Title: METHODS AND COMPOSITIONS FOR THE TREATMENT OF INFLAMMATION, OBESITY AND RELATED METABOLIC DISORDERS

Abstract: This invention provides a method of reducing inflammation in a living cell of a subject. This method increases the level of lipoic acid synthase within the living cell thereby causing the reduction of the inflammation. Various embodiments and variants are contemplated, some of which are set forth in claim format at the end of this document. The aspects, embodiments, and variants so exemplified in the claims format should not be understood as limiting the invention.
METHODS AND COMPOSITIONS FOR THE TREATMENT OF INFLAMMATION, OBESITY AND RELATED METABOLIC DISORDERS

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application is a Patent Cooperation Treaty application and claims the benefit of U.S. Provisional Application No. 60/846,501, filed September 22, 2006, which is relied on herein and incorporated herein by reference in its entirety.

The present application is related to co-pending and commonly owned Patent Cooperation Treaty application having the title: Novel Heterocyclic Compounds as LASY Activators, and having attorney docket number RUS 3.4-027, which was filed on the same date as the present application, and is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

Described herein are methods for treating inflammation, obesity and metabolic disorders, including Type II Diabetes, by regulating the expression of the Lipoic Acid Synthase ("LASY"). Also contemplated are methods and compositions for the identification of new chemical entities that modulate the gene expression of LASY.

LASY is the enzyme that is responsible for the synthesis of endogenous Lipoic acid (LA). Presence of LASY has been established in prokaryotic species and in yeast. However, until recently, it was not known if a mammalian homolog of LASY existed, or even whether mammals had the ability to synthesize LA.

The present invention demonstrates that LASY 1) regulates inflammation in vascular cells, 2) promotes glucose metabolism in skeletal muscle, and that 3) the level of LASY affects the body weight of mammals. The inventors have concluded that LASY prevents controls and/or treats obesity and associated metabolic defects. Described herein are methods of treating inflammation and cardiovascular disease, diabetes and its complications, and obesity by upregulating LASY.
**BRIEF SUMMARY OF THE INVENTION**

In one aspect, the invention provides a method of reducing inflammation in a living cell of a subject. This method increases the level of lipoic acid synthase within the living cell thereby causing the reduction of the inflammation. Various embodiments and variants are contemplated, some of which are set forth in claim format at the end of this document. The aspects, embodiments, and variants so exemplified in the claims format should not be understood as limiting the invention.

In another aspect, the invention provides a method of treating or mitigating an inflammatory disorder, the method including administering to a subject having the disorder an effective amount of an agent that causes an increase in the level of lipoic acid synthase.

In another aspect, the invention provides a method of treating or mitigating an inflammatory disorder associated with increased levels of MCP-1, the method including administering to a subject having the disorder an effective amount of an agent that causes an increase in the level of lipoic acid synthase thereby reducing the level of MCP-1 for the subject.

In yet another aspect, the invention provides a method of improving glucose uptake in a mammal, the method including increasing the level of lipoic acid synthase in the body of the mammal.

In yet another aspect, the invention provides a method of treating or mitigating hyperglycemia in a mammal, the method including increasing the level of lipoic acid synthase in the body of the mammal.

In yet another aspect, the invention provides a method of reducing the weight of a body of a mammal, the method including increasing the level of a lipoic acid synthase in the body of the mammal.

In yet another aspect, the invention provides a method of reducing the level of triglycerides in a body of a mammal, the method including increasing the level of lipoic acid synthase in the body of the mammal. Various embodiments and variants are contemplated, some of which are set forth in claim format at the end of this document.
In yet another aspect, the invention provides a method of treating a metabolic disorder, the method including administering to a subject with the disorder an effective amount of an agent that increases the level of lipoic acid synthase.

In yet another aspect, the invention provides a method of identifying compounds capable of increasing expression of lipoic acid synthase in a living cell, the method including:

a) providing a sample of cells that express lipoic acid synthase;
b) providing a sample of a candidate compound;
c) contacting the cell sample and the compound sample; and
d) measuring a quantitative indicator of expression of said lipoic acid synthase within said cell sample after said contacting step. Various embodiments and variants are contemplated, some of which are set forth in claim format at the end of this document.

In yet another aspect, the invention provides a method of identifying a candidate compound suitable as a pharmaceutical agent for treatment of a disorder treatable by increased expression of lipoic acid synthase, the method including:

a) providing a sample of cells that express lipoic acid synthase;
b) providing a sample of a candidate compound;
c) contacting the cell sample and the compound sample; and measuring a quantitative indicator for the level of expression of the lipoic acid synthase within the treated cell sample; wherein compounds causing a pre-determined increase in the expression of lipoic acid synthase are identified as candidate pharmaceutical agents.

It would be desirable, therefore, to develop a method and system for identifying LASY activators that may be used for the prevention and/or treatment of asthma, inflammation, arthritis, obesity, diabetes, metabolic disorders, and/or cardiovascular disease, such as atherosclerosis, dyslipidemia, and hyperglycemia.

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

Figure 1 illustrates a graph showing that LASY is down-regulated by pro-inflammatory agents.

Figure 2 illustrates a graph showing that inhibition of LASY exacerbates the inflammatory response.

Figure 3 illustrates a graph showing that overexpression of LASY decreases expression of inflammatory genes.

Figure 4 illustrates a graph showing that LASY is down-regulated by glucose.

Figure 5 illustrates a graph showing the basal expression of LASY in skeletal muscle of db/db mice.

Figure 6 illustrates a graph showing that LASY inhibition by siRNA inhibits glucose uptake in human skeletal muscle.

Figure 7 illustrates a graph showing that the basal expression of LASY in adipose tissue of obese mice.

Figure 8 illustrates a graph showing the effect of Compound A on induction of LASY gene expression

Figure 9 illustrates a graph showing the effect of Compound B on body weight in db/db mice.

Figure 10 illustrates a graph showing the effect of Compound B on body weight and food consumption in ob/ob mice.

Figure 11 illustrates a graph showing the effect of Compound B on plasma triglycerides in ob/ob mice.

DETAILED DESCRIPTION OF THE PREFERRED 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 limiting 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 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.

In one aspect, the invention provides a method of reducing inflammation in a living cell of a subject. This method increases the level of lipoic acid synthase within the living cell thereby causing the reduction of the inflammation. Various embodiments and variants are contemplated, some of which are set forth in claim format at the end of this document. The aspects, embodiments, and variants so exemplified in the claims format should not be understood as limiting the invention.

In another aspect, the invention provides a method of treating or mitigating an inflammatory disorder, the method including administering to a subject having the disorder an effective amount of an agent that causes an increase in the level of lipoic acid synthase.

In another aspect, the invention provides a method of treating or mitigating an inflammatory disorder associated with increased levels of MCP-1, the method including administering to a subject having the disorder an effective amount of an agent that
causes an increase in the level of lipoic acid synthase thereby reducing the level of MCP-1 for the subject.

In yet another aspect, the invention provides a method of improving glucose uptake in a mammal, the method including increasing the level of lipoic acid synthase in the body of the mammal.

In yet another aspect, the invention provides a method of treating or mitigating hyperglycemia in a mammal, the method including increasing the level of lipoic acid synthase in the body of the mammal.

In yet another aspect, the invention provides a method of reducing the weight of a body of a mammal, the method including increasing the level of a lipoic acid synthase in the body of the mammal.

In yet another aspect, the invention provides a method of reducing the level of triglycerides in a body of a mammal, the method including increasing the level of lipoic acid synthase in the body of the mammal. Various embodiments and variants are contemplated, some of which are set forth in claim format at the end of this document.

In yet another aspect, the invention provides a method of treating a metabolic disorder, the method including administering to a subject with the disorder an effective amount of an agent that increases the level of lipoic acid synthase.

In another aspect, the invention provides a method of lowering body weight by administering to a subject a peroxiredoxin inducer. As used herein, the term "LASY activator" will be understood by those having ordinary skill in the art as including any compound that increases LASY activity. By way of example, any compound that causes an increase in LASY activity identified by the present method of identifying LASY activators that is described herein is considered to be a LASY activator.

The term "treatment" or "treating" as used herein covers any treatment of a disease in a mammal, particularly a human, and includes: (i) preventing the disease from occurring in a subject which may be predisposed to the disease, but has not yet been diagnosed as having it; (ii) inhibiting the disease, i.e., arresting its development; or (iii) relieving the disease, i.e., causing regression of the disease.

One illustrative LASY activator is Compound A, which has the structure:
Another illustrative LASY activator is Compound B, which has the structure:

In one embodiment, the present invention provides a method of reducing inflammation in a living cell of a subject, which method comprises increasing the level of lipoic acid synthase within said living cell thereby causing said reduction of said inflammation. In certain aspects, the aforementioned cell expresses lipoic acid synthase. In other aspects, the level of lipoic acid synthase is increased by treating said living cell with an external agent.

In another embodiment, the present invention provides a method of treating or mitigating an inflammatory disorder, said method comprising administering to a subject having the disorder an effective amount of an agent that causes an increase in the level of lipoic acid synthase.

In other aspects, the aforementioned inflammation is associated with increased levels of MCP-1. In other aspects, the reduction of inflammation is manifested by reducing the level of MCP-1. In still other aspects, the aforementioned inflammation is a TNFα-induced inflammation and the reduction of inflammation may be manifested by reducing the level of TNFα. In still other embodiments, the inflammation is an AGE-induced inflammation.

Another embodiment provides for a method of treating a disease state which is alleviable by the treatment with a compound that is identified as affecting the activity of the LASY protein or gene (LASY activator), which comprises administering to a subject
in need thereof a therapeutic effective amount said compound or a pharmaceutically acceptable salt thereof. Such disease state includes, but is not limited to metabolic disorders, cardiovascular disorders and inflammatory-induce disease, including but not limited to osteoarthritis, rheumatoid arthritis, obesity, asthma, atherosclerosis, irritable bowel syndrome, Crohn's disease, type 2 diabetes, psoriasis, diabetic nephropathy, retinopathy, and glomerular nephritis.

In another embodiment, the present invention provides a method of treating or mitigating an inflammatory disorder associated with increased levels of MCP-1, said method comprising administering to a subject having the disorder an effective amount of an agent that causes an increase in the level of lipoic acid synthase thereby reducing said level of MCP-1 for said subject.

In another embodiment, the present invention provides a method of improving glucose uptake in a mammal, said method comprising increasing the level of lipoic acid synthase in the body of said mammal. In certain aspects, the step of increasing the levels of lipoic acid synthase is carried out by administering said mammal with an effective amount of an external agent.

In another embodiment, the present invention provides a method of treating or mitigating hyperglycemia in a mammal, said method comprising increasing the level of lipoic acid synthase in the body of said mammal. The step of increasing the levels of lipoic acid synthase can be carried out by administering to the mammal an effective amount of an agent that causes an increased level of lipoic acid synthase. In certain aspects, the aforementioned hyperglycemia is associated with Type II diabetes and/or insulin-resistant diabetes.

In another embodiment, the present invention provides a method of reducing the weight of a body of a mammal, the method comprising increasing the level of a lipoic acid synthase in the body of the mammal. The present invention also provides a method of reducing the level of triglycerides in a body of a mammal, said method comprising increasing the level of lipoic acid synthase in said body of said mammal. The aforementioned step of increasing the levels of the lipoic acid synthase can be carried out by administering to the mammal an effective amount of an agent that increases the level of lipoic acid synthase.
In another embodiment, the present invention provides a method of reducing the level of triglycerides in a body of a mammal, said method comprising increasing the level of lipoic acid synthase in said body of said mammal.

In another embodiment, the present invention provides a method of treating a metabolic disorder, said method comprising administering to a subject with the disorder an effective amount of an agent that increases the level of lipoic acid synthase, wherein the metabolic disorder is selected from the group consisting of diabetes, Type II diabetes, obesity, and hypertension.

In another embodiment, the present invention provides a method of identifying compounds capable of increasing expression of lipoic acid synthase in a living cell, said method comprising:

a) providing a sample of cells that express said lipoic acid synthase;
b) providing a sample of a candidate compound;
c) contacting said cell sample and said compound sample; and
d) measuring a quantitative indicator of expression of said lipoic acid synthase within said cell sample after said contacting step.

The aforementioned quantitative indicator can be the level of mRNA of the lipoic acid synthase. Likewise, the measuring step can include amplifying the mRNA by quantitative PCR.

In another embodiment, the present invention provides a method of identifying a candidate compound suitable as a pharmaceutical agent for treatment of a disorder treatable by increased expression of lipoic acid synthase, said method comprising:

a) providing a sample of cells that express lipoic acid synthase;
b) providing a sample of a candidate compound;
c) contacting said cell sample and said compound sample; and
d) measuring a quantitative indicator for the level of expression of said lipoic acid synthase within said treated cell sample; wherein compounds causing a predetermined increase in the expression of lipoic acid synthase are identified as candidate pharmaceutical agents.

In certain embodiments, the aforementioned disorder is an inflammatory disorder and/or obesity. In still other embodiments, the aforementioned pre-determined level of
increase is at least 300% in comparison with the expression of lipoic acid synthase in untreated cells. Likewise, the quantitative indicator can be the level of mRNA of the lipoic acid synthase. Likewise, the measuring step can include amplifying the mRNA by quantitative PCR.

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.

In one embodiment, the present invention involves the use of a safe and effective amount of one or more identified LASY activators for treating or preventing obesity and inflammation conditions.

The present method includes administering one or more LASY 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.

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

When one or more identified LASY 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 LASY 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.

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.

In particular, the identified LASY 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.

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.

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.

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.

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 LASY 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.

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

An exemplary method of administering one or more identified LASY 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.

The subject identified LASY 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;

The subject identified LASY 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.

The subject identified LASY 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.

In the present method, a subject in need of prevention or treatment of obesity
and/or inflammation conditions is treated with an amount of one or more LASY
activators identified in accordance with the present invention, wherein the amount of the
one or more identified LASY 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
LASY 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.
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.

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.

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.

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 inflammation and obesity and obesity-related conditions, including, but not limited to, type I diabetes, type II diabetes, hyperlipidaemia, hypercholesterolemia, 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.

In some embodiments, it may be useful to include the one or more identified LASY activators as a salt. Those having ordinary skill in the art will recognize the pharmaceutically acceptable salts of the identified LASY activator compounds.

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.

Also included in the invention are the isomeric forms and tautomers and the pharmaceutically-acceptable salts of the identified LASY 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.

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.

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.

As used herein, an "effective amount" means the dose or effective amount to be administered to a patient and the frequency of administration to the subject which is readily determined by one or ordinary skill in the art, by the use of known techniques and by observing results obtained under analogous circumstances. The dose or effective amount to be administered to a patient 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 patient to be treated, and other relevant circumstances.

The phrase "therapeutically-effective" indicates the capability of an agent to prevent, or improve the severity of, the disorder, while avoiding adverse side effects typically associated with alternative therapies. The phrase "therapeutically-effective" is to be understood to be equivalent to the phrase "effective for the treatment, prevention, or inhibition", and both are intended to qualify the amount of the LASY activating compound for use in therapy which will achieve the goal of improvement in the severity of the disorder and the frequency of incidence over treatment, while avoiding adverse side effects typically associated with alternative therapies.

Preferred dosages for the Identified LASY activators are those that are effective to reduce weight in the subject. In another embodiment, an effective dosage is an amount that is effective to upregulate LASY activity in the subject. However, daily dosages 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. Those skilled in the art will appreciate that dosages may also be determined with guidance from Goodman & Goldman's The

The frequency of dose will depend upon the half-life of the active components of the composition. If the active molecules have a short half life (e.g. from about 2 to 10 hours) it may be necessary to give one or more doses per day. Alternatively, if the active molecules have a long half-life (e.g. from about 2 to about 15 days) it may only be necessary to give a dosage once per day, per week, or even once every 1 or 2 months. A preferred dosage rate is to administer the dosage amounts described above to a subject once per day.

For the purposes of calculating and expressing a dosage rate, all dosages that are expressed herein are calculated on an average amount-per-day basis irrespective of the dosage rate. For example, one 100 mg dosage of LASY activator taken once every two days would be expressed as a dosage rate of 50 mg/day. Similarly, the dosage rate of an ingredient where 50 mg is taken twice per day would be expressed as a dosage rate of 100 mg/day.

For purposes of calculation of dosage amounts, the weight of a normal adult human will be assumed to be 70 kg.

The following examples describe embodiments of the invention. Other embodiments within the scope of the 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, together with the examples, be considered to be exemplary only, with the scope and spirit of the invention being indicated by the claims which follow the examples. In the examples, all percentages are given on a weight basis unless otherwise indicated.

**General Methods:**

**LASY Real time PCR screen:**

Human skeletal muscle myoblasts (HSMMs) were grown in 96-well plates in Skeletal Muscle Growth Media (SKGM-2) (Cambrex) for 4 days at 37°C /CO2. On day 4, when the cells were about 70-80% confluent, differentiation media consisting of
DMEM-F12 (Cambrex) with 2% horse serum was added. Cells were allowed to differentiate for 3-5 days before treatments, until myotubules were observed throughout the culture. Treatments with compounds or cytokines and growth factors were done on differentiated cells. All treatments were done for 18 hours unless otherwise indicated.

After treatments, cells were washed with basal media/phosphate-buffered saline (PBS) and cells were harvested by adding Lysis buffer (Stratagene). RNA was extracted from cells using the Absolutely RNA™ 96 microprep kit (Stratagene). Real time quantitative PCR was performed using the LASY primers and Brilliant SYBR Green one-step RT PCR kit (Stratagene). Sequences of the oligonucleotide primers for human LASY are:

5'GGAGCTCGATGTCCAATA 3' (forward primer) and 5'TGGAGGAGGATTCTTGAG 3' (reverse primer). These primers amplify a 150 bp product within the N-terminal half of the human LASY gene. The primers were designed to span exon-intron boundaries so as avoid amplification from genomic DNA.

Amplifications were performed using the Mx3000 instrument (Stratagene). Briefly, the amplification protocol consisted of a 30 minute reverse transcription step at 50°C, followed by a 10 minute denaturation step at 95°C, and 40 cycles of 95°C (30 seconds), 55°C (1 minute) and 72°C (30 seconds). The amplification program was followed by a dissociation program consisting of 81 cycles of incubation in which the temperature is increased by 0.5°C/cycle, beginning at 55°C and ending at 95°C (duration of each cycle was 30 sec). Values obtained for the gene of interest, LASY were normalized to values for a housekeeping gene, Actin.

**LASY knock-down:**

RNA interference (RNAi) was used to study effects of LASY knock down. A web-based software program was used to identify regions within the LASY mRNA sequence that would be optimal for the design of siRNA oligos to knock down LASY. Two siRNA oligos were synthesized (Dharmacon) and efficiency of knock-down was monitored by real-time PCR. Effects of the knock down on TNFα-stimulated inflammation in human microvascular endothelial cells (HMVECs) and on glucose uptake in HSMMs were studied.
The sequences of the two LASY siRNA duplexes are as shown below:

SiRNA1: 5' CAGUCCCGGAAUUACAGAG 3'
        3' GUCAGGGCCUUAAUGUCUC 5'

SiRNA2: 5' CAAGGGCUCACCUUAAGGU 3'
        3'GUUCCGCAGUGGAAUUCCA 5'

The negative control for the siRNA LASY knock-down experiment consisted of a scrambled duplex oligo (Dharmacon). For glucose uptake experiments, HSMM were plated in 24-well plates and allowed to differentiate as described in the previous section. Differentiated cells were treated with siRNA oligos (10 nM) and TransIT-KO reagent (Mirius Corp.) for 24 hrs at 37°C/CO2. Cells were then used for glucose uptake assay or inflammation assay as described below.

LASY Overexpression:

The human LASY gene was amplified from human aortic endothelial cells (HAECs) by Oligo dT priming with Superscript first strand synthesis system (Invitrogen Corp.). The following two LASY primers were used to amplify the full-length gene.

5'-GCCTAGTCCTAAAGAGGAAAT-S' (forward primer)
3'-ACAGCAAGCAACTGTTTAATTG-S' (reverse primer)

The amplification product was cloned by topo cloning into pCR2.1 vector (Invitrogen Corp) and transfected into One Shot E.coli cells (Invitrogen Corp.). Presence of recombinants was verified by PCR and restriction enzyme digestion. Recombinant clones containing the insert were purified. Inserts were then cut out by restriction enzyme digestion with XhoI and KpnI and cloned into the corresponding sites within pBKCMV. Presence of insert in the correct orientation was verified by PCR and sequencing. The plasmid containing the LASY gene (pBKCMV-LASY), was purified in large scale for transfections.

Transfections in HMVECs or HSMMs were done using the Targeffect F-1 reagent with Peptide enhancer (Targeting Sytems, Inc.) using the manufacturer's protocol. Transfections were done in 100 mm plates for all assays. Transfections were done for 24 hours and cells were replated into 96-well plates (for inflammation assay), or 24 well plates (for glucose uptake assay) and incubated for 18 h before treatments.
Glucose Uptake assay:

Cells were washed with DMEM containing 1% BSA and incubated in this medium for 18 hrs. Cells were washed with transport medium (20 mmol/l HEPES, pH 7.4, 140 mol/l NaCl, 5 mmol/l MgCl2, 1 mmol/l CaCl2 and 0.1% (wt/vol) BSA. Next, cells were incubated in transport medium with or without insulin (1 μM/l) for 30 min at 37°C/CO2. This was followed by incubation in transport buffer containing 10 μM/l 2-DG (1.0 μCi/ml) for 30 min at 37°C. Reactions were stopped by aspirating the media and washing cells with PBS containing 20mmol/l D-glucose at 4°C. Cells were solubilized in 0.5% (wt/vol) SDS, and radioactivity was measured by liquid scintillation counting. Data were expressed per milligram of protein, determined by using the bichinchoninic acid method (Pierce Chemical Company).

Cell treatments:

For inflammation and LASY expression assays, HAECs were washed once with treatment medium (basal medium containing 1% FBS). Cells were treated with TNFα (0.05 ng/ml), glycated human serum albumin (US Biologicals) as source of advanced glycation end products (AGEs) (300 μg/ml), or glucose (25 mM) for 18 hours. Cell supernatants were collected and used for MCP-1 ELISA. Cells were harvested for RNA extraction as described below.

RT-PCR:

For real-time and reverse transcriptase (RT)-PCR, cells were washed with 1X Phosphate Buffered Saline (PBS) and harvested by adding cell lysis/binding buffer (Ambion). RNA was extracted from cells according to the manufacturer's protocol (Ambion RNAqueous kit). RNA concentration and quality was determined by using an Agilent Bioanalyser. For RT-PCR, cDNA was synthesis and PCR were performed using Superscript One-Step RT-PCR kit with Platinum Taq (Invitrogen Corporation) using the protocol provided by the manufacturer. The following LASY primers were used in the RT reaction:

5’ CCGGGTATTTGGGAGATATTTTTG3’ (forward primer)
3' GATACGGTCTTTGCAATGTGTT 5' (reverse primer)

Reactions were performed using a standard thermal cycler. RT-PCR end products were analyzed and quantitated by using the Agilent Bioanalyser. Results were expressed as percent of untreated control.

For real-time PCR, the real-time quantitative PCR reaction was performed using Brilliant SYBR Green one-step RT PCR kit (Stratagene) as described above.

MCP-1 ELISA:

MCP-1 ELISA was carried out using Quantikine Human MCP-1 kit as described by the manufacturer (R&D Systems, Inc.). Mouse anti-human MCP-1 was used as the capture antibody and HRP-conjugated goat anti-human MCP-1 was used as detection antibody. Culture media was incubated with the capture antibody (in 96-well plate) for 2 h at room temperature. Wells were washed three times with wash buffer (0.05% Tween-20 in PBS) followed by incubation with detection antibody for 2 h at room temperature. Color development was read at 45 nm in a microplate reader.

Animal experiments:

Two animal models of obesity and type II diabetes, namely, ob/ob and db/db were purchased from Jackson Laboratories. All animal procedures were done in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC). Animals were individually housed, and food intake and water consumption were monitored every day. Body weight was measured once a week using standard laboratory scales. Animals were bled via retro orbital sinus for glucose and lipid measurements. Blood was collected in tubes containing EDTA and immediately centrifuged to separate plasma. Plasma was aliquoted and stored at -20°C. Plasma glucose measurements were done using a glucometer immediately after the bleed. Plasma triglycerides were measured using Infinity Triglyceride Liquid Stable Reagent (Thermo DNA, Inc.). Briefly, blood was collected and spun down for plasma separation. The samples and standard were diluted in Infinity Triglycerides Liquid Stable Reagent and were incubated 5 minutes at 37°C. Absorbance was measured at two wavelengths 520nm for sample reading and 620nm for background subtraction). The concentration
of the sample was automatically extrapolated from the machine using the following equation:

\[
\text{Absorbance of Unknown Triglycerides} = \text{Absorbance of Calibrator} \times \text{Calibrator Value}
\]

Animals were sacrificed at the end of the study by administration of carbon dioxide gas, and tissues were collected and weighed.

**Extraction of RNA from tissues:**

Upon harvest, tissues were wrapped in aluminum foil and snap frozen in liquid nitrogen. For processing, tissue was kept frozen using liquid nitrogen and dry ice. An appropriate size of tissue (100mg for adipose tissue, 30mg for all other tissues) was cut and put into tubes contain a stainless steel bead (5mm) and kit-supplied lysis buffers.

Sample homogenization was achieved using the TissueLyser (Qiagen, Inc.), which included disruption for 2 X 3 minutes at 20-30 Hz. After homogenization, RNA was extracted using the manufacturer's protocols. RNA was extracted from skeletal muscle by using the RNEasy fibrous tissue mini kit (Qiagen, Inc.). RNA from adipose tissue was extracted using the RNeasy lipid tissue mini kit (Qiagen Inc).

**EXAMPLE 1**

**Role of LASY in inflammation:**

The presence of LASY expression in endothelial cells was demonstrated. It was also demonstrated that in the presence of inflammatory cytokines such as tumor necrosis factor α (TNFα) the expression of LASY is down regulated by 50% (Figure 1). This data suggests that down regulation of LASY expression may play a role in TNFα mediated inflammation.
EXAMPLE 2

The role of LASY as an anti-inflammatory protein was also demonstrated. TNFα is known to induce inflammatory gene expression in endothelial cells. One major inflammatory gene induced by TNFα is monocyte chemoattractant protein 1 (MCP-1). MCP-1 has been shown to play a critical role in inflammatory diseases including atherosclerosis, rheumatoid arthritis and asthma. To determine the role of LASY on TNFα mediated induction of MCP-1, we used molecular approaches to reduce LASY expression (LASY knock down). LASY expression was reduced by siRNA (RNAi) gene silencing. Knocking down the LASY gene resulted in exacerbation of the inflammatory response as measured by the levels of MCP-1 by ELISA (Figure 2). Only LASY RNAi, but not control non-specific oligo (C-RNAi), showed this effect. This data demonstrates that LASY plays a protective role in inflammation.

EXAMPLE 3

The anti-inflammatory role of LASY was further demonstrated by overexpression of LASY. Apart from TNFα, glycated proteins containing advanced glycation end products (AGE) also induce inflammatory gene MCP-1 in endothelial cells. AGE has been shown to play a critical role in many diabetic vascular complications. Overexpression of LASY in endothelial cells resulted in significant reduction in TNF-α and AGE-stimulated MCP-1 response (50% and 70% decrease, respectively - compared to mock-transfected control cells - Figure 3). These data support that LASY has a role in protecting cells against inflammation.

EXAMPLE 4

Role of LASY in diabetes:

Diabetes is associated with hyperglycemia (increased plasma glucose). It was demonstrated that high glucose levels down-regulated the expression of LASY. LASY mRNA was measured by real-time PCR. Treatment of human aortic endothelial cells (HAECs) with glucose led to a 60% reduction in LASY mRNA levels (Figure 4).
EXAMPLE 5

Extending the observations from example 4, it was studied if the in vivo expression of LASY was altered in diabetic state, db/db mice were used, a well known model for type 2 diabetes. Skeletal muscle plays a role in insulin-stimulated glucose uptake in the body. In type 2 diabetes, skeletal muscle becomes insulin-resistant and fails to utilize glucose efficiently. LASY mRNA in skeletal muscle tissue from normoglycemic and diabetic mice (db/db) was measured by real-time PCR. The data demonstrate that there was significant down regulation of LASY mRNA levels in skeletal muscle from the diabetic animals compared to their normoglycemic, heterozygous (db/+ ) counterparts and normal control mice (43% and 78%, respectively) (Figure 5).

EXAMPLE 6

Skeletal muscle plays an important role in glucose uptake and utilization, and as shown in Examples 4 and 5, LASY expression is reduced under diabetic conditions in vitro and in vivo. It was next demonstrated that reduced LASY expression resulted in inefficient glucose uptake by skeletal muscle. LASY expression was knocked down using RNAi. The ability of skeletal muscle cells to utilize glucose was measured by using radiolabeled 2-deoxy glucose. Reducing LASY expression by RNAi in skeletal muscle cells significantly reduced glucose uptake (by 68% compared to control RNAi treated cells - Figure 6). Thus, this data demonstrates that LASY plays a preventive role in insulin-resistant state induced in skeletal muscle by diabetes, and that LASY has a protective role in diabetes.

EXAMPLE 7

Role of LASY in obesity:

In addition to skeletal muscle, the adipose tissue plays a role in insulin resistance and obesity. Studies suggest that adipose tissue and adipose tissue-derived hormones and inflammatory cytokines play essential roles in the overall insulin sensitivity in vivo. Adipose tissue plays an active role in energy balance thus control obesity.

To determine if LASY has a role in obesity, basal expression of LASY in a mouse model of obesity was analyzed. LASY mRNA in adipose tissue from control (normal body
weight) and obese mice was measured by real-time PCR. The data demonstrated that there was significant down regulation of LASY mRNA levels in adipose tissue from the obese animals compared to their normal control mice (68% reduction - Figure 7).

EXAMPLE 8

Therapeutic use of LASY induction in obesity

To demonstrate that induction of LASY expression would have a beneficial effect in obesity and related metabolic diseases, an assay to identify LASY activators was designed.

An in vitro high-throughput assay was developed based on real time quantitative PCR to screen for, and identify agents that upregulate LASY mRNA levels. Cells were treated with pharmaceutical agents. Total RNA was extracted from treated cells and used in the real-time PCR assay. Two LASY primers that amplified a 150 bp region within the LASY gene were used in the assay. Chemical compounds that induced LASY mRNA levels were identified. One such compound, (Compound A), induced LASY mRNA levels by 3-6 fold at 100 - 250 µM (Figure 8)

Compounds that were found to induce LASY expression by ≥3 fold were found to be anti-inflammatory (decreased MCP-1 expression in endothelial cells IC₅₀ values: 0.62-2.3 µM). This data supports the earlier experiments that there is an inverse relationship between LASY expression levels and the expression of pro-inflammatory markers.

EXAMPLE 9

This Example demonstrates that a LASY activator shows an anti-obesity effect in animal models of obesity and improves obesity-associated metabolic defects.

Compound B, which induced LASY by 3-6 fold, was tested in two mouse genetic models of obesity and diabetes (db/db and ob/ob). In both models, compound B showed very significant effects on body weight reduction (Figures 9 and 10) compared to vehicle-treated control animals. There was no significant reduction in food intake. Analysis of tissues showed a significant increase in LASY mRNA levels in skeletal muscle in the group treated with compound B (by 57%). This data demonstrates that
induction of LASY is associated with reduction of body weight. Reduction in body weight was also accompanied by a significant reduction in plasma triglyceride levels (Figure 11) and plasma glucose (21%). Thus, LASY induction will have beneficial effects on obesity and associated metabolic defects.
WHAT IS CLAIMED IS:

1. A method of reducing inflammation in a living cell of a subject, which method comprises increasing the level of lipoic acid synthase within said living cell thereby causing said reduction of said inflammation.

2. The method of claim 1, wherein said cell expresses lipoic acid synthase.

3. The method of claim 1, wherein the level of lipoic acid synthase is increased by treating said living cell with an external agent.

4. The method of claim 1, wherein said subject is a cell sample.

5. The method of claim 1, wherein said subject is a living mammal.

6. The method of claim 5, wherein said mammal is a human.

7. The method of claim 1, wherein said inflammation is associated with increased levels of MCP-1.

8. The method of claim 1, wherein said reduction of inflammation is manifested by reducing the level of MCP-1.

9. The method of claim 1, wherein said inflammation is a TNFα-induced inflammation.

10. The method of claim 1, wherein said reduction of inflammation is manifested by reducing the level of TNFα.

11. The method of claim 1, wherein said inflammation is an AGE-induced inflammation.

12. A method of treating or mitigating an inflammatory disorder, said method comprising administering to a subject having the disorder an effective amount of an agent that causes an increase in the level of lipoic acid synthase.

13. The method of claim 12, wherein said inflammatory disorder is associated with increased levels of MCP-1.

14. The method of claim 12, wherein said subject is a mammal.

15. The method of claim 14, wherein said mammal is human.

16. The method of claim 15, wherein said disorder is atherosclerosis.

17. The method of claim 15, wherein said disorder is rheumatoid arthritis.

18. The method of claim 15, wherein said disorder is asthma.
19. The method of claim 16, wherein said disorder is cardiovascular disease.

20. A method of treating or mitigating an inflammatory disorder associated with increased levels of MCP-1, said method comprising administering to a subject having the disorder an effective amount of an agent that causes an increase in the level of lipoic acid synthase thereby reducing said level of MCP-1 for said subject.

21. A method of improving glucose uptake in a mammal, said method comprising increasing the level of lipoic acid synthase in the body of said mammal.

22. The method of claim 21, wherein said step of increasing the levels of lipoic acid synthase is carried out by administering said mammal with an effective amount of an external agent.

23. A method of treating or mitigating hyperglycemia in a mammal, said method comprising increasing the level of lipoic acid synthase in the body of said mammal.

24. The method of claim 23, wherein said step of increasing said levels of the lipoic acid synthase is carried out by administering said mammal with an effective amount of an agent that causes said increased level of lipoic acid synthase.

25. The method of claim 23, wherein said hyperglycemia is associated with Type II diabetes.

26. The method of claim 23, wherein said hyperglycemia is associated with insulin-resistant diabetes.

27. A method of reducing the weight of a body of a mammal, said method comprising increasing the level of a lipoic acid synthase in the body of said mammal.

28. The method of claim 27, wherein said step of increasing said levels of the lipoic acid synthase is carried out by administering said mammal with an effective amount of an agent that increases the level of lipoic acid synthase.

29. A method of reducing the level of triglycerides in a body of a mammal, said method comprising increasing the level of lipoic acid synthase in
said body of said mammal.

30. The method of claim 29, wherein said step of increasing said levels of the lipoic acid synthase is carried out by administering said mammal with an effective amount of an agent that increases the level of said lipoic acid synthase.

31. The method of claim 30, wherein said mammal is a human.

32. A method of treating a metabolic disorder, said method comprising administering to a subject with the disorder an effective amount of an agent that increases the level of lipoic acid synthase.

33. The method of claim 32, wherein said metabolic disorder is diabetes.

34. The method of claim 33, wherein said diabetes is Type II diabetes.

35. The method of claim 32, wherein said metabolic disorder is obesity.

36. The method of claim 32, wherein said metabolic disorder is hypertension.

37. A method of identifying compounds capable of increasing expression of lipoic acid synthase in a living cell, said method comprising:
   a) providing a sample of cells that express said lipoic acid synthase;
   b) providing a sample of a candidate compound;
   c) contacting said cell sample and said compound sample; and
   d) measuring a quantitative indicator of expression of said lipoic acid synthase within said cell sample after said contacting step.

38. The method of claim 37, wherein said quantitative indicator is the level of mRNA of said lipoic acid synthase.

39. The method of claim 38, wherein said measuring step includes amplifying said mRNA by quantitative PCR.

40. A method of identifying a candidate compound suitable as a pharmaceutical agent for treatment of a disorder treatable by increased expression of lipoic acid synthase, said method comprising:
   a) providing a sample of cells that express lipoic acid synthase;
   b) providing a sample of a candidate compound;
   c) contacting said cell sample and said compound sample; and
   d) measuring a quantitative indicator for the level of expression of said lipoic acid synthase.
lipoic acid synthase within said treated cell sample;
wherein compounds causing a pre-determined increase in the expression of lipoic acid synthase are identified as candidate pharmaceutical agents.

41. The method of claim 40, wherein said quantitative indicator is the level of mRNA that encodes lipoic acid synthase.

42. The method of claim 41, wherein said quantitative indicators are obtained by amplifying said mRNA by quantitative PCR.

43. The method of claim 40, wherein said disorder is an inflammatory disorder.

44. The method of claim 40, wherein said pre-determined level of increase is at least 300% in comparison with the expression of lipoic acid synthase in untreated cells.

45. The method of claim 40, wherein said disorder is atherosclerosis.

46. The method of claim 40, wherein said disorder is rheumatoid arthritis.

47. The method of claim 40, wherein said disorder is asthma.

48. The method of claim 40, wherein said disorder is cardiovascular disease.

49. The method of claim 40, wherein said disorder is diabetes.

50. The method of claim 40, wherein said disorder is obesity.
LASY is down-regulated by pro-inflammatory agents

Experiment was done in Human aortic endothelial cells (HAECs)
Control: untreated cells

FIG. 1
Inhibition of LASY exacerbates the inflammatory response
Overexpression of LASY decreases expression of inflammatory genes

FIG. 3
LASY is down-regulated by glucose

Experiment was done in Human aortic endothelial cells (HAECs)
Control: untreated cells

FIG. 4
Basal expression of LASY in skeletal muscle of $db/db$ mice

FIG. 5
LASY inhibition by siRNA inhibits glucose uptake in human skeletal muscle

FIG. 6
Basal expression of LASY in adipose tissue of obese mice

FIG. 7
Effect of Compound A on induction of LASY gene expression

FIG. 8
Compound B: Effects on body weight in \textit{db/db} mice

Body weight compared with baseline values

\begin{figure}
\centering
\includegraphics{figure9}
\caption{FIG. 9}
\end{figure}
Compound B: Effects on body weight and food consumption in \textit{ob/ob} mice

Body weight gain during study period

![Graph showing body weight gain over time for different groups.]

- Vehicle control
- Compound B

\textbf{FIG. 10}
Compound B: Effects on plasma triglycerides in *ob/ob* mice

**FIG. 11**