(51) International Patent Classification: GOIN 33/53 (2006.01)

(21) International Application Number: PCT/IB2008/002140

(22) International Filing Date: 8 August 2008 (08.08.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
1719/CHE/2007 3 August 2007 (03.08.2007) IN
2911/CHE/2007 6 December 2007 (06.12.2007) IN

(71) Applicant (for all designated States except US): DR. REDDY’S LABORATORIES LTD. [IN/IN]; 7-1-27, Ameerpet, Hyderabad, Andhra Pradesh 500 016 (IN).

(71) Inventor (for all designated States except US): MISRA, Parmal [IN/IN]; H. No. 55, Vertex Privilege, Nizampet, Kukatpally, Hyderabad, Andhra Pradesh 500 072 (IN).

(71) Inventor (for all designated States except US): CHAKRABARTI, Ranjan [IN/IN]; Flat 106, My Home Fernhill Apartments, Hyderabad, Andhra Pradesh 500 082 (IN).

(71) Inventor (for all designated States except US): MADHURAREKHA, Chinnaboina [IN/IN]; H. No.: 1-3-2, Kavadigida, Hyderabad, Andhra Pradesh 500 072 (IN).

(71) Inventor (for all designated States except US): HASHAM, Sumera Nikhat [IN/IN]; Plot No. 53/1-Muntaz Villa, P & T Colony, Trimulgherry, Secunderabad, Andhra Pradesh 500015 (IN).

(57) Abstract: A method for identifying an AMPK activator is provided. The method includes providing a sample of cells that include AMPK and perilipin, providing a sample of an AMPK activator-candidate, contacting the cell sample and the activator-candidate, and measuring a quantitative indicator of the AMPK activation within the cell sample after the contacting step.
GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG). Published: — without international search report and to be republished upon receipt of that report — the filing date of the international application is within two months from the date of expiration of the priority period.
MODULATION OF ENDOGENOUS AMPK LEVELS FOR THE TREATMENT OF OBESITY

RELATED APPLICATIONS


BACKGROUND OF THE INVENTION

[00002] The present invention relates generally to prevention and treatment of obesity and related conditions. More specifically, the present invention relates to a method and system for identifying compounds useful for the prevention and treatment of obesity and related conditions.

[00003] Obesity is a condition of energy imbalance where energy input is more than its expenditure. The World Health Organization (WHO) considers obesity as a serious health problem worldwide. This condition often leads to life-threatening diseases, such as cardiovascular diseases and diabetes. In addition to the increased clinical risks, obesity may also result in a reduced quality of life for the affected individual. Obesity is recognized as a major disorder in current society, with serious health and life quality consequences. Improved methods of treatment and/or reliable diagnoses are needed and would be beneficial for patients and their families.

[00004] Recent research indicates that activated AMPK (5'AMP-activated protein kinase) contributes to weight loss. AMPK consists of three proteins (subunits) that together make a functional enzyme that plays a role in cellular energy homeostasis. It is expressed in a number of tissues, including the liver, brain, and skeletal muscle. The net effect of AMPK activation is stimulation of hepatic fatty acid oxidation and ketogenesis, inhibition of cholesterol synthesis, lipogenesis, and triglyceride synthesis, inhibition of adipocyte lipolysis and lipogenesis, stimulation of skeletal
muscle fatty acid oxidation and muscle glucose uptake, and modulation of insulin secretion by pancreatic beta-cells.

AMPK includes a catalytic $\alpha$ subunit, a regulatory $\beta$ subunit, and the scaffolding $\gamma$ subunit imparting stability of the heterotrimer complex. AMPK belongs to a family of energy-sensing enzymes functioning as a fuel gauge that responds to changes in cellular energy stores. When AMPK senses a decrease in high-energy phosphate levels, it inhibits ATP-consuming pathways (e.g., fatty acid synthesis, cholesterol synthesis, and gluconeogenesis) and stimulates ATP-generating processes (e.g., fatty acid oxidation and glycolysis), thus restoring overall cellular energy homeostasis. AMPK is strongly activated by ATP-depleting conditions such as muscle contraction, hypoxia, ischemia, and by inhibitors of glycolysis and uncouplers of oxidative phosphorylation and also by some pharmacological agents. One way in which cells respond to an elevation in AMP levels is to activate the AMPK pathway which is a key pathway in the control of fuel metabolism. When AMPK is activated in cell types such as muscle and liver, these cells reduce fatty acid synthesis and increase fatty acid oxidation. Thus, AMPK plays a key role in energy homeostasis making it an important target for development of drugs to treat obesity and type II diabetes, as well as other conditions and syndromes associated with metabolism.


In cases of lipid metabolism, AMPK results in the phosphorylation and inactivation of acetyl-CoA carboxylase (ACC), a direct AMPK substrate, leading to decreased conversion of acetyl-CoA to

AMPK has also been shown to activate malonyl-CoA decarboxylase in skeletal muscle, further depleting malonyl-CoA. Saha, et. al., J. Biol. Chem. 275:24279-24284 (2000). Additionally, as malonyl-CoA is required for de novo synthesis of fatty acids, decreased malonyl-CoA leads to a reduction in hepatic fatty acid synthesis. Thus, inhibiting the activity of ACC is an efficient way to control fat metabolism as well as obesity. This conclusion is further supported in tests demonstrating that ACC2 knock out mice are resistant to diet-induced obesity and related diabetes, with improved insulin sensitivity. Although ACC inhibitors are able to control diet-induced obesity, existing stored fat in adipose is typically not reduced by this approach.

AMPK activation has been shown to increase glucose transport in muscles. Winder, et al., Am. J. Physiol. 277:E1-E10 (1999). AMPK activation has also been shown to suppress gluconeogenesis in the liver. Fryer, et. al., J. Biol. Chem. 211:25226-25232 (2002).

U.S. Patent No. 7,098,220, to Rault discloses the use of imadazopyridine derivatives as AMPK activators in the treatment of diabetes and the metabolic disorders associated with diabetes. U.S. Patent No. 7,119,205, to Iyengar, discloses the use of thienopyridone as AMPK activators for the treatment of diabetes and obesity. Although these patents show that AMPK activators demonstrate anti-obesity and anti-diabetic activity, neither of these patents recognizes the mechanism by which the AMPK activators work. Accordingly, these patents do not
recognize methods for identifying additional AMPK activators useful in the prevention and/or treatment of obesity and obesity-related conditions, nor do they recognize methods for determining the efficacy of particular AMPK activators for treatment of obesity and obesity-related conditions. Stated differently, these patents do not provide a method for readily identifying AMPK activator-candidates that may be used for the prevention and/or treatment of obesity and obesity-related conditions.

[00012] Perilipin is a protein that coats lipid droplets in adipocytes, the fat-storing cells in adipose tissue. Perilipin acts as a protective coating from the body’s natural lipases, such as hormone-sensitive lipase, that break triglycerides into glycerol and free fatty acids for use in metabolism, a process called lipolysis.

[00013] Perilipin is hyperphosphorylated by protein kinase A (PKA) following β-adrenergic receptor activation. Phosphorylated perilipin changes conformation, exposing the stored lipids to hormone-sensitive lipase-mediated lipolysis.

[00014] Perilipin is an important regulator of lipid storage. Perilipin expression is elevated in obese animals and humans. Perilipin-null mice eat more food than wild-type mice, but gain 1/3 less fat than wild-type mice on the same diet. Additionally, perilipin-null mice are thinner, with more lean muscle mass. Perilipin-null mice also exhibit enhanced leptin production and a greater tendency to develop insulin resistance than wild-type mice.

[00015] It would be desirable, therefore, to develop a method and system for identifying AMPK activators that may be used for the prevention and/or treatment of obesity and obesity-related conditions.

SUMMARY OF THE INVENTION

[00016] Briefly, therefore, in one aspect the present invention is a method for identifying an AMPK activator. The method includes providing a sample of cells that include AMPK and perilipin, providing a sample of an AMPK activator-candidate, contacting the cell sample and the activator-
candidate, and measuring a quantitative indicator of the AMPK activation within the cell sample after the contacting step.

[00017] In another aspect, the invention is a method of screening for an activator of AMPK. The method includes providing at least one cell including AMPK and perilipin, contacting the at least one cell with an AMPK activator-candidate, measuring perilipin phosphorylation, and comparing the phosphorylation in the presence of the activator-candidate with the phosphorylation in the absence of the activator-candidate. In the present method, an increased rate in the phosphorylation in the presence of the activator-candidate, as compared with the rate of phosphorylation in the absence of the activator-candidate, identifies the activator-candidate as an AMPK activator.

[00018] In yet another aspect, the invention is a method of producing an activator of AMPK. The method includes providing at least one cell including AMPK and perilipin, contacting the at least one cell with an AMPK activator-candidate, measuring phosphorylation of perilipin, and comparing the phosphorylation in the presence of the activator-candidate with the phosphorylation in the absence of the activator-candidate. In the present method, an increased rate of phosphorylation in the presence of the activator-candidate, as compared with the rate of phosphorylation in the absence of the activator-candidate, identifies the activator-candidate as an AMPK activator. After identification of the AMPK activator, the method includes producing the AMPK activator. Optionally, the AMPK activator that is produced can be administered to subjects in need of prevention and/or treatment of obesity and related conditions.

[00019] In another aspect, the invention is a kit for identification of AMPK activators useful for the treatment and prevention of obesity and obesity-related conditions. The kit includes an assay including at least one cell including therein AMPK and perilipin.

[00020] In a different aspect, the invention is a kit for identification of AMPK activators useful for the treatment and prevention of obesity and
obesity-related conditions. The kit includes an assay including therein at least AMPK and perilipin.

[00021] In another aspect, the invention is a method of identifying a compound having anti-obesity activity. The method includes providing an assay including AMPK and perilipin, contacting the assay with an AMPK activator-candidate, measuring perilipin phosphorylation, and comparing the phosphorylation in the presence of the activator-candidate with the phosphorylation in the absence of the activator-candidate. In the present method, an increased rate of phosphorylation in the presence of the activator-candidate, as compared with the rate of phosphorylation in the absence of the activator-candidate, identifies the activator-candidate as a compound having anti-obesity activity.

[00022] These and other aspects of the invention will be understood and become apparent upon review of the specification by those having ordinary skill in the art.

BRIEF DESCRIPTION OF THE DRAWINGS

[00023] Figure 1 is diagram of the metabolic pathway of AMPK activation in a cell discovered according to the present invention.

[00024] Figure 2 demonstrates the prospective sites of AMPK phosphorylation in accordance with the present invention.

[00025] Figure 3 demonstrates activation of AMPK resulting in phosphorylation of perilipin as revealed by Western Blotting in accordance with the present invention.

[00026] Figure 4 is an expression profile of SREBP1 C, DGATs, and SCDs in Adipose in accordance with the present invention.

[00027] Figure 5 is an expression profile of ACC and GLUT4 in skeletal muscle in accordance with the present invention.

[00028] Figure 6 is an expression profile of UCP3 in skeletal muscle in accordance with the present invention.
Figure 7 is an expression profile of SREBP1c, ACC, PEPCK AND CPT in liver in accordance with the present invention.

Figure 8 demonstrates the in vivo status of phosphorylation of perilipin protein as discerned using MALDI TOF TOF in accordance with the present invention.

DETAILED DESCRIPTION OF SEVERAL EMBODIMENTS

Reference now will be made in detail to the embodiments of the invention, one or more examples of which are set forth below. Each example is provided by way of explanation of the invention, not limitation of the invention. In fact, it will be apparent to those skilled in the art that various modifications and variations can be made in the present invention without departing from the scope or spirit of the invention. For instance, features illustrated or described as part of one embodiment can be used on another embodiment to yield a still further embodiment. Thus, it is intended that the present invention covers such modifications and variations as come within the scope of the appended claims and their equivalents. Other objects, features and aspects of the present invention are disclosed in or are obvious from the following detailed description. It is to be understood by one of ordinary skill in the art that the present discussion is a description of exemplary embodiments only, and is not intended as limiting the broader aspects of the present invention.

Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. The methods and techniques of the present invention are generally performed according to conventional methods well known in the art. Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics, and protein and nucleic acid chemistry described herein are those well known and commonly used in the art. The nomenclatures used in connection with,
and the laboratory procedures and techniques of, molecular and cellular biology, biochemistry, medicinal, and pharmaceutical chemistry described herein are well-known and commonly used in the art.

[00033] Figure 1 is a diagram of the metabolic pathway of activated AMPK in a cell. This pathway was discovered by the present inventors, resulting in the novel recognition that activated AMPK phosphorylates perilipin, which in turn would lead to enhanced lipolysis, while the released free fatty acid would be metabolized by β-oxidation, through the AMPK regulated ACC2 pathway. At the same time, the de novo synthesis of fatty acid would be inhibited by the AMPK mediated ACC1 pathway. The inventors recognized, therefore, that activated AMPK phosphorylates perilipin, with the net result of the metabolic pathway being a reduction in free fatty acid concentration in the cell, as well as a reduction in the synthesis of free fatty acid in the cell. Moreover, the AMPK-mediated pathway illustrated in Figure 1 does not result in insulin resistance and lipotoxicity as does the PKA mediated pathway. Accordingly, perilipin phosphorylation has been identified by the present inventors as an attractive indicator of AMPK activity, as well as an attractive indicator of anti-obesity and anti-obesity related condition activity.

[00034] In accordance with another aspect, the AMPK activator is envisioned to work as anti-obesity drug by its dual mechanism of action on fat metabolism through ACC inhibition and burning off stored fat in adipose tissue through perilipin inactivation followed by lipolysis by lipases.

[00035] In another aspect, the invention also provides methods for treating obesity or complications associated with it, the methods comprising the step of administering to an animal (e.g., a mammal, including a human) in need thereof an agent that activates AMPK activity in an amount sufficient to increase perilipin concentrations in the cell.

[00036] In accordance with other aspects, present invention also provides a method for increasing the oxidation of fatty acids in a metabolic cell or tissue comprising the step of administering an activator of AMPK in
an amount sufficient to inhibit perilipin activity and thereby stimulate fatty acid oxidation.

[00037] In accordance with other aspects, present invention also provides a method for increasing perilipin activity in a metabolic tissue or cell comprising the step of administering an activator of AMPK in an amount sufficient to mimic.

[00038] As used herein, the term "activity" refers, with respect to a reaction or process, to an observed rate or progression of the reaction or process.

[00039] In one aspect, therefore, the present invention is a method of identifying AMPK activators. The method includes providing a sample of cells that include AMPK and perilipin, providing a sample of an AMPK activator-candidate, contacting the cell sample and the activator-candidate, and measuring a quantitative indicator of the AMPK activation within the cell sample after the contacting step. In at least one embodiment, the quantitative indicator of AMPK activation is phosphorylation of perilipin.

[00040] Without being bound by a particular theory, obesity can be controlled at the peripheral level by targeting simultaneously the AMPK-ACC and AMPK-Perilipin mediated pathways. Thus, a suitable AMPK activator would phosphorylate perilipin, which in turn would lead to enhanced lipolysis, while the released FFA would be metabolized by β-oxidation, through AMPK regulated ACC2 pathway. At the same time, the de novo synthesis of fatty acid would be inhibited by AMPK mediated ACC1 pathway.

[00041] In the present screening method, an increase in the monitored quantitative indicator indicates an upregulation of AMPK activity. As used herein, the term "AMPK activity" refers to the amount of or rate of phosphorylation of perilipin in the sample. Accordingly, in the present method, when the monitored quantitative indicator indicates an increase in AMPK activity upon contact of the cell sample with the candidate
compound, the candidate is shown to be effective in upregulating the AMPK activity, or "activating" AMPK.

[00042] It has been shown that, surprisingly, an upregulation in AMPK activity results in an increase in the phosphorylation of perilipin.

[00043] In some embodiments, the cells are human cells and may be selected from one or more of muscle cells and liver cells. In other embodiments, the cells are animal cells. The contacting step of the present method may be in vitro.

[00044] In another aspect, the present invention is a method of preventing and/or treating obesity and obesity-related conditions. The method includes administering to a subject in need of prevention and/or treatment of obesity and/or obesity-related conditions, an AMPK activator identified by providing a sample of cells that include AMPK and perilipin, providing a sample of an AMPK activator-candidate, contacting the cell sample and the activator-candidate, and measuring a quantitative indicator of the AMPK activation within the cell sample after the contacting step. In at least one embodiment, the quantitative indicator of AMPK activation is phosphorylation of perilipin.

[00045] For ease of reference, the present invention will be described with reference to administration to human subjects. It will be understood, however, that such descriptions are not limited to administration to humans, but will also include administration to other animals, such as mammals, unless explicitly stated otherwise.

[00046] The present method includes administering one or more AMPK activators that are identified by a method described herein to the subject by administration means known in the art. Administration is optionally enteral or parenteral, and administration means contemplated as useful include one or more of topically, buccally, intranasally, orally, intravenously, intramuscularly, sublingually, and subcutaneously. Other administration means known in the art are also contemplated as useful in accordance with the present invention and are discussed in more detail below.
In some embodiments, it may be useful to include the one or more identified AMPK activators as a salt. Those having ordinary skill in the art will recognize the salts of the identified AMPK activator compounds.

In some embodiments, the compound or composition may be in the form of an aqueous composition. The composition may also be nebulized or aerosolized.

The present invention involves the use of a safe and effective amount of one or more identified AMPK activators for phosphorylating perilipin, thereby treating or preventing obesity and obesity-related conditions.

An exemplary method of administering one or more identified AMPK activators is topical, intranasal administration, e.g., with nose drops, nasal spray, or nasal mist inhalation. Other exemplary methods of administration include one or more of topical, bronchial administration by inhalation of vapor and/or mist or powder, orally, intravenously, intramuscularly, and subcutaneously.

Other ingredients which may be incorporated in the present invention include safe and effective amounts of preservatives, e.g., benzalkonium chloride, thimerosal, phenylmercuric acetate; and acidulants, e.g., acetic acid, citric acid, lactic acid, and tartaric acid. The present invention may also include safe and effective amounts of isotonicity agents, e.g., salts, such as sodium chloride, or non-electrolyte isotonicity agents such as sorbitol, mannitol, and lower molecular weight polyethylene glycol.

In the present method, a subject in need of prevention or treatment of obesity and/or obesity-related conditions is treated with an amount of one or more AMPK activators identified in accordance with the present invention, wherein the amount of the one or more identified AMPK activators provides a dosage or amount that is sufficient to constitute a treatment or prevention effective amount.

As used herein, an "effective amount" means the dose or amount of an identified AMPK activator to be administered to a subject and the
frequency of administration to the subject which is readily determined by one of ordinary skill in the art, by the use of known techniques and by observing results obtained under analogous circumstances and has therapeutic action. The dose or effective amount to be administered to the subject and the frequency of administration to the subject can be readily determined by one of ordinary skill in the art by the use of known techniques and by observing results obtained under analogous circumstances. In determining the effective amount or dose, a number of factors are considered by the attending diagnostician, including but not limited to, the potency and duration of action of the compounds used; the nature and severity of the illness to be treated, as well as the sex, age, weight, general health, and individual responsiveness of the subject to be treated, and other relevant circumstances.

[00054] The term "therapeutically-effective" indicates the capability of an agent to prevent, or improve the severity of, the disorder, while avoiding or reducing adverse side effects typically associated with alternative therapies.

[00055] The identified AMPK activators can be supplied in the form of a novel therapeutic composition that is believed to be within the scope of the present invention.

[00056] When one or more identified AMPK activators are supplied along with a pharmaceutically acceptable carrier, a pharmaceutical composition is formed. A pharmaceutical composition of the present invention is directed to a composition suitable for the prevention or treatment of the disorders described herein. The pharmaceutical composition includes at least a pharmaceutically acceptable carrier and one or more identified AMPK activators. Pharmaceutically acceptable carriers include, but are not limited to, physiological saline, Ringer's, phosphate solution or buffer, buffered saline, and other carriers known in the art. Pharmaceutical compositions may also include stabilizers, anti-oxidants, colorants, and diluents. Pharmaceutically acceptable carriers and additives are chosen such that side effects from the pharmaceutical compound are minimized.
and the performance of the compound is not canceled or inhibited to such an extent that treatment is ineffective.

[00057] The term "pharmacologically effective amount" shall mean that amount of a drug or pharmaceutical agent that will elicit the biological or medical agent that will elicit the biological or medical response of a tissue, system, animal, or human that is being sought by a researcher or clinician. This amount can be a therapeutically effective amount.

[00058] The term "pharmaceutically acceptable" is used herein to mean that the modified noun is appropriate for use in a pharmaceutical composition. Pharmaceutically acceptable cations include metallic ions and organic ions. Exemplary metallic ions include, but are not limited to, appropriate alkali metal salts, alkaline earth metal salts, and other physiological acceptable metal ions. Exemplary ions include aluminum, calcium, lithium, magnesium, potassium, sodium, and zinc in their usual valences. Exemplary organic ions include protonated tertiary amines and quaternary ammonium cations, including in part, trimethylamine, diethylamine, N,N'-dibenzylethlenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine) and procaine. Exemplary pharmaceutically acceptable acids include, without limitation, hydrochloric acid, hydroiodic acid, hydrobromic acid, phosphoric acid, sulfuric acid, methanesulfonic acid, acetic acid, formic acid, tartaric acid, maleic acid, malic acid, citric acid, isocitric acid, succinic acid, lactic acid, gluconic acid, glucuronic acid, pyruvic acid, oxalacetic acid, fumaric acid, propionic acid, aspartic acid, glutamic acid, benzoic acid, and the like.

[00059] Also included in the invention are the isomeric forms and tautomers and the pharmaceutically-acceptable salts of the identified AMPK activators. Illustrative pharmaceutically acceptable salts are prepared from formic, acetic, propionic, succinic, glycolic, gluconic, lactic, malic, tartaric, citric, ascorbic, glucuronic, maleic, fumaric, pyruvic, aspartic, glutamic, benzoic, anthranilic, mesylic, stearic, salicylic, p-hydroxybenzoic, phenylacetic, mandelic, embonic (pamoic), methane
sulfonic, ethanesulfonic, benzenesulfonic, pantothenic, toluenesulfonic, 2-hydroxyethanesulfonic, sulfanilic, cyclohexylaminosulfonic, algenic, β-hydroxybutyric, galactaric, and galacturonic acids.

[00060] Suitable pharmaceutically-acceptable base addition salts of compounds of the present invention include metallic ion salts and organic ion salts. Exemplary metallic ion salts include, but are not limited to, appropriate alkali metal (Group IA) salts, alkaline earth metal (Group IA) salts, and other physiological acceptable metal ions. Such salts may be made from the ions and aluminum, calcium, lithium, magnesium, potassium, sodium, and zinc. Exemplary organic salts can be made from tertiary amines and quaternary ammonium salts, including in part, trimethylamine, diethylamine, N.N'-dibenzylethlenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine), and procaine. All of the above salts may be prepared by those skilled in the art by conventional means from the corresponding compound of the present invention.

[00061] The terms "treating" or "to treat" means to alleviate symptoms, eliminate the causation either on a temporary or permanent basis, or to prevent of slow the appearance of symptoms. The term "treatment" includes alleviation, elimination of causation of, or prevention of any of the diseases or disorders described herein. Besides being useful for human treatment, these combinations are also useful for treatment of mammals, including horses, dogs, cats, rats, mice, sheep, pigs, etc.

[00062] The term "subject" for purposes of this application includes any animal. The animal is typically human. A preferred subject is one that is in need of treatment or prevention of one or more of the disorders discussed herein.

[00063] For methods of prevention, the subject is any human or animal subject, and preferably is a subject that is in need of prevention and/or treatment of obesity and obesity-related conditions, including, but not limited to, type I diabetes, type II diabetes, hyperlipidaemia, hypercholesterolaemia, metabolic syndrome, and their cardiovascular
complications. The subject may be a human subject who is at risk of the disorders described herein. The subject may be at risk due to genetic predisposition, sedentary lifestyle, diet, exposure to disorder-causing agents, and/or exposure to pathogenic agents and the like.

[00064] The present pharmaceutical compositions may be administered enterally and/or parenterally. Parenteral administration includes subcutaneous, intramuscular, intradermal, intramammary, intravenous, and other administrative methods known in the art. Enteral administration includes solution, tables, sustained release capsules, enteric, coated capsules, syrups, beverages, foods, and other nutritional supplements. When administered, the present pharmaceutical composition may be at or near body temperature.

[00065] In particular, the identified AMPK activators of the present invention, or compositions in which they are included, can be administered orally, for example, as tablets, coated tablets, dragees, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs. Compositions intended for oral use may be prepared according to any method known in the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavoring agents, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets may contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. These excipients may be, for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, maize starch, or alginic acid; binding agents, for example starch, gelatin or acacia, and lubricating agents, for example magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may be coated by known techniques to delay disintegration and adsorption in the gastrointestinal tract and thereby provide a sustained action over a longer
period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed.

[00066] Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredients are mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredients are present as such, or mixed with water or an oil medium, for example, peanut oil, liquid paraffin, any of a variety of herbal extracts, milk, or olive oil.

[00067] Aqueous suspensions can be produced that contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example, sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethyl-cellulose, sodium alginate, polyvinylpyrrolidone gum tragacanth and gum acacia; dispersing or wetting agents may be naturally-occurring phosphatides, for example lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyoxyethylene sorbitan monooleate.

[00068] The aqueous suspensions may also contain one or more preservatives, for example, ethyl or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, or one or more sweetening agents, such as sucrose or saccharin.

[00069] Oily suspensions may be formulated by suspending the active ingredients in an omega-3 fatty acid, a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol.
Sweetening agents, such as those set forth above, and flavoring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an antioxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, a suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, may also be present.

Syrups and elixirs containing one or more identified AMPK activators may be formulated with sweetening agents, for example glycerol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative, and flavoring and coloring agents.

The subject identified AMPK activators and compositions in which they are included can also be administered parenterally, either subcutaneously, or intravenously, or intramuscularly, or intrasternally, or by infusion techniques, in the form of sterile injectable aqueous or olagenous suspensions. Such suspensions may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above, or other acceptable agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, n-3 polyunsaturated fatty acids may find use in the preparation of injectables;
[00074] The subject identified AMPK activators and compositions in which they are included can also be administered by inhalation, in the form of aerosols or solutions for nebulizers, or rectally, in the form of suppositories prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperature but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials are cocoa butter and poly-ethylene glycols.

[00075] The subject identified AMPK activators and compositions in which they are included can also be administered topically, in the form of creams, ointments, jellies, collyriums, solutions, patches, or suspensions.

[00076] Daily dosages of the identified AMPK activators can vary within wide limits and will be adjusted to the individual requirements in each particular case. In general, for administration to adults, an appropriate daily dosage has been described above, although the limits that were identified as being preferred may be exceeded if expedient. The daily dosage can be administered as a single dosage or in divided dosages.

[00077] Various delivery systems in addition to nutritional supplements include sprays, capsules, tablets, drops, and gelatin capsules, for example.

[00078] Those skilled in the art will appreciate that dosages for the therapeutic use of the identified AMPK activators may also be determined with guidance from Goodman & Goldman's The Pharmacological Basis of Therapeutics, Ninth Edition (1996), Appendix II, pp. 1707-1711.

[00079] Preferred dosages for the identified AMPK activators are those that are effective to increase the phosphorylation of perilipin. In especially preferred embodiments, the dosage should be in a concentration effective to increase the phosphorylation of perilipin such that a reduction in weight is achieved. In yet another embodiment an effective dosage is an amount that is effective to reduce weight in the subject. In another embodiment, an effective dosage is an amount that is effective to upregulate AMPK activity in the subject.
In another aspect, the invention is a method of screening for an activator of AMPK. The method includes providing at least one cell including AMPK and perilipin, contacting the at least one cell with an AMPK activator-candidate, measuring perilipin phosphorylation, and comparing the phosphorylation in the presence of the activator-candidate with the phosphorylation in the absence of the activator-candidate. A difference in the phosphorylation in the presence of the activator-candidate, as compared with the phosphorylation in the absence of the activator-candidate, identifies the activator-candidate as an AMPK activator.

In another aspect, the invention is a method of producing an activator of AMPK. The method includes providing at least one cell including AMPK and perilipin, contacting the at least one cell with an AMPK activator-candidate, measuring perilipin phosphorylation, and comparing the phosphorylation in the presence of the activator-candidate with the phosphorylation in the absence of the activator-candidate. A difference in the phosphorylation in the presence of the activator-candidate, as compared with the phosphorylation in the absence of the activator-candidate, identifies the activator-candidate as an AMPK activator. The method optionally further includes producing the identified AMPK activator for administration to subjects in need of prevention and/or treatment of obesity and obesity-related conditions as described above.

In another aspect, the invention is a pharmaceutical composition for the prevention and/or treatment of obesity and obesity-related conditions. The pharmaceutical composition includes an AMPK activator identified and produced according to any of the previously described methods.

In yet another aspect, the invention is a kit for identification of AMPK activators useful for the prevention and treatment of obesity and obesity-related conditions. The kit includes an assay having at least AMPK and perilipin included therein. In some embodiments, the kit
[00084] In embodiments of the present kit including at least one cell, the cell may be any animal cell. In some embodiments, the cell is at least one human cell. In other embodiments, the at least one cell is a muscle cell. In other embodiments, the at least one cell is a liver cell. In still other embodiments, the kit includes at least one liver cell and at least one muscle cell.

[00085] In another aspect, the invention is a method of identifying one or more compounds that have anti-obesity activity. The method includes providing an assay including AMPK and perilipin, contacting the assay with an AMPK activator-candidate, measuring perilipin phosphorylation and comparing the phosphorylation in the presence of the activator-candidate with the phosphorylation in the absence of the activator-candidate. An increased rate of phosphorylation in the presence of the activator-candidate, as compared with the rate of phosphorylation in the absence of the activator-candidate identifies the AMPK candidate as an AMPK activator having anti-obesity activity.

EXAMPLES

Example 1: AMPK activation:

[00086] AMPK regulates lipid and glucose metabolism. The AMPK activation potential of AMPK activators was evaluated in vitro using a cell based ELISA approach using antibodies specific for p-AMPK in the cell lines HepG2 (derived from liver cells) and L6 (derived from skeletal muscle cells). Both AMPK activators (1 & 2) demonstrated significant AMPK activation in the muscle and liver cell lines. These effects are greater than 200 fold better than that of Metformin and approximately 40 fold better than AICAR, two known AMPK activators having the formulae:
AMPK activator-1 is \( [2-(4\text{-chloro-3-trifluoromethylphenylamino})\text{-thiazol-4-yl}]\text{-acetic acid} \), having the formula:

\[
\begin{align*}
\text{AMPK activator-2 is } & [2-(\text{biphenyl-4-ylamino})\text{-thiazol-4-yl}]\text{-acetic acid}, \text{ having the formula:} \\
\end{align*}
\]

Table 1: AMPK activation of Example 1:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
<th>L6 muscle cells (%) activation</th>
<th>HepG2 cells (%) activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMPK Activator-1</td>
<td>10 ( \mu \text{M} )</td>
<td>92</td>
<td>103</td>
</tr>
<tr>
<td>AMPK Activator-2</td>
<td>10 ( \mu \text{M} )</td>
<td>92</td>
<td>111</td>
</tr>
<tr>
<td>AICAR</td>
<td>250 ( \mu \text{M} )</td>
<td>95</td>
<td>98</td>
</tr>
<tr>
<td>Metformin</td>
<td>2 \text{mM}</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
Example 2: Cell based ELISA for phospho-ACC:

[00089] ACC is the downstream target of AMPK which is inactivated upon its phosphorylation by p-AMPK at Ser-79. The extent of ACC phosphorylation by AMPK activators was evaluated in vitro using a cell based ELISA approach using antibodies specific for p-ACC in the cell lines HepG2 and L6. Both NCEs demonstrated significant ACC phosphorylation in the muscle and liver cell lines. These effects were greater than 200 fold better than that of Metformin and approximately 40 times better than AICAR.

Table 2: Cell based ELISA for phospho-ACC:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
<th>L6 muscle cells (% activation)</th>
<th>HepG2 cells (% activation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMPK Activator-1</td>
<td>10 µM</td>
<td>107</td>
<td>103</td>
</tr>
<tr>
<td>AMPK Activator-2</td>
<td>10 µM</td>
<td>94</td>
<td>105</td>
</tr>
<tr>
<td>AICAR</td>
<td>250 µM</td>
<td>95</td>
<td>95</td>
</tr>
<tr>
<td>Metformin</td>
<td>2 mM</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Example 3: Cell based ELISA for phospho-perilipin:

[00090] 3T3-L1 cells were seeded in 96 well plates and grown to confluency. After 48 hours, differentiation medium was added to cells and incubated for 5-6 days until fat droplets appeared. Drug addition was done in normal DMEM medium and cells were incubated for 24 hours. The treated cells were blotted using phospho-perilipin antibody specific for Ser-492 and the data were read at 450 nM. AMPK activators significantly phosphorylated perilipin in this in vitro assay, which can be clearly observed in Figure 2 and Table 3:
Table 3: Cell-based ELISA for phospho-perilipin:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
<th>% phosphorylation of perilipin (Ser-492)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forskolin</td>
<td>5 µM</td>
<td>100</td>
</tr>
<tr>
<td>AMPK Activator-1</td>
<td>30 µM</td>
<td>46</td>
</tr>
<tr>
<td>AMPK Activator-2</td>
<td>30 µM</td>
<td>36</td>
</tr>
</tbody>
</table>

Example 4:
[00091] Activation of AMPK is an allosteric event mediated by the binding of AMP, its physiological activator, to the γ-subunit of the complex. The binding potential of AMPK activators were evaluated by a radioactive kinase assay performed using purified GST-perilipin protein as a substrate in the presence of γ^{32}P labeled ATP and partially purified rat liver AMPK. The kinase assay revealed that at 150 µm concentration, AMPK activator exhibited ~35% binding compared to 100% binding by 300 µm of AMP.

Example 5:
[00092] The AMPK activator 1 phosphorylated perilipin, in both in vitro and in vivo studies. When treated at 30 mg/kg dose for 28 days, AMPK activator 1 demonstrated 19% reduction in body weight in DIO mice without any significant reduction in food consumption. It reduced 41% PG Plasma glucose in db/db mice at 10 mg/kg dose and also reduced 51% triglycerides (TG) in Zucker fa/fa rats when treated with 30 mg/kg dose. The present method demonstrated that AMPK activator 1 down regulates DGAT2 genes in adipose and ACC in skeletal muscle and also significantly upregulates the UCP3 gene in skeletal muscles. AMPK activator 1 also significantly modulates all AMPK related genes in the liver. AMPK regulates glucose and fat metabolism in the liver and muscle. Initial screening of potential AMPK activating compounds is, therefore, performed in cell lines derived from muscle and liver.
[00093] AMPK activator 1 was evaluated in vitro using a cell based ELISA approach in the cell lines HepG2 and L6. AMPK activator 1
demonstrated significant AMPK activation in both muscle and liver cell lines. These effects are greater than 200-fold better than that of Metformin.

Table 4: AMPK activation of Example 5:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
<th>L6 muscle cells (% activation)</th>
<th>HepG2 cells (% activation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMPK Activator 1</td>
<td>10 µM</td>
<td>91.6</td>
<td>102.7</td>
</tr>
<tr>
<td>Metformin</td>
<td>2mM</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Example 6: AMPK activation by AMPK activator-3:

[00094] AMPK activation potential of AMPK activator-3, [2-(4-trifluoromethyl-phenylamino)-thiazol-4-yl]-acetic acid, having the formula,

![Chemical Structure](image)

was evaluated in vitro using a cell-based ELISA approach in the cell lines HepG2 and L6, the major sites of action by AMPK. AMPK activator-3 demonstrated significant AMPK activation in both muscle and liver cell lines. These effects are greater than 200-fold better than that of metformin.

Table 5: AMPK activation:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
<th>L6 muscle cells (% activation)</th>
<th>HepG2 cells (% activation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMPK Activator-3</td>
<td>10 µM</td>
<td>93.6</td>
<td>141.8</td>
</tr>
<tr>
<td>Metformin</td>
<td>2mM</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Example 7: AMPK activator-3 reduced obesity in mice:

[00095] Obesity and diabetes in man have been linked to excessive caloric intake as well as a high percent of daily intake of fat. C57BL/6J mice fed with a high fat diet for 3 months developed significant body
weight gain accompanied by mild hyperglycemia compared to chow fed mice. This mouse model resembles closely high calorie intake development of obesity in humans. This model was used to investigate body weight lowering potential via the AMPK-ACC-perilipin pathway.

AMPK activator-3 was administered through oral gavage to the animals, once daily for 21 days. After 21 days, AMPK activator-3 was administered twice daily for one week. Control animals received the excipients only. The animals were maintained on the same high fat diet throughout the study. Body weight was monitored at regular intervals.

Table 6: body weight in Example 7:

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight (grams)</th>
<th>% red</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oday</td>
<td>30 day</td>
</tr>
<tr>
<td>Control</td>
<td>40.2 ± 2.3</td>
<td>42.2 ± 2.4</td>
</tr>
<tr>
<td>AMPK Activator-3</td>
<td>40.2 ± 2.6</td>
<td>39.3 ± 3.0</td>
</tr>
</tbody>
</table>

AMPK activator-3 demonstrated a 7% reduction in body weight in the diet induced obesity model after 30 days of treatment compared to the control group. No change in the food intake of the treated animals was observed over the period of the study.

The adipose tissue samples of the treated mice were collected and total protein was extracted. Western analyses of the tissue samples using antibodies specific to phospho-AMPK and phospho-perilipin revealed that there was activation of AMPK followed by in vivo phosphorylation of perilipin (Figure 3). The basal levels of both AMPK and perilipin (non-phosphorylated form) remained unchanged in the treated sets, conclusively indicating the onset of phospho-AMPK-phospho-perilipin pathway.

Example 8: AMPK activator-3 modulates glucose and fat metabolism at the transcriptome level to exert a favorable effect on obesity-related genes:
Upon completion of the study in DIO mice, the animals were sacrificed and liver, skeletal muscle, and adipose tissues were dissected and collected in Eppendorff tubes containing the steal bead (Qiagen laser, Qiagen, Inc.). Total RNA was extracted from these samples, reverse transcribed to generate the cDNA which was used for quantitative PCR with SYBR green based real time primers corresponding to genes of interest. The results of the analyses are represented in Figures 4-7 and Table 8.

Table 8:

<table>
<thead>
<tr>
<th>Tissue</th>
<th>RT</th>
<th>Gene</th>
<th>Control</th>
<th>AMPK Activator-3</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adipose</td>
<td>474</td>
<td>DGAT1</td>
<td>100</td>
<td>105</td>
<td>0.400000</td>
</tr>
<tr>
<td>Adipose</td>
<td>489</td>
<td>DGAT2</td>
<td>100</td>
<td>76</td>
<td>0.003000</td>
</tr>
<tr>
<td>Adipose</td>
<td>491</td>
<td>LASY</td>
<td>100</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>Adipose</td>
<td>490</td>
<td>SCD2</td>
<td>100</td>
<td>66</td>
<td>0.025000</td>
</tr>
<tr>
<td>Adipose</td>
<td>477-2</td>
<td>SREBP1C</td>
<td>100</td>
<td>107</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>493</td>
<td>ACC</td>
<td>100</td>
<td>28</td>
<td>0.040000</td>
</tr>
<tr>
<td>Liver</td>
<td>497</td>
<td>CPT1B</td>
<td>100</td>
<td>93</td>
<td>0.014600</td>
</tr>
<tr>
<td>Liver</td>
<td>492</td>
<td>PEPCK</td>
<td>100</td>
<td>23</td>
<td>0.000100</td>
</tr>
<tr>
<td>Liver</td>
<td>487</td>
<td>SREBP1C</td>
<td>100</td>
<td>47</td>
<td>0.040000</td>
</tr>
<tr>
<td>Muscle</td>
<td>475</td>
<td>ACC</td>
<td>100</td>
<td>45</td>
<td>0.000100</td>
</tr>
<tr>
<td>Muscle</td>
<td>494</td>
<td>CD36</td>
<td>100</td>
<td>89</td>
<td>0.830000</td>
</tr>
<tr>
<td>Muscle</td>
<td>496</td>
<td>DPT1B</td>
<td>100</td>
<td>89</td>
<td>0.840000</td>
</tr>
<tr>
<td>Muscle</td>
<td>476</td>
<td>GLUT4</td>
<td>100</td>
<td>90</td>
<td>0.417000</td>
</tr>
</tbody>
</table>

AMPK activator-3 modulated multiple AMPK regulated genes. In the adipose tissue, AMPK activator-3 may be mediating its effects through DGAT2 down regulation (Figure 4). In skeletal muscle, it down regulates ACC, induces UCP3, but does not modulate GLUT4, CPT1, and CD35.
(Figures 5-6). In liver, it down regulates PEPCK, SREBP1C, and ACC, but does not modulate CPT1 (Figure 7).

[00101] The gene expression profile indicates that AMPK activation favorably modulates glucose and fat metabolism along with having beneficial effects on overall body weight.

Example 9: AMPK activator-3 has favorable effects on plasma lipid profile:

[00102] It is known that obesity is often accompanied by a dysregulation of the total plasma lipid profile. As a corollary, an anti-obesity agent/molecule should be expected to counter dyslipidemic effects. This hypothesis was confirmed when the effects of the AMPK activator-3 were examined in normoglycemic but hyperlipidemic and hyperinsulinemic Zucker fa/fa rats. Male Zucker fa/fa rats, 12 weeks of age, were administered three doses of AMPK activator-3 (10, 30, and 100 mg/kg) and metformin (250 mg/kg) for 23 days. The control animals received the vehicle. After the study period, various parameters as tabulated in Table 9 were monitored. Also, at the end of the experimental regimen, animals were administered Triton WR 1339 (Sigma) at a dose of 250 mg/kg, and plasma triglyceride levels were estimated at 0, 2, 4, 6, 24, 48, and 72 hours respectively.

Table 9: Plasma triglyceride levels on 0 and 23 days after treatment.

<table>
<thead>
<tr>
<th>Group</th>
<th>0 day TG</th>
<th>23 day TG</th>
<th>Percent reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>433 ± 64.43</td>
<td>414.16 ± 37.75</td>
<td></td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>432.19 ± 52.62</td>
<td>289.14 ± 52.45</td>
<td>30</td>
</tr>
<tr>
<td>30 mg/kg</td>
<td>449.72 ± 69.14</td>
<td>178.75 ± 21.79</td>
<td>58</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>418.79 ± 47.63</td>
<td>44.18 ± 7.45</td>
<td>89</td>
</tr>
</tbody>
</table>
Table 10: Plasma free fatty acid levels on day 23

<table>
<thead>
<tr>
<th>Group</th>
<th>23 day FFA</th>
<th>Percent reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.632 ± 0.036</td>
<td></td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>0.567 ± 0.049</td>
<td>10</td>
</tr>
<tr>
<td>30 mg/kg</td>
<td>0.363 ± 0.060</td>
<td>43</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>0.322 ± 0.059</td>
<td>49</td>
</tr>
</tbody>
</table>

Treatment with AMPK activator-3 demonstrated significant lowering of plasma triglycerides, free fatty acids, and insulin levels. Also a significant effect on hepatic TG secretion was observed as depicted in Table 11.

Table 11: Effect on hepatic triglyceride secretion at different times

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>AMPK Activator-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hr</td>
<td>442.15 ± 41.99</td>
<td>196.95 ± 35.00</td>
</tr>
<tr>
<td>2 hr</td>
<td>2298 ± 106.45</td>
<td>1500.78 ± 128.20</td>
</tr>
<tr>
<td>4 hr</td>
<td>4374.98 ± 37.09</td>
<td>2727.26 ± 199.94</td>
</tr>
<tr>
<td>6 hr</td>
<td>5439.66 ± 153.22</td>
<td>4093.80 ± 316.73</td>
</tr>
<tr>
<td>24 hr</td>
<td>6703.38 ± 204.27</td>
<td>4354.42 ± 848.96</td>
</tr>
<tr>
<td>48 hr</td>
<td>5294.65 ± 290.32</td>
<td>2430.70 ± 879.96</td>
</tr>
<tr>
<td>72 hr</td>
<td>1133.34 ± 175.14</td>
<td>280.22 ± 22.74</td>
</tr>
<tr>
<td>AUC</td>
<td>349734</td>
<td>202733</td>
</tr>
<tr>
<td>% reduction</td>
<td>-</td>
<td>42</td>
</tr>
</tbody>
</table>

The overall body weight of the treated animals remained unchanged during the period of the study, while the excipients treated group gained body weight of approximately 70 g.

Example 10: Perilipin undergoes phosphorylation by AMPK in vivo:
[00105] In order to better understand the mechanism of body weight neutrality, the status of the perilipin protein in the treated animals was investigated. Total protein was extracted from adipose tissue of the
control and the treated Zucker fa/fa rats, subjected to 2D gel electrophoresis followed by immunoblotting to identify the perilipin spots. These spots were excised, subjected to tryptic digestion, and then analyzed by MALDI TOF.

As demonstrated in Figure 8, perilipin was hyperphosphorylated at Ser 276 and Ser 492 compared to the control tissues, clearly indicating that perilipin is a substrate of AMPK in vivo.

Example 11: Site specific mutation of Ser 276 and Ser 492 abolishes hyperphosphorylation of perilipin:

Further confirmation of perilipin being utilized as a substrate for AMPK and undergoing hyperphosphorylation at these two specific serine residues was obtained from site directed mutagenesis studies. Perilipin, spanning amino acids 249 to 517 (encompassing 2 phosphorylation sites) was cloned in GST expression vector and the two sites S276 and S492 were mutated by site directed mutagenesis to Alanine. The mutant, as well as the wild-type protein, was expressed following IPTG induction and purified in bound form with GST beads as a fusion protein. This was then subjected to a kinase reaction using commercially available, purified AMPK. The kinase reaction was conducted using kinase buffer, GST fusion protein, 5'AMP (300 µm), hot Y^32P ATP and AMPK enzyme and incubated at 30°C for 30 min. The products were washed and electrophoresed on 10% SDS-PAGE followed by autoradiography and densitometric analyses (Figure 2). Upon mutation of Ser 276 and Ser 492, no hyperphosphorylation of perilipin was observed, confirming that perilipin is a substrate of AMPK.

Other embodiments within the scope of the specification and claims herein will be apparent to one skilled in the art from consideration of the specification or practice of the invention as disclosed herein. It is intended that the specification be considered to be exemplary only, with the scope and spirit of the invention being indicated by the claims.
[00109] All references cited in this specification, including without limitation, all papers, publications, patents, patent applications, presentations, texts, reports, manuscripts, brochures, books, internet postings, journal articles, periodicals, and the like, are hereby incorporated by reference into this specification in their entireties.

[00110] The discussion of the references herein is intended merely to summarize the assertions made by their authors and no admission is made that any reference constitutes prior art. Applicants reserve the right to challenge the accuracy and pertinency of the cited references.

[00111] Although preferred embodiments of the invention have been described using specific terms, devices, and methods, such description is for illustrative purposes only. The words used are words of description rather than of limitation. It is to be understood that changes and variations may be made by those of ordinary skill in the art without departing from the spirit or the scope of the present invention, which is set forth in the following claims. In addition, it should be understood that aspects of the various embodiments may be interchanged both in whole or in part.
WHAT IS CLAIMED IS:

1. A method for the identification of an AMPK activator, the method comprising:
   - providing a sample of cells that include AMPK and perilipin;
   - providing a sample of an AMPK activator-candidate;
   - contacting the cell sample and the activator-candidate; and
   - measuring a quantitative indicator of the AMPK activation within the cell sample after the contacting step.

2. The method according to Claim 1, wherein the quantitative indicator of AMPK activation comprises phosphorylation of perilipin.

3. The method according to Claim 1, wherein the cells are human cells.

4. The method according to Claim 3, wherein the human cells are liver cells.

5. The method according to Claim 3, wherein the human cells are muscle cells.

6. The method according to Claim 1, wherein the contacting step is in vitro.

7. A method of preventing and/or treating obesity and related conditions comprising administering to a subject in need of prevention and/or treatment of obesity and related conditions an AMPK activator identified by the method of Claim 1.

8. The method according to Claim 7, wherein the obesity related conditions are one or more of type I diabetes, type II diabetes,
hyperlipidaemia, hypercholesterolaemia, metabolic syndrome, and their cardiovascular complications.

9. The method according to Claim 7, wherein the subject is a human subject.

10. The method according to Claim 7, wherein the subject is an animal subject.

11. The method according to Claim 7, wherein the administration is in a dosage effective to alleviate or treat obesity and related conditions.

12. A method of screening for an activator of AMPK comprising:
   (a) providing at least one cell including AMPK and perilipin;
   (b) contacting the at least one cell with an AMPK activator-candidate;
   (c) measuring perilipin phosphorylation; and
   (d) comparing the phosphorylation in the presence of the activator-candidate with the phosphorylation in the absence of the activator-candidate; wherein an increased rate of phosphorylation in the presence of the activator-candidate, as compared with the rate of phosphorylation in the absence of the activator candidate, identifies the activator-candidate as an AMPK activator.

13. The method according to Claim 12, wherein the at least one cell is a human cell.

14. The method according to Claim 12, wherein the at least one cell is a liver cell.

15. The method according to Claim 12, wherein the at least one cell is a muscle cell.
16. The method according to Claim 12, wherein the contacting step is conducted in vitro.

17. A method of producing an activator of AMPK comprising:
   (a) providing at least one cell including AMPK and perilipin;
   (b) contacting the at least one cell with an AMPK activator-candidate;
   (c) measuring phosphorylation of perilipin; and
   (d) comparing the phosphorylation in the presence of the activator-candidate with the phosphorylation in the absence of the activator-candidate; wherein an increased rate of phosphorylation in the presence of the activator-candidate, as compared with the rate of phosphorylation in the absence of the activator candidate, identifies the activator-candidate as an AMPK activator; and
   (e) producing the AMPK activator for administration to subjects in need of prevention and/or treatment of obesity and related conditions.

18. The method according to Claim 17, wherein the subject is a human subject.

19. The method according to Claim 17, wherein the obesity-related conditions are one or more of type I diabetes, type II diabetes, hyperlipidaemia, hypercholesterolaemia, metabolic syndrome, and their cardiovascular complications.

20. A pharmaceutical composition for the prevention and/or treatment of obesity and related conditions, the composition comprising an AMPK activator identified according to the method of Claim 17.

21. The pharmaceutical composition according to Claim 20, further comprising a pharmaceutically excipient.
22. A kit for identification of AMPK activators useful for the treatment of obesity and related conditions, the kit comprising:
an assay including at least one cell including therein AMPK and perilipin.

23. The kit according to Claim 22, wherein the at least one cell is a human cell.

24. The kit according to Claim 22, wherein the at least one cell is a liver cell.

25. The kit according to Claim 22, wherein the at least one cell is a muscle cell.

26. The kit according to Claim 22, wherein the assay includes at least one human muscle cell and at least one human liver cell.

27. A kit for identification of AMPK activators useful for the treatment of obesity and related conditions, the kit comprising:
an assay including therein at least AMPK and perilipin.

28. A method of identifying a compound that has anti-obesity activity, the method comprising:
(a) providing an assay including AMPK and perilipin;
(b) contacting the assay with an AMPK activator-candidate;
(c) measuring perilipin phosphorylation; and
(d) comparing the phosphorylation in the presence of the activator-candidate with the phosphorylation in the absence of the activator-candidate; wherein an increased rate of phosphorylation in the presence of the activator-candidate, as compared with the rate of phosphorylation in
the absence of the activator candidate, identifies the activator-candidate as an AMPK activator.
Figure 1: The metabolic pathway of AMPK activation in a cell discovered according to the present invention

PKA mediated pathway leads to the accumulation of FFA resulting in insulin resistance and lipotoxicity. AMPK mediated pathway not only ensures the reduction of FFA by β-oxidation but also inhibits the de novo synthesis of FFA.
Figure 2: The prospective sites of AMPK phosphorylation in accordance with the present invention

<table>
<thead>
<tr>
<th></th>
<th>GST-Plin (aa 249-363)</th>
<th></th>
<th>GST-Plin (aa 364-517)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wild type</td>
<td></td>
<td>Mutant (RRQS276A)</td>
</tr>
<tr>
<td>AMP</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>AMPK</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>phospho-Perilipin(S492)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>Praadipocytes</td>
<td>C2</td>
</tr>
</tbody>
</table>

β-actin
Figure 3: Activation of AMPK resulting in phosphorylation of perilipin as revealed by Western Blotting in accordance with the present invention.

![Western Blot Diagram]

Figure 4: An expression profile of SREBP1C, DGATs, and SCDs in Adipose in accordance with the present invention.

![Expression Profile Diagram]
Figure 5: An expression profile of ACC and GLUT4 in skeletal muscle in accordance with the present invention.

Figure 6: An expression profile of UCP3 in skeletal muscle in accordance with the present invention.
Figure 7: An expression profile of SREBP1c, ACC, PEPCK AND CPT in liver in accordance with the present invention.

![Bar chart showing expression levels of SREBP1c, ACC, PEPCK, and CPT with statistical significance levels.]

Figure 8: In vivo status of phosphorylation of perilipin protein as discerned using MALDI TOF TOF in accordance with the present invention.

![Mass spectra showing phosphorylation status of perilipin protein.]

129 AA

492 AA

Sequence Tag GSEVR

Sequence Tag RRVSD