.

[0015] An aspect of the invention provides methods for detecting the presence of or a predisposition to obesity or an obesity-associated disorder in a human subject. The methods comprise: (a) obtaining a biological sample from a subject; and (b) detecting whether or not there is an alteration in the expression of a Obesity-Signature (OS) gene in the subject as compared to a subject not afflicted with obesity or an obesity-associated disorder. In one embodiment, the Obesity-Signature (OS) gene comprises any gene indicated as being differentially expressed (e.g., up-regulated or downregulated) in any one of Tables 7-8,

and/or Spexin, or a combination of such. In some embodiments, the gene comprises any gene indicated as being differentially expressed (e.g., down-regulated or up-regulated) in any one of Tables 7-8, and/or Spexin, or a combination of such. In one embodiment, the obesity-associated disorder comprises a metabolic disorder, hypertension, congestive heart failure, a lipid related disorder, type II diabetes mellitus, gallbladder disease, osteoarthritis, sleep apnea, cancer, polycystic ovary syndrome (PCOS), or a combination of the listed disorders. In another embodiment, the metabolic disorder comprises hyperglycemia, insulin resistance, hyperinsulinemia, metabolic syndrome, or a combination of such listed. In some embodiments, the lipid related disorder comprises atherosclerosis, coronary heart disease, dyslipidemia, HIV lipidodystrophy, or a combination of those listed disorders. In further embodiments, the cancer comprises endometrial cancer, breast cancer, colon cancer, or a combination of the listed cancers. In one embodiment, the detecting comprises determining in the sample whether expression of at least 2 OS genes, at least 3 OS genes, at least 4 OS genes, at least 5 OS genes, at least 6 OS genes, at least 7 OS genes, at least 8 OS genes, at least 9 OS genes, or at least 10 OS genes indicated as being differentially expressed (e.g., up-regulated or down-regulated) in any one of Tables 7-8, and/or Spexin, or a combination of such, are decreased as compared to expression in a normal sample. In another embodiment, the detecting comprises determining in the sample whether expression of at least 2 OS genes, at least 3 OS genes, at least 4 OS genes, at least 5 OS genes, at least 6 OS genes, at least 7 OS genes, at least 8 OS genes, at least 9 OS genes, or at least 10 OS genes indicated as being differentially expressed (e.g., up-regulated or down-regulated) in any one of Tables 7-8, or a combination of such tables indicated, are increased as compared to expression in a normal sample. In one embodiment, the detecting comprises determining in the sample whether expression of at least 2 OS genes, at least 3 OS genes, at least 4 OS genes, at least 5 OS genes, at least 6 OS genes, at least 7 OS genes, at least 8 OS genes, at least 9 OS genes, or at least 10 OS genes indicated as being differentially expressed (e.g., down-regulated or up-regulated) in any one of Tables 7-8, or a combination of such, are increased as compared to expression in a normal sample. In a further embodiment, the detecting comprises determining in the sample whether expression of at least 2 OS genes, at least 3 OS genes, at least 4 OS genes, at least 5 OS genes, at least 6 OS genes, at least 7 OS genes, at least 8 OS genes, at least 9 OS genes, or at least 10 OS genes indicated as being differentially expressed (e.g., down-regulated or up-regulated) in any one of Tables 7-8, and/or Spexin, or a combination of such indicated, are decreased as compared to expression in a normal sample. In some embodiments, the detecting comprises gene sequencing,

selective hybridization, selective amplification, gene expression a method indicated. In other embodiments, the sample comprises subcutaneous adipose tissue, omental adipose tissue, whole blood, plasma, serum, white blood cells, or mesenteric adipose tissue.

[0016] An aspect of the invention provides for a diagnostic kit for determining whether a sample from a subject exhibits increased or decreased expression of at least 2 or more OS genes, the kit comprising nucleic acid primers that specifically hybridize to a OS gene, wherein the primer will prime a polymerase reaction only when a OS gene in any one of Tables 7-8, and/or Spexin, is present.

[0017] An aspect of the invention provides for methods for identifying a Spexin-modulating compound useful for treating obesity or an obesity-associated disorder in a subject. The methods comprise: (a) administering a test agent to a mouse model of obesity or an obesity-associated disorder; and (b) determining whether the test agent altered serum Spexin levels, serum Leptin levels, a body-weight phenotype, or a combination thereof, as compared to a mouse in the absence of the test agent, thereby identifying a compound useful for treating obesity or an obesity-associated disorder. In one embodiment, the serum Spexin levels are increased. In another embodiment, the serum Leptin levels are decreased. In a further embodiment, the body-weight phenotype is a decrease in adipose tissue mass. In some embodiments, the test agent comprises a polynucleotide, small organic molecule, a small inorganic molecule, a soluble peptide, or an antibody. In other embodiments, the antibody specifically binds to a Spexin protein or a fragment thereof. In further embodiments, the polynucleotide is an antisense RNA that specifically inhibits expression of a Spexin gene that encodes a Spexin protein. In some embodiments, the polynucleotide is an siRNA that specifically targets a Spexin gene that encodes a Spexin protein.

BRIEF DESCRIPTION OF THE FIGURES

[0018] To conform to the requirements for PCT patent applications, many of the figures presented herein are black and white representations of images originally created in color. In the below descriptions and the examples, the colored plots are described in terms of its appearance in black and white. The original color versions can be viewed in Walewski *et al.*, *Obes Surg.* 2010 Jan;20(1):93-107, of which the contents are herein incorporated by reference.

[0019] **FIG. 1** is a log-log plot of gene expression differences between normal fat and obese fat. Data points represent the mean expression ratios (normal [n=8] vs obese fat [n=12]). Colored dots represent genes that have statistically significant differences ($p < 0.05$) in expression values between sample sets (red=genes that are over-expressed in normal fat, green=genes that are over-expressed in obese fat; grey data points represent non-significant data). Ch12;orf39 is Spexin.

[0020] **FIG. 2** is a graph of Spexin (Ch12, orf39) gene expression showing that it is down-regulated 14.8 fold in obese fat vs normal fat. Data represent mean plus SEM.

[0021] **FIG. 3** is a multiple alignment of Spexin homologues from various species. Genebank was queried for human Spexin (NP_085049; SEQ ID NO: 26) and related homologues [chimpanzee (XP001144591; SEQ ID NO: 27), canine (XP_853311; SEQ ID NO: 28), bovine (NP_001068875; SEQ ID NO: 29), mouse (XP_620381; SEQ ID NO: 30), and rat (XP_001076968; SEQ ID NO: 31)]. The histogram on top of each panel indicates the degree of identity of the collection to the majority sequence at each residue position (taller bars = greater identity). The yellow shading within the alignment indicates the amino acid residues that match the majority sequence at each position. Amino acid sequences were aligned using the Clustal W function of MegAlign (DNASTar). Sequence alignment parameters were; gap penalty (10), gap length penalty (0.20), delay divergent sequences (30%), and DNA transition weight (0.50).

[0022] **FIG. 4** shows a Spexin enzyme immunoassay (EIA) standard curve. A dilution series of known Spexin standards is plotted against their corresponding OD values to generate a standard curve for accurate quantitation.

[0023] **FIG. 5** is a bar graph showing that serum Spexin concentrations are lower in obese patients. Serum Spexin levels were assayed by antigen competition EIA in human serum samples from obese and normal weight individuals; (obese; n=7; 1.11 ± 1.67) vs (normal; n=7; 11.60 ± 3.20), ($p < 0.000461$, by T test, 2 tailed with equal variance). Data represent means \pm St Devs in ng/mL.

[0024] **FIG. 6** is a bar graph showing that serum Leptin levels are significantly higher in obese patients. Serum Leptin levels were measured by antigen capture ELISA; obese n=7 (0.69 ± 0.22) vs normals n=6 (0.15 ± 0.15), ($p = 0.0099$, by T test, 2 tailed with equal variance). Data represent mean ODs \pm St Dev, respectively.

[0025] **FIG. 7** is a graph showing the correlation between serum from obese (n=7) and normal weight patients (n=6). Leptin ODs (ordinate) were plotted against Spexin [ng/mL] (abscissa) for each patient sample. The negative correlation ($r=0.9444$) observed between the two circulating peptides is quite strong, with an R^2 of 0.8919.

[0026] **FIG. 8** is a bar graph showing that serum Spexin vs Leptin ratios distinguish Obese vs Normal weight patients. The ratios of Spexin to Leptin are plotted for each patient. Obese (n=7, red) ratios range from 0 to 6.1, and in normal patients (n=6, blue) the ratios range from 19 to 240, with most more than 150.

[0027] **FIG. 9** is a bar graph showing that Spexin is down-regulated 14.8 fold in obese fat vs normal fat. Spexin is referred to as "Peptide A."

[0028] **FIG. 10** is a bar graph of serum Leptin levels. Serum Leptin levels were measured by immunoassay in the same normal (8.53 ± 7.55) and obese patients (37.42 ± 11.56) ($p < 0.01$, by t test, 2 tailed). Data represent mean \pm St Dev.

[0029] **FIG. 11** depicts a schematic of a model of Long Chain Fatty Acid (LCFA) accumulation in Obese Adipocytes. In the adipocytes of normal weight subjects (**FIG. 11A**), LCFA uptake (including facilitated transport) and degradation (including β -oxidation in adipocytes) are in balance, with no net gain or loss of LCFA. As a result, over time, the relative amount of LCFAs in each cell is constant. In adipocytes from obese subjects (**FIG. 11B**), the combination of increased LCFAs by specific transporters (as demonstrated in uptake studies discussed herein), and reduced β -oxidation and fatty acid metabolism (indicated by the expression studies) leads to an accumulation of LCFAs in these adipocytes over time, resulting in enlarged adipocytes and obesity.

[0030] **FIG. 12** is a schematic showing Complexes I – V of the oxidative phosphorylation (Ox-Phos) pathway. These five complexes of the Ox-Phos pathway are responsible for the generation of ATP from energy released by the citric acid cycle. The major subunits of each complex are drawn in the diagram, and boxes indicating key enzymes are labeled with the appropriate EC classification number. Boxes/EC numbers highlighted in red indicate enzymes that are differentially expressed in obesity. One striking result is that the eight genes that are coordinately down-regulated are distributed across all five complexes. (Walewski, et al; 2010). One key point to consider is that while 15 - 50% differences in gene

expression (assuming the protein drops by a corresponding amount) is all that is important in terms of final throughput unless these are rate-limiting enzymes. While the total protein may be lower, there is usually excess functional capacity, assuming enzyme distribution or co-regulatory functions are not important for these proteins in solution. However, in this case, the proteins in question are members of large membrane bound complexes, where the correct stoichiometry of the individual proteins in the overall architecture of the complex is quite important. A 15 - 50% reduction in the amount of an individual protein could reasonably be expected to affect the structural, and consequently functional, properties of the complex. Even more striking, is the coordinate reduction in the expression of key genes across each of the 5 complexes of the electron transport chain.

[0031] **FIG. 13** is a graph showing fatty Acid Uptake Curves. [^3H]-oleic acid uptake by omental adipocytes was studied in 3 obese (dashed curves) and 3 normal wt individuals (solid curves). The curves represent computer fits to the sum of a saturable plus a non-saturable function of the unbound oleic acid concentration. There was no overlap between the two groups.

[0032] **FIG. 14** shows comparisons of obese vs. normal omental fat mRNA expression (Cohort 1). The expression levels of approximately 9,200 genes were queried by microarray. **FIG. 14A** shows a Log-Log plot of all genes assayed in this study. Means of each data point are presented as a scatterplot ($n = 3$ in each group, obese vs. normal). Relative expression data for 566 statistically significant genes ($p < 0.05$, 1.2 fold or greater change in expression) are presented in color (red = up, blue = down). “Red” is depicted as dark grey circles to the top of the light grey circles (above and to the left of the line-of-identity [45°]). “Blue” is depicted as dark grey circles to the bottom of the light grey circles (below and to the right of the line-of-identity [45°]). **FIG. 14B** shows a detailed view of a representative section of scatterplot (centered dark grey square in **FIG. 14A**). Red (up in obese) and blue (down in obese) data points are statistically significant, whereas non-significant data points are represented as grey circles. “Red” is depicted as dark grey circles to the top of the light grey circles. “Blue” is depicted as dark grey circles to the bottom of the light grey circles. **FIG. 14C** shows quartile box plots of means of all genes in normal ($N = 3$) vs. obese ($N = 3$). Striking similarities exist between the two groups.

[0033] **FIG. 15** shows bar graphs of the expression of dodecenoyl-CoA isomerase (DCI) mRNA in normal vs. obese omental fat samples. DCI mRNA expression in obese fat

is reduced versus control fat. In cohort 1, DCI mRNA in omentum of obese individuals demonstrates a 1.59 fold under expression in obese patients (**FIG. 15A**), while in cohort 2 (**FIG. 15B**), very similar results were seen using whole genome arrays, with a 1.64 fold under expression in obese omental fat.

[0034] **FIG. 16** shows bar graphs depicting two adenylate cyclase genes that are involved in hormonal stimulation of lipolysis are under-expressed in obese omental fat. *Adenylate Cyclase 6* is a membrane-associated enzyme and catalyzes the formation of the secondary messenger cyclic adenosine monophosphate (cAMP). This gene was 1.78-fold under-expressed in obese omental fat in cohort 1 (**FIG. 16A**), and 1.64 under-expressed in the cohort 2 samples (**FIG. 16B**). *Adenylate Cyclase activating peptide receptor 1* is a membrane-associated protein and shares significant homology with members of the glucagon/secretin receptor family. This receptor mediates diverse biological actions of adenylate cyclase activating polypeptide 1 and is positively coupled to adenylate cyclase. This gene was 1.74-fold under-expressed in obese omental fat in cohort 1 (**FIG. 16C**), and 1.18 fold under-expressed in the cohort 2 samples (**FIG. 16D**).

[0035] **FIG. 17** is a bar graph showing the biological and technical validation of key gene expression results in Cohort 1 by Alternative Microarray and qRT-PCR in Cohort 2. Key genes which were under-expressed in obese omental fat in cohort 1 were selected for repeat testing in cohort 2 samples. Biological and technical validation was achieved in cohort 2 by both alternative microarray platform and qRT-PCR analysis. The ratio of gene expression in obese vs. normal fat in the Cohort 2 samples is presented for each of 7 genes identified as down-regulated in obesity in our original Cohort 1 study. Our new results, show that all 7 genes are again underexpressed in obese omental fat in the Cohort 2 samples (n=7) vs normal weight controls (n=4). It is striking that for 5 of 7 genes assayed by both technologies, the relative expression ratios are within 10% of each other. The genes illustrated are ECHD (Enoyl Coenzyme A hydratase); ADH1A (Alcohol dehydrogenase 1A); DCI (Dodecenoyl-Coenzyme A delta isomerase); ATP5D (ATP synthase mito F1); CYC1 (cytochrome c-1); NDUFS7 (NADH dehydrogenase Fe-S); COX4I1 (Cytochrome c IV).

[0036] **FIG. 18** shows graphs of the effect of daily Spexin Injection on High-Fat-Diet mouse weights. Two groups of Diet Induced Obesity (DIO) mice, housed under normal conditions and fed a special chow with approximately 60% of calories as fat, were injected daily for 6 days with 0.2 ml of either 1x PBS vehicle (n = 5), or 1x PBS containing Spexin at

a concentration of 2500 ng/mL (n = 5). **FIG. 18A** is a graph of weights per day of PBS injected DIO mice. The PBS injected DIO mice continued to gain weight. **FIG. 18B** is a graph depicting mean body weights of Spexin injected mice. The mice are losing approximately 0.3 g of body weight per day. Setting aside the day 2 body weight, the remaining days show a clear negative correlation between dosing day and body weight ($r = -0.999$).

[0037] **FIG. 19** is a graph showing a comparison of 2 groups of obese, high fat diet (HFD)-fed C57BL/6J mice. One group, with a mean starting weight of 42 grams, was treated with daily intraperitoneal injections of phosphate buffered saline (PBS) plus 0.05% bovine serum albumin (BSA). The second, with a mean starting weight 46 grams, received daily Spexin (Peptide A) injections in 1X PBS plus 0.05% BSA at a dose predicted to minimize weight gain or maintain body weight. The PBS group continued rapid weight gain; the Spexin group gained weight much more slowly. The weight curves of the 2 groups crossed at 6 weeks of treatment. Animals were sacrificed at 7 weeks, at which time the mean weights of the two groups were not different.

[0038] **FIG. 20** is a schematic of human Spexin mRNA expression by tissue. A compilation of Affymetrix human gene expression data from the UCSD Genome Browser indicates that expression of Spexin is limited to a few tissues (for example, CNS nuclei, skeletal muscle, Burkitt's lymphoma [Daudi], and adipose tissue). Spexin expression is low in most human tissues by Affymetrix microarray analysis.

DETAILED DESCRIPTION OF THE INVENTION

[0039] The invention provides, in part, the discovery of genes that are differentially expressed in obese and normal fat, providing the basis for Obesity Signatures (OSs) that can be used as therapeutic targets for obesity. These gene expression studies in human obese fat, thus, can be used to identify therapeutic targets using animal models of obesity. Once the targets are identified, the Obesity Signatures can be used for methods of diagnosis and treatment for obesity and obesity-associated disorders. In one embodiment, genes comprising an Obesity Signature comprise those genes listed in Table 7 and Table 8, Spexin, or a combination thereof.

[0040] The invention provides methods for administering Spexin or a polypeptide comprising SEQ ID NO: 1, or derived from Spexin, or a pharmaceutically acceptable salt

thereof, to an obese subject to treat obesity or an obesity-associated disorder, also provides methods for administering Spexin or a polypeptide comprising SEQ ID NO: 1, or a pharmaceutically acceptable salt thereof, to an obese subject or a subject afflicted with an obesity-associated disorder in order to promote weight loss in the subject. The invention further provides methods for administering Spexin or a polypeptide comprising SEQ ID NO: 1, or a pharmaceutically acceptable salt thereof, to an obese subject or a subject afflicted with an obesity-associated disorder to promote satiety in the subject. The invention also provides methods for detecting the presence of or a predisposition to obesity or an obesity-associated disorder in a human subject. The invention additionally provides a diagnostic kit for determining whether a subject is predisposed to obesity or an obesity-associated disorder or whether a sample from a subject exhibits increased or decreased expression of at least 2 or more Obesity-Signature (OS) genes. The invention further provides methods for identifying compounds useful for treating obesity or an obesity-associated disorder in a subject.

[0041] *Obesity and Obesity-associated Disorders*

[0042] Obesity is characterized by excess body fat and is a chronic disease posing a significant impact on quality of life, productivity, and socioeconomic status. A normal-sized person has between 30 and 35 billion fat cells. When a person gains weight, these fat cells first increase in size and then in number. Obesity is, *de facto*, the increased deposition of long chain fatty acids (LCFA), principally in the form of triglycerides (TG), in adipose and other tissues. Although LCFA were once believed to enter cells exclusively by passive diffusion across plasma membranes, the fact that TG accumulate in specific sites indicated that cellular LCFA uptake involves specific, regulatable mechanisms.

[0043] Obesity has been shown to increase the risk for hypertension, dyslipidemia (high total cholesterol or high levels of triglycerides), type II diabetes, coronary heart disease, stroke, gallbladder disease, osteoarthritis, sleep apnea, and some cancers (for example, endometrial cancer, breast cancer, and colon cancer). In one embodiment, an obesity-associated disorder comprises a metabolic disorder, hypertension, congestive heart failure, a lipid related disorder, type II diabetes mellitus (Non-insulin Dependent Diabetes Mellitus, NIDDM), gallbladder disease, osteoarthritis, sleep apnea, cancer, polycystic ovary syndrome (PCOS), or a combination thereof. In one embodiment, the metabolic disorder comprises hyperglycemia, insulin resistance, hyperinsulinemia, metabolic syndrome, or a combination thereof. In another embodiment, the lipid related disorder comprises atherosclerosis,

coronary heart disease, dyslipidemia, or a combination thereof
cancer comprises endometrial cancer, breast cancer, colon cancer, or a combination thereof.

[0044] Obesity occurs when a subject has a weight and body mass, particularly of fat tissue, above currently accepted standards. The body mass index (BMI; kg/m^2) provides a useful population-level measure of obesity that can be used to estimate the prevalence of obesity within a population and the risks associated with it. For example, a subject is obese with a BMI above the currently accepted standard. When a subject is a human, the current standards for both men and women accepted as “normal” are a BMI of about 20 kg/m^2 to about 24.9 kg/m^2 . In one embodiment, an obese subject has a BMI of 25 kg/m^2 or greater. In another embodiment, an obese subject has a BMI of about 30 kg/m^2 or greater. In further embodiments, an obese subject has a BMI of about 40 kg/m^2 or greater. In some embodiments, the subject is obese when it weighs more than 120% of the normal body weight for its age and height. Even mild obesity, at 20% over desirable weight according to standard height-weight charts, may increase the risk for disease, such as the obesity-associated disorders described herein, and premature death. Normal body weights vary among species and individuals based on height, body build, bone structure, and sex. A subject, according to the invention includes, but is not limited to a human or non-human animal, such as a primate, a dog, a cat, a cow, a horse, a rabbit, a monkey, a mouse, a rat, a pig, a sheep, or a goat.

[0045] *Mouse models of Obesity and Obesity-Associated Disorders*

[0046] Various animal models exist to study obesity (see, for example, Bray, G. A., 1992, Prog. Brain Res. 93:333-341, and Bray, G. A., 1989, Amer. J. Clin. Nutr. 5:891-902). Animals that have mutations leading to syndromes that include obesity symptoms have also been identified. The best studied animal models for obesity are mice. See reviews, e.g., Friedman, J. M. et al., 1991, Mamm. Gen. 1:130-144; Friedman, J. M. and Liebel, R. L., 1992, Cell 69:217-220, which are each incorporated by reference in their entirety. Mice models of obesity have indicated that obesity is a complex trait with a high degree of heritability. Mutations at a number of loci that lead to obese phenotypes have been identified, and include (but are not limited to) the autosomal recessive mutations obese (*ob*), diabetes (*db*), fat (*fat*) and tubby (*tub*).

[0047] The *ob* mutation is found on chromosome 6 while chromosome 4. Each mutation leads to a clinically similar onset of obesity, starting at about one month of age, with a phenotype typified by hyperphagia, severe abnormalities in glucose and insulin metabolism, poor thermoregulation and non-shivering thermogenesis, and extreme torpor and underdevelopment of the lean body mass. In the *db* mouse, elevated triiodothyronine levels have also been reported.

[0048] The *ob* gene and its human homologue were cloned by Zhang, Y. et al., (1994, Nature 372:425-432). The *ob* gene encodes Leptin, a protein of 167 amino acids. It is produced by adipocytes of white adipose tissue, and the level of circulating Leptin is directly proportional to the total amount of fat in the body. In its active form, Leptin is a 16 kDa protein hormone involved in regulating energy intake and energy expenditure, including appetite and metabolism. *ob/ob* mice have mutations in the gene for Leptin, and are responsive to Leptin treatment.

[0049] The *db* locus encodes a high affinity receptor for the *ob* gene product, Leptin (Chen, H. et al., Cell. 1996 Feb 9;84(3):491-5). The *db* gene product, the Leptin receptor, is a single membrane-spanning receptor that is closely related to the gp130 cytokine receptor signal transducing component (Tartaglia, L. A. et al., 1995, Cell 83:1263-1271).

[0050] Another animal model, the *fa/fa* (fatty) rat, is similar to the *ob/ob* mouse and the *db/db* mouse. However, a *fa/fa* rat has a normal capacity for non-shivering thermogenesis but are very sensitive to cold. Torpor seems to play a larger part in the maintenance of obesity in *fa/fa* rats as compared to the *ob/ob* and *db/db* mice.

[0051] ***Overview of Diabetes***

[0052] Obesity, one of the major symptoms in metabolic syndrome, is not only a strong risk factor for the development of Type II diabetes (Abate N, et al., *J Diabetes Complications*, 2000, 14(3):154-74) but also a independent risk factor of coronary heart disease (Wallace AM, et al., *Circulation*, 2001, 104(25):3052-6). The increase of circulating Leptin levels is markedly associated with obesity (Caro JF, et al., *Diabetes*, 1996, 45(11):1455-62; Chu NF, et al., *Int J Obes Relat Metab Disord*, 2000, 24(9):1085-92). The primary physiological function of Leptin is to prevent obesity by regulating food intake and energy balance through activation of its receptors in hypothalamic centers. The elevated

levels of plasma Leptin (also referred to as hyperleptinemia) as diabetes implies a resistance to the action of Leptin.

[0053] Diabetes mellitus is one of the most prevalent chronic disorders affecting the population worldwide. Diabetes mellitus is a disorder of carbohydrate metabolism, characterized by hyperglycemia and glycosuria, which results from inadequate synthesis or utilization of insulin. Hyperleptinemia and hyperinsulinemia are also characteristic of some types of diabetes, such as Type II diabetes (or non-insulin dependent diabetes mellitus, NIDDM; discussed below) Diabetes exists as four categories: Type I diabetes (also referred to as insulin dependent diabetes mellitus, or IDDM), Type II diabetes (NIDDM), gestational diabetes mellitus, and other specific types, wherein each type has a distinct pathogenesis and etiology. In Europe, the United States, and Canada, over 80% of diabetes cases are classified as Type II, and 5-10% are diagnosed as Type I (Carulli L, et al., *Aliment Pharmacol Ther*, 2005, 22 Suppl 2:16-9).

[0054] In Type I diabetes, the pancreas fails to produce insulin, which is essential for glucose homeostasis. This form develops typically in children and adolescents, but can also develop later in life. Type II diabetes results from the inability of insulin-responsive tissues to respond properly to the action of insulin, thus are referred to as being insulin-resistant. Type II diabetes occurs most frequently in adults, but an increase in adolescents diagnosed with Type II diabetes has been observed (Carulli L, et al., *Aliment Pharmacol Ther*, 2005, 22 Suppl 2:16-9; Bouche C, et al., *Endoc Rev*, 2004, 25(5): 807-830).

[0055] Non-insulin dependent diabetes mellitus (NIDDM), or Type II diabetes, occurs predominantly in adults. These individuals produce adequate levels of insulin but display a defect in insulin-mediated utilization and metabolism of glucose in peripheral tissues, such as in skeletal muscle, brown adipose tissue, and white adipose tissue. NIDDM subjects exhibit overproduction of glucose by the liver, thus contributing to the hyperglycemic state associated with the disorder; impairment of glucose-regulated insulin secretion; and resistance to insulin-mediated glucose disposal, thus also contributing to the hyperglycemic status of the afflicted subject. In NIDDM, insulin secretion is often enhanced in order to compensate for insulin resistance. As the disorder prevails, the pancreatic β -cells fail to sustain sufficient insulin secretion to compensate for the impaired insulin responsiveness. However, mechanisms responsible for the failure of β -cell action have yet to be established, but perhaps can be attributed to hyperglycemic effects and/or to the continuous demands

placed on the β -cells by peripheral insulin resistance (Carulli I *Ther*, 2005, 22 Suppl 2:16-9; Bouche C, et al., *Endoc Rev*, 2004, 25(5): 807-830).

[0056] Subjects who display insulin resistance or are diagnosed as a Type II diabetic often exhibit various symptoms that are referred to as metabolic syndrome, or syndrome X. A subject having this syndrome is characterized as exhibiting three or more symptoms selected from the following group: (1) hypertriglyceridemia; (2) abdominal obesity; (3) low high-density lipoprotein cholesterol (HDL); (4) elevated fasting serum glucose levels; (5) high blood pressure; and (6) hyperleptinemia. NIDDM subjects can display a number of the symptoms described. Syndrome X subjects, whether or not overt diabetes mellitus develops, have an increased risk of developing the microvascular and macrovascular complications that occur in NIDDM subjects, such as atherosclerosis and coronary heart disease (Carulli L, et al., *Aliment Pharmacol Ther*, 2005, 22 Suppl 2:16-9).

[0057] Uncontrolled and continuous hyperglycemia connected with diabetes is associated with increased and premature morbidity and mortality. Diabetic subjects, such as those having NIDDM, have a considerable increased risk of macrovascular and microvascular complications, such as coronary heart disease, atherosclerosis, peripheral vascular disease, stroke, hypertension, retinopathy, neuropathy, and nephropathy (Federici M and R Lauro, *Aliment Pharmacol Therapy*, 2005, 22(Suppl 2): 11-15; Desai AS and PT O'Gara, *Indian Heart J*, 2005, 57(4):295-303; Natarajan R and JL Nadler, *Arterioscler Thromb Vasc Biol*, 2004, 24:1542-48). Thus, therapeutic control of obesity, glucose homeostasis, hypertension, and lipid metabolism are critically important in the treatment and clinical management of diabetes mellitus, so as to decrease mortality due to diabetic complications, such as cardiovascular disease.

[0058] *Insulin and Leptin Signaling*

[0059] Binding of insulin to its receptor leads to the activation of several mitogenic and metabolic pathways via insulin receptor substrates (IRS 1-4), Gab-1, and Cbl (Sun XJ, et al., *Nature*, 1995, 377:173-7; Sun XJ, et al., *Nature*, 1991, 352:73-7; Fantin VR, et al., *J Biol Chem*, 1998, 273(17):10726-32; Berg CE, et al., *Biochem Biophys Res Commun*, 2002, 293(3):1021-7; Holgado-Madruga M, et al., *Nature*, 1996, 379:560-4; Ribon V and AR Saltiel, *Biochem J*, 1997, 324(Pt 3):839-45). Insulin binds to its receptor resulting in autophosphorylation of the β -subunits and phosphorylation of the tyrosine residues of the

insulin receptor substrates (such as IRS-1). IRS subsequently] domain of the tyrosine phosphatase, Shp2, as well as the SH3 domain of the adaptor molecule Grb2. Active Grb2 in turn binds to Sos1, which then activates the Ras signaling pathway and downstream transcription of genes. The IRS protein also activates phosphoinositide 3-kinase (PI3K) via binding to its SH2 domain, resulting in increases in intracellular PIP₂ and PIP levels which leads to the activation of phosphatidylinositol phosphate-dependent kinase-1 (PDK-1). This event thus leads to activating the Akt/PKB pathway, which results in intracellular events, such as translocation of the glucose transporter (GLUT4) from an intracellular pool to the cell surface.

[0060] Among the mitogenic and metabolic pathways initiated by the insulin signaling cascade is the phosphatidylinositol 3-kinase (PI3K) pathway (Berman DM, et al., *J Clin Endocrinol Metab*, 2001, 86(1):97-103; Dennis PB, et al., *Mol Cell Biol*, 1996, 16(11):6242-51). In vascular smooth cells (VSMCs), Leptin binds to its receptor and activates both mitogen activated protein kinase (MAPK) and PI3K pathways through phosphorylation of Tyr985 and Tyr1138 residues of the Leptin receptor (Oda A, et al., *Kobe J Med Sci*, 2001, 47(3):141-50). The activated Leptin receptor is known to regulate components of the insulin signaling cascade, such as ERK, Akt, IRS-1, MAP kinase, and PI3-kinase, a cellular action referred to as crosstalk (Niswender KD, et al., *Trends Endocr Metab*, 2004, 15(8):362-9; Myers MG, *Recent Prog Horm Res*, 2004, 59:287-304; Ahima RS and SY Osei, *Physio Behav*, 2004, 81:223-41). The activation of the PI3K pathway can stimulate the phosphorylation of mTOR via activation of Akt (protein kinase B). These directly link the hormones insulin and Leptin (an adipokine) to the mTOR pathway. Activation of mTOR leads to the hyperphosphorylation of p70^{S6K} and the eIF-4E binding protein (4E-BP1) driving translation of the 5' terminal oligopyrimidine tract mRNA and releasing eIF-4E to form the eIF-4F complex, respectively (Brunner L et al., *Obes Relat Metab Disord*, 1997, 21(12):1152-60; Cutfield LS, et al., *J Pediatr*, 2003, 142(2):113-6; Sugiyama H, et al., *J Immunol*, 1996, 157(2):656-60; Volarevic S and G Thomas, *Prog Nucleic Acid Res Mol Biol*, 2001, 65:101-27; Hara K, et al., *J Biol Chem*, 1997, 272(42):26457-63; Graves LM, et al., *Proc Natl Acad Sci U S A*, 1995, 92(16):7222-6).

[0061] *Spexin*

[0062] Spexin is a peptide with GI activity. It was first identified using Markov modeling analysis based on features common to peptide hormones to find new ones in human

proteome sequences (Mirabeau et al., (2007) *Genome Res.*, 17:

contractile effects in a rat stomach explant assay, indicating a biological activity.

[0063] Ch12, orf39 (Spexin; ~ 17,333 bp in humans) maps to locus 12p12.1, which is also defined by microsatellite marker (MS) D12S1042. Locus 12p12.1, which is the site Spexin maps to, has been associated with various phenotypic markers of obesity in various family linkage studies. For example, 12p12.1 has been associated with BMI in whites, with a maximum LOD score of 2.1 (HERITAGE study; Yvon et al; 2001: Obesity Genome Map; Rankinen et al, 2006). MS marker D12S1042 has further been linked to waist circumference, with a maximum LOD score of 2.374 (Oman Family Study, Bayoumi et al, 2008). In addition, linkage with microsatellite marker D12S1042 was significant in a subset of families with high average waist circumference, with a maximum LOD score of 4.45, $p = 0.0045$ for increase in evidence for linkage (Dominican family study; Wang et al; 2009). In one embodiment, a method for detecting the presence of or a predisposition to obesity or an obesity-associated disorder in a human subject comprises obtaining a biological sample from a subject; and detecting whether or not the subject expresses Spexin in combination with displaying phenotypic marker(s) of obesity (BMI, waist circumference, or a combination thereof), as compared to a subject not afflicted with obesity or an obesity-associated disorder. The human Spexin gene is found at a locus which has been linked to obesity-related phenotypes/manifestations in a number of genome-wide association studies. In a further embodiment, a gene in the 12p12.1 locus (and the region defined by microsatellite marker D12S1042), plays a role in obesity. In another embodiment, a genetic alteration in SEQ ID NO:1 (e.g., a deletion, missense mutation, nonsense mutation, or a combination thereof) alters the expression level or primary sequence of spexin message, and/or protein, leading to defective expression and loss of function. Detection of these changes would be the basis for diagnostic assays.

[0064] Spexin is a human hormone, whose under-expression in obesity was identified in obese humans, not mouse models of obesity (see EXAMPLES). In one embodiment, Spexin can be used as a general weight control. In another embodiment, Spexin can be used for appetite regulation. In a further embodiment, Spexin can be used for control of lipodystrophy. In some embodiments, Spexin can be used for reduction of belly fat.

[0065] The polypeptide sequence of human Spexin is depicted in SEQ ID NO: 1. The nucleotide sequence of human Spexin is shown in SEQ ID NO: 2. Sequence information

related to Spexin is accessible in public databases by GenBank
NM_030572 (for mRNA) and NP_085049 (for protein).

[0066] SEQ ID NO: 1 is the human wild type amino acid sequence corresponding to Spexin (residues 1-116), wherein the bolded sequence represents the mature peptide sequence:

```
1 MKGLRSLAAT TLALFLVFVF LGNSSCAPQR LLERRNWTPQ AMLYLKGAQG RRFISDQSRR
61 KDLSDRPLPE RRSNPQLLT IPEAATILLA SLQKSPEDEE KNFDQTRFLE DSSLNW
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[0067] SEQ ID NO: 2 is the human wild type nucleotide sequence corresponding to Spexin (nucleotides 1-638), wherein the underscored bolded “ATG” denotes the beginning of the open reading frame:

```
1 ctgacaagat gtccctgtgg actcccaaac tctactccag atggggaggt gcccttaaca
61 ccaagatttt aaaagctcca atttcagagc aagagtcgaa aactcacaga taaagttata
121 gttattttcag ggttctgaaa agacgcagaa catgaaggga ctcagaagtc tggcagcaac
181 aaccttggtc cttttcctgg tgtttgtttt cctgggaaac tccagctgcg ctccgcagag
241 actggttgag agaaggaact ggactcctca agctatgctc tacctgaaag gggcacaggg
301 tcgccgcttc atctccgacc agagccggag aaaggacctc tccgaccggc cactgccgga
361 aagacgaagc ccaaatcccc aactactaac tattccggag gcagcaacca tcttactggc
421 gtcccttcag aaatcaccag aagatgaaga aaaaaacttt gatcaacca gattcctgga
481 agacagtctg cttaactggt gaaaatatac tggattatgt ttaattatgg ttctattctc
541 tttgaaaaca tgaaccatgt gaataaaacc tttggaccct ttttaaaaaa aaaaaaaaaa
601 aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa
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[0068] *Obesity Signature Genes*

[0069] This invention provides for the discovery that a number human genes have, for the first time, been identified as a cohort of genes involved in obesity. These genes were identified as being differentially expressed in human obese and normal fat. These genes, now that they have been identified, can be used for a variety of useful methods; for example, they can be used to determine whether a subject is predisposed to obesity or an obesity-associated disorder. The genes identified as part of this obesity gene cohort or group (i.e., Obesity Signature genes or “OS genes”) include any gene indicated as being differentially expressed in any one of **Tables 7-8**.

[0070] In one embodiment, the invention provides methods for diagnosing obesity-associated disorder, as well as methods to treat obesity or an obesity-associated disorder, comprising use of nucleic acids, or proteins encoded by nucleic acids, of the Spexin gene. The method diagnoses the underlying cause of obesity and/or an obesity-related disorder.

[0071] In some embodiments, the invention encompasses methods for using OS proteins encoded by a nucleic acid (including, for example, genomic DNA, complementary DNA (cDNA), synthetic DNA, as well as any form of corresponding RNA). For example, an OS protein can be encoded by a recombinant nucleic acid of an OS gene, such as Spexin. The OS proteins of the invention can be obtained from various sources and can be produced according to various techniques known in the art. For example, a nucleic acid that encodes an OS protein can be obtained by screening DNA libraries, or by amplification from a natural source. An OS protein can be a fragment or portion of human Spexin protein. The nucleic acids encoding OS proteins of the invention can be produced via recombinant DNA technology and such recombinant nucleic acids can be prepared by conventional techniques, including chemical synthesis, genetic engineering, enzymatic techniques, or a combination thereof. Non-limiting examples of an OS protein is the polypeptide encoded by the Spexin gene and/or any of the genes listed in any one of Tables 7-8.

[0072] In some embodiments, the invention encompasses use of variants of an OS protein, such as Spexin. Such a variant can comprise a naturally-occurring variant due to allelic variations between individuals (e.g., polymorphisms), mutated alleles related to obesity, or alternative splicing forms. In one embodiment, the invention encompasses methods for using a protein or polypeptide encoded by a nucleic acid sequence of an Obesity Signature (OS) gene, such as the sequence shown in SEQ ID NO: 1, or an OS protein or polypeptide encoded by any of the genes listed in any one of Tables 7-8. In another embodiment, the polypeptide can be modified, such as by glycosylations and/or acetylations and/or chemical reaction or coupling, and can contain one or several non-natural or synthetic amino acids. An example of an OS polypeptide has the amino acid sequence shown in SEQ ID NO: 1. In certain embodiments, the invention encompasses variants of a human protein encoded by an Obesity Signature (OS) gene, such as Spexin. Such variants can include those having at least from about 46% to about 50% identity to SEQ ID NO: 1, or having at least from about 50.1% to about 55% identity to SEQ ID NO: 1, or having at least from about

55.1% to about 60% identity to SEQ ID NO: 1, or having from 65% identity to SEQ ID NO: 1, or having from about 65.1% to about 70% identity to SEQ ID NO: 1, or having at least from about 70.1% to about 75% identity to SEQ ID NO: 1, or having at least from about 75.1% to about 80% identity to SEQ ID NO: 1, or having at least from about 80.1% to about 85% identity to SEQ ID NO: 1, or having at least from about 85.1% to about 90% identity to SEQ ID NO: 1, or having at least from about 90.1% to about 95% identity to SEQ ID NO: 1, or having at least from about 95.1% to about 97% identity to SEQ ID NO: 1, or having at least from about 97.1% to about 99.9% identity to SEQ ID NO: 1.

[0073] *DNA and Polypeptides, Methods, and Purification Thereof*

[0074] The present invention utilizes conventional molecular biology, microbiology, and recombinant DNA techniques available to one of ordinary skill in the art. Such techniques are well known to the skilled worker and are explained fully in the literature. See, e.g. "DNA Cloning: A Practical Approach," Volumes I and II (D. N. Glover, ed., 1985); "Oligonucleotide Synthesis" (M. J. Gait, ed., 1984); "Nucleic Acid Hybridization" (B. D. Hames & S. J. Higgins, eds., 1985); "Transcription and Translation" (B. D. Hames & S. J. Higgins, eds., 1984); "Animal Cell Culture" (R. I. Freshney, ed., 1986); "Immobilized Cells and Enzymes" (IRL Press, 1986); B. Perbal, "A Practical Guide to Molecular Cloning" (1984), and Sambrook, et al., "Molecular Cloning: a Laboratory Manual" (3rd edition, 2001). One skilled in the art can obtain a protein encoded by an OS gene, such as Spexin, or a variant thereof, in several ways, which include, but are not limited to, isolating the protein via biochemical means or expressing a nucleotide sequence encoding the protein of interest by genetic engineering methods. For example, Spexin, or a variant thereof, can be obtained by purifying it from human cells expressing Spexin, or by direct chemical synthesis.

[0075] Host cells which contain a nucleic acid encoding an OS polypeptide (e.g., Spexin), and which subsequently express a protein encoded by an OS gene (e.g., Spexin), can be identified by various procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or immunoassay techniques which include membrane, solution, or chip-based technologies for the detection and/or quantification of nucleic acid or protein. For example, the presence of a nucleic acid encoding a Spexin polypeptide can be detected by DNA-DNA or DNA-

RNA hybridization or amplification using probes or fragments
Spexin polypeptide.

[0076] Amplification methods include, e.g., polymerase chain reaction, PCR (*PCR Protocols, a Guide to Methods and Applications*, ed. Innis, Academic Press, N.Y., 1990 and *PCR Strategies*, 1995, ed. Innis, Academic Press, Inc., N.Y., ligase chain reaction (LCR) (see, e.g., Wu, *Genomics* 4:560, 1989; Landegren, *Science* 241:1077, 1988; Barringer, *Gene* 89:117, 1990); transcription amplification (see, e.g., Kwoh, *Proc. Natl. Acad. Sci. USA* 86:1173, 1989); and, self-sustained sequence replication (see, e.g., Guatelli, *Proc. Natl. Acad. Sci. USA* 87:1874, 1990); Q Beta replicase amplification (see, e.g., Smith, *J. Clin. Microbiol.* 35:1477-1491, 1997), automated Q-beta replicase amplification assay (see, e.g., Burg, *Mol. Cell. Probes* 10:257-271, 1996) and other RNA polymerase mediated techniques (e.g., NASBA, Cangene, Mississauga, Ontario); see also Berger, *Methods Enzymol.* 152:307-316, 1987; Sambrook; Ausubel; U.S. Pat. Nos. 4,683,195 and 4,683,202; Sooknanan, *Biotechnology* 13:563-564, 1995. All the references and patents stated herein are each incorporated by reference in their entireties.

[0077] A guide to the hybridization of nucleic acids is found in e.g., Sambrook, ed., *Molecular Cloning: A Laboratory Manual* (3rd Ed.), Vols. 1-3, Cold Spring Harbor Laboratory, 2001; *Current Protocols In Molecular Biology*, Ausubel, ed. John Wiley & Sons, Inc., New York, 1997; *Laboratory Techniques In Biochemistry And Molecular Biology: Hybridization With Nucleic Acid Probes, Part I. Theory and Nucleic Acid Preparation*, Tijssen, ed. Elsevier, N.Y., 1993. All the references stated herein are each incorporated by reference in their entireties.

[0078] In one embodiment, a fragment of a nucleic acid of the Spexin gene can encompass any portion of at least about 8 consecutive nucleotides of SEQ ID NO: 2. In another embodiment, the fragment can comprise at least about 10 consecutive nucleotides, at least about 15 consecutive nucleotides, at least about 20 consecutive nucleotides, or at least about 30 consecutive nucleotides of SEQ ID NO: 2. Fragments can include all possible nucleotide lengths between about 8 and about 100 nucleotides, for example, lengths between about 15 and about 100 nucleotides, or between about 20 and about 100 nucleotides. Nucleic acid amplification-based assays involve the use of oligonucleotides selected from sequences encoding a polypeptide encoded by an OS gene (e.g., Spexin), or are complimentary to it, to

detect transformants which contain a nucleic acid encoding an e.g., Spexin.

[0079] Methods for detecting and quantifying OS polypeptides (e.g., a Spexin polypeptide) and OS polynucleotides (e.g., a Spexin polynucleotide) in biological samples are known the art. For example, protocols for detecting and measuring the expression of a polypeptide encoded by an OS gene, such as Spexin, using either polyclonal or monoclonal antibodies specific for the polypeptide are well established. Non-limiting examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay using monoclonal antibodies reactive to two non-interfering epitopes on a polypeptide encoded by an OS gene (e.g., Spexin) can be used, or a competitive binding assay can be employed. In one embodiment, expression of, under-, or over-expression of an OS gene product (e.g., a Spexin polypeptide or Spexin mRNA) can be determined. In one embodiment, a biological sample comprises, a blood sample, serum, cells (including whole cells, cell fractions, cell extracts, and cultured cells or cell lines), tissues (including tissues obtained by biopsy), body fluids (e.g., urine, sputum, amniotic fluid, synovial fluid), or from media (from cultured cells or cell lines). In further embodiments, the tissue sample is adipose tissue. In specific embodiments, the adipose tissue is omental adipose, subcutaneous adipose, or mesenteric adipose. The methods of detecting or quantifying Spexin polynucleotides include, but are not limited to, amplification-based assays with signal amplification) hybridization based assays and combination amplification-hybridization assays. For detecting and quantifying Spexin polypeptides, an exemplary method is an immunoassay that utilizes an antibody or other binding agents that specifically bind to a Spexin polypeptide or epitope, for example, ELISA or RIA assays.

[0080] Labeling and conjugation techniques are known by those skilled in the art and can be used in various nucleic acid and amino acid assays. Methods for producing labeled hybridization or PCR probes for detecting sequences related to nucleic acid sequences encoding an OS protein, such as Spexin, include, but are not limited to, oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, a nucleic acid sequence encoding a polypeptide encoded by an OS gene can be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and can be used to synthesize RNA probes *in vitro* by addition of

labeled nucleotides and an appropriate RNA polymerase such :
procedures can be conducted using a variety of commercially available kits (Amersham Pharmacia Biotech, Promega, and US Biochemical). Suitable reporter molecules or labels which can be used for ease of detection include radionuclides, enzymes, and fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, and/or magnetic particles.

[0081] Host cells transformed with a nucleic acid sequence encoding an OS polypeptide, such as Spexin, can be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The polypeptide produced by a transformed cell can be secreted or contained intracellularly depending on the sequence and/or the vector used. Expression vectors containing a nucleic acid sequence encoding an OS polypeptide can be designed to contain signal sequences which direct secretion of soluble polypeptide molecules encoded by an OS gene, such as Spexin, or a variant thereof, through a prokaryotic or eukaryotic cell membrane, or which direct the membrane insertion of a membrane-bound polypeptide molecule encoded by an OS gene or a variant thereof.

[0082] Other constructions can also be used to join a gene sequence encoding an OS polypeptide (e.g., Spexin) to a nucleotide sequence encoding a polypeptide domain which would facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, Wash.). Cleavable linker sequences (i.e., those specific for Factor Xa or enterokinase (Invitrogen, San Diego, Calif.)) can be included between the purification domain and a polypeptide encoded by an OS gene to facilitate purification. One such expression vector provides for expression of a fusion protein containing a polypeptide encoded by an OS gene (e.g., Spexin) and 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification by immobilized metal ion affinity chromatography, while the enterokinase cleavage site provides a means for purifying the polypeptide encoded by an OS gene.

[0083] An OS polypeptide (e.g., Spexin) can be purified from any human or non-human cell which expresses the polypeptide, including those which have been transfected with expression constructs that express an OS protein. A purified OS polypeptide (e.g., Spexin)

can be separated from other compounds which normally associate (e.g., Spexin) in the cell, such as certain proteins, carbohydrates, or lipids, using methods practiced in the art. Non-limiting methods include size exclusion chromatography, ammonium sulfate fractionation, ion exchange chromatography, affinity chromatography, and preparative gel electrophoresis.

[0084] Nucleic acid sequences comprising an OS gene (e.g., Spexin) that encode a polypeptide can be synthesized, in whole or in part, using chemical methods known in the art. Alternatively, an OS polypeptide, such as Spexin, can be produced using chemical methods to synthesize its amino acid sequence, such as by direct peptide synthesis using solid-phase techniques. Protein synthesis can either be performed using manual techniques or by automation. Automated synthesis can be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Optionally, fragments of OS polypeptides can be separately synthesized and combined using chemical methods to produce a full-length molecule. In one embodiment, a fragment of a nucleic acid sequence that comprises an OS gene can encompass any portion of at least about 8 consecutive nucleotides of SEQ ID NO: 2. In one embodiment, the fragment can comprise at least about 10 nucleotides, at least about 15 nucleotides, at least about 20 nucleotides, or at least about 30 nucleotides of SEQ ID NO: 2. Fragments include all possible nucleotide lengths between about 8 and about 100 nucleotides, for example, lengths between about 15 and about 100 nucleotides, or between about 20 and about 100 nucleotides.

[0085] An OS fragment can be a fragment of an OS protein, such as the Spexin protein. For example, the Spexin fragment can encompass any portion of at least about 8 consecutive amino acids of SEQ ID NO: 1. The fragment can comprise at least about 10 consecutive amino acids, at least about 20 consecutive amino acids, at least about 30 consecutive amino acids, at least about 40 consecutive amino acids, at least about 50 consecutive amino acids, at least about 60 consecutive amino acids, at least about 70 consecutive amino acids, or at least about 75 consecutive amino acids of SEQ ID NO: 1. Fragments include all possible amino acid lengths between about 8 and about 100 amino acids, for example, lengths between about 10 and about 100 amino acids, between about 15 and about 100 amino acids, between about 20 and about 100 amino acids, between about 35 and about 100 amino acids, between about 40 and about 100 amino acids, between about 50 and about 100 amino acids, between about

70 and about 100 amino acids, between about 75 and about 100 amino acids, or about 80 and about 100 amino acids.

[0086] A synthetic peptide can be substantially purified via high performance liquid chromatography (HPLC). The composition of a synthetic OS polypeptide can be confirmed by amino acid analysis or sequencing. Additionally, any portion of an amino acid sequence comprising a protein encoded by an OS gene (e.g., Spexin) can be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins to produce a variant polypeptide or a fusion protein.

[0087] ***Screening to Identify Compounds that Ameliorate Obesity***

[0088] The invention provides methods for identifying compounds which can be used for treating obesity or an obesity-associated disorder. The invention also provides methods for identifying compounds which can be used to promote weight loss in an obese subject or a subject afflicted with an obesity-associated disorder. The invention further provides methods for identifying compounds that can be used for promoting satiety in an obese subject or a subject afflicted with an obesity-associated disorder. Since the invention has identified genes that are differentially expressed in obese and normal fat (e.g., Spexin as well as those Obesity Signature genes listed in Tables 7-8), the invention also provides methods for identifying compounds that modulate the expression or activity of an OS gene and/or OS protein, such as Spexin.

[0089] Animal models of obesity, such as *ob/ob* and *db/db* mice, may be used to identify compounds (e.g., test agents) that ameliorate symptoms of obesity and obesity-associated disorders, e.g., excess body fat, elevated serum Leptin levels, decreased serum Spexin levels, or a combination thereof. Such animal models may be used as test substrates for the identification of drugs, pharmaceuticals, therapies, and interventions which may be effective in treating obesity and obesity-associated disorders. For example, animal models may be exposed to a compound, suspected of exhibiting an ability to ameliorate symptoms of obesity and obesity-associated disorders, e.g., excess body fat, elevated serum Leptin levels, decreased serum Spexin levels, or a combination thereof, at a sufficient concentration and for a time sufficient to elicit such an amelioration of symptoms in the exposed animals. The response of the animals to the exposure may be monitored by assessing the reversal of excess

body fat, elevated serum Leptin levels, or decreased serum Spexin levels, or a combination thereof, to treat obesity and obesity-associated disorders.

[0090] A gut explant assay may also be used to identify test agents that ameliorate symptoms of obesity and obesity-associated disorders, e.g., excess body fat, elevated serum Leptin levels, decreased serum Spexin levels, or a combination thereof. According to Mirabeau et al., ((2007) *Genome Res.*, 17: 320-327), strips of stomach fundus muscles can be harvested from rats and mounted in an oxygenated organ bath, where they can be stretched and challenged with bioactive agents, thereby serving as a useful bioassay system. At the beginning of each experiment, acetylcholine chloride is applied to achieve a maximal control contraction, the contractions are recorded. In one embodiment, a synthetic amidated spexin peptide (NWTPQAMLYLKGAQ-amide [SEQ ID NO: 32]; Primm; *see* Mirabeau et al., (2007) *Genome Res.*, 17: 320-327) can be applied. To identify an agonist with properties similar to Spexin, for example, single doses of a test agent can then be applied until reproducible responses, if any, are obtained. To identify an antagonist of Spexin, for example, single doses of a test agent can then be applied until the Spexin responses elicited in the gut explant assay are diminished.

[0091] Test compounds or agents of the invention comprise peptides (such as antibodies or fragments thereof or soluble peptides), small molecules (e.g., small organic and small inorganic molecules), nucleic acids (such as siRNA or antisense RNA), or other agents that can bind to a polypeptide molecule encoded by an OS gene (e.g., Spexin) and/or have a stimulatory or inhibitory effect on the biological activity of a protein encoded by an OS gene or its expression (e.g., Spexin). It will then be determined whether the test agents can promote satiety or weight loss in an obese subject or subject afflicted with an obesity-associated disorder, or whether the test agent can be used in the treatment of obesity or an obesity-associated disorder (e.g., by examining whether there is a change in a body weight phenotype, such as a reduction in body fat mass).

[0092] As used herein, a “Spexin modulating compound” refers to a compound that interacts with the Spexin gene, or the Spexin protein or polypeptide, and modulates its activity and/or its expression. The compound can either increase the activity or expression of a Spexin protein. Conversely, the compound can decrease the activity or expression of a Spexin protein. The compound can be a Spexin agonist or a Spexin antagonist. Some non-limiting examples of Spexin modulating compounds include peptides (such as peptide

fragments comprising SEQ ID NO: 1, or antibodies or fragment (organic or inorganic), and nucleic acids (such as siRNA or antisense RNA specific for a nucleic acid comprising SEQ ID NO: 2). Spexin agonists can be molecules which, when bound to Spexin or its receptor, increase or prolong the activity of the Spexin protein. Spexin agonists can be compounds (e.g., synthetic compounds or other naturally occurring peptides) that elicit the same or similar activity as compared to Spexin. Spexin agonists include, but are not limited to, proteins, nucleic acids, small molecules, or any other molecule which activates the Spexin protein. Spexin antagonists can be molecules which, when bound to the Spexin protein, decrease the amount or the duration of the activity of Spexin. Antagonists include proteins, nucleic acids, antibodies, small molecules, or any other molecule which decrease the activity of the Spexin protein.

[0093] “Modulate” refers to a change in the activity or expression of an OS gene or protein, such as Spexin. For example, modulation can cause an increase or a decrease in protein activity, binding characteristics, or any other biological, functional, or immunological property of an OS protein. In one embodiment, the OS protein is Spexin.

[0094] In one embodiment, a Spexin modulating compound can be a peptide fragment of the Spexin protein that binds to Spexin itself. For example, it can encompass any portion of at least about 8 consecutive amino acids of SEQ ID NO: 1. The fragment can comprise at least about 10 consecutive amino acids, at least about 20 consecutive amino acids, at least about 30 consecutive amino acids, at least about 40 consecutive amino acids, at least about 50 consecutive amino acids, at least about 60 consecutive amino acids, or at least about 75 consecutive amino acids of SEQ ID NO: 1. Fragments include all possible amino acid lengths between and including about 8 and about 100 amino acids, for example, lengths between about 10 and about 100 amino acids, between about 15 and about 100 amino acids, between about 20 and about 100 amino acids, between about 35 and about 100 amino acids, between about 40 and about 100 amino acids, between about 50 and about 100 amino acids, between about 70 and about 100 amino acids, between about 75 and about 100 amino acids, or between about 80 and about 100 amino acids. These peptide fragments can be obtained commercially or synthesized via liquid phase or solid phase synthesis methods (Atherton et al., (1989) Solid Phase Peptide Synthesis: a Practical Approach. IRL Press, Oxford, England). The Spexin peptide fragments can be isolated from a natural source, genetically engineered, or chemically prepared. These methods are well known in the art.

[0095] A Spexin modulating compound can be a protein, (monoclonal, polyclonal, humanized, chimeric, or fully human), or a binding fragment thereof, directed against a polypeptide encoded by the Spexin gene. An antibody fragment can be a form of an antibody other than the full-length form and includes portions or components that exist within full-length antibodies. An antibody fragment can also be an antibody fragment that has been engineered. Antibody fragments can include, but are not limited to, single chain Fv (scFv), diabodies, Fv, (Fab')₂, triabodies, Fc, Fab, CDR1, CDR2, CDR3, combinations of CDR's, variable regions, tetrabodies, bifunctional hybrid antibodies, framework regions, constant regions, and the like (see, Maynard et al., (2000) *Ann. Rev. Biomed. Eng.* 2:339-76; Hudson (1998) *Curr. Opin. Biotechnol.* 9:395-402). Antibodies can be obtained commercially, can be custom generated, or can be synthesized against an antigen of interest according to methods established in the art (e.g., see Beck et al., *Nat Rev Immunol.* 2010 May;10(5):345-52; Chan et al., *Nat Rev Immunol.* 2010 May;10(5):301-16; and Kontermann, *Curr Opin Mol Ther.* 2010 Apr;12(2):176-83, each of which are incorporated by reference in their entireties).

[0096] Inhibition of RNA encoding a polypeptide encoded by an OS gene, e.g., Spexin) can effectively modulate the expression of an OS gene from which the RNA is transcribed. Inhibitors are selected from the group comprising: siRNA; interfering RNA or RNAi; dsRNA; RNA Polymerase III transcribed DNAs; ribozymes; and antisense nucleic acids, which can be RNA, DNA, or an artificial nucleic acid.

[0097] Antisense oligonucleotides, including antisense DNA, RNA, and DNA/RNA molecules, act to directly block the translation of mRNA by binding to targeted mRNA and preventing protein translation. For example, antisense oligonucleotides of at least about 15 bases and complementary to unique regions of the DNA sequence encoding a Spexin polypeptide can be synthesized, e.g., by conventional phosphodiester techniques (Dallas et al., (2006) *Med. Sci. Monit.* 12(4):RA67-74; Kalota et al., (2006) *Handb. Exp. Pharmacol.* 173:173-96; Lutzelburger et al., (2006) *Handb. Exp. Pharmacol.* 173:243-59). Antisense nucleotide sequences include, but are not limited to: morpholinos, 2'-O-methyl polynucleotides, DNA, RNA and the like.

[0098] siRNA comprises a double stranded structure containing from about 15 to about 50 base pairs, for example from about 21 to about 25 base pairs, and having a nucleotide sequence identical or nearly identical to an expressed target gene or RNA within the cell.

The siRNA comprise a sense RNA strand and a complementary annealed together by standard Watson-Crick base-pairing interactions. The sense strand comprises a nucleic acid sequence which is substantially identical to a nucleic acid sequence contained within the target miRNA molecule. "Substantially identical" to a target sequence contained within the target mRNA refers to a nucleic acid sequence that differs from the target sequence by about 3% or less. The sense and antisense strands of the siRNA can comprise two complementary, single-stranded RNA molecules, or can comprise a single molecule in which two complementary portions are base-paired and are covalently linked by a single-stranded "hairpin" area. See also, McMnaus and Sharp (2002) Nat Rev Genetics, 3:737-47, and Sen and Blau (2006) FASEB J., 20:1293-99, the entire disclosures of which are herein incorporated by reference.

[0099] The siRNA can be altered RNA that differs from naturally-occurring RNA by the addition, deletion, substitution and/or alteration of one or more nucleotides. Such alterations can include addition of non-nucleotide material, such as to the end(s) of the siRNA or to one or more internal nucleotides of the siRNA, or modifications that make the siRNA resistant to nuclease digestion, or the substitution of one or more nucleotides in the siRNA with deoxyribonucleotides. One or both strands of the siRNA can also comprise a 3' overhang. As used herein, a 3' overhang refers to at least one unpaired nucleotide extending from the 3'-end of a duplexed RNA strand. For example, the siRNA can comprise at least one 3' overhang of from 1 to about 6 nucleotides (which includes ribonucleotides or deoxyribonucleotides) in length, or from 1 to about 5 nucleotides in length, or from 1 to about 4 nucleotides in length, or from about 2 to about 4 nucleotides in length. For example, each strand of the siRNA can comprise 3' overhangs of dithymidylic acid ("TT") or diuridylic acid ("uu").

[00100] siRNA can be produced chemically or biologically, or can be expressed from a recombinant plasmid or viral vector (for example, see U.S. Patent No. 7,294,504, U.S. Patent No. 7,148,342, and U.S. Patent No. 7,422,896, the entire disclosures of which are herein incorporated by reference). Exemplary methods for producing and testing dsRNA or siRNA molecules are described in U.S. Patent Application Publication No. 2002/0173478 to Gewirtz, and in U.S. Patent Application Publication No. 2007/0072204 to Hannon et al., the entire disclosures of which are herein incorporated by reference.

[00101] RNA polymerase III transcribed DNAs contain p₁ promoter. These DNAs can be transcribed to produce small hairpin RNAs in the cell that can function as siRNA or linear RNAs that can function as antisense RNA. The Spexin modulating compound can contain ribonucleotides, deoxyribonucleotides, synthetic nucleotides, or any suitable combination such that the target RNA and/or gene is inhibited. In addition, these forms of nucleic acids can be single, double, triple, or quadruple stranded. (see for example Bass (2001) *Nature*, 411, 428-429; Elbashir et al., (2001) *Nature*, 411, 494-498; and PCT Publication Nos. WO 00/44895, WO 01/36646, WO 99/32619, WO 00/01846, WO 01/29058, WO 99/07409, WO 00/44914). In one embodiment, short hairpin RNAs can be synthesized exogenously or can be formed by transcribing from RNA polymerase III promoters in vivo. Examples of making and using such shRNAs for gene silencing in mammalian cells are described in, for example, Paddison et al., 2002, *Genes Dev*, 16:948-58; McCaffrey et al., 2002, *Nature*, 418:38-9; McManus et al., 2002, *RNA*, 8:842-50; Yu et al., 2002, *Proc Natl Acad Sci USA*, 99:6047-52). shRNAs are engineered in cells or in an animal to ensure continuous and stable suppression of a desired gene. It is known in the art that siRNAs can be produced by processing a hairpin RNA in the cell.

[00102] A Spexin modulating compound can be a small molecule that binds to the Spexin protein and disrupts its function, or conversely, enhances its function. Small molecules are a diverse group of synthetic and natural substances generally having low molecular weights. They can be isolated from natural sources (for example, plants, fungi, microbes and the like), are obtained commercially, are available as libraries or collections, or can be synthesized. Candidate small molecules that modulate Spexin can be identified via in silico screening or high-through-put screening (HTPS) of combinatorial libraries. Most conventional pharmaceuticals, such as aspirin, penicillin, and many chemotherapeutics, are small molecules, can be obtained commercially, can be chemically synthesized, or can be obtained from random or combinatorial libraries as described below (Werner et al., (2006) *Brief Funct. Genomic Proteomic* 5(1):32-6).

[00103] Knowledge of the primary sequence of a molecule of interest, such as a polypeptide encoded the Spexin gene, and the similarity of that sequence with proteins of known function, can provide information as to the inhibitors or antagonists of the protein of interest, in addition to agonists. Identification and screening of agonists and antagonists is further facilitated by determining structural features of the protein, e.g., using X-ray

crystallography, neutron diffraction, nuclear magnetic resonance techniques for structure determination. These techniques provide for the rational design or identification of agonists and antagonists.

[00104] Test agents, e.g., Spexin modulating compounds, can be screened from large libraries of synthetic or natural compounds (see Wang et al., (2007) *Curr Med Chem*, 14(2):133-55; Mannhold (2006) *Curr Top Med Chem*, 6 (10):1031-47; and Hensen (2006) *Curr Med Chem* 13(4):361-76). Numerous means are currently used for random and directed synthesis of saccharide, peptide, and nucleic acid based compounds. Synthetic compound libraries are commercially available from Maybridge Chemical Co. (Trevillet, Cornwall, UK), AMRI (Albany, NY), ChemBridge (San Diego, CA), and MicroSource (Gaylordsville, CT). A rare chemical library is available from Aldrich (Milwaukee, Wis.). Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant, and animal extracts are available from, e.g., Pan Laboratories (Bothell, Wash.) or MycoSearch (N.C.), or are readily producible. Additionally, natural and synthetically produced libraries and compounds are readily modified through conventional chemical, physical, and biochemical means (Blondelle et al., (1996) *Tib Tech* 14:60).

[00105] Methods for preparing libraries of molecules are well known in the art and many libraries are commercially available. Examples of chemically synthesized libraries are described in Fodor et al., (1991) *Science* 251:767-773; Houghten et al., (1991) *Nature* 354:84-86; Lam et al., (1991) *Nature* 354:82-84; Medynski, (1994) *BioTechnology* 12:709-710; Gallop et al., (1994) *J. Medicinal Chemistry* 37(9):1233-1251; Ohlmeyer et al., (1993) *Proc. Natl. Acad. Sci. USA* 90:10922-10926; Erb et al., (1994) *Proc. Natl. Acad. Sci. USA* 91:11422-11426; Houghten et al., (1992) *Biotechniques* 13:412; Jayawickreme et al., (1994) *Proc. Natl. Acad. Sci. USA* 91:1614-1618; Salmon et al., (1993) *Proc. Natl. Acad. Sci. USA* 90:11708-11712; PCT Publication No. WO 93/20242, dated Oct. 14, 1993; and Brenner et al., (1992) *Proc. Natl. Acad. Sci. USA* 89:5381-5383.

[00106] Small molecule combinatorial libraries can also be generated and screened. A combinatorial library of small organic compounds is a collection of closely related analogs that differ from each other in one or more points of diversity and are synthesized by organic techniques using multi-step processes. Combinatorial libraries include a vast number of small organic compounds. One type of combinatorial library is prepared by means of parallel synthesis methods to produce a compound array. A compound array can be a collection of

compounds identifiable by their spatial addresses in Cartesian that each compound has a common molecular core and one or more variable structural diversity elements. The compounds in such a compound array are produced in parallel in separate reaction vessels, with each compound identified and tracked by its spatial address. Examples of parallel synthesis mixtures and parallel synthesis methods are provided in U.S. Ser. No. 08/177,497, filed Jan. 5, 1994 and its corresponding PCT published patent application W095/18972, published Jul. 13, 1995 and U.S. Pat. No. 5,712,171 granted Jan. 27, 1998 and its corresponding PCT published patent application W096/22529, which are hereby incorporated by reference.

[00107] *Treatment of Obesity and Obesity-Associated Disorders*

[00108] Several well-established obesity treatments ranging from non-pharmaceutical to pharmaceutical intervention are known in the art. Non-pharmaceutical interventions include, but are not limited to, dietary restriction, exercise, psychiatric treatment, and surgical treatments to reduce food consumption (e.g., bariatric surgery) or remove fat (e.g., liposuction). Present pharmacological interventions can induce a weight loss of between 5 to 15 kg. Appetite suppressants and energy expenditure or nutrient-modifying agents are the main focus of pharmacological intervention. Dexfenfluramine (Redux), sibutramine (Meridia), beta3-adrenergic agonists, sympathomimetic adrenergic agents (such as amphetamines (dextroamphetamine)), phentermine, benzphetamine, phendimetrazine, mazindol, diethylpropion, phenylpropanolamine, serotonin (5-HT) reuptake inhibitors (such as sibutramine), and gastrointestinal lipases (such as orlistat) are examples of such pharmacological interventions. *See also*, Bays, (2004) Obesity Research 12(8):1197-1211, and Klonoff et al., J Diabetes Sci Technol. 2008 Sep;2(5):913-8, the contents of each which are incorporated by reference in their entireties. However, if the medication is discontinued, renewed weight gain may ensue. Surgical treatments are comparatively successful, but are complicated, expensive, and have significant risks. Surgical treatments are reserved for patients with extreme obesity and/or with serious medical complications.

[00109] In one embodiment, Spexin is administered to an obese subject to treat obesity or an obesity-associated disorder. In another embodiment, a polypeptide comprising SEQ ID NO: 1, or a pharmaceutically acceptable salt thereof is administered to an obese subject to treat obesity or an obesity-associated disorder. In one embodiment, Spexin is administered to an obese subject or a subject afflicted with an obesity-associated disorder to promote weight

loss in the subject. In another embodiment, a polypeptide comprising a pharmaceutically acceptable salt thereof is administered to an obese subject or a subject afflicted with an obesity-associated disorder to promote weight loss in the subject. In one embodiment, Spexin is administered to an obese subject or a subject afflicted with an obesity-associated disorder to promote satiety in the subject. In another embodiment, a polypeptide comprising SEQ ID NO: 1, or a pharmaceutically acceptable salt thereof is administered to an obese subject or a subject afflicted with an obesity-associated disorder to promote satiety in the subject.

[00110] Spexin, a polypeptide comprising SEQ ID NO: 1, or a pharmaceutically acceptable salt thereof, or a test agent of the invention (e.g., a small organic molecule, a small inorganic molecule, a soluble peptide, or an antibody) can be administered in a therapeutically effective amount. For example, an amount that is sufficient to treat obesity or the obesity-associated disorder, such as by ameliorating symptoms associated with obesity or the obesity-associated disorder (e.g., elevated Leptin levels, decreased spexin levels, and increased body mass), preventing or delaying the onset of obesity or the obesity-associated disorder, and/or also lessening the severity or frequency of symptoms of obesity or the obesity-associated disorder (e.g., elevated Leptin levels, decreased spexin levels, and increased body mass).

[00111] The amount which will be therapeutically effective in the treatment of a particular individual's disorder or condition will depend on the symptoms and severity of the disease, and can be determined by standard clinical techniques. In vitro or in vivo assays may also be used to identify optimal dosage ranges. The precise dose to be used in the formulation will also depend on the route of administration, and the severity of the obesity or the obesity-associated disorder, and should be decided according to the judgment of a practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems, such as a *db/db* mouse, an *ob/ob* mouse, or a High-fat-diet-fed mouse model (e.g., the Diet Induced Obesity (DIO) mouse model).

[00112] Spexin is a peptide hormone that has biological activity in the gut but is noticeably under-expressed by both omental and subcutaneous fat in obese patients (see EXAMPLES). In one embodiment, Spexin is a satiety factor. Spexin, or a small molecule

acting through the same receptor/signaling system, may be use regulate caloric intake.

[00113] A satiety factor is a feedback signal related to energy expenditure and food intake. A satiety factor is involved in regulating appetite, food intake, energy intake or expenditure, or acts as a satiety signal. The satiety factor studied most to date is the hormone Leptin, which is synthesized and secreted predominantly by fat cells. In one embodiment, Spexin is a satiety factor. In a further embodiment, Spexin as a satiety factor can be used for the treatment of obesity. In another embodiment, Spexin can be an alternative and/or complement to surgical treatments to reduce food consumption (e.g., bariatric surgery) or remove fat (e.g., liposuction).

[00114] *Treatment of other body weight disorders*

[00115] Obesity is the most prevalent of body weight disorders. Other body weight disorders, such as anorexia nervosa and bulimia nervosa, also are serious health threats. Further, such body weight disorders as anorexia, lipodystrophy, and cachexia (wasting) are also prominent characteristics of other diseases such as cancer, cystic fibrosis, tuberculosis, congestive heart failure, and AIDS. An aspect of the invention provides for a method of diagnosing a body weight disorder in a subject. In one embodiment, excess of Spexin production or circulating Spexin is responsible for anorexia nervosa, or other eating disorders. In one embodiment, the method for diagnosing a body weight disorder in a human subject comprises: (a) obtaining a biological sample from a subject; and (b) detecting whether or not there is an alteration in the expression of the Spexin gene in the subject as compared to a subject not afflicted with a body weight disorder.

[00116] Wasting is a syndrome characterized by loss of weight, muscle atrophy, fatigue, weakness, and significant loss of appetite in a subject who is not actively trying to lose weight.

[00117] Lipodystrophy is a medical condition characterized by abnormal or degenerative conditions of the body's adipose tissue. It is characterized by a lack of circulating Leptin which may lead to osteosclerosis.

[00118] Spexin may play a role in the pathogenesis of, or treatment of, lipodystrophy in HIV-infected patients. Thus, serum levels of Spexin may be regulated in HIV+ patients. In

one embodiment, a Spexin antagonist is administered to a subject. In another embodiment, a polypeptide directed to the amino acid comprising SEQ ID NO: 1 (e.g., a Spexin antibody) is administered to a subject to treat lipodystrophy. In one embodiment, a Spexin antagonist is administered to a subject or to promote weight gain in the subject. In another embodiment, a polypeptide directed to the amino acid comprising SEQ ID NO: 1 (e.g., a Spexin antibody) is administered to a subject to promote weight gain in the subject.

[00119] Symptoms of these body weight disorders characterized by a lower than normal body weight phenotype (for example, cachexia or lipodystrophy) may be ameliorated by decreasing the level of Spexin gene expression and/or spexin gene product activity. For example, Spexin gene sequences (for example, SEQ ID NO: 2) may be utilized in conjunction with an antisense, a gene “knock-out,” ribozyme and/or a siRNA method to decrease the level of spexin gene expression. In one embodiment, an antibody directed to the Spexin protein can neutralize Spexin activity by way of binding to the Spexin protein.

[00120] *Administration and Dosing*

[00121] Spexin or a Spexin modulating compound can be administered to the subject one time (e.g., as a single injection or deposition). Alternatively, Spexin or a Spexin modulating compound of the invention can be administered once or twice daily to a subject in need thereof for a period of from about 2 to about 28 days, or from about 7 to about 10 days, or from about 7 to about 15 days. It can also be administered once or twice daily to a subject for a period of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 times per year, or a combination thereof. Furthermore, Spexin or a Spexin modulating compound can be co-administrated with another therapeutic, such as dexfenfluramine (Redux), sibutramine (Meridia), a beta3-adrenergic agonist, a sympathomimetic adrenergic agent (such as amphetamines (dextroamphetamine)), phentermine, benzphetamine, phendimetrazine, mazindol, diethylpropion, phenylpropanolamine, a serotonin (5-HT) reuptake inhibitor (such as sibutramine), a gastrointestinal lipase (such as orlistat), a satiety factor, or a combination thereof.

[00122] Spexin or a Spexin modulating compound of the invention can be administered to a subject by any means suitable for delivering Spexin or a Spexin modulating compound to cells of the subject, such as omental adipose cells or subcutaneous adipose cells. For example, Spexin or a Spexin modulating compound can be administered by methods suitable

to transfect cells. Transfection methods for eukaryotic cells include direct injection of the nucleic acid into the nucleus or pronucleus of a cell; electroporation; liposome transfer or transfer mediated by lipophilic materials; receptor mediated nucleic acid delivery, bioballistic or particle acceleration; calcium phosphate precipitation, and transfection mediated by viral vectors.

[00123] The compositions of this invention can be formulated and administered to reduce the symptoms associated with obesity or an obesity-associated disorder by any means that produce contact of the active ingredient with the agent's site of action in the body of a human or non-human subject. They can be administered by any conventional means available for use in conjunction with pharmaceuticals, either as individual therapeutic active ingredients or in a combination of therapeutic active ingredients. They can be administered alone, but are generally administered with a pharmaceutical carrier selected on the basis of the chosen route of administration and standard pharmaceutical practice.

[00124] Pharmaceutical compositions for use in accordance with the invention can be formulated in conventional manner using one or more physiologically acceptable carriers or excipients. The therapeutic compositions of the invention can be formulated for a variety of routes of administration, including systemic and topical or localized administration. Techniques and formulations generally can be found in Remington's Pharmaceutical Sciences, Meade Publishing Co., Easton, Pa (20th ed., 2000), the entire disclosure of which is herein incorporated by reference. For systemic administration, an injection is useful, including intramuscular, intravenous, intraperitoneal, and subcutaneous. For injection, the therapeutic compositions of the invention can be formulated in liquid solutions, for example in physiologically compatible buffers, such as PBS, Hank's solution, or Ringer's solution. In addition, the therapeutic compositions can be formulated in solid form and redissolved or suspended immediately prior to use. Lyophilized forms are also included. Pharmaceutical compositions of the present invention are characterized as being at least sterile and pyrogen-free. These pharmaceutical formulations include formulations for human and veterinary use.

[00125] Pharmaceutical formulations of the invention can comprise Spexin or a Spexin modulating compound (e.g., 0.1 to 90% by weight), or a physiologically acceptable salt thereof, mixed with a pharmaceutically-acceptable carrier. The pharmaceutical formulations of the invention can also comprise Spexin or a Spexin modulating compound of the invention which are encapsulated by liposomes and a pharmaceutically-acceptable carrier. Useful

pharmaceutically-acceptable carriers are water, buffered water 0.3% glycine, or hyaluronic acid.

[00126] Pharmaceutical compositions of the invention can also comprise conventional pharmaceutical excipients and/or additives. Suitable pharmaceutical excipients include stabilizers, antioxidants, osmolality adjusting agents, buffers, and pH adjusting agents. Suitable additives include physiologically biocompatible buffers (e.g., tromethamine hydrochloride), additions of chelants (such as, for example, DTPA or DTPA-bisamide) or calcium chelate complexes (as for example calcium DTPA, CaNaDTPA-bisamide), or, optionally, additions of calcium or sodium salts (for example, calcium chloride, calcium ascorbate, calcium gluconate or calcium lactate). Pharmaceutical compositions of the invention can be packaged for use in liquid form, or can be lyophilized.

[00127] For solid pharmaceutical compositions of the invention, conventional nontoxic solid pharmaceutically-acceptable carriers can be used; for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, or magnesium carbonate.

[00128] Solid formulations can be used for enteral (oral) administration. They can be formulated as, e.g., pills, tablets, powders or capsules. For solid compositions, conventional nontoxic solid carriers can be used which include, e.g., pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, or magnesium carbonate. For oral administration, a pharmaceutically acceptable nontoxic composition is formed by incorporating any of the normally employed excipients, such as those carriers previously listed, and generally 10% to 95% of active ingredient (e.g., peptide). A non-solid formulation can also be used for enteral administration. The carrier can be selected from various oils including those of petroleum, animal, vegetable or synthetic origin, e.g., peanut oil, soybean oil, mineral oil, or sesame oil. Suitable pharmaceutical excipients include e.g., starch, cellulose, talc, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, magnesium stearate, sodium stearate, glycerol monostearate, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol.

[00129] Nucleic acids, peptides, small molecules, or polypeptides of the invention, when administered orally, can be protected from digestion. This can be accomplished either by complexing the nucleic acid, peptide or polypeptide with a composition to render it resistant

to acidic and enzymatic hydrolysis or by packaging the nucleic in an appropriately resistant carrier such as a liposome. Means of protecting compounds from digestion are well known in the art, see, e.g., Fix, Pharm Res. 13: 1760-1764, 1996; Samanen, J. Pharm. Pharmacol. 48: 119-135, 1996; U.S. Pat. No. 5,391,377, describing lipid compositions for oral delivery of therapeutic agents (for example, liposomal delivery). In one embodiment, Spexin or a Spexin modulating compound can be delivered to the alimentary canal or intestine of the subject via oral administration that is can withstand digestion and degradation.

[00130] For oral administration, the therapeutic compositions can take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g., pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate). The tablets can be coated by methods well known in the art. Liquid preparations for oral administration can take the form of, for example, solutions, syrups or suspensions, or they can be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations can be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., ationd oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations can also contain buffer salts, flavoring, coloring and sweetening agents as appropriate.

[00131] Preparations for oral administration can be suitably formulated to give controlled release of the active agent. For buccal administration the therapeutic compositions can take the form of tablets or lozenges formulated in a conventional manner. For administration by inhalation, the compositions for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit can be determined by providing a valve to

deliver a metered amount. Capsules and cartridges of e.g., gela insufflate or can be formulated containing a powder mix of the therapeutic agents and a suitable powder base such as lactose or starch.

[00132] Spexin or a Spexin modulating compound can be delivered in a controlled release system. For example, the polypeptide may be administered using intravenous infusion, an implantable osmotic pump, a transdermal patch, liposomes, or other modes of administration. In one embodiment, a pump may be used (see is Langer, *supra*; Sefton, *CRC Crit. Ref. Biomed. Eng.* 14:201 (1987); Buchwald et al., *Surgery* 88:507 (1980); Saudek et al., *N. Engl. J. Med.* 321:574 (1989)). In another embodiment, polymeric materials can be used (see *Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Pres., Boca Raton, Fla. (1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, *J. Macromol. Sci. Rev. Macromol. Chem.* 23:61 (1983); see also Levy et al., *Science* 228:190 (1985); During et al., *Ann. Neurol.* 25:351 (1989); Howard et al., *J. Neurosurg.* 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in *Medical Applications of Controlled Release*, *supra*, vol. 2, pp. 115-138 (1984)). Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

[00133] The therapeutic compositions can be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection can be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions can take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and can contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient can be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

[00134] Suitable enteral administration routes for the present methods include oral, rectal, or intranasal delivery. Suitable parenteral administration routes include intravascular administration (e.g. intravenous bolus injection, intravenous infusion, intra-arterial bolus injection, intra-arterial infusion and catheter instillation into the vasculature); peri- and intra-tissue injection (e.g., peri-tumoral and intra-tumoral injection, intra-retinal injection, or subretinal injection); subcutaneous injection or deposition including subcutaneous infusion

(such as by osmotic pumps); direct application to the tissue of catheter or other placement device (e.g., a retinal pellet or a suppository or an implant comprising a porous, non-porous, or gelatinous material); and inhalation. For example, Spexin or a Spexin modulating compound of the invention can be administered by injection, infusion, or oral delivery.

[00135] In addition to the formulations described previously, the therapeutic compositions can also be formulated as a depot preparation. Such long acting formulations can be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. For example, the therapeutic compositions can be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

[00136] Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration bile salts and fusidic acid derivatives. In addition, detergents can be used to facilitate permeation. Transmucosal administration can be through nasal sprays or using suppositories. For topical administration, the compositions of the invention are formulated into ointments, salves, gels, or creams as generally known in the art. A wash solution can be used locally to treat an injury or inflammation to accelerate healing. For oral administration, the therapeutic compositions are formulated into conventional oral administration forms such as capsules, tablets, and tonics.

[00137] A composition of the present invention can also be formulated as a sustained and/or timed release formulation. Such sustained and/or timed release formulations can be made by sustained release means or delivery devices that are well known to those of ordinary skill in the art, such as those described in U.S. Pat. Nos.: 3,845,770; 3,916,899; 3,536,809; 3,598,123; 4,008,719; 4,710,384; 5,674,533; 5,059,595; 5,591,767; 5,120,548; 5,073,543; 5,639,476; 5,354,556; and 5,733,566, the disclosures of which are each incorporated herein by reference. The pharmaceutical compositions of the present invention can be used to provide slow or sustained release of one or more of the active ingredients using, for example, hydropropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes, microspheres, or the like, or a

combination thereof to provide the desired release profile in various sustained release formulations known to those of ordinary skill in the art, including those described herein, can be readily selected for use with the pharmaceutical compositions of the invention. Single unit dosage forms suitable for oral administration, such as, but not limited to, tablets, capsules, gel-caps, caplets, or powders, that are adapted for sustained release are encompassed by the present invention.

[00138] In the present methods, Spexin or a Spexin modulating compound can be administered to the subject either as RNA, in conjunction with a delivery reagent, or as a nucleic acid (e.g., a recombinant plasmid or viral vector) comprising sequences which expresses the gene product. Suitable delivery reagents for administration of Spexin or a Spexin modulating compound include the Mirus Transit TKO lipophilic reagent; lipofectin; lipofectamine; cellfectin; or polycations (e.g., polylysine), or liposomes.

[00139] The dosage administered can be a therapeutically effective amount of the composition sufficient to result in amelioration of symptoms of obesity or an obesity-associated disorder in a subject, and can vary depending upon known factors such as the pharmacodynamic characteristics of the active ingredient and its mode and route of administration; time of administration of active ingredient; age, sex, health and weight of the recipient; nature and extent of symptoms; kind of concurrent treatment, frequency of treatment and the effect desired; and rate of excretion.

[00140] In one embodiment, Spexin is administered at a dose so as to achieve a plasma concentration of about 10 ng/ml to about 20 ng/ml in obese patients or patients afflicted with an obesity-associated disorder. In some embodiments, the effective amount of the administered Spexin is at least about 1 ng/ml, at least about 2 ng/ml, at least about 3 ng/ml, at least about 4 ng/ml, at least about 5 ng/ml, at least about 7.5 ng/ml, at least about 10 ng/ml, at least about 15 ng/ml, at least about 20 ng/ml, at least about 25 ng/ml, at least about 30 ng/ml, at least about 35 ng/ml, at least about 40 ng/ml, at least about 45 ng/ml, at least about 50 ng/ml, at least about 60 ng/ml, at least about 70 ng/ml, at least about 80 ng/ml, at least about 90 ng/ml, at least about 100 ng/ml, at least about 125 ng/ml, at least about 150 ng/ml, at least about 175 ng/ml, at least about 200 ng/ml, at least about 250 ng/ml, at least about 300 ng/ml, at least about 350 ng/ml, at least about 400 ng/ml, at least about 450 ng/ml, at least about 500 ng/ml, at least about 600 ng/ml, at least about 700 ng/ml, at least about 800 ng/ml, at least about 900 ng/ml, at least about 1000 ng/ml, at least about 1250 ng/ml, at least about 1500

ng/ml, at least about 1750 ng/ml, at least about 2000 ng/ml, at about 2750 ng/ml, at least about 3000 ng/ml, at least about 3500 ng/ml, at least about 3750 ng/ml, at least about 5000 ng/ml, at least about 7500 ng/ml, or at least about 10,000 ng/ml. In one embodiment, Spexin is administered at a dose of 0.2 ml of Spexin a day (2500ng/mL). In another embodiment, Spexin is administered by daily intraperitoneal (IP) injection.

[00141] In other embodiments, the effective amount of the administered Spexin modulating compound is at least about 0.01 µg/kg body weight, at least about 0.025 µg/kg body weight, at least about 0.05 µg/kg body weight, at least about 0.075 µg/kg body weight, at least about 0.1 µg/kg body weight, at least about 0.25 µg/kg body weight, at least about 0.5 µg/kg body weight, at least about 0.75 µg/kg body weight, at least about 1 µg/kg body weight, at least about 5 µg/kg body weight, at least about 10 µg/kg body weight, at least about 25 µg/kg body weight, at least about 50 µg/kg body weight, at least about 75 µg/kg body weight, at least about 100 µg/kg body weight, at least about 150 µg/kg body weight, at least about 200 µg/kg body weight, at least about 250 µg/kg body weight, at least about 300 µg/kg body weight, at least about 350 µg/kg body weight, at least about 400 µg/kg body weight, at least about 450 µg/kg body weight, at least about 500 µg/kg body weight, at least about 550 µg/kg body weight, at least about 600 µg/kg body weight, at least about 650 µg/kg body weight, at least about 700 µg/kg body weight, at least about 750 µg/kg body weight, at least about 800 µg/kg body weight, at least about 850 µg/kg body weight, at least about 900 µg/kg body weight, at least about 950 µg/kg body weight, or at least about 1000 µg/kg body weight. In one embodiment, the effective amount of the administered Spexin modulating compound is at least about 10 µg/kg body weight.

[00142] In one embodiment, Spexin or a Spexin modulating compound is administered at least once daily. In another embodiment, Spexin or a Spexin modulating compound is administered at least twice daily. In some embodiments, Spexin or a Spexin modulating compound is administered for at least 1 week, for at least 2 weeks, for at least 3 weeks, for at least 4 weeks, for at least 5 weeks, for at least 6 weeks, for at least 8 weeks, for at least 10 weeks, or for at least 12 weeks. In further embodiments, Spexin and/or a Spexin modulating compound is administered in combination with a second therapeutic agent.

[00143] Toxicity and therapeutic efficacy of therapeutic compositions of the present invention can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD₅₀ (the dose lethal to 50% of the

population) and the ED₅₀ (the dose therapeutically effective in dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀. Therapeutic agents that exhibit large therapeutic indices are useful. Therapeutic compositions that exhibit some toxic side effects can be used.

[00144] A therapeutically effective dose of Spexin or a Spexin modulating compound can depend upon a number of factors known to those of ordinary skill in the art. The dose(s) of Spexin or a Spexin modulating compound can vary, for example, depending upon the identity, size, and condition of the subject or sample being treated, further depending upon the route by which the composition is to be administered, if applicable, and the effect which the practitioner desires Spexin or a Spexin modulating compound to have upon the nucleic acid or polypeptide of the invention. These amounts can be readily determined by a skilled artisan.

[00145] *Gene Therapy and Protein Replacement Methods*

[00146] The invention provides methods for treating obesity or an obesity-associated disorder in a subject. In one embodiment, the method can comprise administering to the subject Spexin or a Spexin modulating compound, which can be a polypeptide, small molecule, antibody, or a nucleic acid.

[00147] Various approaches can be carried out to restore the activity or function of an Obesity Signature (OS) genes (e.g., Spexin) in a subject, such as those carrying an altered Spexin gene locus. For example, supplying wild-type Spexin function to such subjects can suppress phenotypic expression of obesity or an obesity related disorder in a subject. Increasing Spexin expression levels or activity can be accomplished through gene or protein therapy.

[00148] A nucleic acid encoding an OS gene, or a functional part thereof (such as Spexin) can be introduced into the cells of a subject. For example, the wild-type Spexin gene (or a functional part thereof) can also be introduced into the cells of the subject in need thereof using a vector as described herein. The vector can be a viral vector or a plasmid. The gene can also be introduced as naked DNA. The gene can be provided so as to integrate into the genome of the recipient host cells, or to remain extra-chromosomal. Integration can occur randomly or at precisely defined sites, such as through homologous recombination. For example, a functional copy of the Spexin gene can be inserted in replacement of an altered

version in a cell, through homologous recombination. Further liposome-mediated transfection, or cationic lipid-mediated transfection. Gene therapy can be accomplished by direct gene injection, or by administering *ex vivo* prepared genetically modified cells expressing a functional polypeptide.

[00149] Delivery of nucleic acids into viable cells can be effected *ex vivo*, *in situ*, or *in vivo* by use of vectors, and more specifically viral vectors (e.g., lentivirus, adenovirus, adeno-associated virus, or a retrovirus), or *ex vivo* by use of physical DNA transfer methods (e.g., liposomes or chemical treatments). Non-limiting techniques suitable for the transfer of nucleic acid into mammalian cells *in vitro* include the use of liposomes, electroporation, microinjection, cell fusion, DEAE-dextran, and the calcium phosphate precipitation method (see, for example, Anderson, Nature, supplement to vol. 392, no. 6679, pp. 25-20 (1998)). Introduction of a nucleic acid or a gene encoding a polypeptide of the invention can also be accomplished with extrachromosomal substrates (transient expression) or artificial chromosomes (stable expression). Cells may also be cultured *ex vivo* in the presence of therapeutic compositions of the present invention in order to proliferate or to produce a desired effect on or activity in such cells. Treated cells can then be introduced *in vivo* for therapeutic purposes.

[00150] Nucleic acids can be inserted into vectors and used as gene therapy vectors. A number of viruses have been used as gene transfer vectors, including papovaviruses, e.g., SV40 (Madzak et al., 1992), adenovirus (Berkner, 1992; Berkner et al., 1988; Gorziglia and Kapikian, 1992; Quantin et al., 1992; Rosenfeld et al., 1992; Wilkinson et al., 1992; Stratford-Perricaudet et al., 1990), vaccinia virus (Moss, 1992), adeno-associated virus (Muzyczka, 1992; Ohi et al., 1990), herpesviruses including HSV and EBV (Margolskee, 1992; Johnson et al., 1992; Fink et al., 1992; Breakfield and Geller, 1987; Freese et al., 1990), and retroviruses of avian (Biandyopadhyay and Temin, 1984; Petropoulos et al., 1992), murine (Miller, 1992; Miller et al., 1985; Sorge et al., 1984; Mann and Baltimore, 1985; Miller et al., 1988), and human origin (Shimada et al., 1991; Helseth et al., 1990; Page et al., 1990; Buchschacher and Panganiban, 1992). Non-limiting examples of *in vivo* gene transfer techniques include transfection with viral (e.g., retroviral) vectors (see U.S. Pat. No. 5,252,479, which is incorporated by reference in its entirety) and viral coat protein-liposome mediated transfection (Dzau et al., Trends in Biotechnology 11:205-210 (1993), incorporated entirely by reference). For example, naked DNA vaccines are generally known in the art; see

Brower, *Nature Biotechnology*, 16:1304-1305 (1998), which is its entirety. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see, e.g., U.S. Pat. No. 5,328,470) or by stereotactic injection (see, e.g., Chen, et al., 1994. *Proc. Natl. Acad. Sci. USA* 91: 3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g., retroviral vectors, the pharmaceutical preparation can include one or more cells that produce the gene delivery system.

[00151] For reviews of gene therapy protocols and methods see Anderson et al., *Science* 256:808-813 (1992); U.S. Pat. Nos. 5,252,479, 5,747,469, 6,017,524, 6,143,290, 6,410,010, 6,511,847; and U.S. Application Publication Nos. 2002/0077313 and 2002/00069, which are all hereby incorporated by reference in their entireties. For additional reviews of gene therapy technology, see Friedmann, *Science*, 244:1275-1281 (1989); Verma, *Scientific American*: 68-84 (1990); Miller, *Nature*, 357: 455-460 (1992); Kikuchi et al., *J Dermatol Sci.* 2008 May;50(2):87-98; Isaka et al., *Expert Opin Drug Deliv.* 2007 Sep;4(5):561-71; Jager et al., *Curr Gene Ther.* 2007 Aug;7(4):272-83; Waehler et al., *Nat Rev Genet.* 2007 Aug;8(8):573-87; Jensen et al., *Ann Med.* 2007;39(2):108-15; Herweijer et al., *Gene Ther.* 2007 Jan;14(2):99-107; Eliyahu et al., *Molecules*, 2005 Jan 31;10(1):34-64; and Altaras et al., *Adv Biochem Eng Biotechnol.* 2005;99:193-260, all of which are hereby incorporated by reference in their entireties.

[00152] Protein replacement therapy can increase the amount of protein by exogenously introducing wild-type or biologically functional protein by way of infusion. A replacement polypeptide can be synthesized according to known chemical techniques or may be produced and purified via known molecular biological techniques. Protein replacement therapy has been developed for various disorders. For example, a wild-type protein can be purified from a recombinant cellular expression system (e.g., mammalian cells or insect cells-see U.S. Pat. No. 5,580,757 to Desnick et al.; U.S. Pat. Nos. 6,395,884 and 6,458,574 to Selden et al.; U.S. Pat. No. 6,461,609 to Calhoun et al.; U.S. Pat. No. 6,210,666 to Miyamura et al.; U.S. Pat. No. 6,083,725 to Selden et al.; U.S. Pat. No. 6,451,600 to Rasmussen et al.; U.S. Pat. No. 5,236,838 to Rasmussen et al. and U.S. Pat. No. 5,879,680 to Ginns et al.), human placenta, or animal milk (see U.S. Pat. No. 6,188,045 to Reuser et al.), or other sources known in the

art. After the infusion, the exogenous protein can be taken up or receptor-mediated mechanism.

[00153] These methods described herein are by no means all-inclusive, and further methods to suit the specific application is understood by the ordinary skilled artisan. Moreover, the effective amount of the compositions can be further approximated through analogy to compounds known to exert the desired effect.

[00154] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Exemplary methods and materials are described below, although methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention.

[00155] All publications and other references mentioned herein are incorporated by reference in their entirety, as if each individual publication or reference were specifically and individually indicated to be incorporated by reference. Publications and references cited herein are not admitted to be prior art.

EXAMPLES

[00156] Examples are provided herein to facilitate a more complete understanding of the invention. The following examples illustrate the exemplary modes of making and practicing the invention. However, the scope of the invention is not limited to specific embodiments disclosed in these Examples, which are for purposes of illustration only, since alternative methods can be utilized to obtain similar results.

Example 1 – IDENTIFICATION OF SPEXIN AS A POTENTIAL SATIETY FACTOR THAT IS APPROXIMATELY 15 FOLD UNDEREXPRESSED IN OMENTAL AND SUBCUTANEOUS FAT IN OBESE PATIENTS

[00157] Human and mouse models of obesity for serum levels of Spexin via radioimmunoassay (RIA) are being tested to see if circulating spexin levels correlate with tissue depot expression levels. Also, western blots of human tissues are being carried out to measure expression levels, and to determine molecular weights of expressed peptides. The genomic sequence and expressed mRNA of Spexin from human sources (both obese and normal) is being characterized to see if sequence variants (e.g., SNPs, insertions, deletions, etc) account for differential expression of Spexin, or if unknown splice variants of Spexin exist, that are not recognized by the probe on the Codelink arrays used in these studies.

[00158] Using whole genome microarray analysis of human fat samples from two different depots, gene expression levels between obese vs. normal weight patients were compared (**FIG. 1**). Several thousand genes that are significantly differentially expressed between the two groups were identified ($p < 0.05$, with no correction for multiple testing). Of all these, the gene with the greatest under expression (14.9 fold-change) in obese fat was Spexin (nor = 24.95 vs obese = 1.68; $p = 0.00292$; “Petide A” in **FIG. 9**), a recently identified secreted peptide hormone that induces stomach contractions in a gut explant assay.

[00159] Since Spexin is significantly under-expressed by both omental and subcutaneous fat in obese patients, and it has biological activity in the gut, without being bound by theory, it is a factor expressed by normal fat tissues to indicate satiety. Without being bound by theory, the

almost complete lack of Spexin expression in obese fat can contribute to the loss of a feedback loop which normally inhibits food intake and energy/lipid storage, leading to obesity.

[00160] Without being bound by theory, Spexin is expressed by adipocytes under normal conditions, to indicate a satiated, or energy saturated state. Therefore, under normal conditions, this signaling would have a dampening effect on energy seeking behavior.

[00161] MATERIALS AND METHODS

[00162] *Patients.* The study population consisted of 11 patients undergoing clinically indicated abdominal laparoscopic surgical procedures, who consented to removal of an omental fat sample during surgery for studies of LCFA transport and a venous blood sample for the measurement of plasma levels of insulin and Leptin. Seven of the patients (all female) were obese, and were undergoing bariatric surgical procedures related to their obesity. The other four patients (all female) were non-obese, and were undergoing a variety of other clinically indicated laparoscopic procedures.

[00163] *Gene Expression Studies*

[00164] *Tissue collection:* Fat samples were collected at the time of bariatric surgery. One to two grams of tissue were placed in RNAlater at -80°C for long-term storage.

[00165] *Isolation of total RNA:* The fat samples were thawed, then homogenized in 5 mls of TRIzol (Invitrogen). After standard phase separation and RNA isolation, the pellet was resuspended in water, RLT lysis buffer, and ethanol for RNA clean-up and on-column DNase treatment (Qiagen). Eluted RNA consistently had A260/A280 ratios > 2.0. The integrity of the total RNA was verified by the presence of robust 18S and 28S peaks in the BioAnalyzer electropherogram.

[00166] *Microarray target labeling and hybridization:* Biotin-labeled cRNA were generated by established procedures. In brief, 2 µg of total RNA were used for synthesizing ds cDNA. This was incubated with biotin labeled 11-UTP in an in-vitro transcription reaction. cRNA was purified by RNeasy columns (Qiagen), and quantified by UV spectrophotometry at 260 nm. The size distribution of the biotin-labeled cRNA was verified on a Bioanalyzer (Agilent). 10 µg of

fragmented cRNA was hybridized overnight on CodeLink Human 10K microarrays. Hybridized cRNAs were detected by Streptavidin- Cy 5 flur (GE Healthcare).

[00167] *Data analysis:* Spot detection was performed with the GenePix Series B scanner (Axon Instruments) and spot quantitation was performed using CodeLink™ Expression Analysis v5.0. Key quantitation parameters are described briefly here. Local background subtraction is carried out on the individual spot intensities, followed by a scaling of each array individually based on the overall array intensity. After median-normalization, individual data sets were uploaded into the GeneSifter microarray data analysis suite for further analysis.

[00168] RESULTS

[00169] *Identification of Ch12; orf39 (Spexin) as significantly under-expressed in Obese fat.* The Tenomodulin data point is identified to validate the data set, as this gene was recently reported to be over-expressed in obese fat samples (**FIG. 1**; Tolppanen, 2007; Saki, 2009). Another biomarker is chitinase, which indicates macrophage activation, reflecting an inflammatory state that is well-known in obesity (**FIG. 1**). Both Spexin and carbonic anhydrase III are significantly over-expressed in normal fat compared to obese fat (**FIG. 1**).

[00170] Ch12;orf39 is a newly identified peptide with GI activity (Mirabeau, 2007; Rucinski, 2010), and a potential satiety factor that is under-expressed in obese fat (**FIG. 1**).

[00171] Table 1 below depicts mRNA expression data for Spexin.

[00172] **Table 1.** Spexin mRNA Expression Data, sample by sample

Group	Condition	N	Mean	SEM	SEM/Mean
1	obese	12	1.6869	+/- 0.4284	25.4%
2	normal	8	24.9532	+/- 5.2260	20.9%

• By Target

Group	Sample	Median Normalized Expression
Obese	Subcu	2.1212
Obese	Subcu	5.5443
Obese	Subcu	1.8812
Obese	Subcu	1.2796
Obese	Omental	0.4901
Obese	Subcu	1.5738
Obese	Omental	0.2394

Obese	Omental	2.6547
Obese	Omental	2.6115
Obese	Omental	0.6285
Obese	Omental	0.3092
Obese	Omental	0.9090
normal	Subcu -1-	22.1529
normal	Subcu -2-	45.3350
normal	Subcu -3-	16.7751
normal	Subcu -4-	29.0327
normal	Omental -1-	19.6793
normal	Omental -2-	8.5981
normal	Omental -3-	10.3961
normal	Omental -4-	47.6567

[00173] Of all the genes that are under-expressed in obese fat, Spexin demonstrates the largest fold-change between obese (1.687) and normal samples (24.95) ($p < 0.00292$) (FIG. 2, Table 2).

[00174] **Table 2.** Spexin mRNA Expression Data, sample by sample (median normalized values for each sample sorted by group [obeses vs normal]) . Statistical analysis by two-tailed t-Test, assuming unequal variance in the 2 sample sets ($p = 0.00292$).

Obese	Normal
2.1212	22.1529
5.5443	45.335
1.8812	16.7751
1.2796	29.0327
0.4901	19.6793
1.5738	8.5981
0.2394	10.3961
2.6547	47.6567
2.6115	
0.6285	
0.3092	
0.909	
1.686875	24.95324

[00175] Spexin is a newly identified peptide with GI activity. Mirabeau et al. (2007) was the first report describing Spexin, and they used Markov modeling analysis based on features common to peptide hormones, to find new ones in human proteome sequences. One of the

hormones they identified was spexin, which demonstrated contractile activity in a rat stomach explant assay, indicating a biological activity. Spexin is secreted. Wan et al. (2010) described brefeldin A (BFA) sensitive secretion of Spexin from transfected cells, indicating a golgi dependent mechanism, and that it plays a role in the biological function of the placenta. Spexin is conserved among various species as indicated in the multiple sequence alignment of Spexin homologues shown in **FIG. 3**.

[00176] REFERENCES

- 1: Mirabeau O, Perlas E, Severini C, Audero E, Gascuel O, Possenti R, Birney E, Rosenthal N, Gross C. Identification of novel peptide hormones in the human proteome by hidden Markov model screening. *Genome Res.* 2007 Mar;17(3):320-7. Epub 2007 Feb 6.
- 2: Rucinski M, Porzionato A, Ziolkowska A, Szyszka M, Macchi V, De Caro R, Malendowicz LK. Expression of the spexin gene in the rat adrenal gland and evidences indicating that spexin inhibits adrenocortical cell proliferation. *Peptides.* 2010 Jan 4.
- 3: Saiki A, Olsson M, Jernås M, Gummesson A, McTernan PG, Andersson J, Jacobson P, Sjöholm K, Olsson B, Yamamura S, Walley A, Froguel P, Carlsson B, Sjöström L, Svensson PA, Carlsson LM.. Tenomodulin is highly expressed in adipose tissue, increased in obesity, and down-regulated during diet-induced weight loss. *J Clin Endocrinol Metab.* 2009 Oct; 94(10): 3987-94.
- 4: Tolppanen AM, Pulkkinen L, Kolehmainen M, Schwab U, Lindström J, Tuomilehto J, Uusitupa M; Finnish Diabetes Prevention Study Group. Tenomodulin is associated with obesity and diabetes risk: the Finnish diabetes prevention study. *Obesity (Silver Spring).* 2007 May;15(5):1082-8.

Example 2 – SPEXIN EXPRESSION IN OBESE AND NORMAL SERUM SAMPLES

[00177] Example 1 discusses the significant under-expression of Spexin in omental and sub-cutaneous fat samples from obese patients. Therefore, it was decided to assay Spexin in serum

samples from obese and normal weight patients, to see if the differences in obese fat gene expression resulted in significant differences in circulating Spexin levels. Leptin levels were also measured since Leptin is known to be a circulating adipokine that is elevated in the obese state. Without being bound by theory, circulating Spexin and Leptin may be “antagonistic” hormones/adipokines that are involved in the regulation of satiety, food intake, and body weight.

[00178] Serum Samples: Serum samples from 7 obese and 7 normal weight human female patients were assayed in these initial studies.

[00179] Spexin Assay: Circulating spexin levels in serum were assayed using the Spexin / NPQ (human, mouse, bovine) EIA kit from Phoenix Pharmaceuticals, Inc, Catalog # EK-023-81, Lot # 601716. This kit measures what is believed to be the processed, bio-active peptide:

Asn-Trp-Thr-Pro-Gln-Ala-Met-Leu-Tyr-Leu-Lys-Gly-Ala-Gln-NH₂ (SEQ ID NO: 3)

[00180] The nature (primary sequence and structure) of circulating spexin in human serum has not been confirmed, nor has it been reported in any literature to date. The assay used has been designed to detect the peptide SEQ ID NO: 3.

[00181] The range of detection of circulating spexin is 0-100 ng/mL. Since this EIA is a competition assay, the measured optical density (OD) at 450 nm is inversely proportional to the amount of free antigen in the sample (see **FIG. 4**).

[00182] With the discovery of significant under-expression of Spexin in omental and subcutaneous fat samples from obese patients (see **FIG. 5**), Spexin concentrations were assayed in serum samples from obese and normal weight patients to see if the reduced gene expression in obese fat resulted in significant differences in circulating Spexin levels. Circulating Spexin is approximately 1/10th lower in concentration in obese serum, which is in reasonable agreement with the 15-fold difference in Spexin gene expression reported above (**FIG. 5**).

[00183] Leptin is known to be elevated in the obese state. Without being bound by theory, circulating Spexin and Leptin may be “antagonistic” or counter-balancing hormones/adipokines that are involved in the regulation of satiety, food intake, and body weight. The magnitude of the difference reported in **FIG. 6** ($0.69/0.15 = 4.6$ -fold increase in circulating Leptin in obese serum)

is equivalent to that reported by Considine, et al. (1996), where the magnitude of increase in circulating Leptin in obese patients was 4.17 fold using a newly developed RIA. Considine, et al. assayed circulating human Leptin in obese (n=139 men and women) vs normal weight men and women (n=136). The mean (+/- SD) Leptin levels were 31.3 +/- 24.1 ng/mL in obese patients, and 7.5 +/- 9.3 ng/mL in normal weight patients. Circulating Leptin levels are significantly higher in obese patients (**FIG. 10**).

[00184] The strong negative correlation ($r = -0.9444$) between Leptin and Spexin in the serum of obese patients and normal weight controls supports the idea that these two peptides play antagonistic roles in the normal regulation of hunger, satiety, body weight and adiposity (**FIG. 7**). Without being bound by theory, each hormone may serve as part of a negative feedback loop, where a rise in one leads to a lowering of the other (and vice versa). In the absence of circulating Spexin, Leptin is over-expressed in the fat, and is over-produced in the serum. Therefore, when Spexin is under expressed in fat, leading to a significant decrease of circulating Spexin, Leptin is over-expressed in the fat of obese patients, resulting in pathophysiologic levels of Leptin in the serum.

[00185] Calculating the ratios of negatively correlated values can magnify the differences in gene expression between groups, and serve as sensitive and reliable diagnostic markers (Gordon et al, 2002, 2003) to distinguish groups. An extension of this idea is presented in **FIG. 8**, where ratios of protein expression confirm the classifications based on BMIs. While this may not seem to add any new information at this time, it may be possible in the future, once more samples are analyzed, to distinguish sub-classes (metabolic syndrome, pre-diabetic, morbidly obese, hyper-lipidemic, etc) of patients in each group with this algorithm.

[00186] References:

- 1: Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL, et al. Serum immunoreactive-Leptin concentrations in normal-weight and obese humans. *N Engl J Med*. 1996 Feb 1;334(5):292-5.
- 2: Gordon GJ, Jensen RV, Hsiao LL, Gullans SR, Blumenstock JE, Richards WG, Jaklitsch MT, Sugarbaker DJ, Bueno R. Using gene expression ratios to predict outcome among patients with mesothelioma. *J Natl Cancer Inst*. 2003 Apr 16;95(8):598-605. PubMed PMID: 12697852.

3: Gordon GJ, Jensen RV, Hsiao LL, Gullans SR, Blumenstock JE, Ramaswamy S, Richards WG, Sugarbaker DJ, Bueno R. Translation of microarray data into clinically relevant cancer diagnostic tests using gene expression ratios in lung cancer and mesothelioma. *Cancer Res.* 2002 Sep 1;62(17):4963-7.

Example 3 – SPEXIN INFUSION TO DETERMINE ROLE OF SPEXIN IN SATIETY

[00187] *Initial dose-determining experiments.* Both normal weight and obese high-fat diet mice will be tested for circulating levels of Leptin, insulin, and Spexin. The Spexin level in human serum is approximately 10 ng/ ml in normal subjects (e.g., non-obese subjects), thus the following four doses will be tested in the initial studies: about 3 ng, about 10 ng, about 30 ng, about 100 ng, about 1 µg, about 10 µg, and about 100 µg..

[00188] Pharmaceutical grade Spexin (a 14 amino acid peptide normally circulating in mice and humans) has been synthesized by conventional solid-state chemistry, and purified to >95% identity by Phoenix Pharmaceuticals (Burlingame, CA). This agent will be solubilized in 1X sterile saline (pH 7.4) at four different concentrations, so that doses of 1.5 µg/kg/QD, 5 µg/kg//QD, 15 µg/kg//QD, or 50 µg/kg/QD can be administered to 5 mice in each dose group. Final total doses of 3 µg/kg/day, 10 µg/kg/day, 30 µg/kg/day, 100 µg/kg/day, or 250 µg/kg/day will be delivered in volumes of 0.2 mls/dose/day via IP injections. For example, to deliver 10 µg total per day, two daily injections of 5 µg each will be administered IP (stock concentrations of Spexin will be 25 µg/ml). Alternatively, to deliver 10 µg per day, a daily injection of 10 µg can also be administered IP (stock concentrations of Spexin will be 50 µg/ml). In a further example, to deliver 50 µg per day, a daily injection of 50 µg can also be administered IP (stock concentrations of Spexin will be 2500 µg/ml).

[00189] Alternatively, five doses of Spexin (3 ng, 10 ng, 30 ng, 100 ng, and 500 ng) will be administered for 30 days via osmotic mini-pump infusions (see Fan et al, *J Nutr.* 2003 Sep;133(9):2707-15). The doses will be selected to range around those calculated to mimic Spexin concentrations seen in normal weight animals. Blood samples will be drawn weekly via tail vein to confirm circulating levels of spexin, Leptin and insulin. Body weights, diet, and water consumption will also be monitored and recorded daily.

[00190] *Administering Effective Dose of Spexin in Various Murine Models of Obesity.*

[00191] Once an effective and safe dose of Spexin is identified, this will be used to test the effects of Spexin in a number of well-known murine models of obesity.

[00192] Mouse Strains to be used include: (1) C57BL/6J background as controls; (2) DIO (Diet Induced Obesity) mice, also known as High Fat Diet Fed Mice; (3) ob/ob; (4) ob/ob treated with Leptin as a positive control; (5) db/db (diabetic mouse); (5) Fat; and (6) Tubby.

[00193] Blood samples will be drawn weekly to confirm circulating levels of Spexin, Leptin and insulin. Body weights, diet, and water consumption will be monitored and recorded daily.

[00194] Spexin dose adjustments (if necessary for a specific strain or model) will be started in new sets of animals.

[00195] At the end of the experiment, heart, liver and epididymal fat pads will be harvested for fatty acid uptake, qRT-PCR and western blot analysis of fatty acid transporters and key enzymes of the oxidative phosphorylation pathway.

Example 4 – LEPTIN EXPRESSION IS SELECTIVELY UPREGULATED IN OBESE OMENTAL FAT

[00196] **Introduction:** Leptin is an adipokine that is expressed by various fat depots. While earlier studies reported that Leptin expression varies between omental, subcutaneous and mesenteric tissues, there is little data comparing expression of Leptin at different tissue depots between obese and normal patients.

[00197] **Methods:** Surgical samples were collected and stored at -80°C in RNAlater. Total RNAs from omental (7 obese, 4 controls) and subcutaneous (5 obese, 4 controls) fat depots were collected from the same patients. cRNAs were generated using standard protocols, and hybridized overnight to Codelink Human Whole Genome microarrays. Median normalized expression data (arbitrary expression units) were analyzed by the GeneSifter Data package. Results are presented as mean +/- SEM.

[00198] Results: Total Leptin expression was not significantly higher in obese vs normal fat, (90.7 +/- 15.1 vs. 77.7 +/- 24.9). There is a large difference in Leptin expression between normal subcutaneous and omental fat (130.5 +/- 30.8 vs. 24.9 +/- 8.6), while in obese samples, the difference in Leptin expression between the depots was less pronounced (118.4 +/- 30.0 vs 71.0 +/- 11.6; sub cu vs. omental). Leptin expression does not change between obese and normals at the subcutaneous depot (130.5 +/- 30.8 vs 118.4 +/- 30.0). However, a dramatic increase in Leptin expression was seen in the obese vs normal omental fat samples (71.0 +/- 11.6 vs 24.9 +/- 8.6).

[00199] Conclusions: The obese state alters Leptin expression in the omental depot, while expression is unchanged in subcutaneous fat from the same patients.

Example 5 – ADIPOCYTE ACCUMULATION OF LONG CHAIN FATTY ACIDS IN OBESITY IS MULTIFACTORIAL, RESULTING FROM INCREASED FATTY ACID UPTAKE AND DECREASED ACTIVITY OF GENES INVOLVED IN FAT UTILIZATION

[00200] Overview. The obesity epidemic causes significant morbidity and mortality. Knowledge of cellular function and gene expression in obese adipose tissue will yield insights into obesity pathogenesis and indicate therapeutic targets. This Example is directed to studying the processes determining fat accumulation in adipose tissue from obese patients.

[00201] Briefly, omental fat was collected from two cohorts of obese bariatric surgery patients and sex matched normal-weight donors. Isolated adipocytes were compared for cell size, volume and long chain fatty acid (LCFA) uptake. Omental fat RNAs were screened by 10K microarray (cohort 1: 3 obese, 3 normal) or Whole Genome microarray (cohort 2: 7 obese, 4 normal). Statistical differences in gene and pathway expression were identified in cohort 1 using GeneSifter Software (Geospiza) with key results confirmed in Cohort 2 samples by microarray, qRT-PCR and pathway analysis.

[00202] Obese omental adipocytes had increased surface area, volume, and V_{\max} for saturable LCFA uptake. Dodecenoyl-coenzyme A delta isomerase (DCI), central to LCFA metabolism, was approximately 1.6 fold under-expressed in obese fat in cohorts 1 and 2.

Additionally, Kyoto Encyclopedia of Genes and Genomics (KEGG) pathway analysis identified the Oxidative Phosphorylation and Fatty Acid Metabolism pathways as having coordinate, non-random down-regulation of gene expression in both cohorts.

[00203] In obese omental fat, saturable adipocyte LCFA uptake was greater than in controls, and expression of key genes involved in lipolysis and β -oxidation and metabolism of fatty acids was reduced. Thus, both increased uptake and reduced metabolism of LCFAs contribute to the accumulation of LCFAs in obese adipocytes.

[00204] **Introduction.** Studies (A1-15) have shown that cellular LCFA uptake occurs by two distinct processes, of which diffusion is the minor component. At the LCFA concentrations typically found between meals, 80-95% of total cellular LCFA uptake is via a saturable, regulatable, facilitated transport process (A7, A8). Studies in animals and patients indicate that regulation of adipocyte LCFA uptake is an important control point for body adiposity (A16-21). However, as in the liver (A22-24), many additional processes also contribute to LCFA and TG accumulation. Therefore, to understand the totality of the processes leading to obesity, it is important to define the global pattern of gene expression in adipose tissue. Published data indicate that at least 50 distinct genes are potentially involved in the establishment and maintenance of obesity (A22).

[00205] This Example discusses multiple processes for determining fat accumulation in adipose tissue from obese patients. Omental adipose tissue was collected from patients undergoing bariatric surgery for the treatment of morbid obesity, and were analyzed as part of an initial, small cohort of samples. The study yielded a pathophysiologic finding and a valuable illustration of the translational research that can be performed via the combined efforts of bariatric surgeons and basic scientists. Both technical and biological validation of the original results were achieved in an ongoing study of a second, larger cohort of patient samples. Also, the observed changes in key genes from the first cohort were validated by using both an alternative microarray platform and qRT-PCR on samples from the second cohort.

[00206] MATERIALS AND METHODS

[00207] Patients

[00208] The initial study population (Cohort 1) consisted of 6 patients undergoing clinically indicated abdominal laparoscopic surgical procedures who consented to removal of an omental fat sample during surgery for studies of LCFA transport. Three of the patients (two males, one female) were obese, and were undergoing bariatric surgical procedures related to their obesity. Despite obesity and the fact that none was on medications that influence glucose metabolism, none of the subjects had elevated fasting blood glucose concentrations. The other three patients (two males, one female) were non-obese, and were undergoing various other clinically indicated laparoscopic procedures. None was diabetic, had a significant chronic inflammatory disease or malignancy, or was on medications that could influence glucose metabolism, and – again - none had an elevated fasting blood glucose. Cohort 2 consisted of 7 obese female bariatric surgery patients and 4 non-obese female controls, all undergoing laparoscopic surgical procedures.

[00209] Physiologic Studies

[00210] **Materials:** 9,10- ^3H oleic acid (OA) was purchased from NEN Life Science Products, type I collagenase for adipocyte isolation from Sigma (St. Louis, MO), fatty acid free bovine serum albumin (BSA) from Boehringer Mannheim (Indianapolis, IN), and human-insulin-specific and human Leptin RIA kits from Linco Research, Inc. (St. Charles, MO).

[00211] **Isolation of adipocytes:** Suspensions of human adipocytes were prepared by collagenase digestion of omental fat samples (A2, A9, A16). Suspensions were maintained at room temperature in Dulbecco's modified Eagle's medium (DMEM) for up to 3 hours until warmed to 37 °C for use (A16), and met established viability criteria (A2, A9,A16). Isolated adipocytes were sized by direct light microscopy at 100X, using an eyepiece reticle with which cell diameters were measured in arbitrary units (1 U = 9.6 μ). The corresponding mean cell surface areas (SA) and cell volumes (Vol) were calculated as described (A25).

[00212] **LCFA uptake studies:** The initial rate of ^3H -OA uptake by omental adipocytes was determined by rapid filtration as described (A1, A2, A16). Briefly, cell suspensions with known cell counts, in 100 μ l of DMEM, were added to 240 μ l of DMEM containing 500 μ M BSA and varying ^3H -OA concentrations, and incubated for 0-30 s at 37 °C. At four specified time points, uptake was stopped (A1, A2), the cells were filtered and washed on the filters, and the filters with the cells were counted by liquid scintillation spectrometry (A2, A16). Adipocyte

[³H]-OA uptake is linear over the initial 30 sec of incubation (A2, A16). The slopes of the cumulative uptake versus time curves, representing initial uptake velocity (V_o), were calculated from four samples obtained in triplicate over this portion of the curve by linear regression.

[00213] Computations and Fitting of Kinetic Data: The unbound oleate concentration ($[OA_u]$) in each test solution was calculated from the OA:BSA molar ratio (v) (A26), using the LCFA:BSA binding constants of Spector et al (A27). The rationale for the use of these particular binding constants rather than several alternatives (A28-30) has been reported in detail previously (A8). Based on prior analyses (e.g. A5-8), measurements of initial oleate uptake velocity at values of v from 0.25-2.0 were fitted to the sum of a saturable and a non-saturable function of the corresponding $[OA_u]$, according to the equation [A1]:

$$UT([OA_u]) = (V_{max} \cdot [OA_u]) / (K_m + [OA_u]) + k \cdot [OA_u],$$

in which $UT([OA_u])$ is the experimental measurement of uptake, in pmol/sec/50,000 cells, at the stipulated $[OA_u]$; V_{max} and K_m are the maximal uptake velocity of the saturable oleic acid uptake component and the value of $[OA_u]$ at one-half the maximal uptake velocity; and k is the rate constant for non-saturable uptake (A2, A7, A8, A19, A21). Data fitting was via the SAAM II version of the Simulation, Analysis and Modeling (SAAM) program (A31), modified for execution on a lap-top PC computer (A32). Prior studies documented that, under the specific conditions employed in the current studies, V_o and derived parameters such as V_{max} are measures of trans-membrane transport, largely unmodified by pre-membrane phenomena such as rate-limiting dissociation from BSA and the effects of the peri-cellular unstirred water layer on substrate availability at the cell surface (A33), or of intracellular binding or metabolism (A1). Studies in which an increase in V_{max} was preceded an increase in adipocyte size early in the development of obesity (A19) and a decrease in V_{max} preceded a reduction in adipocyte size during Leptin-induced weight loss (A17) established that changes in V_{max} did not simply reflect changes in cell volume.

[00214] Statistical considerations: Values for physiologic variables are reported as mean \pm SD, calculated according to standard methods of descriptive statistics (A34). The significance of differences between groups was assessed with Student's 2-tailed t-test, with $\alpha \leq 0.05$ being considered significant.

[00215] Gene Expression Studies

[00216] ***Tissue collection:*** Omental fat samples were collected at the time of laparoscopic surgery. Samples were divided, and 1-2 grams of tissue from each biopsy were placed in RNAlater (Invitrogen, Carlsbad, CA) at -80°C for long-term storage.

[00217] ***Isolation of total RNA:*** The fat samples were thawed, and then homogenized in 15 ml of TRIzol (Invitrogen). After standard phase separation and RNA isolation, the pellet was resuspended in water, RLT lysis buffer, and ethanol for RNA clean-up and on-column DNase treatment (Qiagen). Eluted RNA consistently had A260/A280 ratios > 2.0. The integrity of the total RNA was verified by the presence of robust 18S and 28S peaks in BioAnalyzer electropherograms (Agilent).

[00218] ***Microarray target labeling and hybridization:*** Biotin-labeled cRNAs were generated by established procedures. In brief, 2 µg of total RNA were used for synthesizing ds cDNA. This was incubated with biotin labeled 11-UTP in an in-vitro transcription reaction. cRNA was purified by RNeasy columns (Qiagen), and quantified by UV spectrophotometry at 260 nm. The size distribution of the biotin-labeled cRNA was verified by capillary electrophoresis (Bioanalyzer, Agilent). 10 µg of fragmented cRNA was hybridized overnight on CodeLink Human 10K microarrays (Cohort 1) or Human Whole Genome microarrays (Cohort 2). Hybridized cRNAs were detected by Streptavidin-Cy5 fluor (GE Healthcare).

[00219] ***qRT-PCR validation:*** Expression of seven genes found to be under-expressed by microarray analysis in Cohort 2 was examined in the same samples by qRT-PCR. Individual gene PCR primers were designed using Primer 3 software (v.0.4.0) at <http://fokker.wi.mit.edu/primer3/input.htm> (Table 3).

[00220] Table 3. Primer sequences for qRT-PCR studies.

Gene	Primer	T_m	Sequence (5'→3')	SEQ ID NO:	Product size (nt)
Control (housekeeping) genes					
BBS4	Forward	59.93	atcccaaatcaaaagcaccag	4	157
	Reverse	59.97	ggctttgtgaactgggatgt	5	
MTIF2	Forward	59.99	tcagaaagcccgtagaagt	6	171
	Reverse	59.93	atggcctcaacagaaccatc	7	
PGBD3	Forward	59.99	ccacctgtctgggtacatt	8	211
	Reverse	59.93	gcctgatgtccattgaact	9	
PAICS	Forward	59.96	ctggggagttcaggatgtgt	10	183
	Reverse	59.96	tcagcctgcttcaaggaaat	11	
Target genes					
DCL-SP1	Forward	59.82	acaccctggagaacaccatc	12	185
	Reverse	62.55	ctgtcagcatggcttggaat	13	
ECHD	Forward	59.99	cagcttctcccagactcac	14	218
	Reverse	59.70	atgttgggcaagctctgaat	15	
ADH1A	Forward	60.13	gtgccactgaatgcatcaac	16	195
	Reverse	60.17	ggttttgggaatcaggaggt	17	
ATP5D	Forward	59.85	caaggcaaaacttgagaagg	18	175
	Reverse	60.06	gggcagttcatccagaggt	19	
COX4I1	Forward	59.53	ggcactgaaggagaaggaga	20	204
	Reverse	60.02	gggccgtacacatagtgtt	21	
CYC1	Forward	59.95	ccagctaccatgtccagat	22	166
	Reverse	59.87	tcaggactgaccacttgtgc	23	
NDUFS7	Forward	60.12	agttctctgtggcccatgac	24	197
	Reverse	59.17	ggcatctggctgtagacctt	25	

[00221] Selection criteria included T_m s of approximately 60°C, and PCR product lengths between 150 and 250 bp. First strand cDNAs were synthesized from total RNA samples using the TaqMan Reverse Transcription Reagent kit (Applied Biosystems), with oligo dT as primers. PCRs were performed on the 7300 Real time PCR system (Applied Biosystems), with the SYBR® GREEN PCR Master Mix (Applied Biosystems) in a total volume of 50 µl containing 500 ng cDNA as detailed in the manufacturer's guidelines. PCR conditions were: cycle 1 at 50.0°C for 2 mins, cycle 2 at 95.0°C for 10 mins, followed by 40 cycles (two step; 95.0°C for 0.15 mins, then 60.0°C for 1.00 min.). Each PCR was performed in duplicate. BBS4, MTIF2, PGBD3 AND PAICS were selected as control genes based on their robust expression, with minimal differences between the normal and obese omental fat samples in the Cohort 1 analysis.

The means of their expression levels were used to normalize the expression of target genes DCI-SP1, ECHD, ADH1A, ATP5D, COX4I1, CYC1 and NDUFS7 in all samples. The average fold change (AFC) was computed by using the average difference in the ΔC_t between each test gene and the mean of the 4 control genes for each sample, i.e. $AFC = 2^{-(\text{average } \Delta \Delta C_t)}$.

[00222] *Data analysis:* Microarray spot detection was performed with the GenePix Series B scanner (Axon Instruments) and spot quantitation was performed using CodeLink™ Expression Analysis v4.1 (Cohort 1) or CodeLink™ Expression Analysis v5.0 (Cohort 2). Key quantitation parameters are described briefly below. Local background subtraction is carried out on the individual spot intensities, followed by a scaling of each array individually based on the overall array intensity. After median-normalization, the negative control threshold is calculated using a set of negative control probes as described (A35).

[00223] *Gene ontology and KEGG pathway analysis:* Median-normalized gene expression values were then imported into the GeneSifter gene expression analysis suite (Geospiza, Seattle, WA). Array data for significantly differently expressed genes were overlaid onto ontological pathways (<http://www.geneontology.org/>) (A36) and KEGG pathways (www.genome.jp/kegg/) (A37) using GeneSifter software. The ontological and KEGG pathway analyses provide data on individual genes in the context of that gene's role in described biological/biochemical pathways. A pathway was considered significantly altered from the control gene expression profile if its z-score was less than -2 or greater than 2.

[00224] z-Scores were calculated in GeneSifter as:
$$z\text{-Score} = \frac{\left(r - n \frac{R}{N}\right)}{\sqrt{n \left(\frac{R}{N}\right) \left(1 - \frac{R}{N}\right) \left(1 - \frac{n-1}{N-1}\right)}}$$
 where R = total number of genes meeting selection criteria, N = total number of genes measured, r = number of genes meeting selection criteria with the specified gene ontology (GO) term, and n = total number of genes measured with the specific GO term (38). z-Scores with an absolute value of ≥ 2.0 are considered to indicate significantly altered regulation of the pathway compared with controls. The meaning of the z score depends on the context of the reported score. When reported as a z up score, a positive z-score equal to or greater than 2 indicates that a significant number of genes in the list of differentially expressed genes are up-regulated in the experimental group in

that particular pathway. Conversely, a negative z up score of -2 or less, is also significant, and indicates that fewer than expected genes are over-expressed in the pathway. For z down scores, the interpretation is as follows: a positive z down score indicates that more genes than expected are under-expressed, and a negative z down score indicates that fewer than expected genes are under-expressed in the pathway.

[00225] RESULTS

[00226] **Patients:** The demographic and clinical characteristics of the patients are summarized in Table 4.

[00227] **Table 4.** Demographic and clinical characteristics of study patients

Group	Sex	Age (years)	BMI (kg/m ² BSA)	Insulin (ng/ml)	Leptin (ng/ml)	Glucose (mg/dL)	Cholesterol (mg/dL)	TG (mg/dL)
Cohort 1								
Obese 1	2 M, 1 F	53.7±7.8	42.7±5.7*	9.7±1.5	22.0±4.9**	88.3±7.5****	237±38	213±44
Control 1	2 M, 1 F	50.7±12.6	23.7±0.4	8.6±3.8	2.2±1.0	63.0±5.8	176±19	146±17
Cohort 2								
Obese 2	7 F	51.6±6.0*	48.6±2.6***	ND	ND	92.0±3.1	196±20	130±23
Control 2	4 F	30.0±6.0	22.1±2.3	ND	ND	87.3±10.3	161±6	79±24

* $p<0.05$; ** $p<0.02$; *** $p<0.001$; **** $0.1> p>0.05$ compared with cohort controls

ND not done

[00228] The initial study population (Cohort 1) consisted of three obese and three non-obese subjects, with two males and one female in each group. Cohort 2 consisted of 7 obese and 4 non-obese female patients. The average ages of the obese patients in both cohorts and control patients in Cohort 1 were similar. The non-obese patients in Cohort 2 were younger. Virtually by definition, the BMI was significantly greater in the obese patients than in the control patients in both Cohorts. Values for obese and non-obese subjects in the two cohorts were very similar. In additional studies in Cohort 1, the fasting plasma Leptin concentration was significantly higher in obese than in control subjects. Although mean fasting blood glucose, plasma insulin, and serum cholesterol and triglycerides were all higher in the obese patients than in the controls, only the difference in glucose levels between the obese and non-obese patients of Cohort 1 achieved statistical significance.

[00229] **Adipocyte sizes:** Adipocyte size measurements and the results of adipocyte LCFA uptake studies (Cohort 1 only) are presented in Table 5.

[00230] **Table 5.** Adipocyte measurements and fatty acid uptake kinetics.

Group	Diameter (μm)	Surface area per cell (μm^2)	Volume per cell (pL)	V_{max} (pmol/s/50,000 cells)
Cohort 1				
Obese	94.7 \pm 6.8**	32,717 \pm 9,492****	486 \pm 191	19.6 \pm 4.7*
Control	53.4 \pm 4.4	9,953 \pm 1,659	81 \pm 18	4.8 \pm 2.0
Cohort 2				
Obese 2	101.6 \pm 4.4***	32,799 \pm 2,784***	568 \pm 71***	ND
Control 2	58.1 \pm 5.8	10,910 \pm 2,630	112 \pm 42	ND
Group	K_m (nM)	k (ml/s/50,000 cells)	$V_{\text{max}}/\text{SA} \times 10^8$ (pmol/s/ μ^2)	
Cohort 1				
Obese	149 \pm 15	0.0105 \pm 0.0055	1.20 \pm 0.45	
Control	142 \pm 49	0.0068 \pm 0.0018	0.96 \pm 0.43	
Cohort 2				
Obese 2	ND	ND	ND	
Control 2	ND	ND	ND	

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $0.1 > p > 0.05$ compared with cohort controls

[00231] Adipocytes from obese patients were appreciably larger than those from non-obese controls in both Cohorts. Mean cell diameters were 1.7-1.8 times larger, surface areas 3.0-3.3 times larger, and cell volumes 5.1 - 6.0 times larger in obese than in non-obese patients of both Cohorts.

[00232] **LCFA Uptake Kinetics:** Computer fits of the LCFA uptake curves in the six study subjects in Cohort 1 are illustrated in **FIG. 13**. As in a series reported previously (A16), there was no overlap whatsoever in the curves from obese, compared with non-obese subjects. Both the V_{max} for saturable LCFA uptake and the rate constant (k) for non-saturable uptake were increased in adipocytes from the obese patients. The increase in V_{max} was statistically significant; increases in k , and in the ratio of V_{max} to cell surface area were not. As with some of the biochemical values and measures of adipocyte size, the comparisons between obese and non-obese subjects paralleled those reported in the larger, earlier series (A16). Failure of differences

between groups for some parameters to achieve statistical significance in the present study results principally from the small sizes of the groups comprising Cohort 1.

[00233] *Microarray Analysis:* In Cohort 1, the expression of approximately 10,000 human genes and expressed sequence tags (ESTs) was measured using Codelink Human 10K microarray in each sample of omental fat from obese individuals (n=3) and normal weight donors (n=3). In cohort 2, Codelink Human Whole Genome microarrays queried expression of ~50,000 genes and ESTs in each sample. Thus, samples in Cohort 2 were evaluated for expression of ~5 times as many genes and ESTs as those in Cohort 1. Median normalized expression values were analyzed using the GeneSifter (Geospiza, Seattle, WA) software suite for the identification of differentially expressed genes, and of KEGG pathways with significantly altered gene expression.

[00234] *Measures of quality control of expression data:* In the Cohort 1 studies, a log-log plot of the means of obese (ordinate) versus normal (abscissa) expression values for each data point is seen in **FIG. 14A** (complete data set) and **FIG. 14B** (enlarged section enclosed by the magenta square). The limited data scatter on either side of the line of identity indicates the overall equivalence of the two data sets, while the statistically significant genes (T-test, no correction for multiple testing) indicates the tightness of the data scatter for individual genes. A second indication of this overall equivalence is a comparison of the four mitochondrial ribosomal genes (*L12*, *L38*, *L42* or *S7*) on the chips. None of these four “housekeeping genes” demonstrates significant differences in expression between the two sample sets, showing the overall metabolic equivalence between the obese and normal fat depots. A third measure of overall equivalence can be seen in the corresponding quartile plots (**FIG. 14C**: obese, left, and normal, right), which reveal no systematic differences between the data sets. These three global measures of gene expression indicate that there are no systematic differences between the two data sets, indicating that valid biological conclusions may be drawn if differences in expression are detected for particular individual genes. Highly similar quality control comparisons were obtained from the Cohort 2 samples.

[00235] *Identification of individual genes that are differentially expressed in obese fat:* Pairwise comparisons between the three obese and the three normal samples in Cohort 1 were

performed with minimum fold changes in expression, followed by standard t tests and corrections for multiple testing. Using criteria of an expression difference of ≥ 1.5 -fold and a p value of ≤ 0.05 , 166 differentially expressed genes and ESTs were identified in the Cohort 1 Analyses. However, after applying the Benjamini and Hochberg correction for multiple testing (A39), only one gene from this set, Dodecenoyl-Coenzyme A delta isomerase (3,2 - trans-enoyl-Coenzyme A isomerase) (DCI) demonstrated a statistically significant difference between the two groups in Cohort 1, being under-expressed 1.6 fold in obese fat (**FIG. 15A**). This gene encodes a member of the hydratase/isomerase superfamily. The protein encoded is a key mitochondrial enzyme involved in beta-oxidation of unsaturated fatty acids. It catalyzes the transformation of 3-cis and 3-trans-enoyl-CoA esters arising during the stepwise degradation of cis-, mono-, and polyunsaturated fatty acids to the 2-trans-enoyl-CoA intermediates. For Cohort 2 samples, the results were nearly identical for DCI expression differences between obese and normal omental fat samples (**FIG. 15B**).

[00236] Lipolysis Related Gene Expression in Obese Fat: The initial step in the release of fatty acids from triacylglycerol stores is their hydrolysis via hormonally regulated lipolysis. Two key genes in this process, adenylate cyclase 6 (**FIG. 16A** and **FIG. 16B**) and adenylate cyclase activating peptide receptor 1 (**FIG. 16C** and **FIG. 16D**) are both under-expressed in the obese fat samples in both cohorts. Adenylate cyclase 6 encodes a membrane-associated enzyme that catalyzes the formation of the second messenger cyclic adenosine monophosphate (cAMP). Adenylate cyclase activating polypeptide 1 receptor type I, encodes a membrane-associated receptor protein which mediates diverse biological actions of adenylate cyclase activating polypeptide 1, and is positively coupled to adenylate cyclase. The fact that both of these key genes in the adenylate cyclase signaling cascade are under-expressed in obese omental fat indicates that this tissue may demonstrate reduced responses to physiologic stimulation by lipolytic hormones.

[00237] KEGG Pathway Analysis: To identify a larger group of differentially expressed genes for inclusion in the pathway analysis, all genes in a pair-wise comparison were selected with an uncorrected p value ≤ 0.05 , without correction for multiple testing, since the pathway analysis itself applies a second level statistical filter. Using this uncorrected p value criterion, 612 differentially expressed genes and ESTs between obese and normal fat were identified from

the list of 10,000 that were queried in Cohort 1. This list of differentially expressed genes was subjected to KEGG pathway analysis for the identification of biological pathways with significantly altered gene expression in obese versus normal fat, as indicated by significant z up or z down scores. The complete set of down regulated KEGG pathways found in Cohort 1 is presented in Table 6. No pathways with z up scores had enough differentially expressed genes to be considered biologically significant.

[00238] Table 6. KEGG pathways with significant z down scores.

KEGG pathway	Genes on array	Genes regulated	Up	Down	z score down	
					Cohort 1	Cohort 2
Oxidative phosphorylation	51	8	0	8	4.22	3.67
Synthesis and degradation of ketone bodies	5	2	0	2	3.99	2.95
Fatty acid metabolism	37	6	0	6	2.31	1.59

[00239] Of the pathways with significant z down scores and a sufficient number of genes involved to indicate biological relevance, Oxidative Phosphorylation and Fatty Acid Metabolism, both directly related to energy metabolism and fatty acid biosynthesis and degradation (A37), stand out. Of the genes in each pathway that demonstrate differential expression in Cohort 1, *all* are *under-expressed* in obese fat samples relative to the samples from normal weight individuals (eight genes in the Oxidative Phosphorylation pathway, and six genes in the Fatty Acid Metabolism pathway), in 12 of 14 instances with a fold change of ≥ 1.2 . Results for these 12 genes in Cohort 2 were generally very similar to those described below (Tables 7 and 8).

[00240] Table 7. Downregulated genes in oxidative phosphorylation pathway.

Gene	ID	EC	Complex	Fold change down	
				Cohort 1	Cohort 2
NADH dehydrogenase (ubiquinone) Fe-S protein 7, 29 kDa (NADH-coenzyme Q reductase)	NDUFS7	1.6.5.3; 1.6.99.3	I	1.48	1.31
Succinate dehydrogenase complex, subunit A, flavoprotein (Fp)	SDHA	1.3.5.1	II	1.40	1.16
Cytochrome c-1	CYC1	1.10.2.2	III	1.48	1.37
Cytochrome c oxidase subunit IV isoform 1	COX4H	1.9.3.1	IV	1.51	1.11
ATPase, H ⁺ transporting, lysosomal 50/57 kDa, V1 subunit H	ATP6V1H	3.6.3.14	V	1.55	1.24
ATP synthase, H ⁺ transporting, mitochondrial F0 complex, subunit G	ATP5L	3.6.1.14	V	1.35	1.37
ATP synthase, H ⁺ transporting, mitochondrial F1 complex, delta subunit	ATP5D	3.6.1.14	V	1.51	1.12

[00241] Table 8. Downregulated genes in fatty acid metabolism pathway.

Gene	ID	EC	Fold change down	
			Cohort 1	Cohort 2
Acyl-coenzyme A oxidase 1, palmitoyl	ACOX1	1.3.3.6	1.36	1.02
Alcohol dehydrogenase 1A (class I), alpha polypeptide	ADH1A	1.1.1.1	2.21	1.91
Dodecenoyl-coenzyme A delta isomerase (3,2-trans-enoyl-coenzyme A isomerase)	DCI	5.3.3.8	1.59	1.64
3-Hydroxyacyl-coenzyme A dehydrogenase	ECHD	1.1.1.35	1.59	1.54
Peroxisomal D3, D2-enoyl-CoA isomerase	PECF	5.3.3.8	1.25	1.08

[00242] The individual genes that were under-expressed in the Oxidative Phosphorylation pathway in Cohort 1 with a fold change ≥ 1.2 are presented in Table 7. They include two H⁺ transporting mitochondrial ATP synthases, one lysosomal H⁺ transporting ATPase, two cytochrome c genes, and two dehydrogenases (ubiquinone and flavoprotein). Of the hundreds of proteins that make up the various enzymatic and electron transport complexes found in the inner mitochondrial membrane, the expression of the genes encoding seven of these proteins is down-regulated in obese omental fat. Five of these genes encode proteins that are regulatory enzymes or transport molecules, and these are spread across all 5 of the large complexes comprising the electron transport chain. As a result, even minor down-regulation of each of these, when factored together, can result in an important functional difference in production of ATP by Complex V. The functional organization of these down-regulated genes and their coordinate regulation has been brought into focus by the new pathway analysis employed in this study.

[00243] In complex I, NADH dehydrogenase (1.6.5.3/1.6.99.3) is the first enzyme of the complex that catalyzes the transfer of electrons from NADH to coenzyme Q. In Complex II, succinate dehydrogenase (1.3.5.1) reduces succinate to fumarate. In Complex III, ubiquinol-cytochrome-c reductase (1.10.2.2) is the heme-containing component of the cytochrome b-c1 complex, which accepts electrons from Rieske protein and transfers electrons to cytochrome c in the mitochondrial respiratory chain. In Complex IV, Cox4I1 (1.9.3.1) is one of the nuclear-encoded polypeptide chains of cytochrome c oxidase, the terminal oxidase in mitochondrial electron transport. In complex V, ATPase, H⁺ transporting, mitochondrial F1 complex, delta subunit (ATP6V1H, 3.6.3.14) activates the ATPase activity of the enzyme and couples ATPase activity to proton flow. Genes under-expressed with a fold change ≥ 1.2 in obese fat samples from Cohort 1 that are components of the Fatty Acid Metabolism pathway are presented in Table

77. These genes include *acylCoenzyme A oxidase*, *alcohol dehydrogenase 1A*, *DCI*, another isomerase (*peroxisomal D3,D2-enoyl-CoA isomerase*), and *hydroxyacyl-Coenzyme A dehydrogenase*.

[00244] **qRT-PCR:** Expression of seven genes from either the Oxidative Phosphorylation or Fatty Acid Metabolism pathways which were found to be under-expressed by microarray analysis in Cohort 1 was examined in the Cohort 2 samples by qRT-PCR. The genes are ECHD (3-Hydroxyacyl-Coenzyme A dehydrogenase); ADH1a (Alcohol dehydrogenase 1A); DCI (Dodecenoyl-Coenzyme A delta isomerase); ATP5D (ATP synthase, mito F1); CYC1 (cytochrome c-1); NDUFS7 (NADH dehydrogenase Fe-S); COX4I1 (Cytochrome c oxidase IV) (**FIG. 17**). Seven of these genes were under-expressed in Cohort 2 as assessed with the Whole Human Genome microarray. Thus, there was complete concordance between the Human Whole Genome array and qRT-PC with regard to the direction of regulation of expression of these 7 genes. Also, for the seven genes queried, the direction of difference in expression between obese and controls is also consistent between the two sample sets (Cohort 1 and Cohort 2), since these seven genes are all under- expressed in the obese fat samples. These findings are consistent with a generally reduced energy metabolism in obese fat.

[00245] **DISCUSSION**

[00246] Obesity is the increased deposition of LCFA, principally in the form of TG, in adipose and other tissues. That this deposition is highly selective, and that its extent and turnover differ even between different adipose tissue depots (e.g. A40-43), indicates that there is a complex underlying pathophysiology that involves far more than the passive, un-regulated diffusion of LCFA - the essential building blocks of TG – across cell membranes. Many processes may contribute to TG accumulation: increased LCFA uptake or synthesis, increased LCFA conversion to TG, or increased uptake of pre-formed TG from lipoproteins; or alternatively decreased TG or LCFA removal by decreasing TG lipolysis, decreased LCFA and TG excretion as components of VLDL, or decreased LCFA oxidation (A18, A22-24, A44-46). These multiple processes involve multiple genes, e.g. for plasma membrane and intracellular LCFA transporters; for enzymes of fatty acid or triglyceride synthesis; for receptors, enzymes and other proteins associated the import and/or hydrolysis of pre-formed lipoprotein-

triglycerides, such as the LDL receptor, hepatic lipase, and lipoprotein lipase (LPL); genes associated with VLDL synthesis, assembly and export; and proteins and enzymes of fatty acid oxidation; not to mention numerous transcription factors and other regulatory genes. It would be desirable to assay these processes simultaneously. While the key genes involved in many of these processes have been identified, for other processes, including cellular LCFA uptake, key genes are unknown (A47, A48). Several of the processes, such as cellular LCFA uptake and oxidation, can be assayed directly. While direct quantitation of others is more difficult, a first-order approximation may be obtained via RNA expression studies.

[00247] *Microarray Expression Analysis.* Without being bound by theory, the number of *known* genes potentially involved in obesity pathogenesis is at least 50 (A22). RNA expression microarray technology is an effective approach for analyzing a large number of genes, and also for identifying genes whose role in the process of interest—in this case, obesity—is unknown. Microarrays simultaneously monitor expression of thousands of mRNAs from individual samples. In addition to providing information regarding the expression of pre-selected candidate genes, the high-throughput nature of microarray analysis is ideal for the identification of candidate genes and/or pathways responsible for the pathophysiology of complex diseases such as obesity.

[00248] Data discussed herein was analyzed using a two stage approach. The first stage was to identify individual genes whose expression was significantly different between normal and obese omental fat samples. Using a standard cut-off of 1.5 fold difference in expression, along with Benjamini and Hochberg correction for multiple testing (39), a single gene, Dodecenoyl-Coenzyme A delta isomerase (DCI) or 3,2 trans-enoyl-Coenzyme A isomerase, was identified in Cohort 1 as being under-expressed in obese omental fat. Its under-expression was confirmed in Cohort 2 both by microarray and qRT-PCR expression analyses, providing both biological and technical validation of this result. This gene is a mitochondrial enzyme involved in the β -oxidation of unsaturated fatty acids. Metabolic intermediates produced during the stepwise degradation of unsaturated LCFA enter the Citric Acid Cycle, where they contribute to ATP production by oxidative phosphorylation. Central obesity has been positively associated with an increase in n-6 unsaturated fatty acids, and inversely associated with mono-unsaturated fatty acids (A49).

[00249] **Genomic Organization of *DCI*.** This gene is located at chromosomal locus 16p13.3, encoding a predicted protein of 302 amino acids (ENTREZ [NM_001919]) . Locus 16p13.3 has twice been linked to obesity-related factors, including BMI, both by logarithm of the odds score (LOD score) of microsatellite marker D16S510 in a study of Old Order Amish families (A50) and in a genome scan of African American families enriched for non-diabetic neuropathy (A51). These reports and the reduction of *DCI* expression described herein make this a candidate gene for further study in obesity. The ~50% loss of *DCI* expression is consistent with functional loss of one *DCI* allele, which could reflect microdeletions in this gene region, or single nucleotide polymorphisms (SNPs) adversely affecting the splicing or stability of the *DCI* message.

[00250] **KEGG Pathway Analysis.** The power of microarray analysis is that it allows the simultaneous screening of thousands of genes in each sample, unbiased by candidate gene pre-selection. Recent advances in software allow changes in gene expression to be considered in the context of biological pathways. The Kyoto Encyclopedia of Genes and Genomics (KEGG) consortium (www.genome.jp/kegg ; 37) has established a collection of gene pathways whose gene members are known to interact as parts of a greater whole. Significant changes in pathway member gene expression, especially when the changes are co-ordinate (eg: involving several sequential members of a signaling pathway) indicate that biologically meaningful alterations of pathway regulation and/or function are likely. Moreover, relatively small changes in expression of multiple genes in a pathway, each too small to achieve significance individually, can lead to biologically significant alterations in throughput along a pathway. For example, using a simplistic model, 10% down-regulation of 7 genes in a pathway, where each may be overlooked by single gene analysis, could result in a biologically significant reduction of $\geq 50\%$ in throughput along the pathway.

[00251] **Oxidative Phosphorylation KEGG Pathway.** The Oxidative Phosphorylation pathway captures the energy released by the oxidation of NADH and succinate in the citric acid cycle, producing adenosine triphosphate (ATP) via ATP synthase. Of the 51 genes in this pathway that are included on the arrays used in this study, seven are differentially expressed, and these seven are all under-expressed in obese fat. Data discussed herein show that two protein components of the ATP synthase complex are down-regulated in obese fat: the G subunit of the

ATP synthase F0 complex, and the delta subunits of the F1 complex). These results further show that components of the electron transport chain, including cytochrome c oxidase, and cytochrome c-1, plus NADH dehydrogenase and succinate dehydrogenase (subunit A) are also under-expressed. Without being bound by theory, ATP production can be decreased in obese fat tissues if levels of the corresponding proteins are also diminished. It is important to recognize that each of the five complexes is built up of many subunits, that depend on a delicate macrostructure.

[00252] Data discussed herein showing under-expression of all 7 of the 7 differentially expressed genes involved in oxidative phosphorylation are in agreement with similar findings in monozygotic twins that were discordant for obesity (A52). Microarray analysis of fat biopsies identified 30 genes in the oxidative phosphorylation pathway that were under-expressed in the obese subjects compared with their non-obese twins. A number of these genes were also under-expressed, including ATP5L, ATP5B, ATPV1H, and CYC1. Mustelin et al. also found members of the NADH dehydrogenase complex to be under-expressed in obese fat. Similar data have been reported by Patti et al (A53), who found a coordinated reduction in oxidative metabolism gene expression in skeletal muscle of type II diabetic Mexican-Americans, related to the reduced expression of two transcription factors, nuclear respiratory factor 1 (NRF-1) and PPAR γ co-activator 1, which are known to regulate expression of genes in the oxidative phosphorylation pathway. The genes encoding these transcription factors are not present on the 10K microarrays used in the original studies. In the cohort 2 samples, NRF-1 and PPAR γ co-activator 1 were essentially unchanged, indicating that another mechanism may be responsible for the consistent down-regulation of the oxidative phosphorylation pathway genes in obese omental fat.

[00253] *Fatty Acid Metabolism KEGG Pathway.* The Fatty Acid Metabolism pathway includes the enzymes responsible for the biosynthesis and degradation of LCFAs, leading to the production of Acetyl-CoA, which is then directed into the TCA cycle. From there, oxidation of NADH and succinate produce ATP via ATP synthase in the Oxidative Phosphorylation pathway. Of the 37 genes probed by the arrays, six of six with differential expression were all under-expressed in obese fat. Each of these underexpressed genes plays a role in the metabolism of LCFA, and several have already been reported to play a role in obesity. Acyl-Coenzyme A oxidase 1 (1.3.3.6) expression in omental fat reportedly predicts weight loss outcome after

gastric by-pass surgery (A54). DCI (5.3.3.8), grouped with 3-Hydroxyacyl- Coenzyme A dehydrogenase (1.1.1.35) in **FIG. 12**, plays a key role in LCFA degradation. A higher incidence of childhood obesity has been reported in children with long chain 3-Hydroxyacyl- Coenzyme A dehydrogenase (LCHAD) or trifunctional protein (TFP) deficiency. Placing these children on low fat, high protein and lower carbohydrate diets, resulted in lower energy intake, and increased energy expenditure (A55).

[00254] *Lipolysis Related Gene Expression.* Results discussed herein showing reduced expression of key genes in the cAMP mediated signaling of hormonally stimulated lipolysis are consistent with reports showing a blunted lipolytic response to catecholamine stimulation in obese individuals, especially in abdominal fat (A56) and adipocytes from obese individuals, due in part to reduced expression of hormone sensitive lipase (A57). However, fuller understanding of this complex system awaits ongoing transcriptome studies.

[00255] *ABC-Transporters KEGG Pathway.* One of the genes in this pathway encodes ATP-binding cassette, sub-family D (ALD), member 3 (ABCD3), a member of the superfamily of ATP-binding cassette (ABC) transporters. ABC proteins transport various molecules across extra- and intra-cellular membranes. ABCD3 is a member of the ALD subfamily, which is involved in peroxisomal import of fatty acids and/or fatty acyl-CoAs into the organelle. While little is known about this gene in human obesity, its up-regulation discussed herein is interesting in light of its known role in intracellular LCFA transport. Without being bound by theory, increased peroxisomal LCFA import and subsequent oxidation can partly substitute for the decreased mitochondrial β -oxidation indicated by other findings merits further study.

[00256] *Validation of Results.* It is widely accepted that microarray results analyses require validation. This may involve biological validation in a second, independently collected set of samples, and technological validation by using an alternative microarray platform or an entirely different technology such as qRT-PCR. Validation may also be accomplished by measurements of the proteins encoded by regulated genes, or of biological activity. In the present study, biological validation was achieved by the study of two independently collected sample sets, and technical validation by the use of two different micro-array platforms and qRT-PCR.

[00257] In the early days of micro-array analysis very large sample sizes were considered essential due to concerns about the statistical issues involved in making thousands of simultaneous comparisons on a given sample. As the statistical theory developed for this issue, the numbers required have dropped dramatically. The consensus report of a major conference sponsored by all of the major government science agencies in 2006 concluded that an $n = 5$ per group was sufficient for most micro-array analyses (A58). Since that conference numbers have dropped further. An appreciable number of microarray papers have been published in reputable journals based on studies in even smaller cohorts, including those in which $n=3$ per group (e.g. A59-61). The present study is well within the published 2006 guidelines.

[00258] Because different cell populations within a tissue may express particular genes at different rates, it was once considered important to fractionate tissues into purified cell populations before performing micro-array analyses. This may still be useful in specific instances, but gene expression array studies have tended to move away from this since cell isolation procedures themselves can introduce major artifactual changes in gene expression. This has been especially well studied in whole blood vs. PBMC comparisons, where changes in RNA expression due to experimental manipulation can seriously mask what was going on in vivo (A62). Many currently believe that cell fractionation is essential only in specific situations. The present studies were performed in unfractionated adipose tissue samples. Without being bound by theory, increased macrophage infiltration into obese fat influencing comparisons with results in non-obese fat seem very unlikely in a study, such as this one, in which the key results are *down-regulation* of multiple biologically relevant genes in the obese fat samples. Further, the expression ratio of 23 macrophage-specific genes in unfractionated obese vs. non-obese omental fat averaged 1.1 ± 0.06 , strongly arguing against the possibility that the observed down-regulation of DCI and related genes reflected macrophage infiltration.

[00259] ***Working Model of Accumulation of Long Chain Fatty Acids in Obese Adipocytes.*** Many laboratories have examined the balance between LCFA uptake, LCFA disposition, and cellular TG content in the liver as it relates to the pathogenesis of hepatic steatosis (A18, A22-24, A44-46). Similar considerations apply to adipocytes and the pathophysiology of obesity. In adipocytes of normal weight subjects, allowing for meal-related diurnal variation, LCFA uptake (including facilitated transport) and degradation (including β -oxidation) or elimination (e.g.

lipolysis) are in balance. Consequently, the net amount of fat in each cell is essentially constant over time, resulting in the relatively stable weights seen in non-obese subjects. In contrast, in adipocytes from obese subjects, the combination of increased facilitated LCFA uptake, shown in the uptake studies discussed herein, and reduced β -oxidation, lipolysis and LCFA metabolism, indicated by gene expression analysis, leads to accumulation of LCFAs and TG over time.

[00260] The result of the observed changes in LCFA kinetics and expression of metabolic genes will be chronic accumulation of LCFA and TG, resulting in enlarged adipocytes and significant weight gain over time. The strength of this study is the combination of the high-throughput first stage, where thousands of genes are queried; and the systems biology approach of the second stage, where established pathways that contain a statistically significant number of genes with altered expression are identified. The combination of microarray analysis with extensive pathway filtering of identified genes is increasingly considered a state-of-the-art approach, which should guide future experimental validation. Without being bound by theory, synergistic effects of drug cocktails whose components are aimed at various targets in these pathways can work at lower individual doses, with less toxicity, and in more patients, than drugs that target only one of a pathway's many gene components. The interface between bariatric surgery and basic science may prove to be the optimal place to carry out this critically important translational research.

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Example 6 – SPEXIN DOSE RESPONSE EXPERIMENT IN C57BL/6J DIO MICE

[00262] The effect of Spexin on weight loss will be investigated by daily IP injections of Spexin (about 3 µg/kg/day, about 10 µg/kg/day, about 30 µg/kg/day, and about 100 µg/kg/day) in Diet-Induced-Obesity (DIO) mice, where all of the known signaling systems are intact. Both normal weight and DIO mice will be tested for circulating levels of Leptin, insulin, and Spexin.

[00263] Pharmaceutical grade Spexin (a 14 amino acid peptide normally circulating in mice and humans) has been synthesized by conventional solid-state chemistry, and purified to >95% identity by Phoenix Pharmaceuticals (Burlingame, CA). This agent will be solubilized in 1X sterile saline (pH 7.4) at four different concentrations, so that doses of 10 µg/kg/day/QD, 50 µg/kg/day/QD, 250 µg/kg/day/QD, or 500 µg/kg/day/QD can be administered to 5 mice in each

dose group. Final total doses of 10 ng/day, 50 ng/day, 250 ng/day, or 500 ng/day, will be delivered in volumes of 0.2 mls/dose/day via IP injections. For example, to deliver 10 ng total per day, two daily injections of 5 ng each will be administered IP (stock concentrations of Spexin will be 25 ng/ml). Alternatively, to deliver 10 ng per day, a daily injection of 10 ng can also be administered IP (stock concentrations of Spexin will be 50 ng/ml). The doses are calculated to result in physiologic levels of Spexin with some latitude introduced to allow for the rough estimates based on the current data available and the preliminary characterization of Spexin's basic pharmacologic parameters.

[00264] C57BL/6J DIO mice from Jackson Labs, whose average weight is approximately 30% greater than their age-matched controls at 17 weeks, will be acclimated to laboratory conditions prior to initiating the Spexin dosing studies.

[00265] Experimental Outlines

[00266] *Spexin Dose-Response Experiment.* The objective of this experiment will be to determine dose(s) of Spexin to be used in subsequent studies. The effects of Spexin will be followed by daily monitoring of body weights, food and water consumption, and their plasma will be assayed for Leptin, insulin and Spexin periodically.

Mouse Strain	Number
DIO/60% HFD (untouched neg controls)	5
DIO/60% HFD (saline injection neg controls)	5
DIO/60% HFD (5 animals @ at each of 4 different doses) (doses = 10, 50, 250, and 500 ngs Spexin/ injection/QD)	20
C57BL/6J (saline injection neg controls)	5
C57BL/6J (Spexin at 30 ngs/injection/QD)	5
Total	40

[00267] *Spexin Dosing in Various Murine Models of Obesity.*

Mouse Strain	Saline	Spexin	Leptin (as + control)
C57BL/6J background as controls	6	6	
DIO/60% HFD	6	6	
ob/ob	6	6	
ob/ob treated with leptin as positive control			6
db/db	6	6	
Fat	6	6	
Tubby	6	6	
Additional animals for dose- adjusted experiments	6	6	
sub-totals	42	42	6
Total	90		

Grand total = 130 animals

[00268] The studies described herein will be expanded by measuring and/or analyzing Spexin and its actions on the hypothalamus via traditional neuroscience techniques, such as immunohistochemistry, and by functional imaging, such as PET scans, of living animals in the absence or presence Spexin, as well as in the absence or presence Spexin under a variety of physiological conditions.

Example 7 – SPEXIN EXPERIMENT IN C57BL/6J DIO MICE

[00269] Obesity has reached epidemic proportions in adults and children in the US, and is linked to a myriad of co-morbidities, including insulin resistance, the metabolic syndrome, type 2 diabetes, and fatty liver disease. Whole human genome microarrays were used to identify individual genes and biological pathways whose expression is altered in omental and subcutaneous fat samples from obese patients. The gene with the most dramatic under expression in obese fat was Spexin, a recently identified peptide of unknown function, the expression of which has never been reported in fat. The data indicate that Spexin message is highly expressed in omental and subcutaneous fat from normal weight human subjects, but is dramatically reduced (~15-fold) in obese fat samples ($p < 0.00292$). Serum Spexin peptide levels

are approximately 10X lower in obese than in non-obese patients ($p < 0.0002$), and Spexin and Leptin levels in the serum of obese patients and normal weight controls demonstrate a strong negative correlation ($r = -0.92$). These results in human patients and parallel findings in high fat diet-fed (HFD) obese mice indicate that Spexin plays a role in the regulation of food consumption, energy metabolism and body weight.

[00270] Spexin was administered at 10 $\mu\text{g/kg}$ body weight/ day IP to obese HFD-fed C57BL/6J mice. The injections reduced food consumption and body weight in mice with HFD-induced obesity, while the corresponding control HFD-mice, receiving only vehicle, continued to gain weight. It was previously shown that regulation of adipocyte LCFA uptake is an important control point for body adiposity, Spexin was subsequently administered for 7 weeks at a dose to maintain body weight in obese HFD-fed mice (**FIG. 19**).

What is claimed is:

1. A method for treating obesity or an obesity-associated disorder in a subject, the method comprising administering to a subject an effective amount of Spexin, thereby treating obesity or an obesity-associated disorder in the subject.
2. A method for treating obesity or an obesity-associated disorder in a subject, the method comprising administering to a subject in need thereof a polypeptide comprising SEQ ID NO: 1, or a pharmaceutically acceptable salt thereof, thereby treating obesity or an obesity-associated disorder in the subject.
3. A method for promoting satiety in an obese subject or a subject afflicted with an obesity-associated disorder, the method comprising administering to a subject an effective amount of Spexin, thereby promoting satiety in an obese subject.
4. A method for promoting satiety in an obese subject or a subject afflicted with an obesity-associated disorder, the method comprising administering to a subject in need thereof a polypeptide comprising SEQ ID NO: 1, or a pharmaceutically acceptable salt thereof, thereby promoting satiety in an obese subject.
5. A method of promoting weight loss in an obese subject or a subject afflicted with an obesity-associated disorder, the method comprising:
 - a) administering to a subject an effective amount of Spexin; and
 - b) determining whether Spexin decreased the subject's body mass as compared to the subject's body mass prior to treatment with Spexin,

thereby promoting weight loss in the obese subject or the subject afflicted with an obesity-associated disorder.

6. A method of promoting weight loss in an obese subject or a subject afflicted with an obesity-associated disorder, the method comprising:

- a) administering to a subject in need thereof a therapeutic amount of a polypeptide comprising SEQ ID NO: 1 or a pharmaceutically acceptable salt thereof; and
- b) determining whether the polypeptide comprising SEQ ID NO: 1 decreased the subject's body mass as compared to the subject's body mass prior to treatment with polypeptide comprising SEQ ID NO: 1,

thereby promoting weight loss in the obese subject or the subject afflicted with an obesity-associated disorder.

7. A method of decreasing serum Leptin levels in an obese subject or a subject afflicted with an obesity-associated disorder, the method comprising:

- a) administering to a subject an effective amount of Spexin; and
- b) determining whether Spexin decreased the subject's serum Leptin levels as compared to the subject's serum Leptin levels prior to treatment with Spexin,

thereby decreasing serum Leptin levels in the obese subject or the subject afflicted with an obesity-associated disorder.

8. A method of decreasing serum Leptin levels in an obese subject or a subject afflicted with an obesity-associated disorder, the method comprising:
 - a) administering to a subject in need thereof a therapeutic amount of a polypeptide comprising SEQ ID NO: 1 or a pharmaceutically acceptable salt thereof; and
 - b) determining whether the polypeptide comprising SEQ ID NO: 1 decreased the subject's serum Leptin levels as compared to the subject's serum Leptin levels prior to treatment with polypeptide comprising SEQ ID NO: 1,thereby decreasing serum Leptin levels in the obese subject or the subject afflicted with an obesity-associated disorder.
9. The method of any one of claims 1-8, wherein the subject is a human or non-human animal.
10. The method of claim 9, wherein the non-human animal is a mouse, rat, dog, or cat.
11. The method of any one of claims 1-8, wherein the obesity-associated disorder comprises a metabolic disorder, hypertension, congestive heart failure, a lipid related disorder, type II diabetes mellitus, gallbladder disease, osteoarthritis, sleep apnea, cancer, polycystic ovary syndrome (PCOS), or a combination thereof.

12. The method of claim 11, wherein the metabolic disorder comprises hyperglycemia, insulin resistance, hyperinsulinemia, metabolic syndrome, or a combination thereof.
13. The method of claim 11, wherein the lipid related disorder comprises atherosclerosis, HIV lipodystrophy, coronary heart disease, dyslipidemia, or a combination thereof.
14. The method of claim 11, wherein the cancer comprises endometrial cancer, breast cancer, colon cancer, or a combination thereof.
15. The method of any one of claims 1-8, wherein the subject has a Body Mass Index (BMI) greater than about 25 kg/m².
16. The method of any one of claims 1-8, wherein the subject has a Body Mass Index (BMI) greater than about 30 kg/m².
17. The method of any one of claims 1-8, wherein the subject has a Body Mass Index (BMI) greater than about 35 kg/m².
18. The method of any one of claims 1, 3, 5, or 7, wherein the subject displays a decrease in adipose tissue mass after treatment with Spexin.
19. The method of claims 1, 3, 5, or 7, wherein the amount administered results in at least about 1 ng/ml in the serum.
20. The method of claims 1, 3, 5, or 7, wherein the amount administered results in at least about 3 ng/ml in the serum.

21. The method of claims 1, 3, 5, or 7, wherein the amount administered results in at least about 10 ng/ml in the serum.
22. The method of claims 1, 3, 5, or 7, wherein the amount administered results in at least about 30 ng/ml in the serum.
23. The method of claims 1, 3, 5, or 7, wherein the amount administered results in at least about 100 ng/ml in the serum.
24. The method of claims 1, 3, 5, or 7, wherein the amount administered results in at least about 250 ng/ml in the serum.
25. The method of claims 1, 3, 5, or 7, wherein the amount administered results in at least about 500 ng/ml in the serum.
26. The method of claims 1, 3, 5, or 7, wherein Spexin is administered at least once daily or at least twice daily.
27. The method of claims 1, 3, 5, or 7, wherein Spexin is administered for at least 1 week, for at least 2 weeks, for at least 3 weeks, for at least 4 weeks, for at least 5 weeks, for at least 6 weeks, for at least 8 weeks, for at least 10 weeks, for at least 12 weeks, for at least 24 weeks, or for at least 48 weeks.
28. The method of claims 1, 3, 5, or 7, wherein Spexin is administered for at least 1 year, for at least 1.5 years, for at least 2 years, for at least 2.5 years, or for at least 5 years.

29. A method for detecting the presence of or a predisposition to obesity or an obesity-associated disorder in a human subject, the method comprising:
- (a) obtaining a biological sample from a subject; and
 - (b) detecting whether or not there is an alteration in the expression of a Obesity-Signature (OS) gene in the subject as compared to a subject not afflicted with obesity or an obesity-associated disorder.
30. The method of claim 29, wherein the Obesity-Signature (OS) gene comprises any gene indicated as being up-regulated in any one of Tables 7-8, or a combination thereof.
31. The method of claim 29, wherein the Obesity-Signature (OS) gene comprises Spexin, any gene indicated as being down-regulated in any one of Tables 7-8, or a combination thereof.
32. The method of claim 29, wherein the detecting comprises detecting in the sample whether there is an increase in a OS mRNA, a OS polypeptide, or a combination thereof.
33. The method of claim 29, wherein the detecting comprises detecting in the sample whether there is a decrease in a OS mRNA, a OS polypeptide, or a combination thereof.
34. The method of claim 29, wherein the obesity-associated disorder comprises a metabolic disorder, hypertension, congestive heart failure, a lipid related disorder,

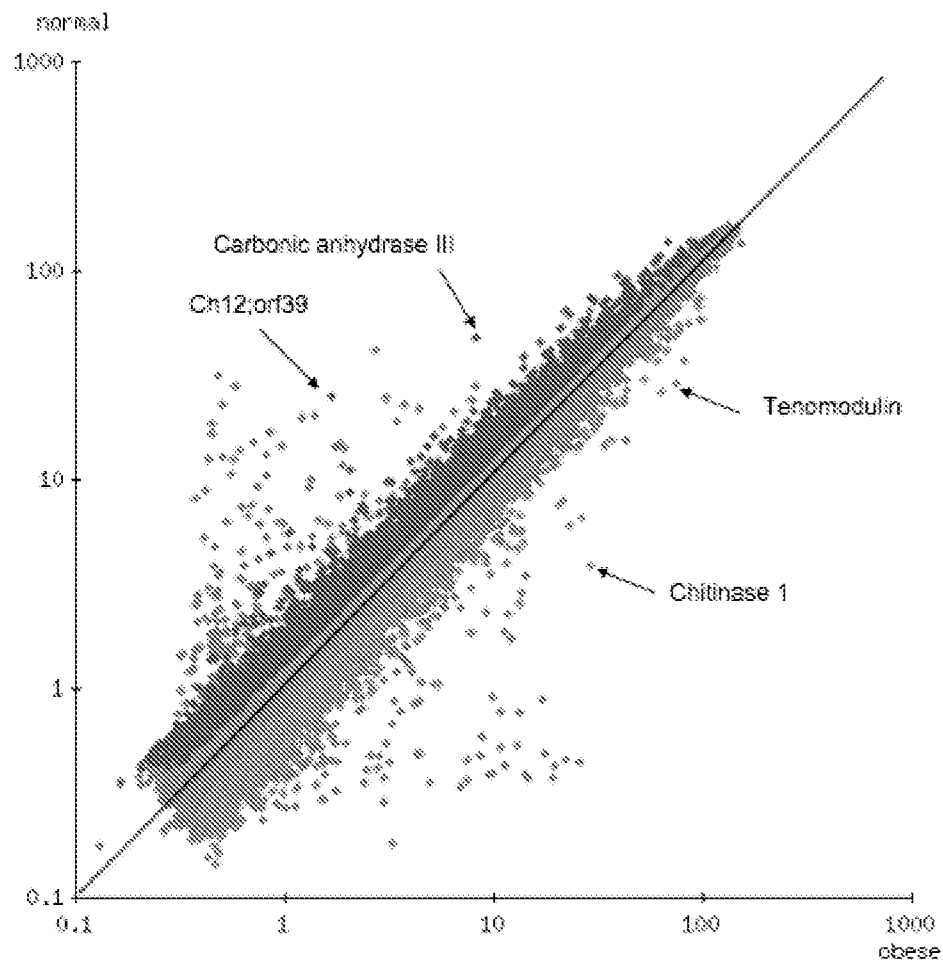
type II diabetes mellitus, gallbladder disease, osteoarthritis, sleep apnea, cancer, polycystic ovary syndrome (PCOS), or a combination thereof.

35. The method of claim 34, wherein the metabolic disorder comprises hyperglycemia, insulin resistance, hyperinsulinemia, metabolic syndrome, or a combination thereof.
36. The method of claim 34, wherein the lipid related disorder comprises atherosclerosis, coronary heart disease, dyslipidemia, HIV lipidodystrophy, or a combination thereof.
37. The method of claim 34, wherein the cancer comprises endometrial cancer, breast cancer, colon cancer, or a combination thereof.
38. The method of claim 29, wherein the detecting comprises determining in the sample whether expression of at least 2 OS genes, at least 3 OS genes, at least 4 OS genes, at least 5 OS genes, at least 6 OS genes, at least 7 OS genes, at least 8 OS genes, at least 9 OS genes, or at least 10 OS genes indicated as being down-regulated in any one of Tables 7-8, Spexin, or a combination thereof, are decreased as compared to expression in a normal sample.
39. The method of claim 29, wherein the detecting comprises determining in the sample whether expression of at least 2 OS genes, at least 3 OS genes, at least 4 OS genes, at least 5 OS genes, at least 6 OS genes, at least 7 OS genes, at least 8 OS genes, at least 9 OS genes, or at least 10 OS genes indicated as being up-regulated in any one of Tables 7-8, or a combination thereof, are increased as compared to expression in a normal sample.

40. The method of claim 29, wherein the detecting comprises gene sequencing, selective hybridization, selective amplification, gene expression analysis, or a combination thereof.
41. The method of claim 29, wherein the sample comprises subcutaneous adipose tissue, omental adipose tissue, whole blood, plasma, serum, white blood cells, or mesenteric adipose tissue.
42. A diagnostic kit for determining whether a sample from a subject exhibits increased or decreased expression of at least 2 or more OS genes, the kit comprising nucleic acid primers that specifically hybridize to a OS gene, wherein the primer will prime a polymerase reaction only when a OS gene in any one of Tables 7-8, Spexin, or a combination thereof, is present.
43. A method for identifying a Spexin-modulating compound useful for treating obesity or an obesity-associated disorder in a subject, the method comprising:
- (a) administering a test agent to a mouse model of obesity or an obesity-associated disorder; and
 - (b) determining whether the test agent altered serum Spexin levels, serum Leptin levels, a body-weight phenotype, or a combination thereof, as compared to a mouse in the absence of the test agent,
- thereby identifying a compound useful for treating obesity or an obesity-associated disorder.

44. The method of claim 43, wherein the serum Spexin levels are increased.
45. The method of claim 43, wherein the serum Leptin levels are decreased.
46. The method of claim 43, wherein the body-weight phenotype is a decrease in adipose tissue mass.
47. The method of claim 43, wherein the test agent comprises a polynucleotide, small organic molecule, a small inorganic molecule, a soluble peptide, or an antibody.
48. The method of claim 47, wherein the antibody specifically binds to a Spexin protein or a fragment thereof.
49. The method of claim 47, wherein the polynucleotide is an antisense RNA that specifically inhibits expression of a Spexin gene that encodes a Spexin protein.
50. The method of claim 47, wherein the polynucleotide is an siRNA that specifically targets a Spexin gene that encodes a Spexin protein.

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**FIG. 1**

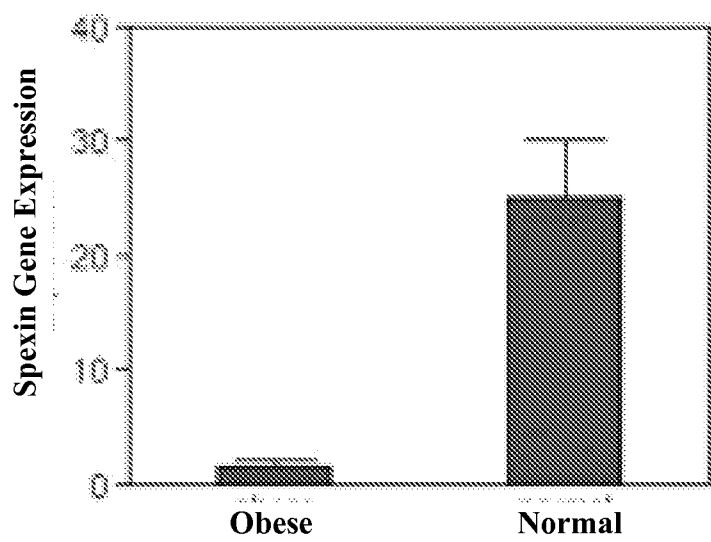
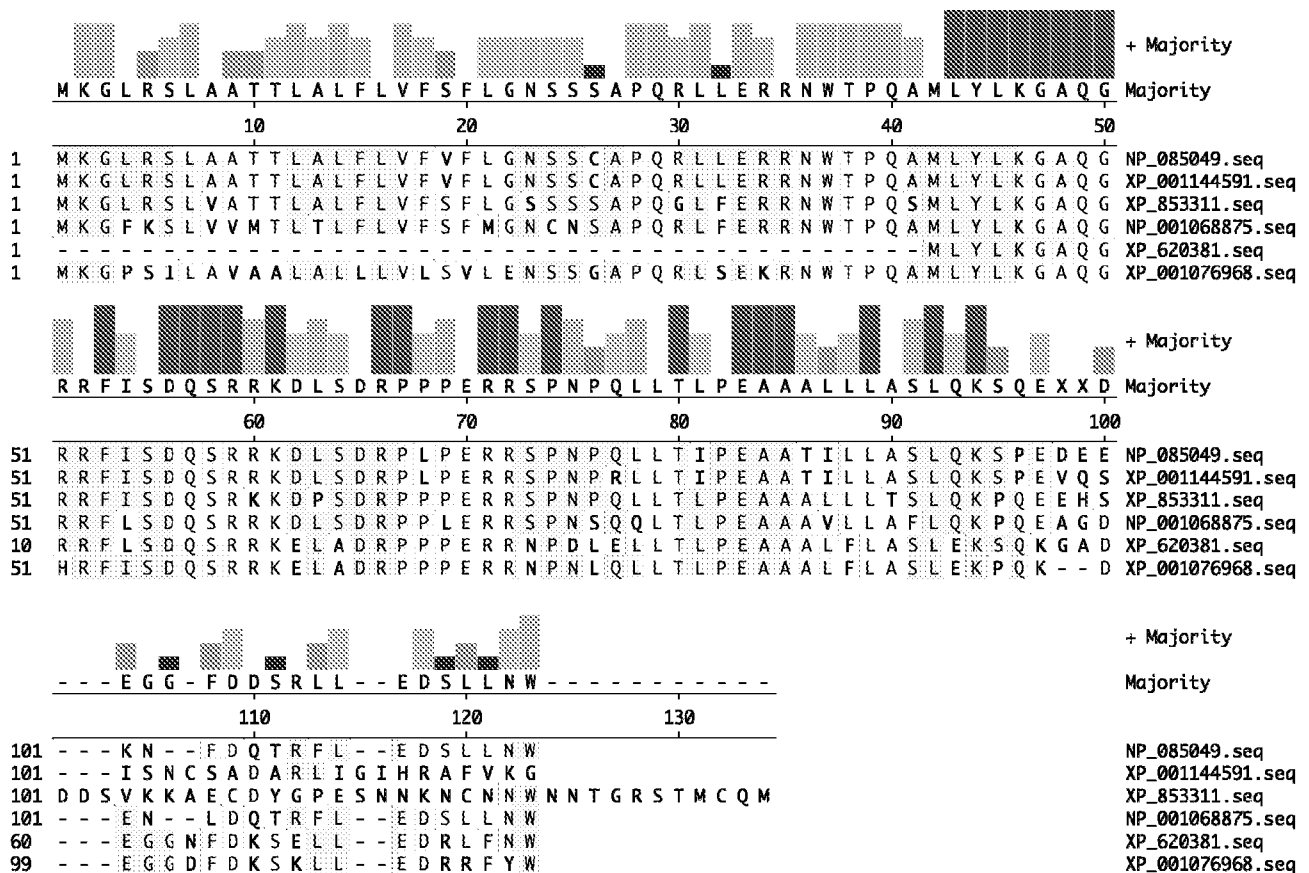
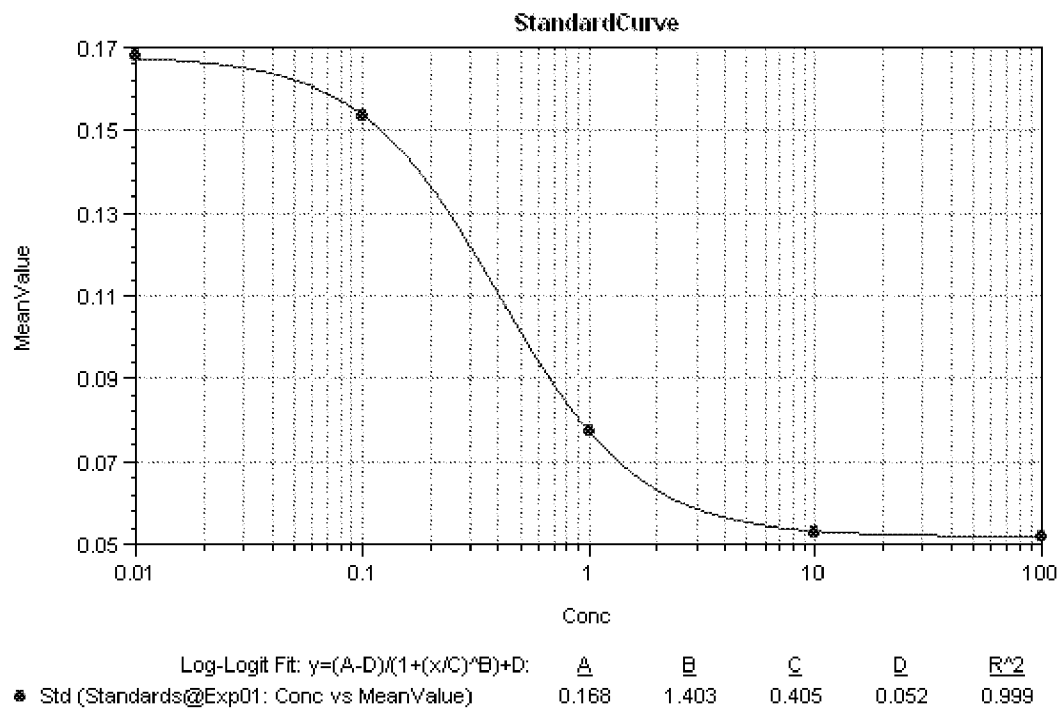
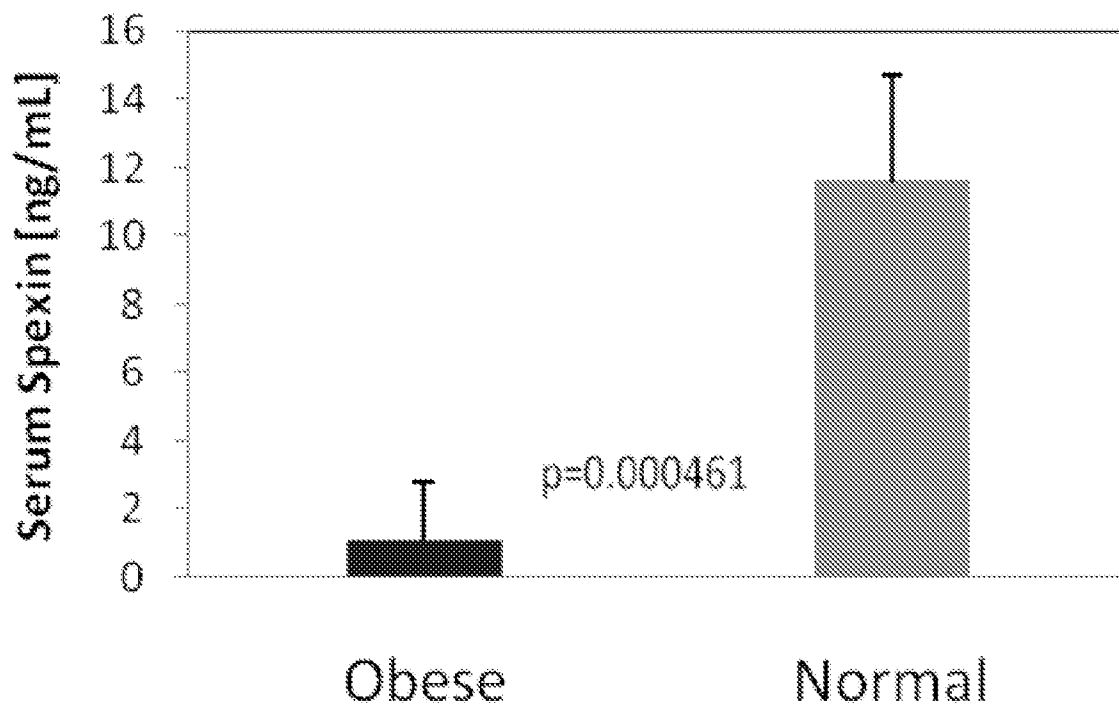


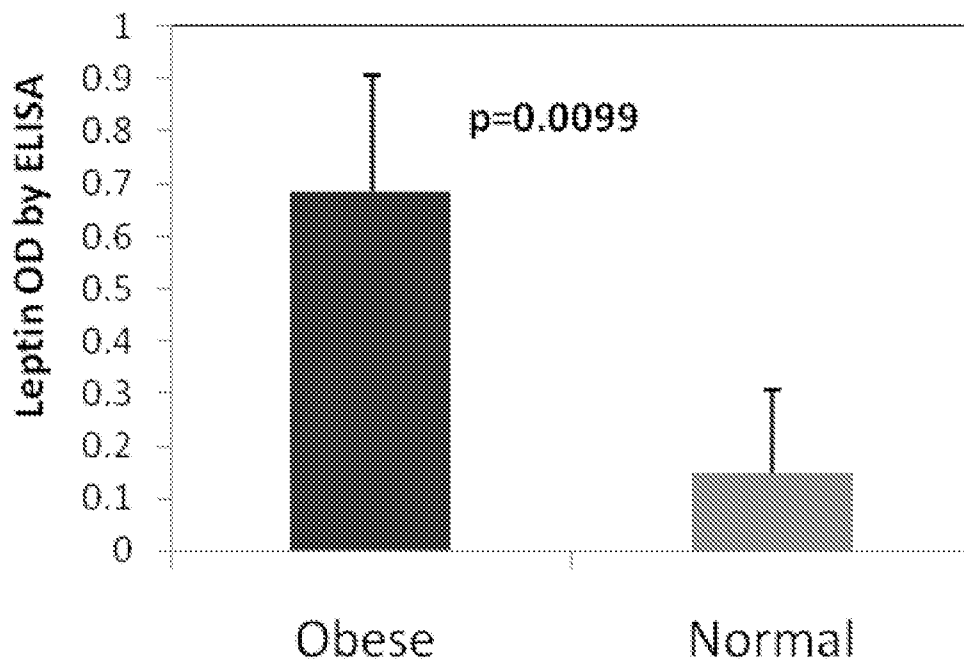
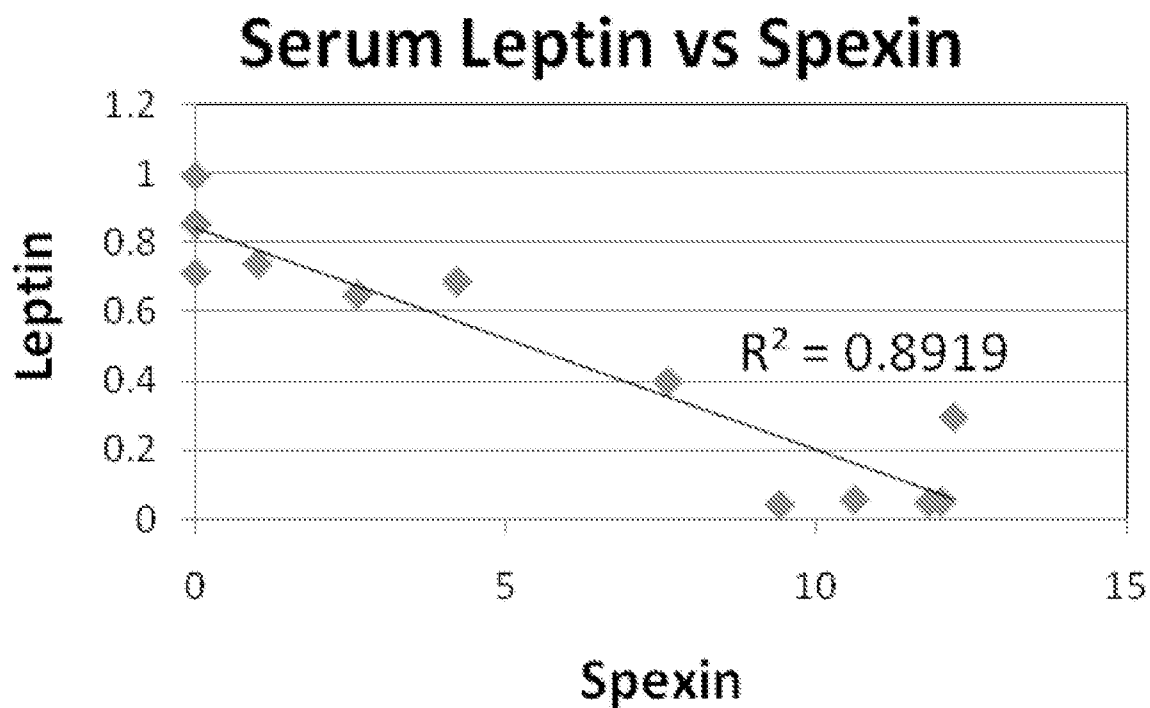
FIG. 2



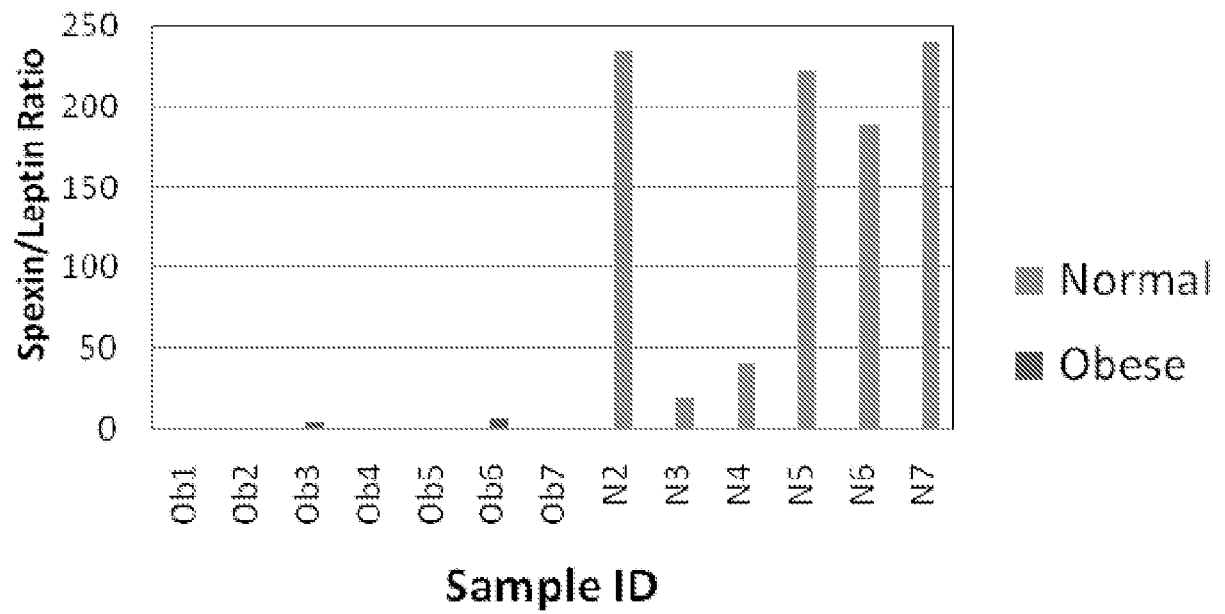
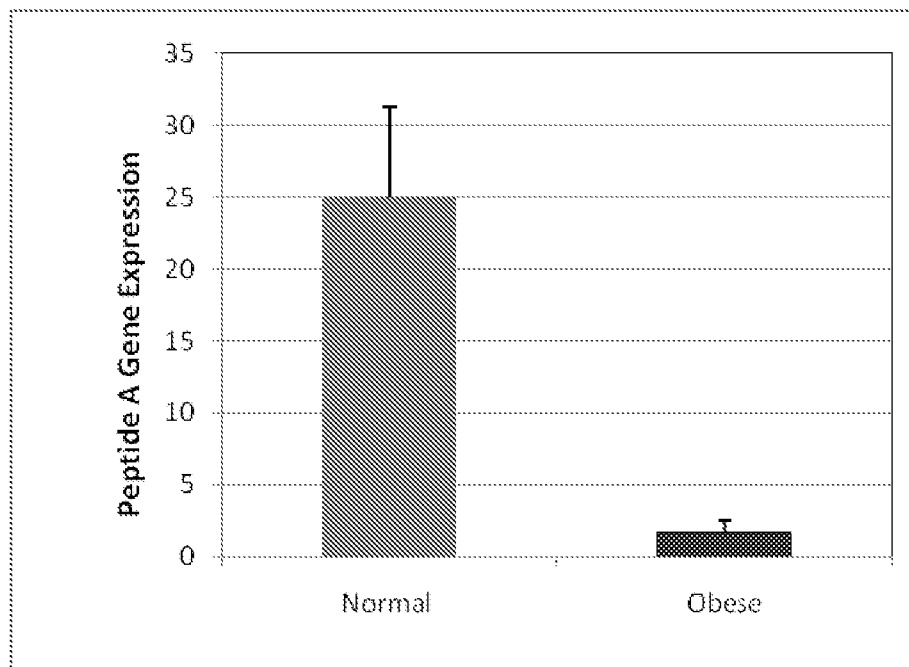
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**FIG. 4****FIG. 5**

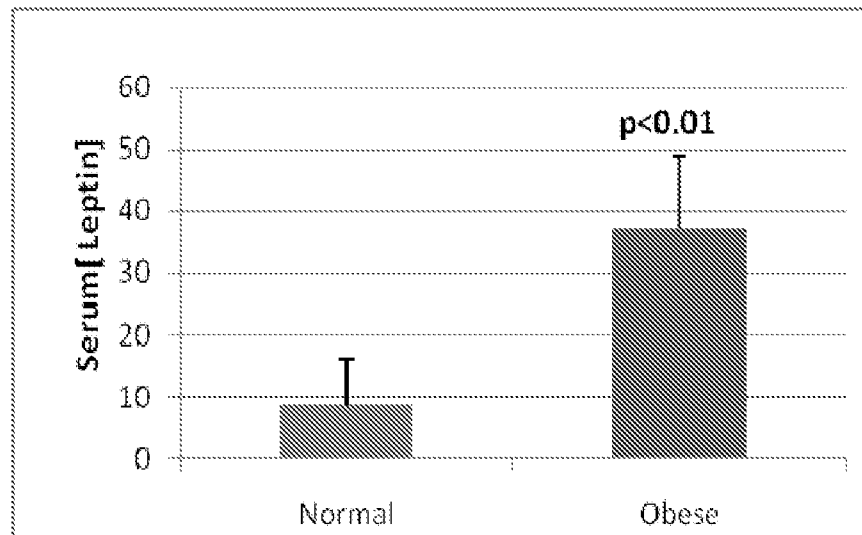
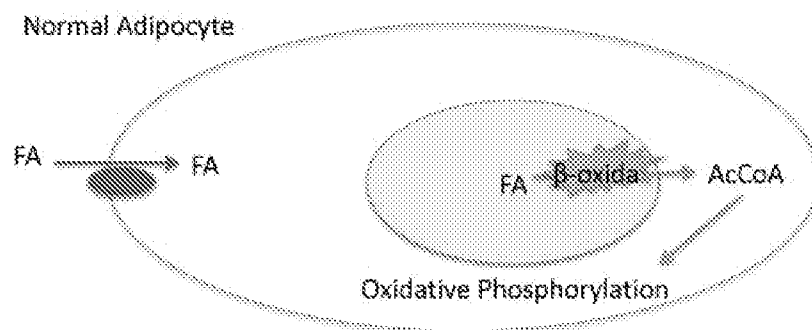
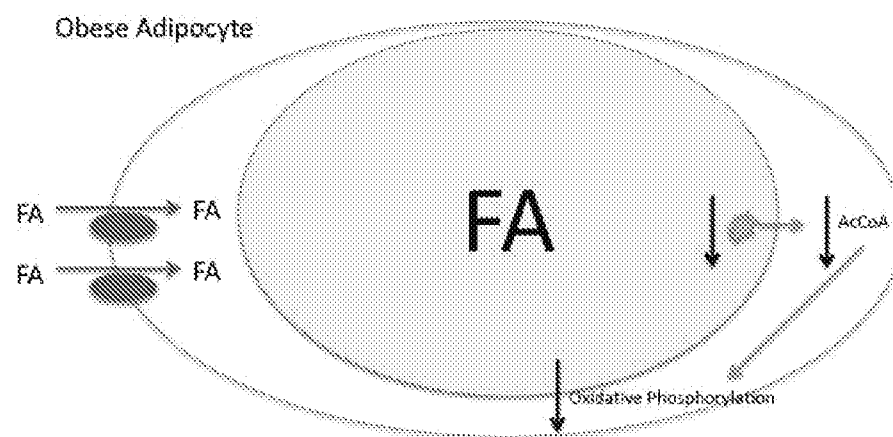
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**FIG. 6****FIG. 7**

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**FIG. 8****FIG. 9**

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**FIG. 10****A****B****FIG. 11**

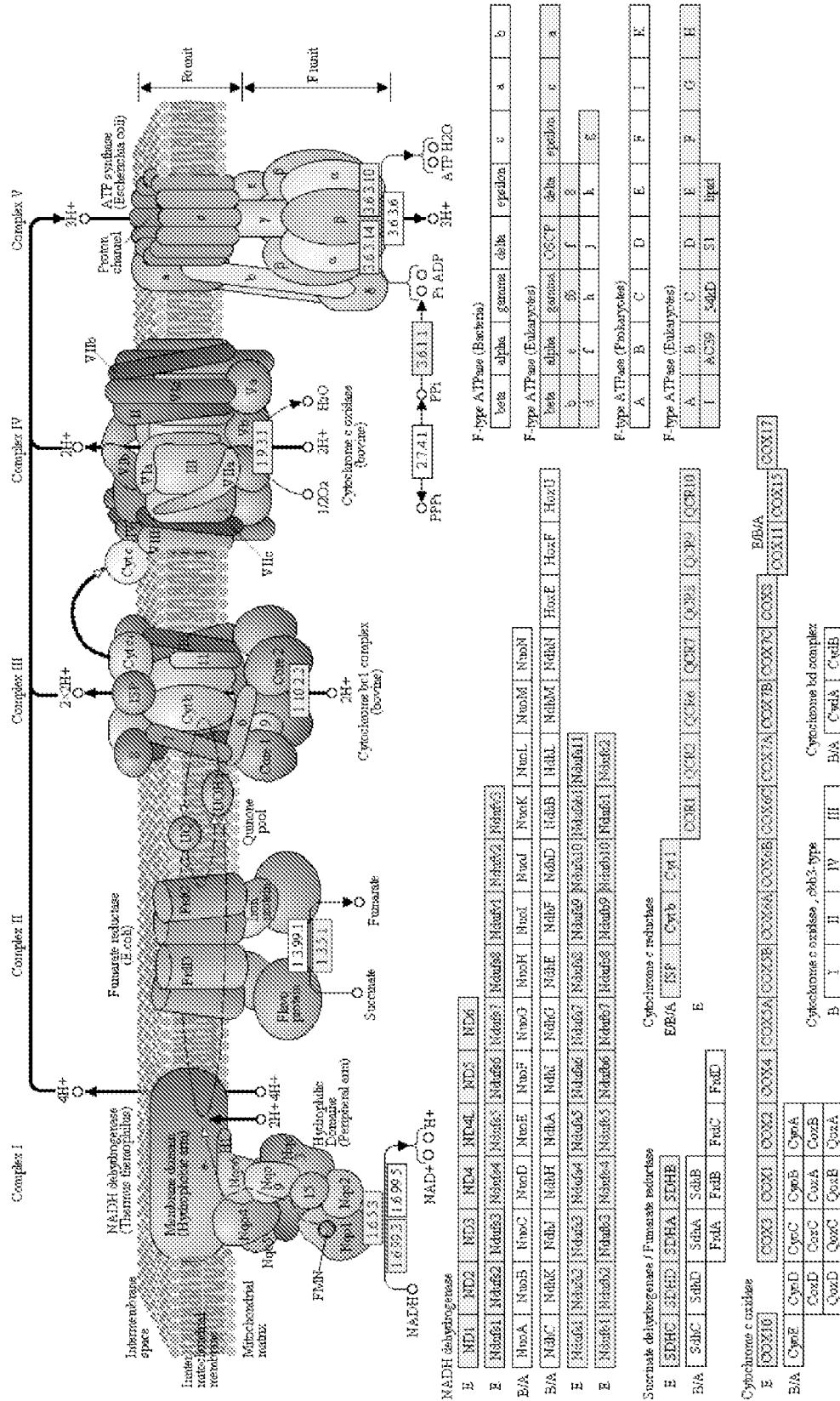
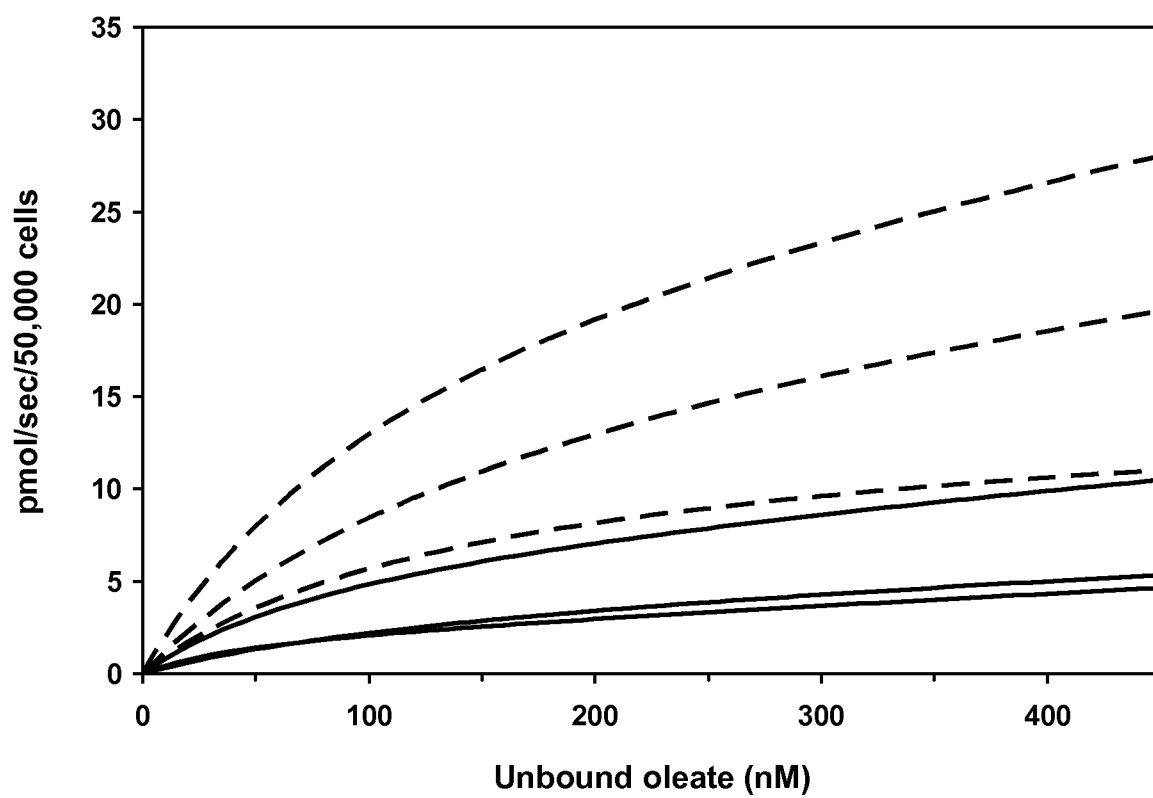


FIG. 12

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**FIG. 13**

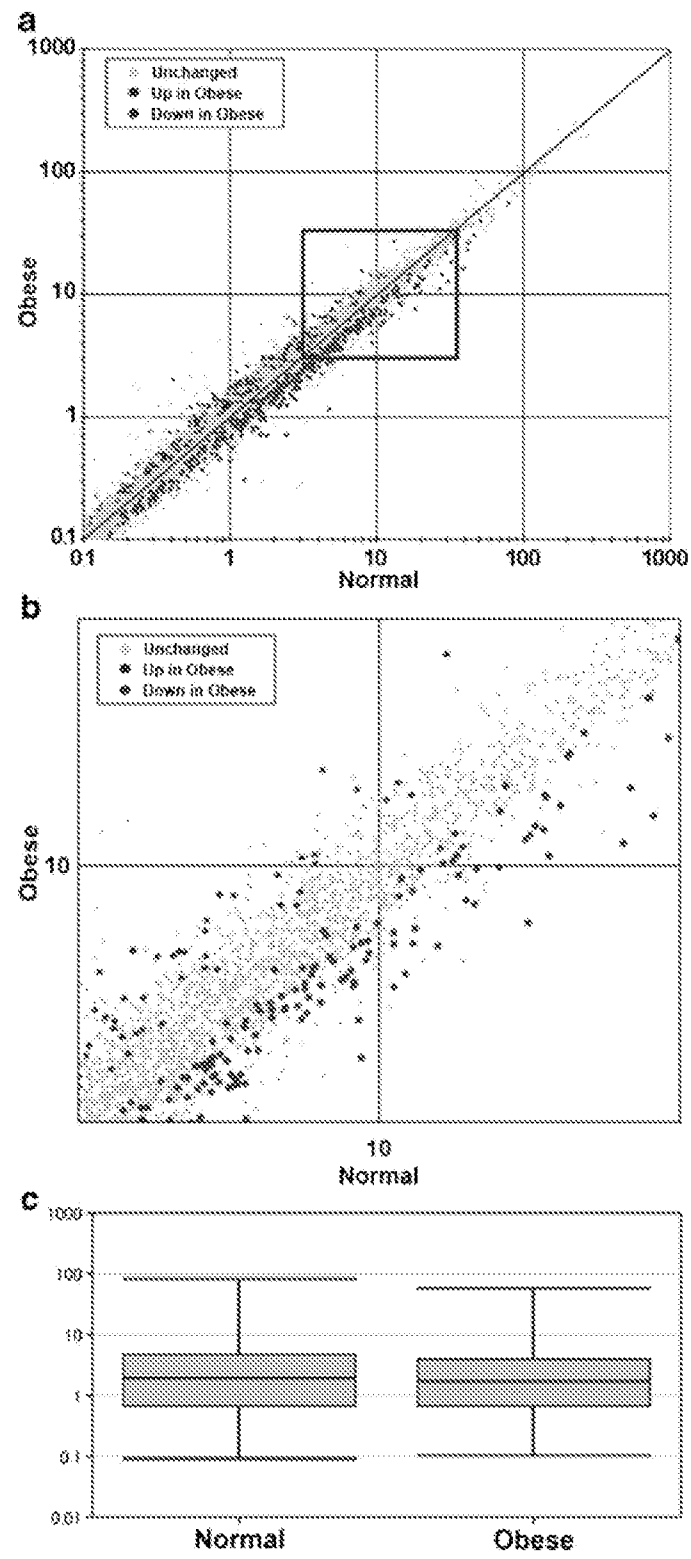
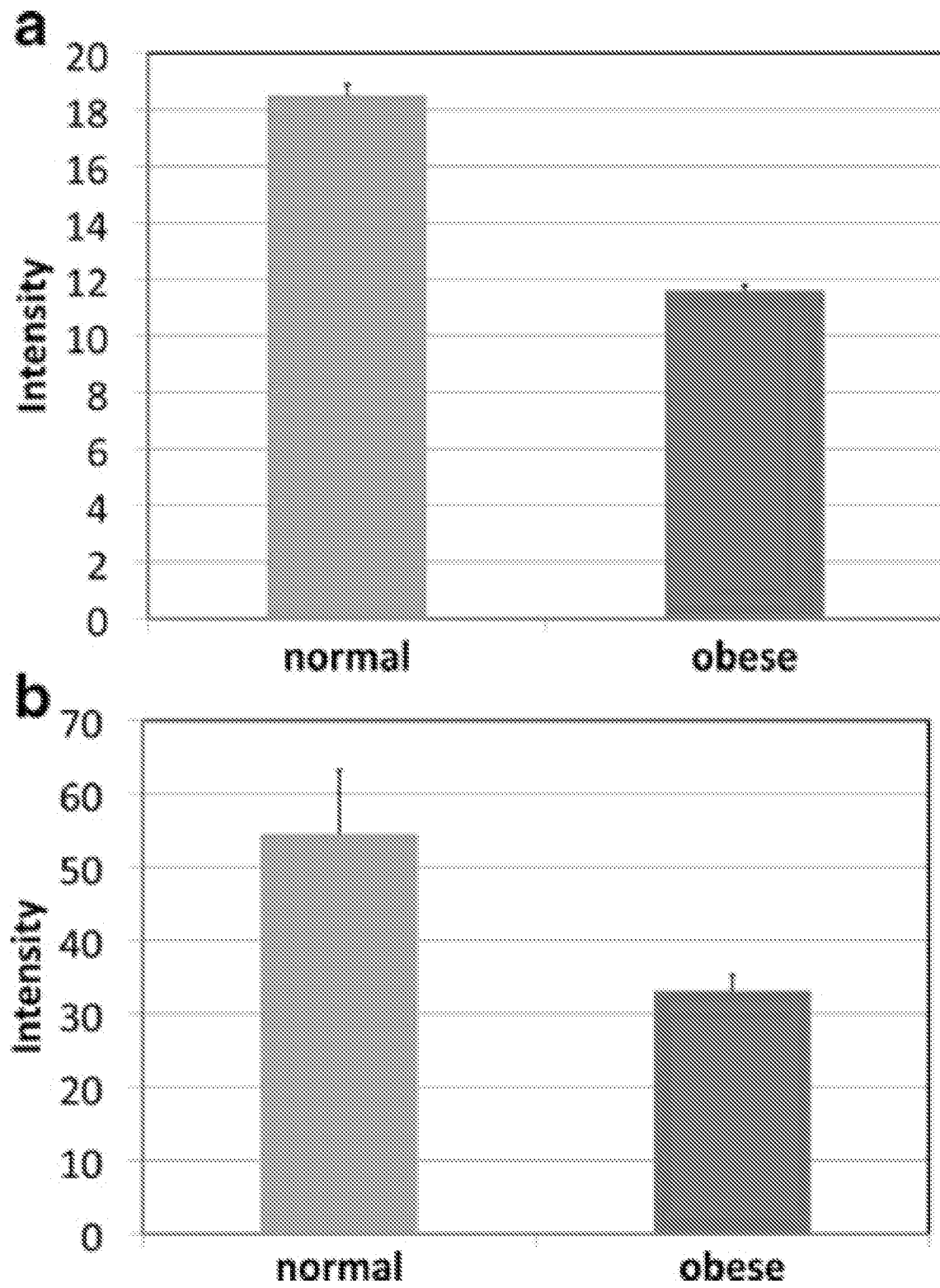
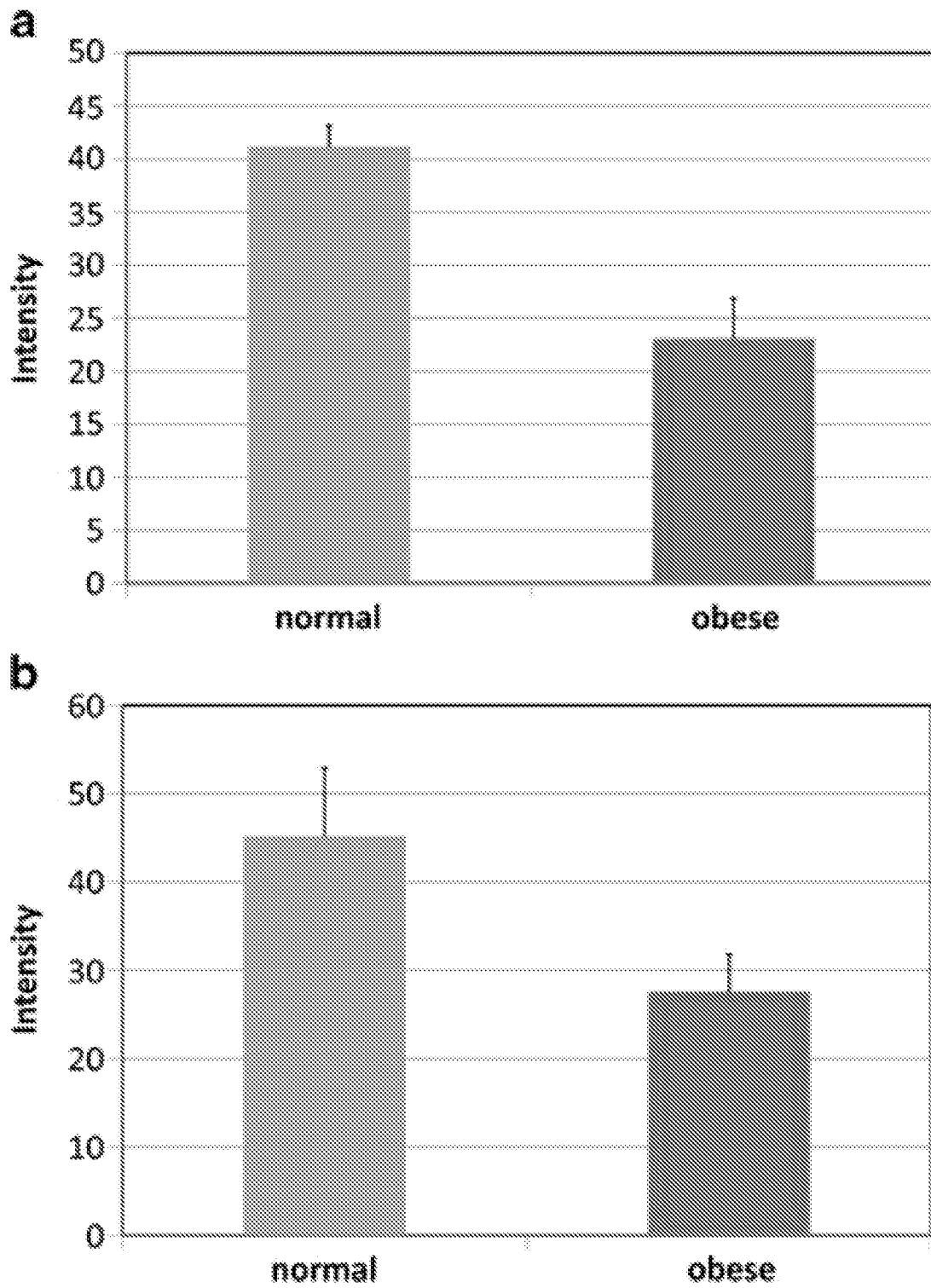


FIG. 14

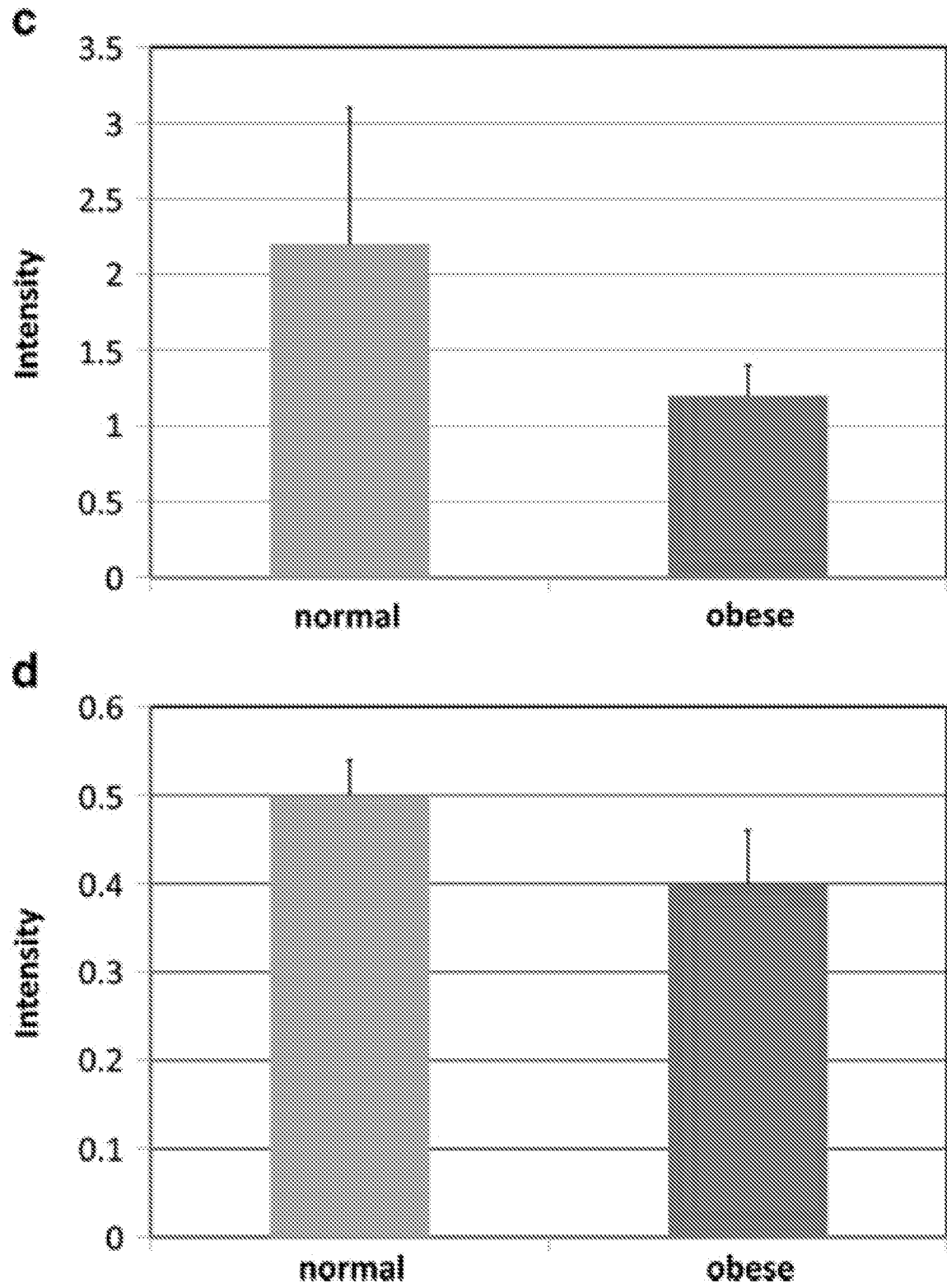
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**FIG. 15**

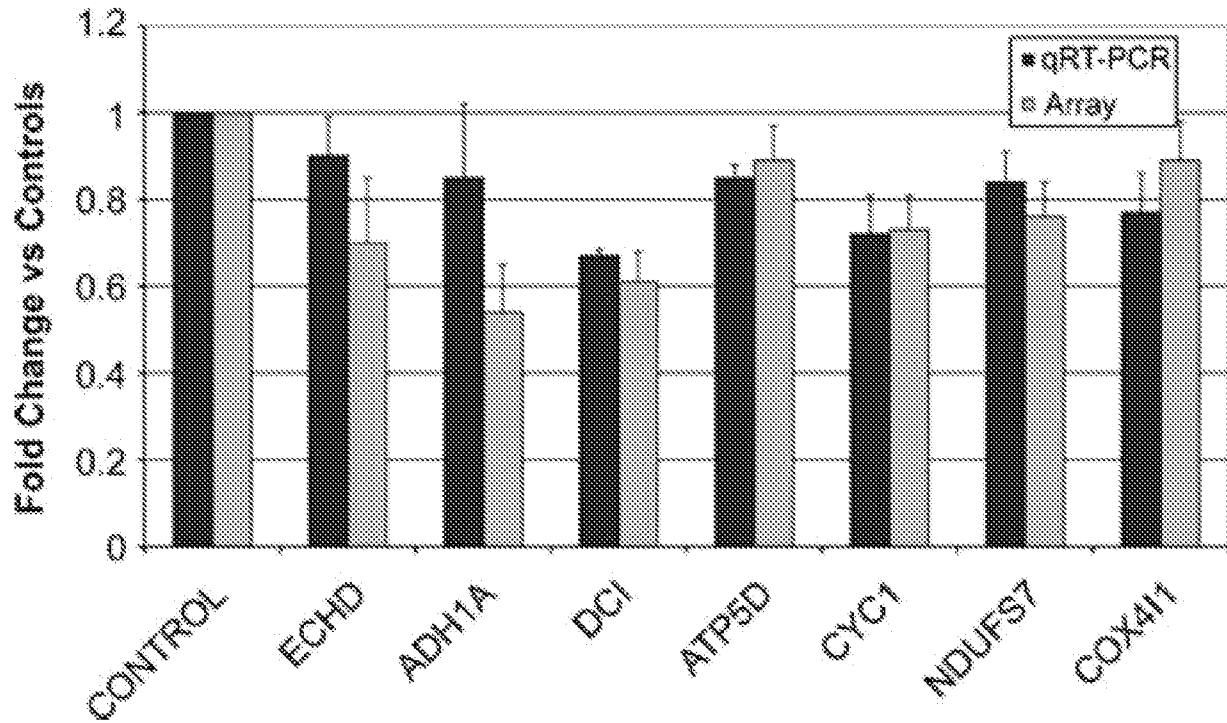
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**FIGS. 16A-B**

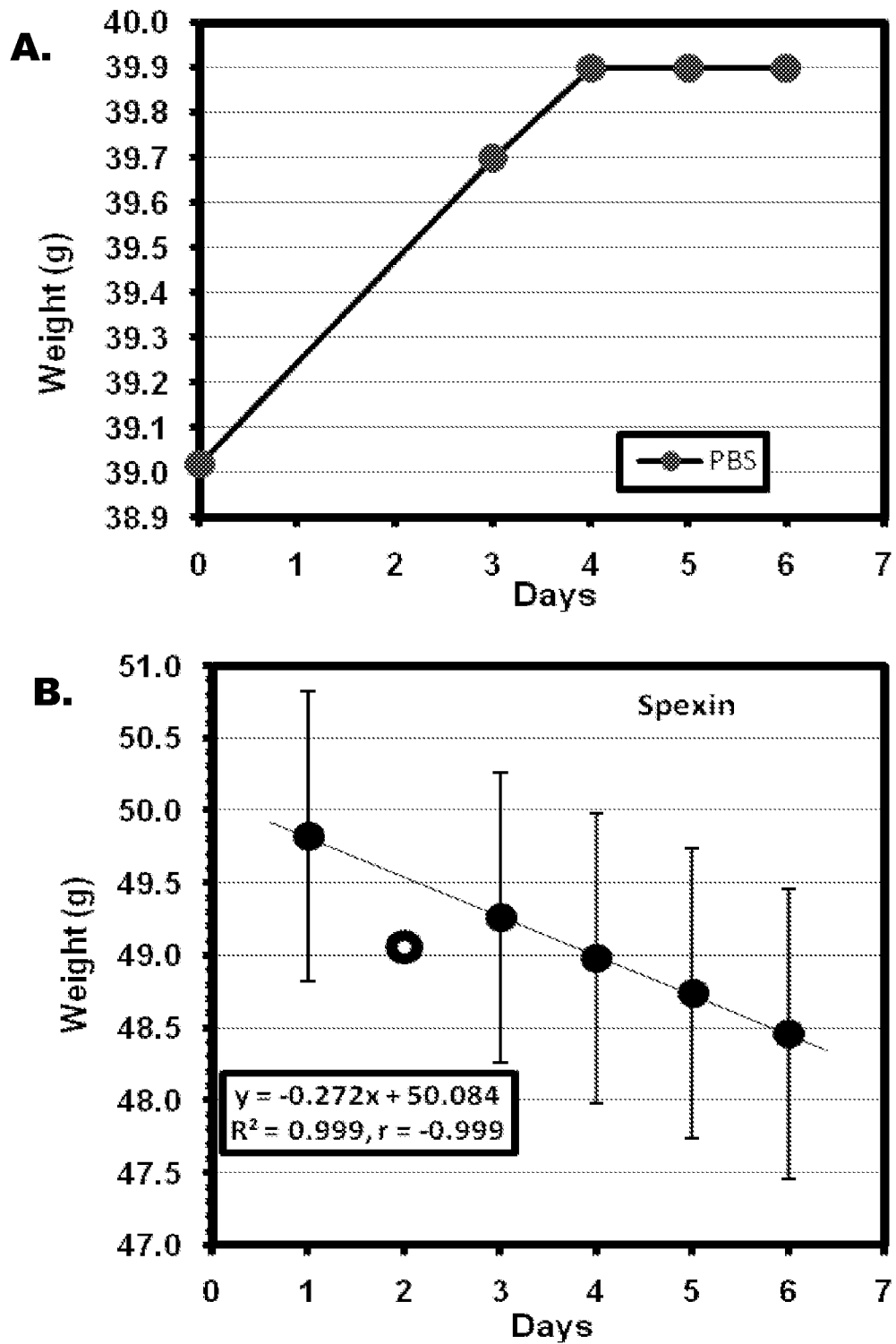
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**FIGS. 16C-D**

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**FIG. 17**

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**FIG. 18**

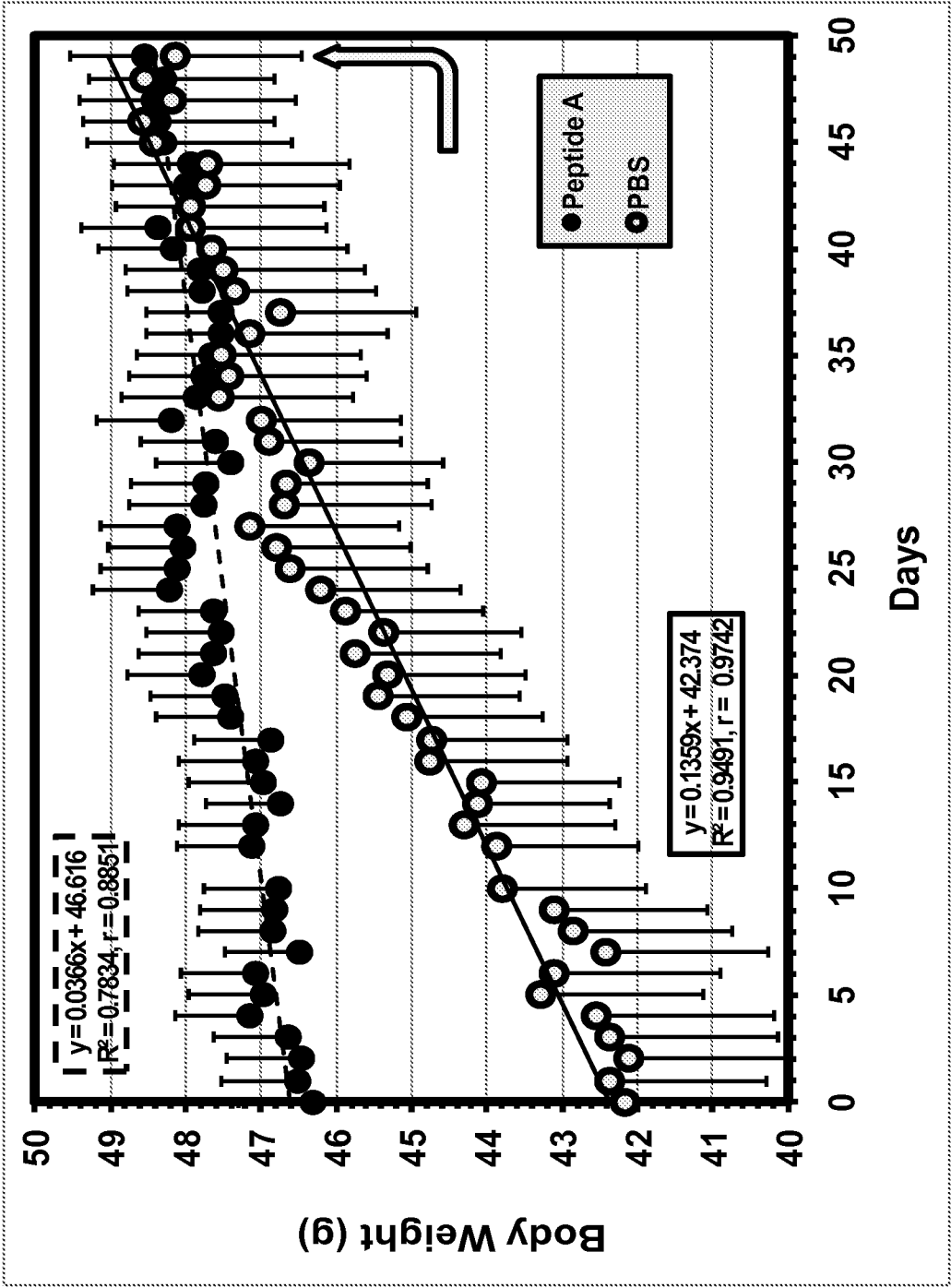


FIG. 19

Human Spexin
mRNA Expression
by Tissue
(UCSD Genome Browser compilation of
Affymetrix data)

Ratios	Absolute
testis seminiferous tubule	
testis interstitial	
testis germ cell	
testis Leydig cell	
testis	
ovary	
placenta	
liver	
fetal liver	
kidney	
lung	
fetal lung	
bronchial epithelial cells	

The U133A and GNF1H chip sets
together complete the Affymetrix
Human array set

Ratios	Absolute	Ratios	Absolute
whole blood		trachea	
BM-CD34+		uterus corpus	
BM-CD105+ endothelial		uterus	
BM-CD33+ myeloid		smooth muscle	
BM-CD71+ early erythroid		tongue	
bone marrow		skeletal muscle	
lymph node		cardiac myocytes	
tonsil		heart	
thymus		atrioventricular node	
dorsal root ganglion		pancreatic islets	
superior cervical ganglion		pancreas	
trigeminal ganglion		salivary gland	
ciliary ganglion		prostate	
spinal cord		adrenal cortex	
medulla oblongata		adrenal gland	
pons		pituitary gland	
olfactory bulb		thyroid	
globus pallidus		fetal thyroid	
caudate nucleus		adipocyte	
subthalamic nucleus		skin	
thalamus		appendix	
hypothalamus		colorectal adenocarcinoma	
amygdala		leukemia chronic myelogenous(k562)	
cerebellum peduncles		lymphoma Burkitts Daudi	
cerebellum		leukemia promyelocytic(hl60)	
cingulate cortex		lymphoma Burkitts Raji	
prefrontal cortex		721 B lymphoblasts	
occipital lobe		leukemia lymphoblastic(molt-4)	
parietal lobe		PB-CD19+ Bcells	
temporal lobe		PB-CD8+ Tcells	
whole brain		PB-CD4+ Tcells	
fetal brain		PB-CD56+ NK Cells	
		PB-CD14+ monocytes	
		PB-BDCA4+ dendritic cells	

FIG. 20

GNF Expression Atlas 2 Data from U133A and GNF1H Chips