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(54) **METHODS AND COMPOSITIONS FOR
MODULATING CARBOHYDRATE
METABOLISM**

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of application No. 09/339,472, filed on Jun. 23, 1999,
now abandoned, and which is a continuation-in-part
of application No. PCT/US98/17883, filed on Aug.
28, 1998, which is a continuation-in-part of applica-
tion No. 09/103,754, filed on Jun. 24, 1998, now Pat.
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(57) **ABSTRACT**

Methods and compositions for modulating carbohydrate metabolism in a host are provided. In the subject methods, diacylglycerol acyltransferase (DGAT) activity (specifically DGAT1 activity) is modulated, e.g., reduced or enhanced, to achieve a desired insulin and/or leptin sensitivity, thereby modulating carbohydrate metabolism, e.g., increasing or decreasing blood glucose levels, glucose uptake into cells and assimilation into glycogen. Also provided are pharmaceutical compositions for practicing the subject methods. The subject methods and compositions find use in a variety of applications, including the treatment of hosts suffering conditions associated with abnormal carbohydrate metabolism, such as obesity or diabetes.

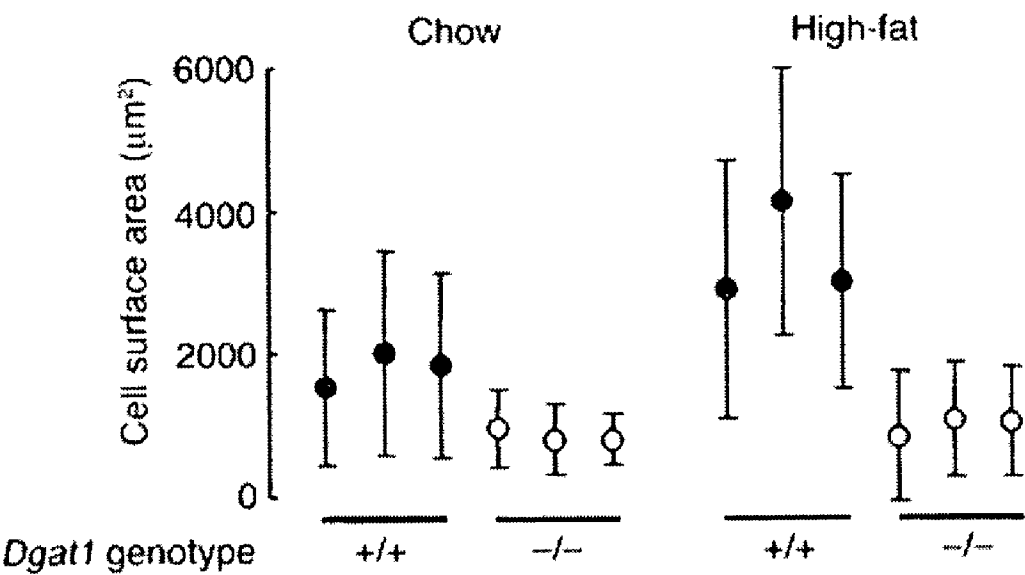
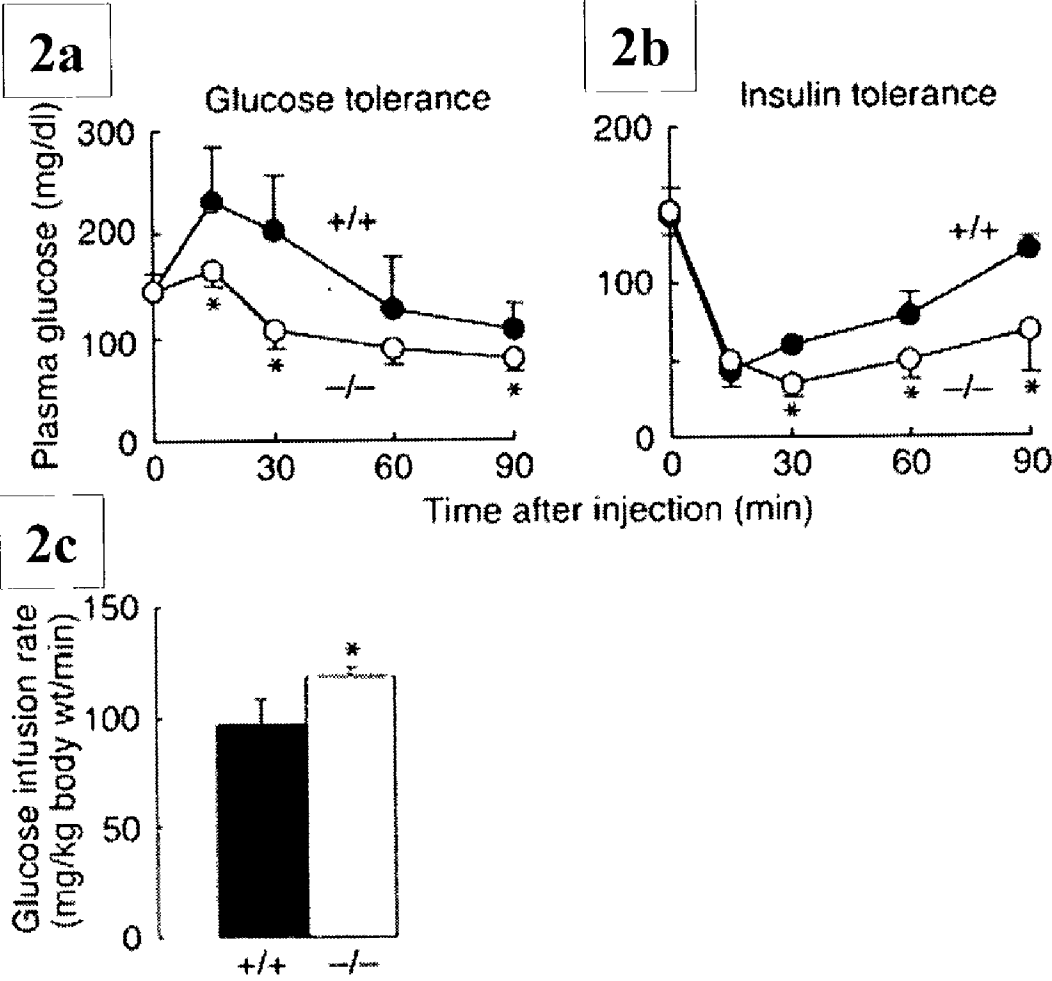
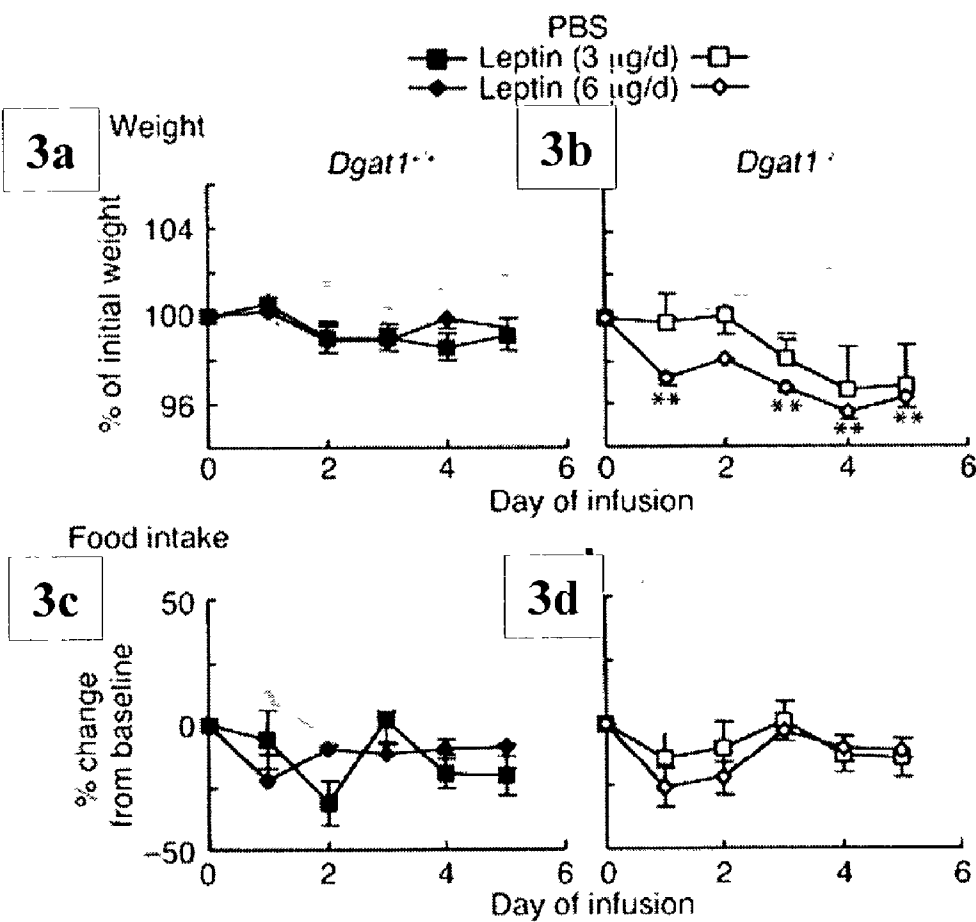


FIG. 1



FIGS. 2a-2c



FIGS. 3a-3d

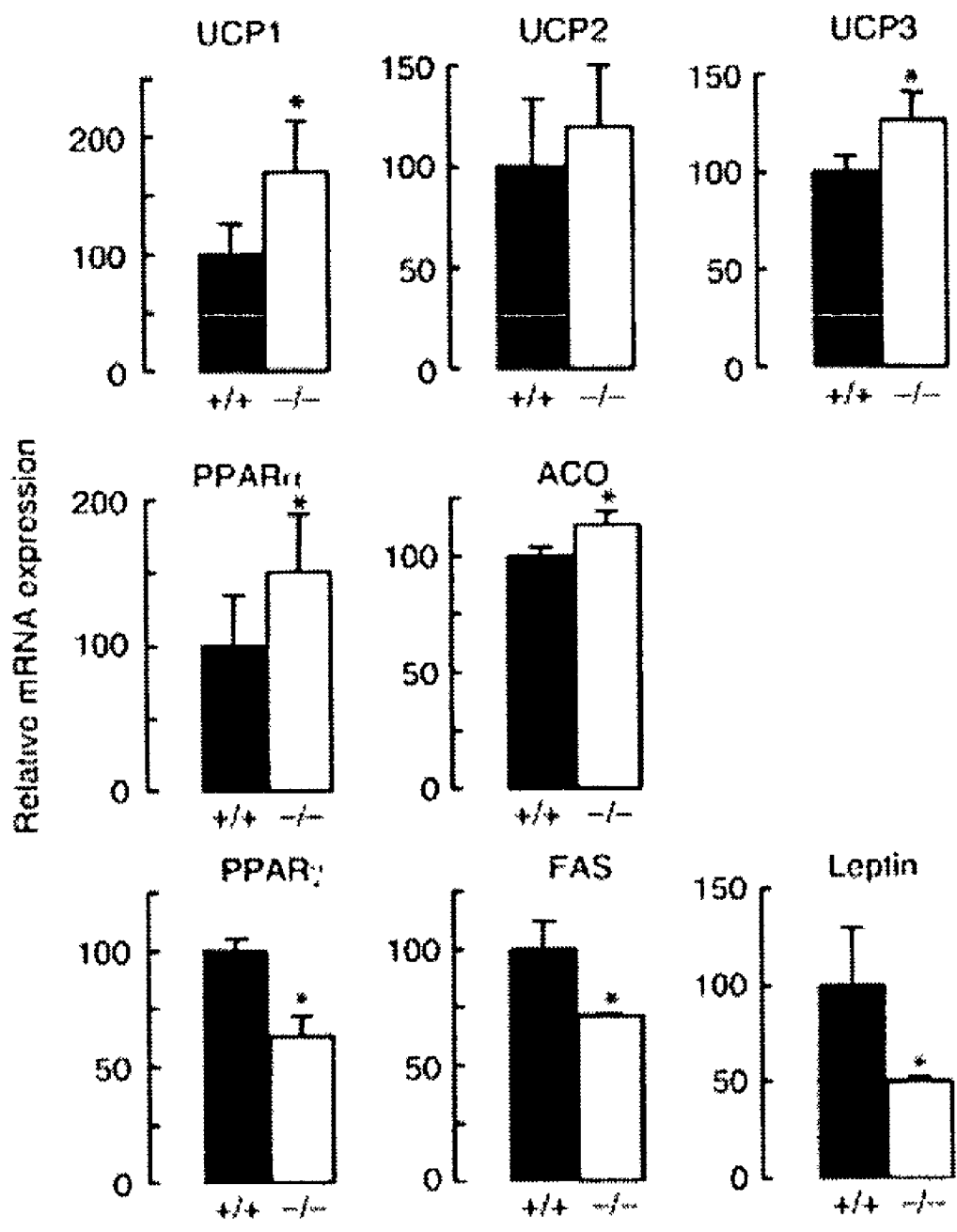
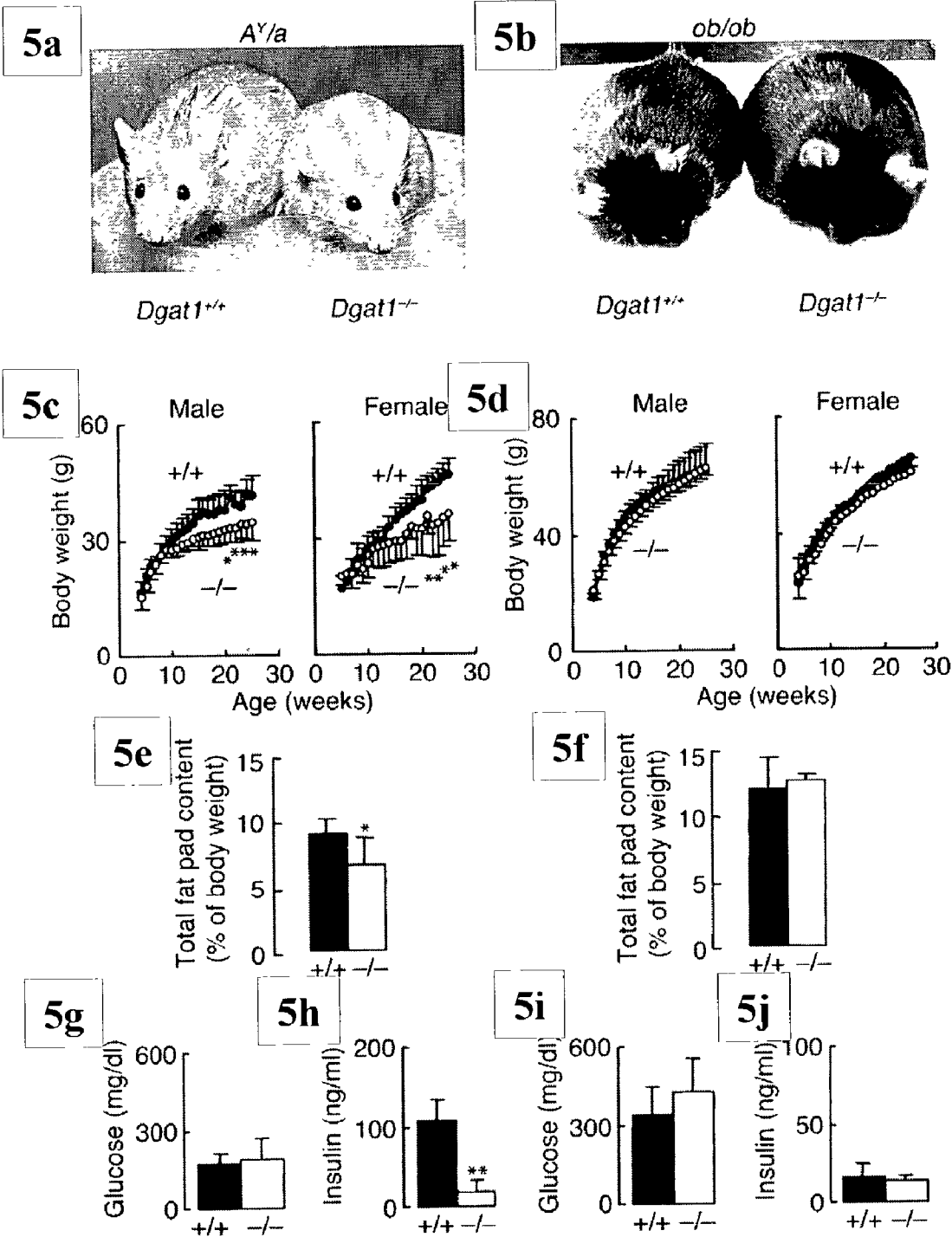


FIG. 4



FIGS. 5a-5j

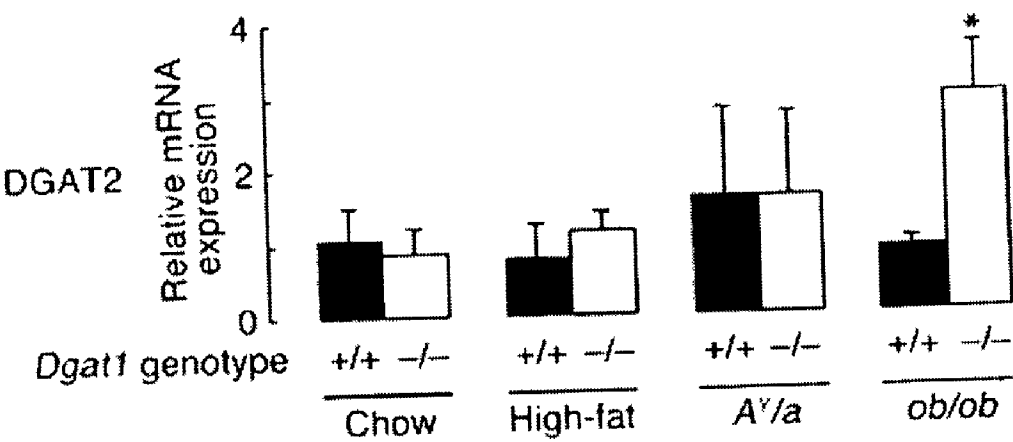


FIG. 6

METHODS AND COMPOSITIONS FOR MODULATING CARBOHYDRATE METABOLISM

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of application Ser. No. 10/040,315 filed Oct. 29, 2001; which application is: (a) a continuation-in-part of application Ser. No. 09/339,472 filed on Jun. 23, 1999, which application claims priority to the filing date of U.S. Provisional Patent Application Serial No. 60/107,771 filed Nov. 9, 1998; and (b) a continuation-in-part of PCT application Ser. No. PCT/US98/17883, filed Aug. 28, 1998, which application is a continuation in part of application Ser. No. 09/103,754, now U.S. Pat. No. 6,344,548, filed Jun. 24, 1998; the disclosures of which applications are herein incorporated by reference.

ACKNOWLEDGMENT OF GOVERNMENT SUPPORT

[0002] This invention was made with Government support under Grant Nos. DK-56084 and DK-26356 awarded by the National Institutes of Health. The Government has certain rights in this invention.

INTRODUCTION

[0003] 1. Field of the Invention

[0004] The invention relates generally to methods of treating conditions associated with abnormal carbohydrate metabolism, such as obesity and diabetes. Specifically, the invention relates to methods of modulating insulin and/or leptin sensitivity in a host.

[0005] 2. Background of the Invention

[0006] A human that weighs greater than about 20% more than an ideal weight is considered obese, and, as such, is highly susceptible the health problems including coronary artery disease, stroke, and certain cancers. Within the United States about 24% of men and 27% of women are considered mildly to severely obese. While partially effective treatments for obesity based on diet, lifestyle and surgery have been developed, no effective drug-based treatment for human obesity is currently available.

[0007] Obesity in humans is commonly associated with altered sensitivity to insulin and/or leptin, which are circulatory hormones that modulate energy homeostasis. In general, leptin, the product of the obese gene, acts in the central nervous system to regulate food intake, energy metabolism and body weight. Insulin, however, mainly functions to regulate the concentration of blood sugar and blood lipids through the promotion of glucose and lipid intake into cells for utilization and storage.

[0008] Leptin is secreted by adipocytes and acts in the hypothalamus through the leptin receptor (OB-R). Despite the fact that rodents with mutations in either the leptin or OB-R genes are profoundly obese and diabetic (Coleman et al., *Diabetologia* 14, 141-148, 1978), most obese humans do not have mutations in these genes (Considine et al., *Diabetes* 19, 992-994, 1996). The observation that the vast majority of obese humans have chronically elevated serum leptin levels (Maffei, et al. (1995) *Nature Med.* 1, 1155-1161) has led to proposal that a primary cause of obesity is "leptin resis-

tance" in that obese individuals, while capable of generating a large amount of circulatory leptin, are unable to properly respond to it. The mechanisms by which a subject becomes leptin resistant are not understood.

[0009] Insulin is secreted by the pancreas in response to an increase in blood glucose, for example after glucose has entered the bloodstream from the intestine after a carbohydrate-rich meal. Insulin stimulates glucose uptake by muscle tissue, where the glucose is converted to glucose 6-phosphate, which is then used to make glycogen. As a consequence of the accelerated uptake of glucose from the blood, blood glucose concentration falls to the normal level, slowing insulin release from the pancreas. There is a closely adjusted feedback relationship between the rate of insulin secretion and blood glucose concentration, which holds blood glucose concentration nearly constant despite large fluctuations in dietary intake.

[0010] In addition to stimulating glucose uptake, insulin also stimulates the storage of excess carbohydrates as fat. It activates both the oxidation of glucose 6-phosphate to pyruvate via glycolysis and the oxidation of pyruvate to acetyl-CoA. If not oxidized further for energy production, this acetyl-CoA is used for fatty acid synthesis in the liver, and these fatty acids are exported as triglycerols of plasma lipoproteins (VLDLs) to adipose tissue. Insulin stimulates triacylglycerol (TAG) synthesis in adipocytes, using fatty acids released from the VLDL TAGs. The fatty acids are ultimately derived from the glucose taken from the blood by the liver.

[0011] As such, insulin regulates the conversion of excess blood glucose into two storage forms: glycogen (in liver and muscle) and TAG (in adipose tissue).

[0012] Many obese individuals have elevated levels of insulin as well as elevated levels of blood sugar and/or blood lipid, and, as such, are thought to be "insulin resistant" in that these individuals, while capable of generating a large amount of circulatory insulin, are unable to respond properly to the insulin. The causative mechanisms of insulin resistance have not yet been fully elucidated, and examples of diseases caused by insulin resistance include diabetes, obesity, diabetic microangiopathies (diabetic nephropathy, diabetic neuropathy, and diabetic retinopathy), impaired glucose tolerance, hyperinsulinemia, hyperlipemia, arteriosclerosis, hypertension, obesity, ischemic heart diseases, ischemic brain disorders, and peripheral arterial embolism (Tamio Teramoto, et al., (1995) *Biomedicine & Therapeutics* 29, 8-96).

[0013] It is clear that insulin and leptin signaling play key roles in maintaining energy homeostasis in normal individuals and that most obese individuals have altered sensitivity to these hormones, leading to excessive food consumption, low energy usage and weight gain. A need therefore exists for therapies based on modulating the sensitivity of a subject to leptin and/or insulin. This invention meets these, and other, needs.

SUMMARY OF THE INVENTION

[0014] Methods and compositions for modulating carbohydrate metabolism in a host are provided. In the subject methods, diacylglycerol acyltransferase (DGAT) activity (specifically DGAT1 activity) is modulated, e.g., reduced or

enhanced, to achieve a desired insulin and/or leptin sensitivity, thereby modulating carbohydrate metabolism, e.g., increasing or decreasing blood glucose levels, glucose uptake into cells and assimilation into glycogen. Also provided are pharmaceutical compositions for practicing the subject methods, etc. The subject methods and compositions find use in a variety of applications, including the treatment of hosts suffering conditions associated with abnormal carbohydrate metabolism, such as obesity or diabetes.

BRIEF DESCRIPTION OF THE FIGURES

[0015] FIG. 1 is a graph showing decreased adipocyte size in $Dgat1^{-/-}$ mice. Each circle represents the mean adipocyte surface area of one female mouse. More than 100 adipocytes were measured per mouse. For high-fat experiments, mice were fed a high-fat diet for 10 weeks.

[0016] FIGS. 2a-2c are graphs showing increased insulin sensitivity in $Dgat1^{-/-}$ mice. (FIG. 2a) Glucose tolerance test. (FIG. 2b) Insulin tolerance test. (FIG. 2c) Hyperinsulinemic-euglycemic clamp study. $n=5-6$ chow-fed male mice per genotype in each experiment. $*P<0.05$ versus $Dgat1^{+/+}$ mice.

[0017] FIGS. 3a-3d are graphs showing increased weight loss in response to leptin infusion in $Dgat1^{-/-}$ mice. (FIG. 3a and FIG. 3b) Body weight. (FIG. 3c and FIG. 3d) Food intake. Sex-, age-, and weight-matched mice were used. $n=6-8$ chow-fed mice per genotype. Error bars represent SEM. $**P<0.01$ versus $Dgat1^{+/+}$ mice receiving the same dose of leptin.

[0018] FIG. 4 is a panel of graphs showing expression of leptin-regulated genes in $Dgat1^{-/-}$ mice. The expression of UCP1 was examined in BAT. The expression of other genes was examined in WAT. For PPAR α and leptin, results were obtained with real-time PCR. For other genes, results were obtained with Northern blotting. $n=4-6$ chow-fed male mice per genotype. $*P<0.05$ versus $Dgat1^{+/+}$ mice.

[0019] FIGS. 5a-5j are a series of figures showing the effects of DGAT1 deficiency on energy and glucose metabolism in Agouti yellow (A^Y/a) and leptin-deficient (ob/ob) mice. $n=8-12$ mice per genotype for growth curves, $n=5$ chow-fed male mice per genotype for fat pad content, and $n=4-6$ chow-fed male mice per genotype for plasma glucose and insulin concentrations. $*P<0.05$, $**P<0.01$ versus $Dgat1^{+/+}$ mice.

[0020] FIG. 6 is a graph showing increased DGAT2 mRNA expression in WAT of leptin-deficient $Dgat1^{-/-}$ mice. Results were obtained with real-time PCR. $n=4-6$ male mice per genotype. $*P<0.05$ versus ob/ob $Dgat1^{+/+}$ mice.

DEFINITIONS

[0021] Unless defined otherwise, 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. Still, certain elements are defined below for the sake of clarity and ease of reference.

[0022] As used herein, the terms "condition associated with abnormal carbohydrate metabolism" and "disorder associated with abnormal carbohydrate metabolism" are used interchangeably to refer to any disorder that is caused by an alteration in carbohydrate metabolism. Such disorders

include, but are not limited to: blood glucose-related disorders, including diabetic microangiopathies (diabetic nephropathy, diabetic neuropathy, and diabetic retinopathy), impaired glucose tolerance, hyperinsulinemia, hyperlipemia, arteriosclerosis, hypertension, obesity, ischemic heart diseases, ischemic brain disorders, peripheral arterial embolism, diabetes mellitus, diabetes insipidus, gestational diabetes, diabetes insipidus, diabetes mellitus, nephrogenic diabetes insipidus, diabetes mellitus, lipodystrophic diabetes mellitus, non-insulin-dependent diabetes mellitus, type 1 diabetes and type 2 diabetes; and disorders relating to obesity, including diabetes, type 2 diabetes, hypertension, stroke, myocardial infarction or congestive heart failure, prostate and colon cancer, gallstones, cholecystitis, gout, gouty arthritis, osteoarthritis, degenerative arthritis of the knees, hips, or the lower back, apnea, and pickwickian syndrome. In general, a condition associated with abnormal carbohydrate metabolism is caused, or is otherwise associated with, an abnormal, e.g., decreased sensitivity to insulin and/or leptin. In certain embodiments, a condition associated with leptin and/or insulin over-activity, e.g., anorexia, is also encompassed by this term.

[0023] The term "phenomenon associated with carbohydrate metabolism" as used herein refers to a structural, molecular, or functional characteristic associated with carbohydrate metabolism, particularly such a characteristic that is readily assessable in a mammalian host. Such characteristics include, but are not limited to: increased or decreased lipid levels, adipocyte size, blood glucose levels, glucose disposal, glucose tolerance, glucose uptake, glycogen synthesis rate, insulin tolerance, weight loss, food intake, triacylglycerol levels, energy expenditure, weight levels, weight gain, activity levels or an alteration in lipid content and the like.

[0024] The term "phenomenon associated with sensitivity to insulin and/or leptin" as used herein refers to a structural, molecular, or functional characteristic associated with carbohydrate metabolism, particularly such a characteristic that is readily assessable in a mammalian host. Such characteristics include, but are not limited to: increased or decreased lipid levels, adipocyte size, blood glucose levels, glucose disposal, glucose tolerance, glucose uptake, glycogen synthesis rate, insulin tolerance, weight loss, food intake, triacylglycerol levels, energy expenditure, weight levels, weight gain, activity levels or an alteration in lipid content and the like.

[0025] As used herein, the terms "determining," "measuring," and "assessing," and "assaying" are used interchangeably and include both quantitative and qualitative determinations.

[0026] The terms "polypeptide" and "protein", used interchangeably herein, refer to a polymeric form of amino acids of any length, which can include coded and non-coded amino acids, chemically or biochemically modified or derivatized amino acids, and polypeptides having modified peptide backbones. The term includes fusion proteins, including, but not limited to, fusion proteins with a heterologous amino acid sequence, fusions with heterologous and homologous leader sequences, with or without N-terminal methionine residues; immunologically tagged proteins; fusion proteins with detectable fusion partners, e.g., fusion

proteins including as a fusion partner a fluorescent protein, β -galactosidase, luciferase, etc.; and the like.

[0027] The terms “polynucleotide” and “nucleic acid molecule” are used interchangeably herein to refer to polymeric forms of nucleotides of any length. The polynucleotides may contain deoxyribonucleotides, ribonucleotides, and/or their analogs. Nucleotides may have any three-dimensional structure, and may perform any function, known or unknown. The term “polynucleotide” includes single-, double-stranded and triple helical molecules. “Oligonucleotide” generally refers to polynucleotides of between about 5 and about 100 nucleotides of single- or double-stranded DNA. However, for the purposes of this disclosure, there is no upper limit to the length of an oligonucleotide. Oligonucleotides are also known as oligomers or oligos and may be isolated from genes, or chemically synthesized by methods known in the art.

[0028] As used herein the term “isolated,” when used in the context of an isolated compound, refers to a compound of interest that is in an environment different from that in which the compound naturally occurs. “Isolated” is meant to include compounds that are within samples that are substantially enriched for the compound of interest and/or in which the compound of interest is partially or substantially purified.

[0029] As used herein, the term “substantially pure” refers to a compound that is removed from its natural environment and is at least 60% free, preferably 75% free, and most preferably 90% free from other components with which it is naturally associated.

[0030] By “transgenic animal” is meant a non-human animal, usually a mammal, having a non-endogenous (i.e., heterologous) nucleic acid sequence present as an extrachromosomal element in a portion of its cells or stably integrated into its germ line DNA (i.e., in the genomic sequence of most or all of its cells). Heterologous nucleic acid is introduced into the germ line of such transgenic animals by genetic manipulation of, for example, embryos or embryonic stem cells of the host animal according to methods well known in the art. A “transgene” is meant to refer to such heterologous nucleic acid, e.g., heterologous nucleic acid in the form of an expression construct (e.g., for the production of a “knock-in” transgenic animal) or a heterologous nucleic acid that upon insertion within or adjacent a target gene results in a decrease in target gene expression (e.g., for production of a “knock-out” transgenic animal).

[0031] A “knock-out” of a gene means an alteration in the sequence of the gene that results in a decrease of function of the target gene, preferably such that target gene expression is undetectable or insignificant. Transgenic knock-out animals can comprise a heterozygous knock-out of a target gene, or a homozygous knock-out of a target gene. “Knock-outs” as used herein also include conditional knock-outs, where alteration of the target gene can occur upon, for example, exposure of the animal to a substance that promotes target gene alteration, introduction of an enzyme that promotes recombination at the target gene site (e.g., Cre in the Cre-lox system), or other method for directing the target gene alteration postnatally.

[0032] A “knock-in” of a target gene means an alteration in a host cell genome that results in altered expression (e.g.,

increased (including ectopic) or decreased expression) of a target gene, e.g., by introduction of an additional copy of the target gene, or by operatively inserting a regulatory sequence that provides for enhanced expression of an endogenous copy of the target gene. “Knock-in” transgenics can comprise a heterozygous knock-in of the target gene or a homozygous knock-in of a target gene. “Knock-ins” also encompass conditional knock-ins.

[0033] By “operably linked” is meant that a DNA sequence and a regulatory sequence(s) are connected in such a way as to permit gene expression when the appropriate molecules (e.g., transcriptional activator proteins) are bound to the regulatory sequence(s).

[0034] By “operatively inserted” is meant that a nucleotide sequence of interest is positioned adjacent a nucleotide sequence that directs transcription and translation of the introduced nucleotide sequence of interest.

[0035] The term “therapeutic agent” as used herein refers to any molecule, e.g., protein or small molecule, pharmaceutical compound, antibody, antisense molecule, ribozyme, and the like, useful in the treatment of a disease or condition, e.g., a condition associated with abnormal carbohydrate metabolism. For example, therapeutic agents of the invention include molecules that inhibit, ameliorate, or relieve symptoms of a condition associated with abnormal carbohydrate metabolism.

[0036] The term “unit dosage form” as used herein refers to physically discrete units suitable as unitary dosages for subjects (e.g., animals, usually humans), each unit containing a predetermined quantity of agent(s) in an amount sufficient to produce the desired effect in association with a pharmaceutically acceptable diluent, carrier or vehicle. The specifications for the novel unit dosage forms of the present invention will depend on a variety of factors including, but not necessarily limited to, the particular agent employed and the effect to be achieved, and the pharmacodynamics associated with each compound in the host.

[0037] The terms “treatment”, “treating” and the like are used herein to generally mean obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. “Treatment” as used herein covers any treatment of a disease in a mammal, particularly a human, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; or (c) relieving the disease, i.e., causing regression of the disease.

[0038] The terms “subject,” “host,” “patient,” and “individual” are used interchangeably herein to refer to any mammalian subject for whom diagnosis or therapy is desired, particularly humans. Other subjects may include cattle, dogs, cats, guinea pigs, rabbits, rats, mice, horses, and so on.

DETAILED DESCRIPTION OF THE INVENTION

[0039] Methods and compositions for modulating carbohydrate metabolism in a host are provided. In the subject

methods, diacylglycerol acyltransferase (DGAT) activity (specifically DGAT1 activity) is modulated, e.g., reduced or enhanced, to achieve a desired insulin and/or leptin sensitivity, thereby modulating carbohydrate metabolism, e.g., increasing or decreasing blood glucose levels, glucose uptake into cells and assimilation into glycogen, etc. Also provided are pharmaceutical compositions for practicing the subject methods. The subject methods and compositions find use in a variety of applications, including the treatment of hosts suffering conditions associated with abnormal carbohydrate metabolism, such as obesity or diabetes.

[0040] Before the subject invention is described further, it is to be understood that the invention is not limited to the particular embodiments of the invention described below, as variations of the particular embodiments may be made and still fall within the scope of the appended claims. It is also to be understood that the terminology employed is for the purpose of describing particular embodiments, and is not intended to be limiting. Instead, the scope of the present invention will be established by the appended claims.

[0041] In this specification and the appended claims, the singular forms “a,” “an” and “the” include plural reference unless the context clearly dictates otherwise. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs.

[0042] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the, context clearly dictates otherwise, between the upper and lower limit of that range, and any other stated or intervening value, in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[0043] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs. Although any methods, devices and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, the preferred methods, devices and materials are now described.

[0044] All publications mentioned herein are incorporated herein by reference for the purpose of describing and disclosing the subject components of the invention that are described in the publications, which components might be used in connection with the presently described invention.

[0045] In further describing the invention, representative methods of modulating sensitivity of a host to insulin and/or leptin and compositions for performing the methods are described first. Following this, a detailed description of representative applications in which the subject methods find use is provided. Next, representative kits that find use in practicing the subject methods are further described.

[0046] Methods of Modulating Sensitivity to Insulin and/or Leptin

[0047] The invention provides methods of modulating sensitivity to insulin and/or leptin in a host. In general, the

methods involve administering to a host an effective amount of one or more active agents that modulate DGAT1 activity in the host to modulate sensitivity to insulin and/or leptin in the host.

[0048] By DGAT1 activity is meant the activity of a DGAT1 protein, where representative DGAT1 proteins are disclosed in Cases et al., Proc. Nat'l Acad. Sci. USA (1998) 95:13018-13023 and Genbank Accession Nos.: AAC63997, AF059202; as well as U.S. Pat. Nos. 6,100,077 and 6,344,548 and the priority applications to the present application (listed above); the disclosures of which are herein incorporated by reference.

[0049] As DGAT1 activity is modulated in certain embodiments of the invention, DGAT1 activity is increased or decreased in these embodiments. In many embodiments, DGAT1 activity is increased or decreased by at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 80%, at least about 90%, or more, as compared to a baseline DGAT1 activity level, e.g., that observed in the host prior to administration of the active agent. In certain embodiments, DGAT1 activity may be decreased by at least about 100%, by at least about 300%, by at least about 5-fold, by at least about 10-fold, by at least about 50-fold, or by at least about 100-fold, or more, as compared to a baseline DGAT1 activity level, e.g., that observed in the host prior to administration of the active agent.

[0050] Upon administration of a DGAT1 modulatory agent to a host, insulin and/or leptin activity is usually increased or decreased by at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 80%, at least about 90%, or more, when compared to a control in the absence of an effective amount of the active agent. In certain embodiments, upon administration of a DGAT1 modulatory agent, especially a DGAT1 inhibitory agent, to a host, insulin and/or leptin activity may be increased by at least about 100%, by at least about 300%, by at least about 5-fold, by at least about 10-fold, by at least 50-fold, or by at least 100-fold as compared to a control in the absence of an effective amount of the active agent.

[0051] Sensitivity to insulin and/or leptin is usually measured in terms of an assessment of a phenomenon associated with carbohydrate metabolism of a host, e.g., levels of blood glucose or fatty acids, in response to a specific dose of insulin and/or leptin, especially after consumption of carbohydrates.

[0052] In some embodiments where the desired modulation of sensitivity to insulin and/or leptin is an increase in sensitivity to insulin and/or leptin, one or more agents that decreases DGAT1 activity is administered to the host: For example, in certain embodiments, one or more agents that decreases DGAT1 activity is administered to the host. In these embodiments, the agent is typically a DGAT1 inhibitor.

[0053] In some embodiments where the desired modulation of sensitivity to insulin and/or leptin is a decrease in

sensitivity to insulin and/or leptin, one or more agents that increase DGAT1 activity is administered to the host. For example, in certain embodiments, one or more agents that increases DGAT1 activity is administered to the host.

[0054] For the modulation of DGAT 1 activity in a host, an effective amount of an active agent(s) that modulates the activity, e.g., reduces the activity of DGAT1 in vivo, is administered to the host. The active agent may be one or a mixture of a variety of different compounds, including: polynucleotide compositions (e.g., coding sequences, antisense compositions, siRNA compositions, etc.), polypeptide, including antibody, compositions, naturally occurring or synthetic small molecule compounds, etc.

[0055] In certain embodiments, the active agents administered to the host are polynucleotide or nucleic acid compositions. The nucleic acids may be coding sequences, e.g., genes, gene fragments etc., which may be present in expression vectors, where such vectors generally have convenient restriction sites located near the promoter sequence to provide for the insertion of nucleic acid sequences. Transcription cassettes may be prepared that include a transcription initiation region, the target gene or fragment thereof, and a transcriptional termination region.

[0056] The transcription cassettes may be introduced into a variety of vectors, e.g. plasmid; retrovirus, e.g. lentivirus; adenovirus; and the like, where the vectors are able to transiently or stably be maintained in the cells, usually for a period of at least about one day, more usually for a period of at least about several days to several weeks.

[0057] In yet other embodiments of the invention, the active agent is an agent that modulates, and generally decreases or down regulates, the expression of DGAT1 in the host. Antisense molecules can be used to down-regulate expression of a gene in cells. The anti-sense reagent may be antisense oligonucleotides (ODN), particularly synthetic ODN having chemical modifications from native nucleic acids, or nucleic acid constructs that express such anti-sense molecules as RNA. The antisense sequence is complementary to the mRNA of the targeted gene, and inhibits expression of the targeted gene products. Antisense molecules inhibit gene expression through various mechanisms, e.g. by reducing the amount of mRNA available for translation, through activation of RNase H, or steric hindrance. One or a combination of antisense molecules may be administered, where a combination may comprise multiple different sequences.

[0058] Antisense molecules may be produced by expression of all or a part of the target gene sequence in an appropriate vector, where the transcriptional initiation is oriented such that an antisense strand is produced as an RNA molecule. Alternatively, the antisense molecule is a synthetic oligonucleotide. Antisense oligonucleotides will generally be at least about 7, usually at least about 12, more usually at least about 20 nucleotides in length, and not more than about 500, usually not more than about 50, more usually not more than about 35 nucleotides in length, where the length is governed by efficiency of inhibition, specificity, including absence of cross-reactivity, and the like. It has been found that short oligonucleotides, of from 7 to 8 bases in length, can be strong and selective inhibitors of gene expression (see Wagner et al. (1996), *Nature Biotechnol.* 14:840-844).

[0059] A specific region or regions of the endogenous sense strand mRNA sequence is chosen to be complemented

by the antisense sequence. Selection of a specific sequence for the oligonucleotide may use an empirical method, where several candidate sequences are assayed for inhibition of expression of the target gene in an in vitro or animal model. A combination of sequences may also be used, where several regions of the mRNA sequence are selected for antisense complementation.

[0060] Antisense oligonucleotides may be chemically synthesized by methods known in the art (see Wagner et al. (1993), supra, and Milligan et al., supra.) Preferred oligonucleotides are chemically modified from the native phosphodiester structure, in order to increase their intracellular stability and binding affinity. A number of such modifications have been described in the literature, which alter the chemistry of the backbone, sugars or heterocyclic bases.

[0061] Among useful changes in the backbone chemistry are phosphorothioates; phosphorodithioates, where both of the non-bridging oxygens are substituted with sulfur; phosphoramidites; alkyl phosphotriesters and boranophosphates. Achiral phosphate derivatives include 3'-O'-5'-S-phosphorothioate, 3'-S-5'-O-phosphorothioate, 3'-CH₂-5'-O-phosphonate and 3'-NH-5'-O-phosphoramidate. Peptide nucleic acids replace the entire ribose phosphodiester backbone with a peptide linkage. Sugar modifications are also used to enhance stability and affinity. The α -anomer of deoxyribose may be used, where the base is inverted with respect to the natural β -anomer. The 2'-OH of the ribose sugar may be altered to; form 2'-(-methyl or 2'-O-allyl sugars, which provides resistance to degradation without comprising affinity. Modification of the heterocyclic bases must maintain proper base pairing. Some useful substitutions include deoxyuridine for deoxythymidine, 5-methyl-2'-deoxycytidine and 5-bromo-2'-deoxycytidine for deoxycytidine. 5-propynyl-2'-deoxyuridine and 5-propynyl-2'-deoxycytidine have been shown to increase affinity and biological activity when substituted for deoxythymidine and deoxycytidine, respectively.

[0062] As an alternative to anti-sense inhibitors, catalytic nucleic acid compounds, e.g. ribozymes, anti-sense conjugates, etc. may be used to inhibit gene expression. Ribozymes may be synthesized in vitro and administered to the patient, or may be encoded on an expression vector, from which the ribozyme is synthesized in the targeted cell (for example, see International patent application WO 9523225, and Beigelman et al. (1995), *Nucl. Acids Res.* 23:4434-42). Examples of oligonucleotides with catalytic activity are described in WO 9506764. Conjugates of anti-sense ODN with a metal complex, e.g. terpyridylCu(II), capable of mediating mRNA hydrolysis are described in Bashkin et al. (1995), *Appl. Biochem. Biotechnol.* 54:43-56.

[0063] Alternatively, gene expression can be modified by gene silencing using double-strand RNA (Sharp (1999) *Genes and Development* 13: 139-141). RNAi, otherwise known as double-stranded RNA interference (dsRNAi) or small interfering RNA (siRNA), has been extensively documented in the nematode *C. elegans* (Fire, A., et al, *Nature*, 391, 806-811, 1998) and an identical phenomenon occurs in plants, in which it is usually referred to as post-transcriptional gene silencing (PTGS) (Van Blokland, R., et al., *Plant J.*, 6: 861-877, 1994; deCarvalho-Niebel, F., et al., *Plant Cell*, 7: 347-358, 1995; Jacobs, J. J. M. R. et al., *Plant J.*, 12: 885-893, 1997; reviewed in Vaucheret, H., et al., *Plant J.*, 16:

651-659, 1998). The phenomenon also occurs in fungi (Romano, N. and Masino, G., *Mol. Microbiol.*, 6: 3343-3353, 1992, Cogoni, C., et al., *EMBO J.*, 15: 3153-3163; Cogoni, C. and Masino, G., *Nature*, 399: 166-169, 1999), in which it is often referred to as "quelling". RNAi silencing can be induced many ways in plants, where a nucleic acid encoding an RNA that forms a "hairpin" structure is employed in most embodiments. Alternative strategies include expressing RNA from each end of the encoding nucleic acid, making two RNA molecules that will hybridize. Current strategies for RNAi induced silencing in plants are reviewed by Carthew et al (*Curr Opin Cell Biol.* 2001 13:244-8). RNAi is also described in WO 02/44321 and WO 01/68836; the priority documents of which are herein incorporated by reference.

[0064] Also of-interest are polypeptide, e.g., proteinaeous, active agents. Specific polypeptide agents include proteins or active fragments thereof, e.g., DGAT1 proteins, etc.

[0065] A specific type of polypeptide active agent of interest is an antibody agent that modulates DGAT1 activity in the host. The antibodies may be monoclonal or polyclonal, and produced according to methods known in the art. Antibody fragments, such as Fv, F(ab')₂ and Fab may be prepared by cleavage of the intact protein, e.g. by protease or chemical cleavage.

[0066] Alternatively, a truncated gene is designed. For example, a chimeric gene encoding a portion of the F(ab')₂ fragment would include DNA sequences encoding the CH1 domain and hinge region of the H chain, followed by a translational stop codon to yield the truncated molecule.

[0067] Consensus sequences of H and L J regions may be used to design oligonucleotides for use as primers to introduce useful restriction sites into the J region for subsequent linkage of V region segments to human C region segments. C region cDNA can be modified by site directed mutagenesis to place a restriction site at the analogous position in the human sequence.

[0068] Expression vectors include plasmids, retroviruses, YACs, EBV derived episomes, and the like. A convenient vector is one that encodes a functionally complete human CH or CL immunoglobulin sequence, with appropriate restriction sites engineered so that any VH or VL sequence can be easily inserted and expressed. In such vectors, splicing usually occurs between the splice donor site in the inserted J region and the splice acceptor site preceding the human C region, and also at the splice regions that occur within the human CH exons. Polyadenylation and transcription termination occur at native chromosomal sites downstream of the coding regions. The resulting chimeric antibody may be joined to any strong promoter, including retroviral LTRs, e.g. SV-40 early promoter, (Okayama et al. (1983) *Mol. Cell. Bio.* 3:280), Rous sarcoma virus LTR (Gorman et al. (1982) *P.N.A.S.* 79:6777), and moloney murine leukemia virus LTR (Grosschedl et al. (1985) *Cell* 41:885); native Ig promoters, etc.

[0069] Naturally occurring or synthetic small molecule compounds of interest as active agents include numerous chemical classes, though typically they are organic molecules, preferably small organic compounds having a molecular weight of more than 50 and less than about 2,500

daltons. Candidate agents include functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. The candidate agents often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Candidate agents are also found among biomolecules including peptides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof. Of particular interest are those agents identified by the screening assays of the subject invention, as described above.

[0070] In certain embodiments, in addition to the DGAT1 modulatory active agent, a leptin and/or insulin modulatory active agent, e.g., an agent that enhances or inhibits leptin and/or insulin activity, is administered. For example, in certain embodiments where a DGAT1 inhibitory agent is administered, a leptin activity enhancing agent (e.g. leptin or an activity mimetic thereof) may also be administered, such that both a DGAT1 inhibitory agent and a leptin activity enhancing agent are administered to the host. In certain other embodiments where a DGAT1 inhibitory agent is administered, an insulin activity enhancing agent (e.g. insulin or an activity mimetic thereof) may also be administered, such that both a DGAT1 inhibitory agent and an insulin activity enhancing agent are administered to the host. Such embodiments include those embodiments where one wishes to modulate sensitivity to insulin and/or leptin in way that modulates a phenomenon associated with carbohydrate metabolism, e.g., blood glucose levels.

[0071] In practicing the subject methods, an effective amount of the active agent is administered to a host, where the term "effective amount" means a dosage sufficient to produce a desired result, where the desired result is the desired modulation, e.g., enhancement, reduction, of DGAT1 activity and a desired modulation in sensitivity to insulin and or leptin.

[0072] In practicing the subject methods, the active agent or agents are typically administered to the host in a physiologically acceptable delivery vehicle, e.g., as a pharmaceutical preparation. A variety of representative formulations, dosages, routes of administration for candidate agents, nucleic acid delivery vehicles and nucleic acid formulations for nucleic acid delivery are described below.

[0073] Formulations, Dosages, and Routes of Administration

[0074] The invention provides formulations, including pharmaceutical formulations, that include an agent which modulates sensitivity to insulin and/or leptin in a host. In general, a formulation comprises an effective amount of an agent that modulates DGAT 1 (and/or leptin) activity in a host. An "effective amount" refers to an amount that is sufficient to produce a desired result, e.g., reduction of weight to normal levels, reduction of blood glucose levels, stabilization of blood glucose levels etc. In many embodiments, the desired result is at least a reduction or increase in a phenotype as compared to a control such that the phenotype is more similar to normal.

[0075] In certain embodiments, the agent that modulates sensitivity to insulin and/or leptin is administered before,

with, or after administration of insulin and/or leptin, or mimetics thereof. Mimetics of insulin and of leptin activity are well known the art (see, for example, U.S. Pat. Nos. 6,444,641, 6,335,316, 5,952,297, 5,700,662, 5,698,669, 6,420,340, 7 6,355,635 and 3 6,420,339; the disclosures of which are herein incorporated by reference).

[0076] Formulations

[0077] In the subject methods, the active agent(s) may be administered to the host using any convenient means capable of resulting in the desired reduction in of a carbohydrate metabolism-related phenotype.

[0078] Thus, the agent can be incorporated into a variety of formulations for therapeutic administration. More particularly, the agents of the present invention can be formulated into pharmaceutical compositions by combination with appropriate, pharmaceutically acceptable carriers or diluents, and may be formulated into preparations in solid, semi-solid, liquid or gaseous forms, such as tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, inhalants and aerosols.

[0079] In pharmaceutical dosage forms, the agents may be administered in the form of their pharmaceutically acceptable salts, or they may also be used alone or in appropriate association, as well as in combination, with other pharmaceutically active compounds. The following methods and excipients are merely exemplary and are in no way limiting.

[0080] For oral preparations, the agents can be used alone or in combination with appropriate additives to make tablets, powders, granules or capsules, for example, with conventional additives, such as lactose, mannitol, corn starch or potato starch; with binders, such as crystalline cellulose, cellulose derivatives, acacia, corn starch or gelatins; with disintegrators, such as corn starch, potato starch or sodium carboxymethylcellulose; with lubricants, such as talc or magnesium stearate; and if desired, with diluents; buffering agents, moistening agents, preservatives and flavoring agents.

[0081] The agents can be formulated into preparations for injection by dissolving, suspending or emulsifying them in an aqueous or nonaqueous solvent, such as vegetable or other similar oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids or propylene glycol; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives.

[0082] The agents can be utilized in aerosol formulation to be administered via inhalation. The compounds of the present invention can be formulated into pressurized acceptable propellants such as dichlorodifluoromethane, propane, nitrogen and the like.

[0083] Furthermore, the agents can be made into suppositories by mixing with a variety of bases such as emulsifying bases or water-soluble bases. The compounds of the present invention can be administered rectally via a suppository. The suppository can include vehicles such as cocoa butter, carbowaxes and polyethylene glycols, which melt at body temperature, yet are solidified at room temperature.

[0084] Unit dosage forms for oral or rectal administration such as syrups, elixirs, and suspensions may be provided wherein each dosage unit, for example, teaspoonful, table-

spoonful, tablet or suppository, contains a predetermined amount of the composition containing one or more inhibitors. Similarly, unit dosage forms for injection or intravenous administration may comprise the inhibitor(s) in a composition as a solution in sterile water, normal saline or another pharmaceutically acceptable carrier.

[0085] The term "unit dosage form," as used herein, refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of compounds of the present invention calculated in an amount sufficient to produce the desired effect in association with a pharmaceutically acceptable diluent, carrier or vehicle. The specifications for the novel unit dosage forms of the present invention depend on the particular compound employed and the effect to be achieved, and the pharmacodynamics associated with each compound in the host.

[0086] Other modes of administration will also find use with the subject invention. For instance, an agent of the invention can be formulated in suppositories and, in some cases, aerosol and intranasal compositions. For suppositories, the vehicle composition will include traditional binders and carriers such as, polyalkylene glycols, or triglycerides. Such suppositories may be formed from mixtures containing the active ingredient in the range of about 0.5% to about 10% (w/w), preferably about 1% to about 2%.

[0087] Intranasal formulations will usually include vehicles that neither cause irritation to the, nasal mucosa nor significantly disturb ciliary function. Diluents such as water, aqueous saline or other known substances can be employed with the subject invention. The nasal formulations may also contain preservatives such as, but not limited to, chlorobutanol and benzalkonium chloride. A surfactant may be present to enhance absorption of the subject proteins by the nasal mucosa.

[0088] An agent of the invention can be administered as injectables. Typically, injectable compositions are prepared as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. The preparation may also be emulsified or the active ingredient encapsulated in liposome vehicles.

[0089] Suitable excipient vehicles are, for example, water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. In addition, if desired, the vehicle may contain minor amounts of auxiliary substances such as wetting or emulsifying agents or pH buffering agents. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in the art. See, e.g., Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., 17th edition, 1985; Remington: The Science and Practice of Pharmacy, A. R. Gennaro, (2000) Lippincott, Williams & Wilkins. The composition or formulation to be administered will, in any event, contain a quantity of the agent adequate to achieve the desired state in the subject being treated.

[0090] The pharmaceutically acceptable excipients, such as vehicles, adjuvants, carriers or diluents, are readily available to the public. Moreover, pharmaceutically acceptable auxiliary substances, such as pH adjusting and buffering agents, tonicity adjusting agents, stabilizers, wetting agents and the like, are readily available to the public.

[0091] Dosages

[0092] Although the dosage used will vary depending on the clinical goals to be achieved, a suitable dosage range is one which provides up to about 1 μg to about 1,000 μg or about 10,000 μg of an agent that modulates insulin and/or leptin resistance in a host, and in embodiments, modulates carbohydrate metabolism or treats a host suffering from a condition associated with abnormal carbohydrate metabolism.

[0093] Those of skill will readily appreciate that dose levels can vary as a function of the specific compound, the severity of the symptoms and the susceptibility of the subject to side effects. Preferred dosages for a given compound are readily determinable by those of skill in the art by a variety of means.

[0094] Routes of Administration

[0095] Conventional and pharmaceutically acceptable routes of administration include intranasal, intramuscular, intratracheal, intratumoral, subcutaneous, intradermal, topical application, intravenous, rectal, nasal, oral and other parenteral routes of administration. Routes of administration may be combined, if desired, or adjusted depending upon the agent and/or the desired effect. The composition can be administered in a single dose or in multiple doses.

[0096] The agent can be administered to a host using any available conventional methods and routes suitable for delivery of conventional drugs, including systemic or localized routes. In general, routes of administration contemplated by the invention include, but are not necessarily limited to, enteral, parenteral, or inhalational routes.

[0097] Parenteral routes of administration other than inhalation administration include, but are not necessarily limited to, topical, transdermal, subcutaneous, intramuscular, intraorbital, intracapsular, intraspinal, intrasternal, and intravenous routes, i.e., any route of administration other than through the alimentary canal. Parenteral administration can be carried to effect systemic or local delivery of the agent. Where systemic delivery is desired, administration typically involves invasive or systemically absorbed topical or mucosal administration of pharmaceutical preparations.

[0098] The agent can also be delivered to the subject by enteral administration. Enteral routes of administration include, but are not necessarily limited to, oral and rectal (e.g., using a suppository) delivery.

[0099] Methods of administration of the agent through the skin or mucosa include, but are not necessarily limited to, topical application of a suitable pharmaceutical preparation, transdermal transmission, injection and epidermal administration. For transdermal transmission, absorption promoters or iontophoresis are suitable methods. Iontophoretic transmission may be accomplished using commercially available "patches" which deliver their product continuously via electric pulses through unbroken skin for periods of several days or more.

[0100] By treatment is meant at least: an amelioration of the symptoms associated with the pathological condition afflicting the host, where amelioration is used in a broad sense to refer to at least a reduction in the magnitude of a parameter, e.g. symptom, associated with the pathological condition being treated, such as a obesity and psychological

trauma associated therewith. As such, treatment also includes situations where the pathological condition, or at least symptoms associated therewith, are completely inhibited, e.g. prevented from happening, or stopped, e.g. terminated, such that the host no longer suffers from the pathological condition, or at least the symptoms that characterize the pathological condition.

[0101] A subject polynucleotide can be delivered as a naked polynucleotide, or associated with (complexed with) a delivery vehicle. "Associated with", or "complexed with", encompasses both covalent and non-covalent interaction of a polynucleotide with a given delivery vehicle.

[0102] Nucleic Acid Delivery Vehicles

[0103] In certain embodiment, an agent is a nucleic acid. Nucleic acids may be delivered using several different vehicles, including viral and non-viral delivery vehicles.

[0104] Viral Delivery Vehicles

[0105] A subject polynucleotide can be associated with viral delivery vehicles. As used herein, a "viral delivery vehicle" intends that the polynucleotide to be delivered is encapsidated in a viral particle.

[0106] Numerous viral genomes useful in in vivo transformation and gene therapy are known in the art, or can be readily constructed given the skill and knowledge in the art. Included are replication competent, replication deficient, and replication conditional viruses. Viral vectors include adenovirus, mumps virus, a retrovirus, adeno-associated virus, herpes simplex virus (HSV), cytomegalovirus (CMV), vaccinia virus, and poliovirus, and non-replicative mutants/variants of the foregoing. In some embodiments, a replication-deficient virus is capable of infecting slowly replicating and/or terminally differentiated cells, since the respiratory tract is primarily composed of these cell types. For example, adenovirus efficiently infects slowly replicating and/or terminally differentiated cells. In some embodiments, the viral genome itself, or a protein on the viral surface, is specific or substantially specific for cells of the targeted cell. A viral genome can be designed to be target cell-specific by inclusion of cell type-specific promoters and/or enhancers operably linked to a gene(s) essential for viral replication.

[0107] Where a replication-deficient virus is used, as the viral genome, the production of virus particles containing either DNA or RNA corresponding to the polynucleotide of interest can be produced by introducing the viral construct into a recombinant cell line which provides the missing components essential for viral replication and/or production. Preferably, transformation of the recombinant cell line with the recombinant viral genome will not result in production of replication-competent viruses, e.g., by homologous recombination of the viral sequences of the recombinant cell line into the introduced viral genome. Methods for production of replication-deficient viral particles containing a nucleic acid of interest are well known in the art and are described in, for example, Rosenfeld et al., *Science* 252:431-434, 1991 and Rosenfeld et al., *Cell* 68:143-155, 1992 (adenovirus); U.S. Pat. No. 5,139,941 (adeno-associated virus); U.S. Pat. No. 4,861,719 (retrovirus); and U.S. Pat. No. 5,356,806 (vaccinia virus). Methods and materials for manipulation of the mumps virus genome, characterization of mumps virus genes responsible for viral fusion and viral replication, and the structure and sequence of the mumps

viral genome are described in Tanabayashi et al., *J. Virol.* 67:2928-2931, 1993; Takeuchi et al., *Archiv. Viro.* 128:177-183, 1993; Tanabayashi et al., *Virol.* 187:801-804, 1992; Kawano et al., *Virol.*, 179:857-861, 1990; Elango et al., *J. Gen. Virol.* 69:2893-28900, 1988.

[0108] Non-Viral Delivery Vehicles

[0109] A subject polynucleotide can be administered using a non-viral delivery vehicle. "Non-viral delivery vehicle" (also referred to herein as "non-viral vector") as used herein is meant to include chemical formulations containing naked or condensed polynucleotides (e.g., a formulation of polynucleotides and cationic compounds (e.g., dextran sulfate)), and naked or condensed polynucleotides mixed with an adjuvant such as a viral particle (i.e., the polynucleotide of interest is not contained within the viral particle, but the transforming formulation is composed of both naked polynucleotides and viral particles (e.g., adenovirus particles) (see, e.g., Curiel et al. 1992 *Am. J. Respir. Cell Mol. Biol.* 6:247-52)). Thus "non-viral delivery vehicle" can include vectors composed of polynucleotides plus viral particles where the viral particles do not contain the polynucleotide of interest. "Non-viral delivery vehicles" include bacterial plasmids, viral genomes or portions thereof, wherein the polynucleotide to be delivered is not encapsidated or contained within a viral particle, and constructs comprising portions of viral genomes and portions of bacterial plasmids and/or bacteriophages. The term also encompasses natural and synthetic polymers and co-polymers. The term further encompasses lipid-based vehicles. Lipid-based vehicles include cationic liposomes such as disclosed by Felgner et al (U.S. Pat. Nos. 5,264,618 and 5,459,127; *PNAS* 84:7413-7417, 1987; *Annals N.Y. Acad. Sci.* 772:126-139, 1995); they may also consist of neutral or negatively charged phospholipids or mixtures thereof including artificial viral envelopes as disclosed by Schreier et al. (U.S. Pat. Nos. 5,252,348 and 5,766,625).

[0110] Non-viral delivery vehicles include polymer-based carriers. Polymer-based carriers may include natural and synthetic polymers and co-polymers. Preferably, the polymers are biodegradable, or can be readily eliminated from the subject. Naturally occurring polymers include polypeptides and polysaccharides. Synthetic polymers include, but are not limited to, polylysines, and polyethyleneimines (PEI; Boussif et al., *PNAS* 92:7297-7301, 1995) which molecules can also serve as condensing agents. These carriers may be dissolved, dispersed or suspended in a dispersion liquid such as water, ethanol, saline solutions and mixtures thereof. A wide variety of synthetic polymers are known in the art and can be used.

[0111] "Non-viral delivery vehicles" further include bacteria. The use of various bacteria as delivery vehicles for polynucleotides has been described. Any known bacterium can be used as a delivery vehicle, including, but not limited to non-pathogenic strains of *Staphylococcus*, *Salmonella*, and the like.

[0112] Formulations for Nucleic Acid Delivery

[0113] The polynucleotide to be delivered can be formulated as a DNA- or RNA-liposome complex formulation. Such complexes comprise a mixture of lipids which bind to genetic material (DNA or RNA) by means of cationic charge (electrostatic interaction). Cationic liposomes which may be

used in the present invention include 3β -[N-(N', N'-dimethyl-aminoethane)-carbamoyl]-cholesterol (DC-Chol), 1,2-bis(oleoyloxy-3-trimethylammonio-propane (DOTAP) (see, for example, WO 98/07408), lysinylphosphatidylethanolamine (L-PE), lipopolyamines such as lipospermine, N-(2-hydroxyethyl)-N,N-dimethyl-2,3-bis(dodecyloxy)-1-propanaminium bromide, dimethyl dioctadecyl ammonium bromide (DDAB), dioleoylphosphatidyl ethanolamine (DOPE), dioleoylphosphatidyl choline (DOPC), N(1,2,3-dioleoyloxy) propyl-N,N,N-triethylammonium (DOTMA), DOSPA, DMRIE, GL-67, GL-89, Lipofectin, and Lipofectamine (Thiery et al. (1997) *Gene Ther.* 4:226-237; Felgner et al., *Annals N.Y. Acad. Sci.* 772:126-139, 1995; Eastman et al., *Hum. Gene Ther.* 8:765-7.73, 1997). Polynucleotide/lipid formulations described in U.S. Pat. No. 5,858,784 can also be used in the methods described herein. Many of these lipids are commercially available from, for example, Boehringer-Mannheim, and Avanti Polar Lipids (Birmingham, Ala.). Also encompassed are the cationic phospholipids found in U.S. Pat. Nos. 5,264,618, 5,223,263 and 5,459,127. Other suitable phospholipids which may be used include phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingomyelin, phosphatidylinositol, and the like. Cholesterol may also be included.

[0114] Utility

[0115] The subject compositions and methods of modulating sensitivity to insulin and/or leptin in a host find use in a variety of protocols. In some embodiment, these protocols involve administering to a host an effective amount of one or more active agents that alter DGAT1 activity in the host and thereby modulate carbohydrate metabolism in the host. In other embodiments, these protocols involve administering to a host suffering from a condition associated with abnormal carbohydrate metabolism an effective amount of one or more active agents that modulate DGAT activity in the host and treat the host for the condition.

[0116] By treatment is meant at least an amelioration of a symptom associated with the pathological condition afflicting the host, where amelioration is used in a broad sense to refer to at least a reduction in the magnitude of a parameter, e.g., symptom, associated with the pathological condition being treated. Treatment also includes outcomes where the pathological condition, or at least symptoms associated therewith, are completely inhibited, e.g. prevented from happening, or stopped, e.g., terminated, such that the host no longer suffers from the pathological condition, or at least the symptoms that characterize the pathological condition. For example, where the disease condition is marked by the presence of variable blood glucose levels, treatment includes at least a reduction in the variability of blood glucose levels, including a restoration of stable blood glucose levels of a normal host.

[0117] In certain other embodiments, insulin, leptin or a mimetic thereof is administered before, at the same time as, or after the DGAT activity modulating agent in order to modulate carbohydrate metabolism or treat a condition associated with abnormal carbohydrate metabolism. It is expected that such combinations, e.g., a formulation including insulin and/or leptin and a DGAT modulatory agent will be efficacious in treating a disease relating to abnormal carbohydrate metabolism.

[0118] A variety of hosts may be used in the subject methods. Generally such hosts are mammals or mammalian,

where these terms are used broadly to describe organisms which are within the class mammalia, including the orders carnivore (e.g., dogs and cats), rodentia (e.g., mice, guinea pigs, and rats), and primates (e.g., humans, chimpanzees, and monkeys). In many embodiments, the hosts will be humans.

[0119] Of particular interest is treatment and prevention of diseases relating to abnormal carbohydrate metabolism associated with increased sensitivity to insulin and/or leptin. Such diseases include blood glucose-related disorders (diabetes) and obesity, including, blood glucose-related disorders, including diabetic microangiopathies (diabetic nephropathy, diabetic neuropathy, and diabetic retinopathy), impaired glucose tolerance, hyperinsulinemia, hyperlipemia, arteriosclerosis, hypertension, obesity, ischemic heart diseases, ischemic brain disorders, peripheral arterial embolism, diabetes mellitus, diabetes insipidus, gestational diabetes, diabetes innocus, diabetes insipidus, nephrogenic diabetes insipidus, diabetes intermittens, diabetes mellitus; insulin-dependent diabetes mellitus, lipotrophic diabetes mellitus, non-insulin-dependent diabetes mellitus, type 1 diabetes and type 2 diabetes; and disorders relating to obesity, including diabetes, type 2 diabetes, hypertension, stroke, myocardial infarction or congestive heart failure, prostate and colon cancer, gallstones, cholecystitis, gout, gouty arthritis osteoarthritis, degenerative arthritis of the knees, hips, or the lower back, apnea, and pickwickian syndrome.

[0120] The subject methods also find use in the modulation of carbohydrate metabolism in hosts not suffering from a particular condition but in which the modulation of carbohydrate metabolism is nonetheless desired. Subject treatment methods are typically performed on hosts with such disorders or on hosts with a desire to avoid contracting such disorders. As such, the invention also contemplates preventing or reducing the risk of a disease relating to abnormal carbohydrate metabolism in a host by administering a pharmaceutical composition, and for achieving a desired outcome that is not abnormal, but otherwise desirable, for example weight loss of a non-obese host, or a decrease in blood glucose levels in a non-diabetic host.

[0121] In particular, modulation of glucose uptake and glycogen synthesis in muscle or liver cells, or lipid (e.g. triacylglycerol), transport, synthesis, and storage e.g. in adipocyte cells is of interest.

[0122] Kits

[0123] Also provided by the subject invention are kits for practicing the subject methods, as described above. The subject kits at least include one or more of a pharmaceutical preparation comprising at least one active agent that modulates DGAT activity, such as those representative DGAT1 modulatory agents described above. Other optional components of the kit include: a syringe or another administration device, and glucose-monitoring devices such as glucose test strips, etc. Also included in the kit may be insulin, leptin, or an activity mimetic thereof. The various components of the kit may be present in separate containers or certain compatible components may be pre-combined into a single container, as desired. In many embodiments, kits with unit doses of the active agent, e.g., in oral or injectable doses, are provided. In many embodiments the subject composition is contained within a media, such as a media suitable for injection into a human bloodstream.

[0124] In addition to above-mentioned components, the subject kits typically further include instructions for using the components of the kit to practice the subject methods treating a host suffering from a disease relating to abnormal carbohydrate metabolism by administering to said host an effective amount of one or more active agents that modulate DGAT in the host to modulate sensitivity to insulin and/or leptin sensitivity in the host and treat the host for the condition. The instructions for practicing the subject methods are generally recorded on a suitable recording medium. For example, the instructions may be printed on a substrate, such as paper or plastic, etc. As such, the instructions may be present in the kits as a package insert, in the labeling of the container of the kit or components thereof (i.e., associated with the packaging or subpackaging) etc. In other embodiments, the instructions are present as an electronic storage data file present on a suitable computer readable storage medium, e.g. CD-ROM, diskette, etc. In yet other embodiments, the actual instructions are not present in the kit, but means for obtaining the instructions from a remote source, e.g. via the internet, are provided. An example of this embodiment is a kit that includes a web address where the instructions can be viewed and/or from which the instructions can be downloaded. As with the instructions, this means for obtaining the instructions is recorded on a suitable substrate.

[0125] Animal Models for Modulated Sensitivity to Insulin and/or Leptin

[0126] The invention further provides a non-human animal model for modulated sensitivity to insulin and/or leptin. In general, the non-human animal model is characterized by having abnormal DGAT activity.

[0127] A non-human animal may be any animal, e.g., a mammal or avian species that can serve as an animal model for testing therapies sensitivity to insulin and/or leptin. In many embodiments the non-human animal is a laboratory animal, usually a rodent, e.g., mouse, rat, hamster, guinea pig or the like. Other suitable laboratory animals are rabbits, cats, dogs, small monkeys, and apes. In addition, certain farm animals are also often employed as laboratory animals, notably chickens, goats, sheep, and pigs. Mice suitable for use in the present invention can be produced from any of a variety of background strains including, but not necessarily limited to, the strains C.B-17, C3H, BALB/c, C57131/6, AKR, BA, B10, 129, etc. Non-human animals are readily available from researchers or commercial suppliers, such as Jackson Laboratories (Bar Harbor, Me.), Charles River Breeding Laboratories (Wilmington, Mass.), Taconic Farms (Germantown, N.Y.), to mention a few such suppliers.

[0128] DGAT activity, including DGAT1 and/or DGAT2 activity, may be modified in animals by a variety of methods. In many embodiments, these methods involve modifying the expression of DGAT in a transgenic animal. In many embodiments, the expression of a DGAT endogenous to the animal is reduced in an animal. This may be accomplished through knockout strategies, where a nucleic acid insertion into an endogenous gene inactivates the gene (described in U.S. Pat. Nos. 5,487,992; 5,627,059; 5,631,153; and 6,204,061), or by other methods e.g. antisense, inhibitory RNA (RNAi), ribozyme or co-suppression technologies, as is known in the art (e.g. Hannon et al., Nature 418:244-51, 2002; Ueda, J Neurogenet. 15:193-204, 2001; Review. Lin-

denbach et al., Mol Cell. 9:925-7, 2002; Brantl, Biochim Biophys Acta. 1575:15-25, 2002; Zhang et al., Ann N Y Acad Sci. 923:210-33, 2000). In other embodiments, an endogenous or exogenous DGAT is over-expressed in an animal. In these embodiments, a DGAT, leptin or leptin receptor coding sequence (for example, a coding sequence provided by one of the following NCBI accession: NM_010046 (SEQ ID NO:1), XM_035370 (SEQ ID NO:2), NM_053437 (SEQ ID NO:3), AJ318490 (SEQ ID NO:4), AF221132 (SEQ ID NO:5), AF468649 (SEQ ID NO:6), AY093657 (SEQ ID NO: 7), AF384161 (SEQ ID NO:8), NM_012079 (SEQ ID NO:9), AF384163 (SEQ ID NO:10), AF384162 (SEQ ID NO:11), AF078752 (SEQ ID NO:12), is cloned into an expression cassette in an appropriate vector, and transferred into the genome of an animal to make a transgenic animal. In certain embodiments, the animal is homozygous for a defect in a DGAT gene (DGAT1 or DGAT2), and in many embodiments, the subject animal is homozygous for a knockout in one of these genes.

[0129] Cloning technology, cloning strategies, expression cassettes, and suitable vectors for performing animal transformation are well known in the art (Ausubel, et al, *Short Protocols in Molecular Biology*, 3rd ed., Wiley & Sons, 1995; Sambrook, et al., *Molecular Cloning: A Laboratory Manual*, Second Edition, (1989) Cold Spring Harbor, N.Y.). Methods of generating transgenic, non-human animals, particularly transgenic, non-human mammals, are also known in the art. See, e.g., U.S. Pat. Nos. 6,268,545; 6,255,554; 6,222,094; 5,387,742, 4,736,866 and 5,565,186 and 6,204,43; "Transgenic Animal Technology" C. A. Pinkert, ed. (1997) Acad. Press; "Gene Knockout Protocols" M. J. Tymms, et al., eds. (2001) Humana Press; and "Gene Targeting: A Practical Approach" A. L. Joyner, ed. (2000) Oxford Univ. Press.

[0130] One method for producing a transgenic animal employs embryonic stem (ES) cells obtained from an animal to be transformed, e.g. mouse, rat, guinea pig, etc. In these methods, ES cells are grown on an appropriate fibroblast-feeder layer or grown in the presence of appropriate growth factors, such as leukemia inhibiting factor (LIF). When ES cells have been transformed, they may be used to produce transgenic animals. After transformation, the cells are plated onto a feeder layer in an appropriate medium. Cells containing the construct may be detected by employing a selective medium. After sufficient time for colonies to grow, they are picked and analyzed for the occurrence of homologous recombination or integration of the construct. Those colonies that are positive may then be used for embryo manipulation and blastocyst injection. Blastocysts are obtained from 4 to 6 week old superovulated females. The ES cells are trypsinized, and the modified cells are injected into the blastocoel of the blastocyst. After injection, the blastocysts are returned to each uterine horn of pseudopregnant females. Females are then allowed to go to term and the resulting litters screened for mutant cells having the construct. By providing for a different phenotype of the blastocyst and the ES cells, chimeric progeny can be readily detected. Progeny of transgenic animals may be screened for the presence of the modified gene and males and females having appropriate modified genomes are mated to produce homozygous progeny.

[0131] In certain embodiments, transgenic animals may be inter-crossed or may contain more than one genetic modification

in order to produce a subject animal model. For example, an animal overexpressing DGAT1 may be bred with-an animal knockout of a leptin-encoding gene to produce an a subject-animal model containing an increase in DGAT activity and a decrease in leptin activity and an animal overexpressing leptin may also be bred with an animal knockout of a DGAT-encoding gene to produce an a subject animal model containing a decrease in DGAT activity and an increase in leptin activity, etc. Animals with abnormal DGAT expression may be intercrossed with animals containing abnormal expression of other genes (e.g. obese, agouti yellow) in order to produce a subject animal model.

[0132] In embodiments where DGAT is over-expressed in a subject animal, DGAT activity is increased more than about 1.5-fold, more than about 2-fold, more than about 3-fold, more than about 5-fold, more than about 10-fold or even more than about 100-fold in a subject animal, as compared to an animal in which DGAT expression is not increased.

[0133] In embodiments where DGAT expression is decreased in a subject animal, DGAT expression is decreased by more than about 30%, more than about 50%, more than about 70%, more than about 90%, more than about 95% or even more than about 98%, about 99% or 99.5% in a subject animal, as compared to an animal in which DGAT is not decreased.

[0134] The subject animals have abnormal sensitivity to insulin and/or leptin relative to a normal animal of the same species. In certain embodiments, the features exhibited by the subject animals include, but are not limited to, increased or decreased blood glucose, increased or decreased blood insulin, increased or decreased body weight, increased or decreased physical activity, increased or decreased fat pad content, and increased or decreased sensitivity to insulin and/or leptin. In these embodiment, subject animal features may be increased or decreased by about 10% or more, about 20% or more, about 30% or more, about 50% or more, about 70% or more, about 80% or more, about 90% or more, or even about 95% or more as compared to a normal animal of the same species. Such animals find use in a variety of applications, including the screening methods described below.

[0135] Screening Assays

[0136] The invention further provides methods of screening a candidate agent for an activity that modulates sensitivity to insulin and/or leptin, e.g. stimulators or inhibitors of sensitivity to insulin and/or leptin. These screening assays typically provide for qualitative/quantitative measurements of a phenomenon associated with sensitivity to insulin and/or leptin in the presence of a particular candidate agent. The screening methods be performed in vivo, in vitro or in a cell free system, which are readily developed by those of skill in the art. Test agents that have a desirable effect in any subject screening assay method find use in a variety of applications, including modulating sensitivity to insulin and/or leptin in a host, modulating carbohydrate metabolism in a host, or treating a disorder associated with carbohydrate metabolism.

[0137] Using the above screening methods, a variety of different agents may be identified. Such agents may target

the DGAT enzyme itself, or an expression regulatory factor thereof. Such agents may be inhibitors or promoters of DGAT activity, where inhibitors are those agents that result in at least a reduction of DGAT activity as compared to a control and enhancers result in at least an increase in DGAT activity as compared to a control. Specific screening assay methods are described below.

[0138] In vivo Assays

[0139] The invention provides in vivo methods of screening a candidate agent for an activity that modulates sensitivity to insulin and/or leptin. In general, the method involves administering a candidate agent to an animal, particularly a subject transgenic animal and determining the effect of the candidate agent on sensitivity of the animal to insulin and/or leptin.

[0140] The invention further provides in vivo methods of screening a candidate agent for an activity that modulates carbohydrate metabolism. In general, the method involves administering a candidate agent to an animal, particularly a subject transgenic animal and determining the effect of the candidate agent on a phenomenon associated with carbohydrate metabolism.

[0141] The invention further provides in vivo methods of screening a candidate agent for an activity that reduces a symptom of a condition associated with abnormal carbohydrate metabolism. In general, the method involves administering a candidate agent to an animal, particularly a subject transgenic animal and determining the effect of the candidate agent on a phenomenon associated with carbohydrate metabolism.

[0142] In many embodiments the determining step of the in vivo assay method involves measuring a certain phenomenon associated with sensitivity to insulin and/or leptin or a, phenomenon associated with carbohydrate metabolism including, but not limited to: insulin sensitivity or tolerance, leptin sensitivity or tolerance, lipid composition, adipocyte size, blood glucose levels, glucose-disposal, glucose tolerance, insulin tolerance, weight loss, food intake, triacylglycerol content or composition, energy expenditure, weight, weight gain, activity or an alteration in lipid content and the like.

[0143] In certain embodiments, the subject animals are placed on a high fat diet, or a glucose-rich diet, before, during or after administration of the candidate agent, and compared to similarly fed controls.

[0144] In vivo assays of the invention include controls, where suitable controls include a sample in the absence of the test agent. Generally a plurality of assay mixtures is run in parallel with different agent concentrations to obtain a differential response to the various concentrations. Typically, one of these concentrations serves as a negative control, i.e. at zero concentration or below the level of detection.

[0145] A candidate agent of interest is one that modulates, i.e., reduces or increases, DGAT activity, DGAT expression, lipid (e.g. TAG) composition, adipocyte size, blood glucose levels, glucose disposal, glucose tolerance, insulin tolerance, weight loss, food intake, triacylglycerol content or composition, energy expenditure, weight, weight gain, activity or alters lipid content etc., by at least about 10%, at least about

20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 80%, at least about 90%, or more, when compared to a control in the absence of the test agent. In general, a candidate agent will cause a subject animal to be more similar to an equivalent animal that is not altered in DGAT activity.

[0146] In vitro Assays

[0147] The invention provides in vitro methods of screening a candidate agent for an activity that modulates sensitivity to insulin and/or leptin. In general, the methods involve contacting a cell with abnormal DGAT activity with a candidate agent and determining the effect of the agent on the cell in order to assess the candidate agent's activity that modulates sensitivity to insulin and/or leptin.

[0148] In many embodiments, the cell with abnormal DGAT activity is an in which DGAT gene expression has been modified as compared to an unaltered cell. Methods for altering gene expression in a cell are well known to one of skill in the art (discussed in Ausubel, et al., *Short Protocols in Molecular Biology*, 3rd ed., Wiley & Sons, 1995; Sambrook, et al., *Molecular Cloning: A Laboratory Manual*, Second Edition, (1989) Cold Spring Harbor, N.Y.). These methods may involve DGAT overexpression via introduction of a genetic construct designed to express DGAT coding sequences, or may involve downregulating DGAT expression via knockout strategies (described in U.S. Pat. Nos. 5,487,992; 5,627,059; 5,631,153; and 6,204,061), or by other methods e.g. antisense, inhibitory RNA (RNAi), ribozyme or co-suppression technologies, as is known in the art (e.g. Hannon et al., *Nature* 418:244-51, 2002; Ueda, *J Neurogenet.* 15:193-204, 2001; Review. Lindenbach et al., *Mol Cell.* 9:925-7, 2002; Brantl, *Biochim Biophys Acta.* 1575:15-25, 2002; Zhang et al., *Ann N Y Acad Sci.* 923:210-33, 2000).

[0149] In embodiments where DGAT is overexpressed in a cell, DGAT expression is increased more than about 1.5-fold, more than about 2-fold, more than about 3-fold, more than about 5-fold, more than about 10-fold or even more than about 100-fold in the cell, as compared to a cell in which DGAT is not increased.

[0150] In embodiments where DGAT expression is decreased in a cell, DGAT expression is decreased by more than about 30%, more than about 50%, more than about 70%, more than about 90%, more than about 95% or even more than about 98%, about 99% or 99.5% in the, as compared to a cell in which DGAT is not decreased.

[0151] Methods for measuring insulin sensitivity in a cell are well known in the art, and generally involve contacting a cell with insulin or a mimetic, and assaying glucose uptake (or, for example, 2-deoxyglucose, 3-O-methylglucose, hexose, methylaminoisobutyric acid etc.), glycogen synthesis, or the expression of certain genes such as glycogen synthase. Many cell lines may be used for these methods, including fibroblast cell lines, muscle cell lines, and hepatoma cell lines. Exemplary publications describing insulin sensitivity assays are: Klein et al. (*Bioessays*, 24:382-8, 2002), Crook et al. (*Diabetes* 44:314-20, 1995), Saribia et al. (*Biochem Cell Biol* 68:536-42, 1990), Meienhofer et al., (*Eur J Biochem* 169:237-43, 1987), Grunfeld et al. (*Endocrinology*

113:1763-70, 1983) and several other assays would be immediately apparent to one of skill in the art.

[0152] Methods for measuring leptin sensitivity are also well known the art, and may be performed on a number of cell lines, including human breast cells, pituitary cells, hepatoma cells and pancreatic cells. Exemplary publications describing insulin sensitivity assays are: Smith et al., (*Domest Anim Endocrinol.* 22:145-54, 2002), Tsumanuma et al., (*Pituitary.* 3:211-20, 2000), Kaser et al., (*Int J Obes Relat Metab Disord.* 25:1633-9, 2001), Tanizawa et al., (*Endocrinology.* 138:4513-6, 1997) and Gainsford et al., (*Proc Natl Acad Sci* 93:14564-8, 1996) and several other assays would be immediately apparent to one of skill in the art.

[0153] In certain embodiments the subject cell is a cell from a subject model animal. In these embodiments a cell from a subject animal model is isolated and may be cultured to produce a cell that has altered DGAT activity.

[0154] In certain embodiments the determining step of the in vitro assay method involves measuring DGAT activity, DGAT expression, lipid (e.g. TAG) biosynthesis, deposition or secretion and the like.

[0155] In vitro assays of the invention include controls, where suitable controls include a sample in the absence of the test agent. Generally a plurality of assay mixtures is run in parallel with different agent concentrations to obtain a differential response to the various concentrations. Typically, one of these concentrations serves as a negative control, i.e. at zero concentration or below the level of detection.

[0156] A test agent of interest is one that modulates, i.e., reduces or increases, DGAT activity, DGAT expression, lipid (e.g. TAG) biosynthesis, deposition or secretion, or sensitivity to insulin and/or leptin by at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 80%, at least about 90%, or more, when compared to a control in the absence of the test agent. In general, a test agent will cause a subject cell to be more similar to an equivalent cell that is not altered in DGAT activity.

[0157] Cell Free Assays

[0158] The invention provides cell free methods of screening a candidate agent for an activity that modulates sensitivity to insulin and/or leptin. In general, the methods involve admixing an extract of a cell (or a synthetic mimetic thereof) with DGAT activity, or isolated DGAT polypeptide, with a candidate agent and determining the effect of the agent on the extract in order to assess the candidate agent's activity that modulates sensitivity to insulin and/or leptin. In many embodiments the assay methods involve measuring DGAT activity, lipid (e.g. TAG) biosynthesis, or and the like,

[0159] Cell free assays of the invention include controls, where suitable controls include a sample in the absence of the candidate agent. Generally a plurality of assay mixtures is run in parallel with different agent concentrations to obtain a differential response to the various concentrations. Typically, one of these concentrations serves as a negative control, i.e. at zero concentration or below the level of detection.

[0160] A test agent of interest is one that modulates, i.e., reduces or increases, DGAT activity, lipid biosynthesis or the like, by at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 80%, at least about 90%, or more, when compared to a control in the absence of the test agent. In general, a candidate agent will cause a subject extract to be more similar to an equivalent extract from a cell that is not altered in DGAT activity.

[0161] A variety of other reagents may be included in the screening assay. These include reagents like salts, neutral proteins, e.g. albumin, detergents, etc that are used to facilitate optimal protein-protein binding and/or reduce non-specific or background interactions. Reagents that improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, anti-microbial agents, etc. may be used.

[0162] Candidate Agents

[0163] The terms "candidate agent," "test agent," "agent," "substance" and "compound" are used interchangeably herein and describe a variety of agents that may be screened using the above methods.

[0164] Candidate agents encompass numerous chemical classes, though typically they are organic molecules, preferably small organic compounds having a molecular weight of more than 50 and less than about 2,500 daltons. Candidate agents comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. The candidate agents often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Candidate agents are also found among biomolecules including peptides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof.

[0165] Candidate agents include those found in large libraries of synthetic or natural compounds. For example, synthetic compound libraries are commercially available from Maybridge Chemical Co. (Trevillet, Cornwall, UK), ComGenex (South San Francisco, Calif.), and MicroSource (New Milford, Conn.). 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 Pan Labs (Bothell, Wash.) or are readily producible. Additionally, natural or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical means, and may be used to produce combinatorial libraries. Known pharmacological agents may be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification, amidification, etc. to produce structural analogs. New potential therapeutic agents may also be created using methods such as rational drug design or computer modeling. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides and oligopeptides.

[0166] Screening may be directed to known pharmacologically active compounds and chemical analogs thereof, or to new agents with unknown properties such as those created through rational drug design.

[0167] A variety of other reagents may be included in screening assays. These include reagents like salts, neutral proteins, e.g. albumin, detergents, etc that are used to facilitate optimal protein-protein binding and/or reduce non-specific or background interactions. Reagents that improve the efficiency of the assay, such as protease inhibitors, anti-microbial agents, etc. may be used. The mixture of components is added in any order that provides for the requisite binding. Incubations are performed at any suitable temperature, typically between 4 and 40° C. Incubation periods are selected for optimum activity, but may also be optimized to facilitate rapid high-throughput screening. Typically between 0.1 and 1 hour will be sufficient.

[0168] Candidate agents may also include biopolymers, including nucleic acids (e.g. DNA, RNA, cDNA, plasmids and this like), for example those encoding DGAT1 or DGAT2, or antisense DGAT1 or DGAT2 nucleic acids and the like, carbohydrates, lipids (e.g. lipids that inhibit the activity of DGAT) and proteins and polypeptides, (such as DGAT1 or DGAT2 or an antibody specific for DGAT1 or DGAT2).

[0169] In particular embodiments, the candidate agent may be niacin, or other agents known in the art, e.g. those described in Lesnik et al. (Arch Dermatol Res 1992;284(2):100-5).

[0170] Agents that have an effect in an assay method of the invention may be further tested for cytotoxicity, bioavailability, and the like, using well known assays. Agents that have an effect in an assay method of the invention may be subjected to directed or random and/or directed chemical modifications, such as acylation, alkylation, esterification, amidification, etc. to produce structural analogs. Such structural analogs include those that increase bioavailability, and/or reduced cytotoxicity. Those skilled in the art can readily envision and generate a wide variety of structural analogs, and test them for desired properties such as increased bioavailability and/or reduced cytotoxicity and/or ability to cross the blood-brain barrier.

[0171] The following examples are presented for purposes of illustration only, and are not to be construed as limiting on the scope of the invention in any way.

EXPERIMENTAL

[0172] Materials and Methods

[0173] Mice: *Dgat1*^{-/-} mice (about 95% C57BL/6 and 5% 129/SvJae background) were generated previously (Smith, et al.(2000) Nat. Genet. 25:87-90). Wild-type (*Dgat1*^{+/+}), *ob/ob*, and *A^Y/a* mice (all in C57BL/6 background) were from the The Jackson Laboratory (Bar Harbor, Me. USA). *A^Y/a* mice are obese and insulin resistant, reflecting the antagonism of melanocyte-stimulating hormone in the hypothalamus. They are also severely leptin resistant. Genotyping for *Dgat1* and *ob* was performed as described (Smith et al., supra). *A^Y/a* mice were identified by their yellow fur. Mice were housed in a pathogen-free barrier facility (12-hour light/12-hour dark cycle) and fed rodent chow (Ralston Purina Co., St. Louis, Miss., USA). For high-fat diet experi-

ments, mice were fed a Western-type diet containing 21% fat by weight (Harlan Teklad Laboratory, Madison, Wis., USA) for 4 weeks unless stated otherwise. All experiments were approved by the Committee on Animal Research of the University of California, San Francisco.

[0174] Tissue lipid analysis. Tissue lipids were extracted and separated by TLC as described (Smith et al., supra). Triglycerides and diacylglycerol were scraped from the TLC plate, and heptadecanoic acid (about 10% of total fatty acids) was added as an internal standard. Methyl esters were synthesized with the addition of methanolic HCl/toluene (4:1 vol/vol) to the TLC adsorbent for 12 hours at 37° C., extracted with hexane, and analyzed with gas-liquid chromatography (HP6890 Gas Chromatograph; Hewlett-Packard, Palo Alto, Calif., USA). Methyl esters were separated with a 10-ft glass column (10% SP-2330 on 100/120 Chromosorb WAW; Supelco, St. Louis, Mo., USA) at 175° C. for 5 minutes and increasing to 210° C. at a rate of 2.5° C./min. The weight of triglycerides and diacylglycerol in the samples was calculated with reference to the internal standard.

[0175] Adipocyte size determination: Adipose tissue was obtained from the reproductive fat pads of 14-week-old male mice. The samples were fixed in paraformaldehyde, embedded in paraffin, cut into 5- μ m sections, and stained with hematoxylin and eosin. Images of the histology sections were analyzed with Adobe Photoshop 5.0.1 (Adobe Systems Inc., San Jose, Calif., USA) and Image Process Tool Kit (Reindeer Games, Gainesville, Fla., USA) as described (Chen and Farese, J. Lipid Res. 43:986-9, 2002).

[0176] Glucose metabolism studies: Glucose (1 g/kg body wt) or bovine insulin (1 U/kg body wt; Sigma Chemical Co., St. Louis, Mo., USA) was injected intraperitoneally, and glucose concentrations were measured with a glucometer (Accu-Chek; Roche Diagnostics Corp., Indianapolis, Ind., USA). The hyperinsulinemic-euglycemic clamp studies were performed as described (Ferriera Diabetes. 50:1064-1068, 2001) with slight modifications. Weight-matched 10- to 14-week-old male mice were fasted for 4 hours before the clamp studies. Insulin was infused at 20 mU/kg/min, and plasma glucose concentration was clamped at 120 mg/dl. For *ob/ob* and *A^Y/a* mice, nonfasting blood samples for glucose and insulin measurements were obtained at noon. Insulin was measured with a rat insulin RIA kit (Linco Research Inc., St. Charles, Mo., USA).

[0177] Leptin infusion studies: Mice were infused with recombinant human leptin (gift from F. Chehab, University of California, San Francisco) with a microosmotic pump (Alza model 1002; DURECT Corp., Cupertino, Calif., USA), inserted subcutaneously into the interscapular region. Plasma leptin levels were measured by Anilytics Inc. (Gaithersburg, Md., USA).

[0178] Northern blots. White adipose tissue (WAT) was obtained from the reproductive fat pads and brown adipose tissue (BAT) from the interscapular region of 10- to 14-week-old male mice. Total RNA was isolated, and pooled RNA samples (10 μ g) were subjected to electrophoresis and blot hybridization with ³²P-labeled cDNA probes. Blots were rehybridized with a β -actin probe (Ambion Inc., Austin, Tex., USA) for loading normalization. Probes for uncoupling protein 2 (UCP-2) and UCP-3 were generated by PCR with WAT cDNA and the following primers: 5'-GTCGAT-

TCCGCCCTCGGTG-3' (SEQ ID NO:13), 5'-GAGG-GAAAGTGATGAGATCT-3' (SEQ ID NO:14) (UCP-2); 5'-GTCGGACACAGCCTTCTGC-3', (SEQ ID NO:15)5'-ACCTTGGACCGCCAGCCGG-3' (SEQ ID NO:16) (UCP-3). The remaining probes were gifts from M. Reitman, NIH, Bethesda, Md., USA (UCP-1); B. Staels, Institut National de la Santé et de la Recherche Médicale (Lille, France) (acyl CoA oxidase); B. Spiegelman, Dana-Farber Cancer Institutes, Boston, Mass., USA (peroxisome proliferator-activated receptor); and I. Shimomura, M. Brown, and J. Goldstein, University of Texas Southwestern, Dallas, Tex., USA (fatty acid synthase). Signals were quantified with a PhosphorImager (Bio-Rad Laboratories, Hercules, Calif., USA).

[0179] Real-time PCR: Tissues were homogenized, and total RNA was extracted. RNA (1 µg) was reverse-transcribed in a 20-µl reaction containing oligo(dT)₁₂₋₁₈ primer and Superscript II enzyme (Invitrogen Corp., Carlsbad, Calif., USA). Primer and probe sequences (Table 1) were selected with Primer Express (Perkin-Elmer Applied Biosystems, Foster City, Calif., USA). The PCR reaction (50 µl) contained 1 µl of cDNA, 1× Gold buffer, 4 mM MgCl₂, 500 µM dNTP, primers (200 nM), 100 nM probe (labeled with 6-carboxyfluorescein), and 1.25 U of AmpliTaq Gold DNA polymerase (Perkin-Elmer Applied Biosystems). Real-time PCR was performed with the ABI Prism 7700 System (Perkin-Elmer Applied Biosystems). Expression levels were calculated by the comparative cycle of threshold detection method, according to the manufacturer. Expression of β-actin was used for loading normalization.

[0180] Statistical methods: Data are shown as mean±SD unless stated otherwise. Measurements were compared with the t test or Mann-Whitney rank-sum test. Differences in body weight or food intake were compared with ANOVA, followed by the Tukey-Kramer test.

taining the mouse DGAT gene was isolated from a genomic 129/Sv library. Short and long arms of homologous sequences were amplified by PCR from this clone and subcloned into pNTKloxP to generate a gene targeting vector. The vector contained a neomycin resistance gene for positive selection and a thymidine kinase gene for negative selection. Upon homologous recombination, the vector was designed to interrupt the DGAT coding sequences at amino acid 360 of the 498-amino acid murine protein. The entire C-terminus, including a highly conserved region common to all ACAT gene family members is deleted. The gene targeting vector was electroporated into RF8 embryonic stem cells by electroporation, and several targeted clones were identified by Southern blotting (targeting frequency of ~1 in 300).

[0182] One of these targeted clones was injected into C57BL/6 blastocysts and chimeras were generated; male chimeras subsequently passed the DGAT knockout mutation through the germline to their offspring. The resultant mice, which were heterozygous for the DGAT gene disruption, were intercrossed to generate mice that were homozygotes.

[0183] Inactivation of the DGAT-gene in the homozygote knockouts was verified by examining DGAT mRNA which was found to be absent in the knockout mice. In activation of the DGAT gene was also verified by studying DGAT activity in tissues using an assay that measures the incorporation of [14C]oleoyl.CoA into triglycerides. The results from the activity assays show that DGAT activity is virtually gone from every nearly every tissue tested.

EXAMPLE II

Altered Lipid Composition in Tissues of Dgat1^{-/-} Mice

[0184] Dgat1^{-/-} mice had a 30-40% reduction of triglyceride levels in WAT and skeletal muscle (Table 2). Liver

TABLE 1

Real-time PCR primer and probe sequences				
Table 1				
Gene:	Primer pair or probe		Sequence	
Actin	5'	5'	5'-CATCTTGGCCTCACTGTCCA-3'	SEQ ID NO:17
	3'	5'	5'-GGGCCGGACTCATCGTACT-3'	SEQ ID NO:18
	Probe	5'	5'-CTTCCAGCAGATGTGGATCAGCAAGC-3'	SEQ ID NO:19
DGAT2	5'	5'	5'-AGTGGCAATGCTATCATCATCGT-3'	SEQ ID NO:20
	3'	5'	5'-AAGGAATAAGTGGGAACCCAGATCA-3'	SEQ ID NO:21
	Probe	5'	5'-CCTGGCAAGAACGCAGTCACCTG-3'	SEQ ID NO:22
Leptin	5'	5'	5'-TCTCCGAGACCTCCTCCATCT-3'	SEQ ID NO:23
	3'	5'	5'-TTCCAGGACGCCATCCAG-3'	SEQ ID NO:24
	Probe	5'	5'-TCCCTGCCTCAGACCAGTGGCCT-3'	SEQ ID NO:25
PPAR	5'	5'	5'-CAGGAGAGCAGGGATTGCA-3'	SEQ ID NO:26
	3'	5'	5'-CCTACGCTCAGCCCTCTTCAT-3'	SEQ ID NO:27
	Probe	5'	5'-AGAGGCCTCCCTCCTACGCTTGG-3'	SEQ ID NO:28

EXAMPLE I

Preparation and Characterization of DGAT Knockout Mice

[0181] DGAT knockout mice were generated using standard techniques of gene targeting. A mouse P1 clone con-

triglyceride levels trended lower in chow-fed Dgat1^{-/-} mice. On a high-fat diet, however, Dgat1^{-/-} mice had significantly lower liver triglyceride levels than Dgat1^{+/+} mice (28±16 versus 157±28 mg/g tissue weight, P<0.05). Unexpectedly, levels of diacylglycerol, a substrate for the DGAT reaction, were not elevated, and in fact tended to be lower in Dgat1^{-/-}

tissues (Table 2). DGAT1 deficiency also altered the fatty acid composition of triglycerides in WAT and skeletal muscle, resulting in a relative decrease in monounsaturated (16:1 and 18:1) fatty acids and a relative increase in saturated (16:0 and 18:0) fatty acids (Table 2).

body weight and food intake. Leptin infusion achieved comparable levels of increase in plasma leptin levels in Dgat1^{+/+} and Dgat1^{-/-} mice (0.7±0.1 versus 0.5±0.2 ng/ml for 6 μg/day, P>0.05). In young (10- to 14-week-old) Dgat1^{+/+} mice, leptin administration suppressed the normal

TABLE 2

Lipid composition of tissues in Dgat1 and Dgat1 ^{+/+} mice									
	Triglycerides	Diacylglycerol	Fatty acid composition of triglycerides (% of total fatty acids)						
			(% of tissue weight)	16:0	16:1	18:0	18:1	18:2w6	18:3w6
White adipose tissue									
+/+	32.4 ± 7.9	0.06 ± 0.02	20.0 ± 0.6	8.4 ± 1.2	1.8 ± 0.2	37.3 ± 1.2	25.3 ± 1.9	0.3 ± 0.3	0.7 ± 0.1
-/-	20.3 ± 3.0 ^A	0.04 ± 0.01	26.4 ± 1.5 ^B	4.5 ± 1.4 ^B	3.8 ± 1.0 ^B	34.2 ± 1.3 ^A	25.3 ± 3.4	0.1 ± 0.3	0.9 ± 0.4
Skeletal muscle									
+/+	4.3 ± 1.9	0.008 ± 0.002	21.0 ± 2.0	7.7 ± 1.1	<0.001	38.0 ± 1.9	24.3 ± 2.8	0.2 ± 0.2	1.1 ± 0.6
-/-	2.4 ± 0.5 ^A	0.006 ± 0.003	27.3 ± 0.7 ^B	3.1 ± 1.1 ^B	<0.001	33.5 ± 1.7 ^A	23.3 ± 2.8	0.3 ± 0.2	1.3 ± 0.4
Liver									
+/+	0.2 ± 0.1	0.013 ± 0.003	ND	ND	ND	ND	ND	ND	ND
-/-	0.1 ± 0.0	0.003 ± 0.001 ^A							

Twelve to sixteen-week-old male mice fed a chow diet were used
ND, not determined.
^AP < 0.05,
^BP < 0.01 versus Dgat1^{+/+}
n = 3 per genotype.

EXAMPLE III

Decreased Adipocyte Size in Dgat1^{-/-} Mice

[0185] Concomitant with the decreased triglyceride levels in WAT, Dgat1^{-/-} mice had smaller adipocytes than Dgat1^{+/+} mice on both chow and high-fat diets (FIG. 1). Adipocytes from Dgat1^{-/-} mice developed minimal hypertrophy in response to a high-fat diet, whereas those from Dgat1^{+/+} mice doubled in size. This protection from diet-induced adipocyte hypertrophy in Dgat1^{-/-} mice mirrored their weight response to a high-fat diet. In addition to having smaller adipocytes, male Dgat1^{-/-} mice had a lower mean DNA content in reproductive fat pads than Dgat1^{+/+} mice (169±36 versus 273±50 μg/fat pad, P<0.05), showing that Dgat1^{-/-} mice also had fewer adipocytes.

EXAMPLE IV

Increased Insulin Sensitivity in Dgat1^{-/-} mice

[0186] Glucose- and insulin-tolerance tests were performed. Dgat1^{-/-} mice had lower plasma glucose concentrations than Dgat1^{+/+} mice after a glucose load (FIG. 2a) or an insulin injection (FIG. 2b), showing that Dgat1^{-/-} mice have increased insulin sensitivity. This was confirmed in hyperinsulinemic-euglycemic clamp studies, which showed that Dgat1^{-/-} mice required an approximately 20% higher glucose infusion rate than Dgat1^{+/+} mice to maintain euglycemia (FIG. 2c).

EXAMPLE V

Increased Weight Loss in Response to Leptin Infusion in Dgat1^{-/-} Mice

[0187] Leptin was subcutaneously infused into Dgat1^{+/+} mice and Dgat1^{-/-} mice and measured their response in

weight gain seen in control (PBS-infused) mice (FIG. 3a). The same doses of leptin caused an additional 3% weight loss in age-matched Dgat1^{-/-} mice (FIG. 3a), indicating an enhanced response to leptin.

[0188] Dgat1^{-/-} mice consistently ate more than Dgat1^{+/+} mice at baseline (25.3%±1.6% versus 19.5%±3.2% of body weight, P<0.05). During leptin infusion, Dgat1^{-/-} mice continued to eat more than Dgat1^{+/+} mice (19.8%±3% versus 15.8%±2.4% of body weight after 5 days of leptin (6 μg/day) infusion, P<0.05). Expressed as percentage of change from baseline, reduction in food intake in response to leptin infusion was comparable in Dgat1^{-/-} and Dgat1^{+/+} mice (FIG. 3, c and d). Similarly, the absolute reduction in food intake per day was similar in Dgat1^{+/+} and Dgat1^{-/-} mice in response to leptin infusion in several different experiments (not shown). These results imply that the increased weight loss in leptin-treated Dgat1^{-/-} mice resulted from increased energy expenditure rather than reduced food intake.

EXAMPLE VI

Expression of Leptin-Regulated Genes in Dgat1^{-/-} Mice

[0189] The expression of several leptin-regulated genes in BAT and WAT of Dgat1^{-/-} mice was measured (FIG. 4). In Dgat1^{-/-} BAT, UCP 1 expression was increased by approximately 70% versus controls. In WAT, Dgat1^{-/-} mice had increased levels of UCP3 expression, and UCP2 expression levels trended higher. The expression of genes involved in fatty acid oxidation (peroxisome proliferator-activated receptor α[PPARα] and acyl CoA oxidase) was also higher in Dgat1^{-/-} WAT. In contrast, Dgat1^{-/-} mice had decreased levels of expression for genes involved in adipogenesis

(PPAR γ) and lipid synthesis (fatty acid synthase) in WAT. Dgat1^{-/-} mice also had an approximate 50% reduction in leptin (ob) mRNA expression. These results show that Dgat1^{-/-} mice have increased leptin sensitivity at baseline.

EXAMPLE VII

DGAT1 Deficiency Protects Against Obesity and Insulin Resistance in A^{Y/a} Mice but not in ob/ob Mice

[0190] DGAT1 deficiency protects against obesity and insulin resistance induced by high-fat feeding. We introduced DGAT1 deficiency into A^{Y/a} and ob/ob mice through breeding. DGAT1 deficiency protected against obesity in A^{Y/a} mice (FIG. 5a), decreasing body weight (FIG. 5b) and fat pad content (FIG. 5c) by approximately 20% at 25 weeks. A^{Y/a} Dgat1^{-/-} and A^{Y/a} Dgat1^{+/-} mice had similar plasma glucose levels (FIG. 5d), but DGAT1 deficiency reduced plasma insulin levels by approximately 80% in A^{Y/a} mice (FIG. 5e), most likely by increasing their insulin sensitivity. In contrast, DGAT1 deficiency had no apparent effects in ob/ob mice. In the setting of leptin deficiency, both Dgat1^{-/-} and Dgat1^{+/-} mice became obese (FIG. 5f) and diabetic, with similar growth curves (FIG. 5g), fat pad content (FIG. 5h), and plasma glucose (FIG. 5i) and insulin (FIG. 5j) levels. DGAT1 deficiency also had no apparent effect on the obesity and diabetes of mice lacking functional leptin receptors (db/db mice, not shown).

EXAMPLE VIII

Increased DGAT2 mRNA Expression in WAT of Leptin-Deficient Dgat1^{-/-} Mice

[0191] DGAT2 mRNA expression in WAT of Dgat1^{-/-} mice in different backgrounds and conditions was measured (FIG. 6). DGAT2 expression was not increased in Dgat1^{-/-}

mice at baseline (chow) or after 15 weeks of a high-fat diet. DGAT2 expression was also not increased in A^{Y/a} Dgat1^{-/-} mice. In contrast, DGAT2 expression was elevated approximately threefold in leptin-deficient Dgat1^{-/-} mice. This suggests that leptin normally downregulates DGAT2 expression in WAT and that the upregulation of DGAT2 expression may compensate for the loss of DGAT1 in leptin-deficient Dgat1^{-/-} mice.

[0192] It is evident from the above results and discussion that the subject invention provides an important method for modulating sensitivity to insulin and/or leptin which method may be used for the treatment of diseases and conditions associated with altered sensitivity to leptin and/or insulin, e.g. conditions associated with abnormal carbohydrate metabolism such as diabetes and obesity. As such, the subject methods and systems find use in a variety of different applications, including research, industry, and medicine. Accordingly, the present invention represents a significant contribution to the art.

[0193] All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. The citation of any publication is for its disclosure prior to the filing date and should not be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention.

[0194] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it is readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

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Gly	Val	Met	His	Arg	Asp	Leu	Lys	Pro	Glu	Asn	Phe	Leu	Phe	Asp	Ser	145	150	155
Pro	Lys	Asp	Asp	Ala	Lys	Leu	Lys	Ala	Thr	Asp	Phe	Gly	Leu	Ser	Val	165	170	175
Phe	Tyr	Lys	Pro	Gly	Gln	Tyr	Leu	Tyr	Asp	Val	Val	Gly	Ser	Pro	Tyr	180	185	190
Tyr	Val	Ala	Pro	Glu	Val	Leu	Lys	Lys	Cys	Tyr	Gly	Pro	Glu	Ile	Asp	195	200	205
Val	Trp	Ser	Ala	Gly	Val	Ile	Leu	Tyr	Ile	Leu	Leu	Ser	Gly	Val	Pro	210	215	220
Pro	Phe	Trp	Ala	Glu	Thr	Glu	Ser	Gly	Ile	Phe	Arg	Gln	Ile	Leu	Gln	225	230	235
Gly	Lys	Leu	Asp	Phe	Lys	Ser	Asp	Pro	Trp	Pro	Thr	Ile	Ser	Glu	Ala	245	250	255
Ala	Lys	Asp	Leu	Ile	Tyr	Lys	Met	Leu	Glu	Arg	Ser	Pro	Lys	Lys	Arg	260	265	270
Ile	Ser	Ala	His	Glu	Ala	Leu	Cys	His	Pro	Trp	Ile	Val	Asp	Glu	Gln	275	280	285
Ala	Ala	Pro	Asp	Lys	Pro	Leu	Asp	Pro	Ala	Val	Leu	Ser	Arg	Leu	Lys	290	295	300
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Asn	Asp	Gly	Lys	Ile	Asp	Phe	Ser	Glu	Phe	Thr	Ala	Met	Met	Arg	Lys	450	455	460
Gly	Asp	Gly	Val	Gly	Arg	Ser	Arg	Thr	Met	Met	Lys	Asn	Leu	Asn	Phe	465	470	475
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Glu Asp Tyr Glu Asp Val Trp Arg Glu Ile Gln Ile Met His His Leu	65	70	75
Ser Glu His Pro Asn Val Val Arg Ile Lys Gly Thr Tyr Glu Asp Ser	85	90	95
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What is claimed is:

1. A method of modulating sensitivity of a host to insulin and/or leptin, said method comprising:

administering to said host an effective amount of one or more active agents that alter DGAT1 activity in said host to modulate sensitivity of said host to insulin and/or leptin.

2. The method according to claim 1, wherein said agent decreases DGAT1 activity and said method is a method of increasing sensitivity of a host to insulin.

3. The method according to claim 1, wherein said agent decreases DGAT1 activity and said method is a method of increasing sensitivity of a host to leptin.

4. The method according to claim 1, wherein said agent increases DGAT1 activity and said method is a method of decreasing sensitivity of a host to insulin.

5. The method according to claim 1, wherein said agent increases DGAT1 activity and said method is a method of decreasing sensitivity of a host to leptin.

6. A method of modulating carbohydrate metabolism in a host, said method comprising:

administering to said host an effective amount of one or more active agents that modulate DGAT1 activity in said host and modulate carbohydrate metabolism in said host.

7. The method according to claim 6, wherein said carbohydrate is glucose.

8. The method according to claim 7, wherein said active agent modulates glucose uptake into cells of said host.

9. The method according to claim 8, wherein said cells are selected from muscle and liver cells.

10. The method according to claim 7, wherein said active agent increases glycogen synthesis in cells of said host.

11. The method according to claim 10, wherein said cells are selected from muscle and liver cells.

12. The method according to claim 6, wherein said carbohydrate is fat.

13. The method according to claim 12, wherein said active agent modulates triacylglycerol synthesis.

14. The method according to claim 13, wherein said active agent modulates levels of stored triacylglycerol in cells of said host.

15. The method according to claim 14, wherein said cells are adipocyte cells.

16. A method of treating a host suffering from a condition associated with abnormal carbohydrate metabolism, said method comprising:

administering to said host an effective amount of one or more active agents that modulate DGAT activity in said host and treat said host for said condition.

17. The method according to claim 16, wherein said agent modulates sensitivity to insulin and/or leptin.

18. The method according to claim 16, wherein said agent decreases DGAT1 activity in said host and the condition is obesity.

19. The method according to claim 18, wherein said treatment comprises a reduction of host weight and/or body fat.

20. The method according to claim 16, wherein said agent decreases DGAT activity in said host and the condition is diabetes.

21. The method according to claim 20, wherein said agent treatment comprises a stabilization of blood glucose levels.

22. The method according to claim 16, wherein said agent increases DGAT1 activity in said host and said condition is anorexia.

23. The method according to claim 16, wherein said host is a human.

24. The method according to claim 16, wherein said administering step comprises co-administering insulin and/or leptin or an activity mimetic thereof with an agent that modulates DGAT1 activity.

25. A pharmaceutical preparation comprising a DGAT1 activity modulatory agent in a pharmaceutically acceptable vehicle.

26. The pharmaceutical preparation according to claim 25, wherein said DGAT1 activity modulatory agent increases DGAT1 activity and decreases insulin and/or leptin sensitivity.

27. The pharmaceutical preparation according to claim 25, wherein said DGAT1 activity modulatory agent decreases DGAT1 activity and increases insulin and/or leptin sensitivity.

28. The pharmaceutical preparation according to claim 25, further comprising insulin, leptin or a mimetic thereof.

29. A kit comprising:

(a) a pharmaceutical preparation comprising at least one active DGAT1 modulatory agent; and

(b) instructions for treating a host suffering from a condition associated with abnormal carbohydrate metabolism by administering to said host an effective amount of one or more active DGAT1 modulatory agents to treat said host for said condition.

30. The kit according to claim 29, wherein said one or more active DGAT1 modulatory agents reduce DGAT1 activity and said instructions are instructions for treating obesity and/or diabetes.

31. The kit according to claim 29, wherein said kit further includes insulin and/or leptin.

* * * * *