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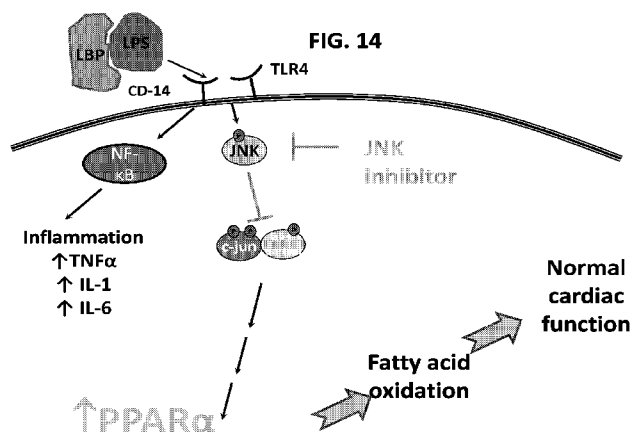
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(54) Title: Methods for Treating and Preventing Cardiac Dysfunction in Septic Shock



(57) **Abstract:** Cardiac dysfunction during sepsis is due, at least in part, to cardiac energy deficiency. It has been discovered that lipopolysaccharide (LPS)-mediated cardiac dysfunction is prevented or treated by treatments that improve FA oxidation (FAO), despite the persistence of inflammation. The present invention relates to methods for increasing or maintaining cardiac function in a subject, by administering to the subject a therapeutically effective amount of an agent that increases fatty acid oxidation in the heart.

METHODS FOR TREATING AND PREVENTING CARDIAC DYSFUNCTION IN SEPTIC SHOCK

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of United States Provisional Patent Application Serial No. 61/445,002, entitled “Methods for Treating and Preventing Cardiac Dysfunction in Septic Shock,” filed on February 21, 2011, the entire contents of which are hereby incorporated by reference as if fully set forth herein, under 35 U.S.C. §119 (e).

STATEMENT OF GOVERNMENTAL INTEREST

[0002] This invention was made with Government support under Contract Nos. HL45095, HL73029, and T32 HL007343 awarded by National Heart, Lung, and Blood Institute of NIH. The Government has certain rights in the invention.

BACKGROUND OF THE INVENTION

[0003] Sepsis is a major cause of death in intensive care units and is associated with cardiac dysfunction. Septic shock is characterized by hypotension, ischemia, and multiple organ failure and can lead to increased mortality. Cardiac dysfunction is a definitive consequence of severe sepsis and is characterized by impaired contractility, diastolic dysfunction, as well as reduced cardiac index and ejection fraction (EF). The mechanisms that underlie myocardial depression during septic shock are not well known. However, in an effort to further understand the complexity of cardiac dysfunction, the effects of sepsis have been reproduced in experimental models. The most common models used include injection of lipopolysaccharide (LPS), which is a bacterial cell wall component that induces pathophysiological consequences similar to those found during septic shock.

[0004] Cardiac septic shock is a severe condition, which besides its inflammatory component is characterized by dramatic reduction in metabolic rate. Given the high mortality associated with sepsis and the inability of anti-inflammatory treatment to improve mortality, other means of treating cardiac dysfunction associated with septic shock are needed. Therefore, there is a great

need for new methods and compositions to treat and prevent cardiac dysfunction associated with septic shock.

SUMMARY OF THE INVENTION

[0005] A first set of embodiments of the invention is directed to a method for increasing or maintaining cardiac function in a subject in need of such treatment, by administering to the subject a therapeutically effective amount of an agent that increases fatty acid oxidation (hereinafter “FAO”) in the heart. In certain embodiments, the subject has sepsis or is at risk of developing sepsis, or the subject has heart failure, or is at risk of developing heart failure.

[0006] In certain embodiments of the above method for increasing or maintaining cardiac function, the agent that increases fatty acid oxidation in the heart is a PPAR agonist selected from the group comprising PPAR α agonists, PPAR γ agonists, dual PPAR α and PPAR γ agonists, or combinations thereof. The PPAR α agonist is selected from the group comprising Alpha WY-14643, GW9578, GW-590735, K-111, LY-674, KRP-101, DRF-10945, LY518674, Propanoic Acid 2-[4-[3-[2,5-dihydro-1-[(4-methylphenyl)methyl]-5-oxo-1H-1,2,4-triazol-3-yl]propyl]phenoxy]-2-methyl, fibrates, fenofibrate, clofibrate, and bezafibrate. The PPAR γ agonist may be selected from the group comprising thiazolidinedione, rosiglitazone, pioglitazone, MCC-555, GL-262570, englitazone, darglitazone, isaglitazone, JTT-501, T-895645, R-119702, N,N-2344, YM-440, thiazolidinedione, GI 262570, R-483 and rivoglitazone.

[0007] In certain other embodiments of the above method for increasing or maintaining cardiac function, the agent is an inhibitor of either c-Jun N-terminal kinase 1 or 2 (JNK1 or JNK2 inhibitors) that increases fatty acid oxidation in the heart, including the JNK inhibitor SP600125. In another embodiment FAP is increased by reducing translation of mRNA encoding either JNK1 or JNK2, such as by administering therapeutically effective amounts of an antisense nucleic acid, siRNA, shRNA, micro RNA (miRNA), ribozyme, microRNA mimic, supermir, and aptamer that specifically hybridizes to JNK thereby reducing its expression.

[0008] Other embodiments include methods for increasing or maintaining cardiac function in a subject by administering a therapeutically effective amount of an agent that increases fatty acid oxidation in the heart such as a PPAR α -coactivator-1 (PGC-1), an estrogen-related receptor (ERR) α , or a combination thereof.

[0009] Other embodiments are directed to various pharmaceutical compositions and kits including formulations comprising therapeutically effective amounts of agents that increase fatty acid oxidation in the heart and are effective in increasing or maintaining cardiac function in a subject. The formulations may comprise PPAR α agonists, PPAR γ agonists, dual PPAR α and PPAR γ agonists, or combinations thereof, JNK inhibitors, or antisense nucleic acids, siRNAs, shRNAs, microRNAs (miRNA), ribozymes, microRNA mimics, supermirs, and aptamers, PPAR α -co-activator-1 (PGC-1), estrogen-related receptor (ERR) α , or a combination thereof. Administration can be orally or intravenously. In certain embodiments the compositions are formulated for slow release to minimize the need for repeated delivery and to maintain a steady concentration of drugs.

[0010] Another set of embodiments is directed to methods for treating or preventing cardiac dysfunction in a subject having sepsis or at risk of developing sepsis, by administering therapeutically effective amounts of an agent that increases fatty acid oxidation in the heart. The above embodiments of the methods include administering the agent before cardiac function is diminished. The embodiments also comprise methods wherein the subject is treated with at least two members selected from the group comprising JNK inhibitors, PPAR α agonists, PPAR γ agonists, and dual PPAR α and PPAR γ agonists. In certain embodiments the two agents comprise a JNK inhibitor and a PPAR agonist. In certain embodiments the PPAR γ agonist is either rosiglitazone administered in amounts of from about 0.1 to about 20mg/day, and pioglitazone, administered in amounts of from about 0.1 to about 45 mg/day.

[0011] These and other features, aspects, and advantages of the present invention will become better understood with regard to the following description, appended claims, and accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] The present invention is illustrated by way of example, and not by way of limitation, the Figures.

[0013] Figure 1 (FIG. 1): Sepsis is associated with increased inflammation but anti-inflammatory treatments do not prevent septic shock. Knockout animal models that are resistant

to cardiac septic shock and anti-inflammatory therapies that were applied to humans failed to prevent septic shock.

[0014] Figure 2 (FIG. 2): Sepsis is associated with reduced fatty acid oxidation. Effects of LPS on the expression of fatty acid metabolism-associated genes.

[0015] Figure 3 (FIG. 3): Effects of LPS on (A) cardiac function, (B) plasma interleukin 6, and (C)-(D) gene expression levels of cardiac inflammatory cytokines and proteins that are associated with fatty acid metabolism. LPS induced inflammation and reduced cardiac function and expression of lipid metabolism-related genes in C57BL/6 mice.

[0016] Figure 4 (FIG. 4): (A) Modulation of PPAR α fatty acid and glucose utilization during the progression of heart failure. (B) Effects of LPS in the expression of cardiac genes that are associated with glucose metabolism. Heart failure reduces FAO and increases glucose utilization.

[0017] Figure 5 (FIG. 5): (A)-(B) Several miRs (microRNAs) have been associated with heart failure related cardiac dysfunction in C57BL/6 mice. (C) Cardiac microRNA changes that occur in heart failure are not observed in sepsis-mediated heart dysfunction in C47BL/6 mice.

[0018] Figure 6 (FIG. 6): Effects of LPS in gene expression levels of α - and β -myosin heavy chain (MHC) isoforms in the hearts of C57BL/6 mice. LPS reduces both α MHC and β MHC.

[0019] Figure 7 (FIG. 7): Effects of LPS in the phosphorylation/activation of JNK and c-Jun in hearts of C57BL/6 mice. JNK and c-Jun are activated in LPS-treated C57BL/6 mice.

[0020] Figure 8 (FIG. 8): (A)-(B) Protein levels of JNK2 (A) and p-cJun (B) in AC16 cells that were treated with adenovirus-expressing constitutively active JNK. (C) Effects of adenovirus-mediated expression of a constitutively active JNK in PPAR α gene expression levels in a human cardiomyocyte cell line (AC16). (D) P-JNK levels in LPS-treated AC16 cells. (E) LPS-treatment reduces PPAR α expression in a human cardiomyocyte cell line. The effect of LPS on PPAR α gene expression is prevented by combined treatment of cells with LPS and JNK inhibitor (SP600125).

[0021] Figure 9 (FIG. 9): Effects of LPS in cardiac function and in PPAR α gene expression levels of JNK2 $^{-/-}$ and C57BL/6 mice. PPAR α gene expression is reduced and JNK pathway is

activated. (A) LPS does not affect cardiac function which is already reduced in JNK2^{-/-} mice. (B) LPS dramatically reduces PPAR α gene expression in JNK2^{-/-} mice and in normal mice. (C) Effects of LPS in the phosphorylation/activation of JNK and c-Jun in hearts of JNK2^{-/-} mice.

[0022] Figure 10 (FIG. 10): Outline of the protocol for the treatment of C57BL/6 mice with JNK inhibitor (SP600125) and LPS.

[0023] Figure 11 (FIG. 11): Effects of JNK inhibitor in the phosphorylation/activation of (A) c-Jun, (B) PPAR α gene expression levels and (C) cardiac function. JNK inhibitor blocked the effects of LPS on PPAR α gene expression and cardiac function in C57BL/6 mice.

[0024] Figure 12 (FIG. 12): Effects of JNK inhibitor in (A) cardiac fatty acid oxidation, (B) PGC1- α and (C) Cpt1 gene expression levels. Cardiac function improvement by JNK inhibitor is associated with increased FAO.

[0025] Figure 13 (FIG. 13): Effects of LPS and combined LPS and JNK inhibitor treatments in the expression of inflammatory cytokines genes (A) TNF α ; (B) IL-6; and (C) IL-1 α . Inflammation was not reduced in LPS-treated C57BL/6 mice that were injected with JNK inhibitor.

[0026] Figure 14 (FIG. 14): Schematic representation of the proposed pathway for the beneficial effect of JNK inhibition in the prevention of LPS-mediated PPAR α gene downregulation and in the improvement of fatty acid oxidation and cardiac function. JNK inhibitor increases PPAR α which in turn normalizes cardiac function in LPS-treated C57BL/6 mice.

[0027] Figure 15 (FIG. 15): Increased (A) fatty acid oxidation and (B) metabolic rate in mice with constitutive cardiomyocyte-specific PPAR γ expression in PPAR α deficiency background. FAO is increased despite reduction of PPAR α .

[0028] Figure 16 (FIG. 16): Effects of LPS in (A) PPAR α gene expression and in (B) cardiac function of α MHC-PPAR γ mice. Constitutive expression of PPAR γ protects from LPS-mediated cardiac dysfunction despite PPAR α downregulation.

[0029] Figure 17 (FIG. 17): Effects of LPS in cardiac expression of inflammatory cytokines genes (A) IL-1 α ; (B) IL-6; and (C) TNF α of α MHC-PPAR γ mice. Improved heart function in LPS-treated α MHC-PPAR γ mice is not associated with reduced inflammation.

[0030] Figure 18 (FIG. 18): Protocol for the treatment of C57BL/6 mice with rosiglitazone and LPS.

[0031] Figure 19 (FIG. 19): Effects of rosiglitazone in cardiac (A) PPAR α and (B) PGC1 α gene expression, in cardiac (C) fatty acid oxidation and in (D) cardiac function of LPS-treated C57BL/6 mice. Rosiglitazone increases FAO and protects from LPS-mediated cardiac dysfunction in normal mice.

[0032] Figure 20 (FIG. 20): Effects of rosiglitazone in cardiac expression of inflammatory cytokines genes (A) TNF- α and (B) IL-1 α of LPS-treated C57BL/6 mice. Inflammation was not affected by rosiglitazone treatment.

[0033] Figure 21 (FIG. 21): Schematic representation of the role of rosiglitazone as a therapeutic in order to activate PPAR γ and prevent LPS-mediated cardiac dysfunction by increasing fatty acid oxidation.

[0034] Figure 22 (FIG. 22): Fractional shortening (A) and cardiac ATP content (B) of LPS-treated C57BL/6 and α MHC-PPAR γ mice. (C) Fractional shortening of C57BL/6 mice treated with LPS and rosiglitazone. (D) Palmitate oxidation rate in hearts of LPS-treated α MHC-PPAR γ mice and of C57BL/6 mice treated with LPS and rosiglitazone or JNKin.

[0035] Figure 23 (FIG. 23): LPS inhibits cardiac fatty acid oxidation and impairs cardiac function - (A) PPAR α mRNA levels in hearts of 10- to 12-week-old C57BL/6 mice that were treated with 5 mg/kg LPS. $n = 5$; **, $p < 0.01$. (B, C) Photographs of echocardiograms (B) and fractional shortening (C) of 10- to 12-week-old C57BL/6 mice that were treated with 5 mg/kg LPS $n = 5$; **, $p < 0.01$. (D) [3H]palmitic acid oxidation in cardiac muscle of C57BL/6 mice treated with 5 mg/kg LPS. $n = 4$; *, $p < 0.05$. (E) ATP levels in hearts obtained from C57BL/6 mice that were treated with 5 mg/kg LPS; $n = 4$; *, $p < 0.05$. (F) Western blot analysis of pAMPK and total AMPK obtained from hearts of 10- to 12-week-old C57BL/6 mice that were treated with 5 mg/kg LPS. (G) Mitochondrial structure analysis and intracellular arrangement by

electron microscopy of cardiac tissue from LPS-treated C57BL/6 mice showed that LPS treatment did not affect either the morphology or the intracellular arrangement of mitochondria as compared to control mice that were treated with saline.

[0036] Figure 24 (FIG. 24): Constitutive expression of PPAR γ in cardiomyocytes stimulates cardiac fatty acid oxidation and prevents LPS-mediated heart dysfunction despite elevated inflammation. (A) Western blot analysis of pAMPK, total AMPK, pJNK and total JNK obtained from hearts of 10- to 12-week-old α MHC-PPAR γ mice that were treated with 5 mg/kg LPS. (B) The left bar represents the control and the right bar reflects LPS. ERR α , perilipin 2, perilipin 5, PGC-1 α , PGC-1 β , AOX, PDK4, PPAR α , PPAR δ , Cpt-1 and CD36 mRNA levels in hearts of 10- to 12-week-old α MHC-PPAR γ mice that were treated with 5 mg/kg LPS. $n = 5$; *, $p < 0.05$, **, $p < 0.01$. (C) [3H]palmitic acid oxidation in cardiac muscle of α MHC-PPAR γ mice treated with 5 mg/kg LPS. $n = 4$; *, $p < 0.05$. (D) ATP levels in hearts obtained from α MHC-PPAR γ mice that were treated with 5mg/kg LPS; $n=4$; *, $p < 0.05$. (E, F) Photographs of echocardiograms (E) and fractional shortening (F) of 10 weeks old α MHC-PPAR γ mice that were treated with 5 mg/kg LPS $n = 4$. (G-I) IL-1 α (G), IL-6 (H) and TNF α (I) mRNA levels in hearts of 10 weeks old α MHC-PPAR γ mice that were treated with 5 mg/kg LPS. $n = 5$; **, $p < 0.01$.

[0037] Figure 25 (FIG. 25): Rosiglitazone-mediated activation of PPAR γ prevents LPS-induced reduction in cardiac fatty acid oxidation and improves heart function despite elevated inflammation - (A, B) Photographs of echocardiograms (A) and fractional shortening (B) of 10-12 weeks old C57BL/6 mice that were treated with 5 mg/kg LPS or a combination of LPS and 35 mg/kg rosiglitazone (rosi). Control cells were treated with saline; $n = 4$; *, $p < 0.05$. (C) Western blot analysis of pAMPK, total AMPK, pJNK and total JNK obtained from hearts of C57BL/6 mice that were treated with 5 mg/kg LPS or a combination of LPS and 35 mg/kg rosiglitazone (Rosi). Control cells were treated with saline. (D) [3H]palmitic acid oxidation in cardiac muscle of 10-12 weeks old C57BL/6 mice that were treated with 5 mg/kg LPS or a combination of LPS and 35 mg/kg rosiglitazone (Rosi). Control cells were treated with saline; $n = 4$; *, $p < 0.05$. (E) Bar 1 of 4 depicts the control. Bar 2 of 4 depicts Rosi. Bar 3 of 4 depicts LPS. Bar 4 of 4 depicts a combination of Rosi and LPS. PPAR α , PPAR γ , PPAR δ , ERR α , AOX, PGC-1 α , PGC-1 β , CD36, perilipin 2, perilipin 5 and PDK4 mRNA levels in hearts of C57BL/6 mice that were treated with 5 mg/kg LPS or a combination of LPS and 35 mg/kg rosiglitazone (Rosi). Control

cells were treated with saline; n=5; *, p<0.05, **, p<0.01, +, p<0.001. (F-H) TNF α (F), IL-1 α (G) and IL-6 (H) mRNA levels in hearts of C57BL/6 mice that were treated with 5 mg/kg LPS or a combination of LPS and 35 mg/kg rosiglitazone (Rosi). Control cells were treated with saline; n=5; *, p<0.05; **, p<0.01, +; p<0.001.

[0038] Figure 26 (FIG. 26): Rosiglitazone improves survival of LPS-treated C57BL/6 mice – Survival curves of C57BL/6 mice that were treated with saline (n=10), 35mg/kg/day rosiglitazone (n=10), 5 mg/kg LPS (n=14) or combination of rosiglitazone and LPS (n=12).

[0039] Figure 27 (FIG. 27): Proposed model – (A) Schematic model that explains the role of the activation of PPAR γ in the induction of fatty acid oxidation and prevention of LPS-mediated cardiac dysfunction. Binding of the complex that consists of LPS and lipopolysaccharide binding protein (LBP) on the TLR4 and CD14 receptors leads to downregulation of PPAR α , which eventually causes inhibition of fatty acid oxidation and cardiac dysfunction. In addition, inflammatory pathways are activated via stimulation of the NF- κ B signaling pathway. (B) Activation of PPAR γ prevents sepsis-mediated down-regulation of cardiac fatty acid oxidation, thus it protects cardiac function in sepsis despite increased levels of inflammatory cytokines.

[0040] Figure 28 (FIG. 28): Administration of PPAR α agonist WY-14643 improved fractional shortening by 25%.

[0041] In the Summary of the Invention above and in the Detailed Description of the Invention, and the claims below, and in the accompanying drawings, reference is made to particular features of the invention. It is to be understood that the disclosure of the invention in this specification includes all possible combinations of such particular features. For example, where a particular feature is disclosed in the context of a particular aspect or embodiment of the invention, or a particular claim, that feature can also be used, to the extent possible, in combination with and/or in the context of other particular aspects and embodiments of the invention, and in the invention generally. For the purposes of explanation, numerous specific details are set forth in order to provide a thorough understanding of the present invention. It will be apparent, however, to one skilled in the art that the present invention may be practiced without these specific details.

DETAILED DESCRIPTION

[0042] It has now been discovered that lipopolysaccharide (LPS)-mediated cardiac dysfunction can be treated by administering agents that improve FAO, despite the persistence of inflammation, thereby providing a focused therapy for those in heart failure. These results have strong therapeutic implications for treating diseases or disorders associated with cardiac dysfunction during sepsis.

Definitions

[0043] Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well-known and commonly used in the art. The methods and techniques of the present invention are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. *See, e.g.*, Sambrook *et al.* Molecular Cloning: A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989); Ausubel *et al.*, Current Protocols in Molecular Biology, Greene Publishing Associates (1992, and Supplements to 2002); Harlow and Lane Antibodies: A Laboratory Manual Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1990); Principles of Neural Science, 4th ed., Eric R. Kandel, James H. Schwartz, Thomas M. Jessell editors. McGraw-Hill/Appleton & Lange: New York, N.Y. (2000). Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art.

[0044] The terms "individual," "subject," and "patient" are used interchangeably herein and refer to any mammalian subject for whom diagnosis, treatment, or therapy is desired, particularly humans. A "subject" as used herein generally refers to any living multicellular organism. Subjects include, but are not limited to animals (*e.g.*, cows, pigs, horses, sheep, dogs and cats) and plants, including hominoids (*e.g.*, humans, chimpanzees, and monkeys). The term includes transgenic and cloned species. The term "patient" refers to both human and veterinary subjects.

[0045] "Administering" shall mean delivering in a manner which is affected or performed using any of the various methods and delivery systems known to those skilled in the art.

Administering can be performed, for example, orally, or intravenously, via implant, transmucosally, transdermally, intradermally, intramuscularly, subcutaneously, or intraperitoneally. Administering can also be performed, for example, once, a plurality of times, and/or over one or more extended periods.

[0046] The phrase "therapeutically effective amount" means an amount sufficient to produce a therapeutic result. Generally the therapeutic result is an objective or subjective improvement of a disease or condition, achieved by inducing or enhancing a physiological process, blocking or inhibiting a physiological process, or in general terms performing a biological function that helps in or contributes to the elimination or abatement of the disease or condition. For example, eliminating or reducing or mitigating the severity of a disease or set of one or more symptoms. The full therapeutic effect does not necessarily occur by administration of one dose and may occur only after administration of a series of doses. Thus, a therapeutically effective amount may be administered in one or more administrations. A therapeutic effective amount further includes an amount to maintain cardiac function in a subject with sepsis or is at risk of having sepsis.

[0047] A subject is "at a high risk of sepsis" where a subject has a condition that reduces the ability to fight serious infections. These conditions include having a weakened immune system—due to use of drugs that suppress the immune system (such as chemotherapy drugs or corticosteroids) or due to certain disorders (such as cancer, AIDS, and immune disorders, spinal cord injuries and certain blood disorders). The risk is also increased in people who are more likely to have bacteria enter their bloodstream. Such people include those who have a medical device inserted into the body (such as a catheter inserted into a vein or the urinary tract, drainage tubes, or breathing tubes). When medical devices are inserted, they can move bacteria into the body. Bacteria may also collect on the surface of such devices, making infection and sepsis more likely. The longer the device is left in place, the greater the risk. Other conditions also increase the risk of sepsis: (i) injecting recreational drugs where the drugs and needles used are rarely sterile; (ii) having an artificial (prosthetic) joint or heart valve or certain heart valve abnormalities as bacteria tend to lodge and collect on these structures and can be released into the blood stream; and (iii) having an infection that persists despite treatment with antibiotics: as

some bacteria that cause infections and sepsis are resistant to antibiotics and that can cause sepsis. A physician will be able to identify patients for whom intervention by the methods of the present invention is warranted.

[0048] Treating a disease means taking steps to obtain beneficial or desired results, including clinical results, such as mitigating, alleviating or ameliorating one or more symptoms of a disease; diminishing the extent of disease; delaying or slowing disease progression; ameliorating and palliating or stabilizing a metric (statistic) of disease. “Treatment” refers to the steps taken.

[0049] “Mitigating” means reducing or ameliorating a disease or symptom of a disease. For example, mitigation can be achieved by administering a therapeutic agent before the phenotypic expression of the disease (*i.e.* prior to the appearance of symptoms of the disease) Mitigation includes making the effects of disease less severe by avoiding, containing, reducing or removing it or a symptom of it. Mitigating an enumerated disease as described herein comes within the definition of “treating” an enumerated disease before symptoms occur. Amounts of therapeutic agents that mitigate a disease are herein referred to as “therapeutically effective amounts.”

[0050] “Significantly higher” or “significantly increased” is at least about a 15% increase over control levels or pretreatment levels whereas “significantly reduced” is at least about a 15% decrease over control levels or pretreatment levels.

[0051] By “maintaining cardiac function” is meant that the level of cardiac function remains essentially unchanged, *i.e.* further loss of cardiac function is prevented.

[0052] “Agent” means PPAR α agonists, PPAR γ agonists, dual PPAR α and PPAR γ agonists, or combinations thereof or JNK inhibitors such as SP600125. The agent may be an antisense nucleic acid, siRNA, shRNA, microRNA (miRNA), ribozyme, microRNA mimic, supermir, or aptamer that specifically hybridizes to JNK1 or 2 and known JNK1 and 2 inhibitors. An agent may be a PPAR α -coactivator-1 (PGC-1), estrogen-related receptor (ERR) α or combination thereof. PPAR α agents may be Alpha WY-14643, GW9578, GW-590735, K-111, LY-674, KRP-101, DRF-10945, LY518674, Propanoic Acid 2-[4-[3-[2,5-dihydro-1-[(4-methylphenyl)methyl]-5-oxo-1H-1,2,4-triazol-3-yl]propyl]phenoxy]-2-methyl, fibrate, fenofibrate, clofibrate, and bezafibrate. PPAR γ agonists may be hiazolidinedione, rosiglitazone, pioglitazone, MCC-555, GL-262570, englitazone, darglitazone, isaglitazone, JTT-501, T-

895645, R-119702, N,N-2344, YM-440, thiazolidinedione, GI 262570, R-483 and rivoglitazone. These “agents” increase cardiac function or maintain cardiac function in subjects with sepsis by increasing fatty acid oxidation in the heart.

[0053] “MiR” also “micro RNA” means a newly discovered class of small non-coding RNAs that are key negative regulators of gene expression. Like conventional protein-encoding RNA, miRs are transcribed by RNA polymerase II and their expression is controlled by transcriptional factors. The mature miRs inhibit target mRNA translation or promote their degradation by directly binding to specific miR binding sites in the 3'-untranslated region (3'-UTR) of target genes.

[0054] “siRNA” means small interfering RNA, sometimes known as short interfering RNA or silencing RNA, and is a class of double-stranded RNA molecules, 20-25 nucleotides in length, that play a variety of roles in biology. The most notable role of siRNA is its involvement in the RNA interference (RNAi) pathway, where it interferes with the expression of a specific gene.

[0055] “shRNA” means a small hairpin RNA or short hairpin RNA, that is a sequence of RNA that makes a tight hairpin turn that can be used to silence gene expression via RNA interference. shRNA uses a vector introduced into cells and utilizes the U6 or H1 promoter to ensure that the shRNA is always expressed. This vector is usually passed on to daughter cells, allowing the gene silencing to be inherited. The shRNA hairpin structure is cleaved by the cellular machinery into siRNA, which is then bound to the RNA-induced silencing complex (RISC). This complex binds to and cleaves mRNAs which match the siRNA that is bound to it. shRNA is transcribed by RNA polymerase III. shRNA production in a mammalian cell can sometimes cause the cell to mount an interferon response as the cell seeks to defend itself from what it perceives as viral attack.

[0056] “Ribozyme” is an RNA molecule with a well-defined tertiary structure that enables it to catalyze a chemical reaction. Ribozyme means ribonucleic acid enzyme. It may also be called an RNAenzyme or catalytic RNA. It contains an active site that consists entirely of RNA. Many natural ribozymes catalyze either the cleavage of one of their own phosphodiester bonds (self-cleaving ribozymes), or the cleavage of bonds in other RNAs. Some have been found to catalyze the aminotransferase activity of the ribosome.

[0057] “Supermirs” refer to single stranded, double stranded or partially double stranded oligomers or polymers of RNA or DNA or both, which has a nucleotide sequence that is substantially identical to a miRNA and that is antisense with respect to its mRNA target.

[0058] “Aptamers” are oligonucleic acid or peptide molecules that bind to a specific target molecule. Aptamers are usually created by selecting them from a large random sequence pool, but natural aptamers also exist in riboswitches. Aptamers can be used for both basic research and clinical purposes as macromolecular drugs. Aptamers can be combined with ribozymes to self-cleave in the presence of their target molecule. These compound molecules have additional research, industrial and clinical applications.

[0059] “ERR α ” or Estrogen-related receptor alpha, also known as NR3B1 (nuclear receptor subfamily 3, group B, member 1), is a nuclear receptor that in humans is encoded by the *ESRRA* (Estrogen Related Receptor Alpha) gene.

[0060] “c-Jun N-terminal kinase (JNK) is a member of the mitogen-activated protein kinase (MAPK) family and plays an essential role in TLR-mediated inflammatory responses.

[0061] “Treating” a subject afflicted with a disorder shall mean causing the subject to experience a reduction, delayed progression, regression or remission of the disorder and/or its symptoms. In one embodiment, recurrence of the disorder and/or its symptoms is prevented. In the preferred embodiment, the subject is cured of the disorder and/or its symptoms.

Background

[0062] Cardiac dysfunction during sepsis is due, at least in part, to cardiac energy deficiency. It has now been discovered that lipopolysaccharide (LPS)-mediated cardiac dysfunction can be both treated (mitigated and prevented) by administering agents that improve FAO. These agents include PPAR agonists and JNK inhibitors. As will be described in detail, the addition of JNK inhibitors and PPAR agonists administered within 24 hours of LPS prevented the typical LPS-induced increase in FAO and as a result, also prevented cardiac dysfunction. Thus, increasing FAO preferably within 24 hours of diagnosing sepsis, upregulated PPAR α expression, maintained or increased FAO, and rescued cardiac function despite elevated expression of cardiac inflammatory markers. Without being bound by theory, PPAR γ activation may

compensate for LPS-mediated reduction of PPAR α thereby restoring FAO and cardiac function. Because the agents were able to prevent the expected loss of cardiac function even after sepsis had begun and even though inflammation persisted, certain claims are directed to a method for mitigating and preventing cardiac dysfunction as well as treating it in sepsis by increasing FAO.

Embodiments of the Invention

[0063] Cardiac dysfunction is a definitive consequence of severe sepsis and is characterized by impaired contractility, diastolic dysfunction, as well as reduced cardiac index and ejection fraction (EF). In an effort to investigate cardiac dysfunction, the effects of septic shock have been reproduced in experimental models of sepsis. The most common models include injection of lipopolysaccharide (LPS). LPS is a bacterial cell wall component that induces pathophysiological consequences similar to those found during septic shock. With the use of this LPS model, it has now been discovered that LPS-mediated cardiac dysfunction can be both treated and prevented by administration of with agents that improve fatty acid oxidation in the heart. Optimal results can be achieved if the therapeutic agents (JNK inhibitors and PPAR agonists) are administered within 24 hours after a subject is diagnosed as having sepsis or is at risk of developing sepsis.

[0064] As is described in the Summary of the Invention, some embodiments are directed to methods for increasing or maintaining cardiac function in a subject heart in a subject in need of such treatment, particularly by increasing fatty acid oxidation in the heart, for example in a subject that has sepsis or is at risk of developing sepsis, or has heart failure, or is at risk of developing heart failure. By increasing or maintaining cardiac function is meant that the post-treatment level of cardiac function is “significantly higher” or “significantly increased” to at least about a 15% increase over control levels or pretreatment levels.

[0065] The desired increase in fatty acid oxidation in the heart is a result of administering therapeutically effective amounts of agents as described herein, including PPAR agonists, JNK inhibitors (*e.g.*, SP600125), or antisense nucleic acids, siRNAs, micro RNAs (miRNA), short hairpin RNAs (shRNA), ribozymes, microRNA mimics, supermirs, and aptamers that specifically hybridize to JNK1 or JNK2, thereby reducing its expression and PPAR α -coactivators-1, estrogen-related receptors, or combinations thereof.

[0066] PPAR α agonists that increase fatty acid oxidation include those in the group comprising Alpha WY-14643, GW9578, GW-590735, K-111, LY-674, KRP-101, DRF-10945, LY518674, Propanoic Acid 2-[4-[3-[2,5-dihydro-1-[(4-methylphenyl)methyl]-5-oxo-1H-1,2,4-triazol-3-yl]propyl]phenoxy]-2-methyl, fibrates, fenofibrate, clofibrate, and bezafibrate.

[0067] PPAR γ agonists increase FAO in type 2 diabetic human muscle cells. PPAR γ agonists that will prevent or alleviate sepsis-mediated heart dysfunction and mortality, include those selected from the group comprising thiazolidinedione, rosiglitazone, pioglitazone, MCC-555, GL-262570, englitazone, darglitazone, isaglitazone, JTT-501, T-895645, R-119702, N,N-2344, YM-440, thiazolidinedione, GI 262570, R-483, and rivoglitazone.

[0068] Other embodiments are directed to methods for treating or preventing cardiac dysfunction in a subject having sepsis or at risk of developing sepsis, by administering therapeutically effective amounts of an agent that increases fatty acid oxidation in the heart. The above embodiments of the methods include administering the agent before cardiac function is diminished. The embodiments also comprise methods wherein the agent comprises at least two members selected from the group comprising JNK inhibitors, PPAR α agonists, PPAR γ agonists, and dual PPAR α and PPAR γ agonists. In certain embodiments the two agents comprise a JNK inhibitor and a PPAR agonist. Embodiments can also comprise methods for treating or preventing cardiac dysfunction in a subject having heart failure, by administering therapeutically effective amounts of an agent that increases fatty acid oxidation in the heart.

[0069] Certain embodiments of the invention provide pharmaceutical compositions and kits including the agents that increase fatty acid oxidation described herein. In preferred embodiments, the pharmaceutical formulations comprise therapeutically effective amounts of at least two of the following groups: (i) JNK1 or a JNK2 inhibitors including antisense nucleic acids, siRNAs, shRNAs, microRNAs (miRNA), ribozymes, microRNA mimics, supermirs, and aptamers; (ii) PPAR agonists selected from the group comprising PPAR agonists, PPAR γ agonists, dual PPAR α and PPAR γ agonists; and (iii) PPAR α -co-activator-1 (PGC-1) and (iii) estrogen-related receptor (ERR), which amounts treat or prevent cardiac dysfunction in a subject having heart failure or sepsis or at risk of developing them; or in amounts that increase cardiac function also in the patient having heart failure or sepsis or at risk of developing them. It has

been reported that MiR-520b decreases the levels of p-JNK and siMEKK2 abolishes levels of p-JNK in hepatoma cells.²¹⁶

[0070] The amount of PPAR agonist and JNK inhibitors to administer will vary as described below. The 2005 Physician's Desk Reference (PDR) describes administering an oral formulation of rosiglitazone in amounts of from about 8 mg/day to about 20 mg/day for treating diabetes (page 1442), and pioglitazone in amounts of from about 8 mg/day to about 45 mg/day (Page 3185). Pioglitazone is preferred as it has fewer deleterious effects on blood lipids.

[0071] In the experiments described herein, an injectable formulation of rosiglitazone was made that was administered intraperitoneally in amounts of 35 mg/kg, however lower doses of 8.5 mg/kg- 16.5 mg/kg were also effective. Data not shown. The efficacy of the intraperitoneally injectable form of rosiglitazone shows that it need not be administered orally to be effective. Routine experimentation will determine the optimal dose of rosiglitazone and pioglitazone, and the optimal formulation and route of administration. In certain embodiments this dose range is from about 0.1 mg/day to about 45 mg/day. Determination of the optimal doses of other PPAR agonists and JNK inhibitors can be accomplished by beginning with administering FDA approved doses and formulations for each respective agent that has been FDA-approved, or for FDA-approved agents that are similar. For example, the following agents are FDA approved: (i) Fibrates including Fenofibrate (43 mg daily FDA approved), Clofibrate (500 mg daily FDA approved), and Bezafibrate (400 mg daily FDA approved) and Gemfibrozil (600 mg daily); and (ii) Thiazolidinedione which is a generic term that includes rosiglitazone and, pioglitazone (15 mg, FDA approved).

[0072] Treatment of acute cardiac function associated with sepsis is short term, therefore drugs that have long term toxic side effects can be used at the physician's discretion. The progress of this therapy is easily monitored by conventional techniques and assays that may be used to adjust dosage to achieve a desired therapeutic effect.

[0073] To summarize, cardiac function can be increased in a subject, and sepsis-mediated heart dysfunction can be prevented or treated by stimulation of FA oxidation in cardiomyocytes: (i) via inhibition of JNK by JNK inhibitors (*e.g.*, SP600125), or antisense nucleic acids, siRNAs, micro RNAs (miRNA), short hairpin RNAs (shRNA), ribozymes, microRNA mimics, supermirs, and

aptamers, that specifically hybridize to JNK thereby reducing its expression; (ii) via activation of PPAR γ using agonists such as rosiglitazone and dual PPAR α / PPAR γ agonists; (iii) via activation of PPAR α using agonists (alpha agonists and dual PPAR α / PPAR γ); (iv) via activation of both PPAR α / PPAR γ with agonists; and (v) via PPAR α -coactivator-1 (PGC-1) and/or estrogen related receptor (ERR) α , to activate PPAR α . The agents are preferably administered as soon as possible after a subject is identified as being at risk of developing sepsis or as having sepsis, preferably before any cardiac function has been lost or diminished.

Overview

[0074] It is well established that nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B)¹⁴ and c-Jun N-terminal kinases (JNK)^{15, 16} are targets of LPS stimulus, and they are both inducers of production of inflammatory response-related cytokines, such as TNF α and interleukin IL-1 and IL-8. However, anti-inflammatory driven therapies, such as administration of corticosteroids,¹⁷⁻¹⁹ IL-1 receptor antagonists,^{20, 21} or anti-TNF α ²² have not improved patients' mortality. Therefore, besides inflammation, sepsis-mediated effects must also involve other pathophysiologic mechanisms.

[0075] Studies involving LPS administration have resulted in the conclusion that LPS administration leads to profound changes in cardiac energy balance with a dramatic reduction in fatty acid oxidation.²³⁻²⁵ Hearts rely mostly on fatty acid catabolism for their energy needs.³² In fact, approximately 70% of the produced energy results from fatty acid oxidation. In pathologic conditions that are characterized by reduced cardiac work such as heart failure, it has been documented that fatty acid oxidation is reduced^{33,34} and glucose oxidation is increased. In contrast, LPS-mediated reduction in cardiac fatty acid oxidation is not compensated by increased glucose catabolism.³⁵ Thus, sepsis is accompanied by cardiac energy production deficiency.

[0076] Fatty acid oxidation involves peroxisomes and mitochondria, and it is regulated by several enzymes and transcription factors. Nuclear receptors, particularly PPARs, have a major role in the control of fatty acid oxidation. Different PPARs have different tissue distribution and modulate different physiological functions. The PPARs play a key role in various aspects of the regulation of a large number of genes, the products of which genes are directly or indirectly crucially involved in lipid and carbohydrate metabolism.

[0077] PPARs have attracted considerable scientific attention in the last few years in part because of their emergence as the molecular target of a new group of Type II (non-insulin dependent) diabetes (NIDDM) medicines, the glitazones. PPARs are nuclear transcription factors that can be activated by ligands and belong to the class of nuclear hormone receptors. Three PPAR isoforms exist: (i) PPAR α , (ii) PPAR γ , and (iii) PPAR δ (identical to PPAR β), and are encoded by different genes.¹⁹⁶ In humans, PPAR γ exists in three variants, PPAR γ_1 , PPAR γ_2 , and PPAR γ_3 , which are the result of alternative use of promoters and differential mRNA splicing.

[0078] A marked reduction of cardiac PPAR α accompanies LPS administration.^{28, 43} Recent studies²⁰⁰ showed that JNK inhibits PPAR α gene expression. PPAR α -mediated fatty acid oxidation in the heart and other tissues relies on the activation of peroxisomal and mitochondrial enzymes such as, acyl-CoA oxidase (AOX) and carnitine palmitoyl-transferase I (CptI). A major co-activator of PPAR α -mediated fatty acid oxidation, at the transcriptional level, is the PPAR γ -coactivator-1 (PGC-1).⁴⁴ Many different factors that are known to increase PPAR α expression, include glucocorticoids⁴⁵, farnesoid X receptor (FXR)⁴⁶, AMP-activated protein kinase (AMPK)⁴⁷⁻⁴⁹, estrogen related receptor (ERR) α ⁵⁰, retinoic acid⁵¹, retinoid X receptor (R α R)⁵², phorbol-12-myristate-13-acetate⁵³, exercise training⁵⁴, heat shock factor-1⁵⁵ and others.

[0079] PPAR α expression is downregulated by other factors including heart failure⁵⁶, myocardial infarction,⁵⁷ hypoxia,^{58, 59} IL-1 β ,⁶⁰ IL-6,⁶⁰ PPAR δ ,^{61, 62} NF- κ B,⁶³ glucose,^{64, 65} insulin,⁶⁶ Akt,⁶⁷ c-Myc,⁶⁸ the Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway,⁶⁹ reactive oxygen species,⁶³ growth hormone,⁷⁰ androgens,⁷¹ and angiotensin II.⁷² PPAR α gene expression levels and subsequent fatty acid oxidation are upregulated by estrogen related receptor (ERR) α , which acts in conjunction with PGC-1 α and binds directly to the PPAR α promoter.⁵⁰ In addition, ERR α gene expression is induced by PGC-1 α ^{50, 73} at the transcriptional level indicating a positive feedback loop in the coordination of PGC-1 α and ERR α towards increase of the PPAR α gene expression.

[0080] PPAR γ , besides its well-known role as a major regulator of lipogenesis,^{39, 40} also contributes to fatty acid oxidation in cardiac⁴¹ and skeletal⁴² muscle. Mice with constitutive PPAR γ expression specifically in the hearts of PPAR α -/- mice (α MHC-PPAR γ /PPAR α ^{-/-}) have elevated fatty acid oxidation levels, as well as improved cardiac function and survival as compared to the cardiolipectoxic α MHC-PPAR γ mice.⁴¹ These observations indicated that an

overexpression of PPAR γ can substitute for PPAR α . PPAR γ agonists have also been shown to increase fatty acid oxidation in type 2 diabetic human muscle cells.⁴² Thus, one method to overcome a marked reduction in PPAR α might be via overexpression of other members of the PPAR gene family.

[0081] In both PPAR α -dependent and -independent mechanisms, PGC-1 is an important component for fatty acid oxidation. PGC-1 is a coactivator of several cellular energy metabolism-related transcription factors such as PPARs, estrogen related receptors (ERR), LXR, thyroid hormone receptor, retinoid receptors, glucocorticoid receptor, estrogen receptor and others.¹²¹ PGC-1 α expression is downregulated by LPS and the JNK signaling pathway.¹²²

Summary of Results

[0082] The results herein show that certain agents that increase fatty acid oxidation in the heart affected by sepsis ultimately increase or maintain cardiac function with the above-described therapeutic implications. Sepsis-mediated heart dysfunction can be prevented and treated by stimulation of FAO in cardiomyocytes: (i) via inhibition of JNK by JNK inhibitors (*e.g.*, SP600125), (Examples 4 and 9) or antisense nucleic acids, siRNAs, micro RNAs (miRNA), short hairpin RNAs (shRNA), ribozymes, microRNA mimics, supermirs, and aptamers, that specifically hybridize to JNK thereby reducing its expression; (ii) via activation of PPAR γ using agonists such as rosiglitazone and dual PPAR α / PPAR γ agonists; (Examples 5 and 10-12) (iii) via activation of PPAR α using agonists (alpha agonists and dual PPAR α / PPAR γ) (Example 8); (iv) via activation of both PPAR α / PPAR γ with agonists; and (v) via PPAR α -coactivator-1 (PGC-1) and/or estrogen related receptor (ERR) α , to activate PPAR α . Details are set forth in the Examples.

Therapeutic Oligonucleotides

[0083] Based on these known sequences of the targeted miRs or mRNA and the genes encoding them, therapeutic oligonucleotides can be engineered using methods known in the art. These oligonucleotides include antisense DNA or RNA (or chimeras thereof), small interfering RNA (siRNA), micro RNA (miRNA), short hairpin RNA, ribozymes, microRNA mimic, supermir, and aptamers. Different combinations of these therapeutic agents can be formulated for administration to a subject using methods well known in the art.

[0084] The siRNA Oblimersen (Genasense^R) has been given to patients for up to six cycles of 7 days at a 3 mg/kg/day dose with no severe adverse effects. Oligonucleotides are relatively safe, and have been administered at doses of up to 15 mg/kg to non-human primates. Webb MS, *et al. Antisense Nucleic Acid Drug Dev.* 2001;11:155; O'Brien S, *et al. J. Clin. Oncol.* 2007; 25: 1114. Therapeutic oligonucleotides have been administered in amounts ranging from about 0.1 mg/kg to about 50 mg/kg, and can be delivered for example, intravenously. MOLECULAR THERAPY Vol. 13, No. 4, April 2006. Administration of the therapeutic agents or compositions of this invention, may be accomplished using any of the conventionally accepted modes of administration, and doses vary based on the severity and type of disorder, and on the patient, doses may be on the lower or higher end of the spectrum.

[0085] The nucleic acid sequences of the human JNK suitable for targeting are in the public domain are:

Homo sapiens JNK1 NCBI Reference Sequence: NG_029053.1.

Homo sapiens JNK2 NCBI Reference Sequence: NG_029059.1

Homo sapiens JNK3 NCBI Reference Sequence: NG_013325.1.

[0086] Since JNK3 is expressed mostly in neural tissues, it is not considered a major target in the heart.

[0087] The mRNA and gene sequences encoding JNK are set forth by accession numbers.

[0088] For JNK1:

JNK1 GenBank: L26318.1 (Human protein kinase (JNK1) mRNA), mRNA: BC144063 (*Homo sapiens* mitogen-activated protein kinase 8, mRNA), Gene map locus: 10q11.2, JNK1 α 1: NP_002741 (mitogen-activated protein kinase 8 isoform JNK1 α 1 [*Homo sapiens*]), JNK1 α 2: NP_620637 (mitogen-activated protein kinase 8 isoform JNK1 α 2 [*Homo sapiens*]), JNK1 β 1: NP_620634, JNK1 β 2: NP_620635.

[0089] For JNK2:

JNK2 GenBank: U09759.1, mRNA: U09759.1, Gene map locus: 5q35, JNK2 α 1: NP_620707, JNK2 α 2: NP_002743, JNK2 β 1: NP_620708, JNK2 β 2: NP_620709, JNK2 γ : NP_001128516. JNK3 GenBank: BC035057.1 (*Homo sapiens* mitogen-activated protein kinase 10, mRNA), mRNA: BC022492 (*Homo sapiens* mitogen-activated protein kinase 10, mRNA), Gene map locus: 4q21.3.

[0090] For JNK3:

JNK3 α 1: AAC50605 (JNK3 α 1 protein kinase [*Homo sapiens*]), and JNK3 α 2: AAC50604.

[0091] The nucleotide sequences of mouse c-Jun N-terminal kinase JNK1, -2, and -3 cDNAs have been deposited in DDBJ/EMBL/GenBank with accession nos. AB005663, AB005664, and AB005665, respectively. It has been reported that MiR-520b decreases the levels of p-JNK in human hepatoma cells.²¹⁶ Therefore the same MiR can be used to inhibit JNK in embodiments of the present invention.

[0092] The oligonucleotides that may be used as agents herein are synthesized *in vitro* and do not include compositions of biological origin. Based on these known sequences of the targeted miRs or mRNA and the genes encoding them, therapeutic oligonucleotides can be engineered using methods known in the art. These oligonucleotides include antisense DNA or RNA (or chimeras thereof), small interfering RNAs (siRNA), micro RNAs (miRNA), short hairpin RNAs, ribozymes, microRNA mimics, supermirs, and aptamers. Different combinations of these therapeutic agents can be formulated for administration to a subject using methods well known in the art. Certain embodiments of the present invention involve the therapeutic use of antisense nucleic acids or inhibitory RNAs such as small interfering RNA (siRNA) or short hairpin RNAs (shRNA) to reduce or inhibit expression and hence the biological activity of the certain targeted miRs or mRNA.

[0093] Therapeutic nucleic acids include, *e.g.*, small interfering RNA (siRNA), shRNA, micro RNA (miRNA), antisense oligonucleotides, ribozymes, microRNA mimics, supermirs, and aptamers. These nucleic acids act via a variety of mechanisms. siRNA or miRNA can down-regulate intracellular levels of specific proteins through a process termed RNA interference (RNAi). Following introduction of siRNA or miRNA into the cell cytoplasm, these double-stranded RNA constructs can bind to a protein termed RISC. RNA-Induced Silencing Complex, or RISC, is a multiprotein complex that incorporates one strand of a small interfering RNA (siRNA) or micro RNA (miRNA). RISC uses the siRNA or miRNA as a template for recognizing complementary mRNA. When it finds a complementary strand, it activates RNase and cleaves the RNA. This process is important both in gene regulation by microRNAs and in defense against viral infections, which often use double-stranded RNA as an infectious vector. RNAi can provide down-regulation of specific proteins by targeting specific destruction of the corresponding mRNA that encodes for protein synthesis.

[0094] The therapeutic applications of RNAi are extremely broad, since siRNA and miRNA constructs can be synthesized with any nucleotide sequence directed against a gene or mRNA encoding a target protein. To date, siRNA constructs have shown the ability to specifically down-regulate target proteins in both *in vitro* and *in vivo* models and they are currently being evaluated in clinical studies.

[0095] Antisense oligonucleotides and ribozymes can also inhibit mRNA translation into protein. In the case of antisense constructs, these single stranded deoxynucleic acids have a complementary sequence to that of the target protein mRNA and can bind to the mRNA by Watson-Crick base pairing. This binding either prevents translation of the target mRNA and/or triggers RNase H degradation of the mRNA transcripts. Consequently, antisense oligonucleotides have tremendous potential for specificity of action (*i.e.*, down-regulation of a specific disease-related protein). To date, these compounds have shown promise in several *in vitro* and *in vivo* models, including models of inflammatory disease, cancer, and HIV (reviewed in Agrawal, Trends in Biotech. 14:376-387 (1996)). Antisense can also affect cellular activity by hybridizing specifically with chromosomal DNA. Advanced human clinical assessments of several antisense drugs are currently underway.

[0096] It is desirable to optimize the stability of the phosphodiester internucleotide linkage and minimize its susceptibility to exonucleases and endonucleases in serum. (Zelphati, O., *et al.*, Antisense. Res. Dev. 3:323-338 (1993); and Thierry, A. R., *et al.*, pp 147-161 in Gene Regulation: Biology of Antisense RNA and DNA (Eds. Erickson, R P and Izant, J G; Raven Press, NY (1992)).

[0097] Therapeutic nucleic acids being currently being developed do not employ the basic phosphodiester chemistry found in natural nucleic acids, because of these and other known problems. Modifications have been made at the internucleotide phosphodiester bridge (*e.g.*, using phosphorothioate, methylphosphonate or phosphoramidate linkages), at the nucleotide base (*e.g.*, 5-propynyl-pyrimidines), or at the sugar (*e.g.*, 2'-modified sugars) (Uhlmann E., *et al.* Antisense: Chemical Modifications. Encyclopedia of Cancer, Vol. X., pp 64-81 Academic Press Inc. (1997)). Others have attempted to improve stability using 2'-5' sugar linkages (*see, e.g.*, U.S. Pat. No. 5,532,130).

[0098] Small interfering RNA (siRNA) has essentially replaced antisense ODN and ribozymes as the next generation of targeted oligonucleotide drugs under development. SiRNAs are RNA duplexes normally 16-30 nucleotides long that can associate with a cytoplasmic multi-protein complex known as RNAi-induced silencing complex (RISC). RISC loaded with siRNA mediates the degradation of homologous mRNA transcripts; therefore siRNA can be designed to knock down protein expression with high specificity. Unlike other antisense technologies, siRNA function through a natural mechanism evolved to control gene expression through non-coding RNA. This is generally considered to be the reason why their activity is more potent *in vitro* and *in vivo* than either antisense ODN or ribozymes. A variety of RNAi reagents, including siRNAs targeting clinically relevant targets, are currently under pharmaceutical development, as described, *e.g.*, in de Fougères, A. *et al.*, Nature Reviews 6:443-453 (2007).

[0099] While the first described RNAi molecules were RNA:RNA hybrids comprising both an RNA sense and an RNA antisense strand, it has now been demonstrated that DNA sense:RNA antisense hybrids, RNA sense:DNA antisense hybrids, and DNA:DNA hybrids are capable of mediating RNAi (Lamberton, J. S, and Christian, A. T., (2003) Molecular Biotechnology 24:111-119). Thus, the invention includes the use of RNAi molecules comprising any of these different types of double-stranded molecules. In addition, it is understood that RNAi molecules may be used and introduced to cells in a variety of forms. Accordingly, as used herein, RNAi molecules encompasses any and all molecules capable of inducing an RNAi response in cells, including, but not limited to, double-stranded oligonucleotides comprising two separate strands, *i.e.* a sense strand and an antisense strand, *e.g.*, small interfering RNA (siRNA); double-stranded oligonucleotide comprising two separate strands that are linked together by non-nucleotidyl linker; oligonucleotides comprising a hairpin loop of complementary sequences, which forms a double-stranded region, *e.g.*, shRNAi molecules, and expression vectors that express one or more polynucleotides capable of forming a double-stranded polynucleotide alone or in combination with another polynucleotide.

[0100] A "single strand siRNA compound" as used herein, is a siRNA compound which is made up of a single molecule. It may include a duplexed region, formed by intra-strand pairing, *e.g.*, it may be, or include, a hairpin or pan-handle structure. Single strand siRNA compounds may be antisense with regard to the target molecule.

[0101] A single strand siRNA compound may be sufficiently long that it can enter the RISC and participate in RISC mediated cleavage of a target mRNA. A single strand siRNA compound is typically at least 14, and in other embodiments at least 15, 20, 25, 29, 35, 40, or 50 nucleotides in length. In certain embodiments, it is less than 200, 100, or 60 nucleotides in length.

[0102] Hairpin siRNA compounds will have a duplex region equal to or at least 17, 18, 19, 29, 21, 22, 23, 24, or 25 nucleotide pairs. The duplex region will may be equal to or less than 200, 100, or 50, in length. In certain embodiments, ranges for the duplex region are 15-30, 17 to 23, 19 to 23, and 19 to 21 nucleotides pairs in length. The hairpin may have a single strand overhang or terminal unpaired region. In certain embodiments, the overhangs are 2-3 nucleotides in length. In some embodiments, the overhang is at the sense side of the hairpin and in some embodiments on the antisense side of the hairpin.

[0103] A "double stranded siRNA compound" as used herein, is a siRNA compound which includes more than one, and in some cases two, strands in which interchain hybridization can form a region of duplex structure.

[0104] The antisense strand of a double stranded siRNA compound may be equal to or at least, 14, 15, 16 17, 18, 19, 25, 29, 40, or 60 nucleotides in length. It may be equal to or less than 200, 100, or 50, nucleotides in length. Ranges may be 17 to 25, 19 to 23, and 19 to 21 nucleotides in length. As used herein, term "antisense strand" means the strand of a siRNA compound that is sufficiently complementary to a target molecule, *e.g.* a target RNA.

[0105] The sense strand of a double stranded siRNA compound may be equal to or at least 14, 15, 16 17, 18, 19, 25, 29, 40, or 60 nucleotides in length. It may be equal to or less than 200, 100, or 50, nucleotides in length. Ranges may be 17 to 25, 19 to 23, and 19 to 21 nucleotides in length.

[0106] The double strand portion of a double stranded siRNA compound may be equal to or at least, 14, 15, 16 17, 18, 19, 20, 21, 22, 23, 24, 25, 29, 40, or 60 nucleotide pairs in length. It may be equal to or less than 200, 100, or 50, nucleotides pairs in length. Ranges may be 15-30, 17 to 23, 19 to 23, and 19 to 21 nucleotides pairs in length.

[0107] In many embodiments, the siRNA compound is sufficiently large that it can be cleaved by an endogenous molecule, *e.g.*, by Dicer, to produce smaller siRNA compounds, *e.g.*, siRNAs agents.

[0108] The sense and antisense strands may be chosen such that the double-stranded siRNA compound includes a single strand or unpaired region at one or both ends of the molecule. Thus, a double-stranded siRNA compound may contain sense and antisense strands, paired to contain an overhang, *e.g.*, one or two 5' or 3' overhangs, or a 3' overhang of 1-3 nucleotides. The overhangs can be the result of one strand being longer than the other, or the result of two strands of the same length being staggered. Some embodiments will have at least one 3' overhang. In one embodiment, both ends of a siRNA molecule will have a 3' overhang. In some embodiments, the overhang is 2 nucleotides.

[0109] In certain embodiments, the length for the duplexed region is between 15 and 30, or 18, 19, 20, 21, 22, and 23 nucleotides in length, *e.g.*, in the ssiRNA compound range discussed above. ssiRNA compounds can resemble in length and structure the natural Dicer processed products from long dsRNAs. Embodiments in which the two strands of the ssiRNA compound are linked, *e.g.*, covalently linked are also included. Hairpin, or other single strand structures which provide the required double stranded region, and a 3' overhang are also within the invention.

[0110] The siRNA compounds described herein, including double-stranded siRNA compounds and single-stranded siRNA compounds can mediate silencing of a target RNA, *e.g.*, mRNA, *e.g.*, an mRNA transcript of a gene that encodes a protein. A gene may also be targeted. In general, the RNA to be silenced is an endogenous gene or a pathogen gene. In addition, RNAs other than mRNA, *e.g.*, tRNAs, and viral RNAs, can also be targeted.

[0111] As used herein, the phrase "mediates RNAi" refers to the ability to silence, in a sequence specific manner, a target RNA. While not wishing to be bound by theory, it is believed that silencing uses the RNAi machinery or process and a guide RNA, *e.g.*, an ssiRNA compound of 21 to 23 nucleotides.

[0112] In one embodiment, a siRNA compound is "sufficiently complementary" to a target RNA, *e.g.*, a target mRNA, such that the siRNA compound silences production of protein encoded by the target mRNA. In another embodiment, the siRNA compound is "exactly complementary" to a target RNA, *e.g.*, the target RNA and the siRNA compound anneal, for example to form a hybrid made exclusively of Watson-Crick base pairs in the region of exact complementarity. A "sufficiently complementary" target RNA can include an internal region (*e.g.*, of at least 10 nucleotides) that is exactly complementary to a target RNA. Moreover, in certain embodiments, the siRNA compound specifically discriminates a single-nucleotide difference. In this case, the siRNA compound only mediates RNAi if exact complementarity is found in the region (*e.g.*, within 7 nucleotides of) the single-nucleotide difference.

Micro RNAs (miRNAs)

[0113] "MicroRNAs" (miRNAs or miRs) have been implicated in a number of biological processes including regulation of developmental timing, apoptosis, fat metabolism, and hematopoietic cell differentiation among others. In certain embodiments, miRNAs are small, non-protein coding RNAs of about 18 to about 25 nucleotides in length that regulate gene expression in a sequence-specific manner. miRNAs act as repressors of target miRNAs by promoting their degradation, when their sequences are perfectly complementary, or by inhibiting translation, when their sequences contain mismatches. miRNAs are transcribed by RNA polymerase II (pol II) or RNA polymerase III (pol III; see Qi et al. (2006) Cellular & Molecular Immunology Vol. 3:411-419) and arise from initial transcripts, termed primary miRNA transcripts (pri-miRNAs), that are generally several thousand bases long and are derived from individual miRNA genes, from introns of protein coding genes, or from poly-cistronic transcripts that often encode multiple, closely related miRNAs. *See* review of Carrington et al. (2003). Pri-miRNAs are processed in the nucleus by the RNase Drosha into about 70- to about 100-nucleotide hairpin-shaped precursors (pre-miRNAs). Following transport to the cytoplasm, the hairpin pre-miRNA is further processed by Dicer to produce a double-stranded miRNA (Lee et al., 1993). The mature miRNA strand is then incorporated into the RNA-induced silencing complex (RISC), where it associates with its target mRNAs by base-pair complementarity. In the relatively rare cases in which a miRNA base pairs perfectly with an mRNA target, it promotes

mRNA degradation. More commonly, miRNAs form imperfect heteroduplexes with target mRNAs, affecting either mRNA stability or inhibiting mRNA translation.

Short Hairpin RNAs (shRNAs)

[0114] In certain embodiments of the invention, a small hairpin RNA or short hairpin RNA (shRNA) is a sequence of RNA that makes a tight hairpin turn that can be used to silence gene expression via RNA interference. shRNA is transcribed by RNA polymerase III. shRNA production in a mammalian cell can sometimes cause the cell to mount an interferon response as the cell seeks to defend itself from what it perceives as viral attack.

Ribozymes

[0115] According to another embodiment of the invention, targeted mRNA is inhibited by ribozymes, which have specific catalytic domains that possess endonuclease activity (Kim and Cech, *Proc Natl Acad Sci USA*. 1987 December; 84(24):8788-92; Forster and Symons, *Cell*. 1987 Apr. 24; 49(2):211-20). For example, a large number of ribozymes accelerate phosphoester transfer reactions with a high degree of specificity, often cleaving only one of several phosphoesters in an oligonucleotide substrate (Cech *et al.*, *Cell*. 1981 December; 27(3 Pt 2):487-96; Michel and Westhof, *J Mol. Biol.* 1990 Dec. 5; 216(3):585-610; Reinhold-Hurek and Shub, *Nature*. 1992 May 14; 357(6374):173-6). This specificity has been attributed to the requirement that the substrate bind via specific base-pairing interactions to the internal guide sequence ("IGS") of the ribozyme prior to chemical reaction.

[0116] At least six basic varieties of naturally-occurring enzymatic RNAs are known presently. Each can catalyze the hydrolysis of RNA phosphodiester bonds in trans (and thus can cleave other RNA molecules) under physiological conditions. In general, enzymatic nucleic acids act by first binding to a target RNA. Such binding occurs through the target binding portion of an enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through complementary base-pairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a target RNA will destroy its ability to direct synthesis of an encoded protein. After an enzymatic nucleic acid has bound and

cleaved its RNA target, it is released from that RNA to search for another target and can repeatedly bind and cleave new targets.

[0117] The enzymatic nucleic acid molecule may be formed in a hammerhead, hairpin, a hepatitis delta virus, group I intron or RNaseP RNA (in association with an RNA guide sequence) or Neurospora VS RNA motif, for example. Specific examples of hammerhead motifs are described by Rossi *et al.* Nucleic Acids Res. 1992 Sep. 11; 20(17):4559-65. Examples of hairpin motifs are described by Hampel *et al.* (Eur. Pat. Appl. Publ. No. EP 0360257), Hampel and Tritz, Biochemistry 1989 Jun. 13; 28(12):4929-33; Hampel *et al.*, Nucleic Acids Res. 1990 Jan. 25; 18(2):299-304 and U.S. Pat. No. 5,631,359. An example of the hepatitis virus motif is described by Perrotta and Been, Biochemistry. 1992 Dec. 1; 31(47):11843-52; an example of the RNaseP motif is described by Guerrier-Takada *et al.*, Cell. 1983 December; 35(3 Pt 2):849-57; Neurospora VS RNA ribozyme motif is described by Collins (Saville and Collins, Cell. 1990 May 18; 61(4):685-96; Saville and Collins, Proc Natl Acad Sci USA. 1991 Oct. 1; 88(19):8826-30; Collins and Olive, Biochemistry. 1993 Mar. 23; 32(11):2795-9); and an example of the Group I intron is described in U.S. Pat. No. 4,987,071. Ribozyme constructs need not be limited to specific motifs mentioned herein.

[0118] Methods of producing a ribozyme targeted to any polynucleotide sequence are known in the art. Ribozymes may be designed as described in Int. Pat. Appl. Publ. No. WO 93/23569 and Int. Pat. Appl. Publ. No. WO 94/02595, and synthesized to be tested *in vitro* and *in vivo*, as described therein.

[0119] Ribozyme activity can be optimized by altering the length of the ribozyme binding arms or chemically synthesizing ribozymes with modifications that prevent their degradation by serum ribonucleases (see *e.g.*, Int. Pat. Appl. Publ. No. WO 92/07065; Int. Pat. Appl. Publ. No. WO 93/15187; Int. Pat. Appl. Publ. No. WO 91/03162; Eur. Pat. Appl. Publ. No. 92110298.4; U.S. Pat. No. 5,334,711; and Int. Pat. Appl. Publ. No. WO 94/13688, which describe various chemical modifications that can be made to the sugar moieties of enzymatic RNA molecules), modifications which enhance their efficacy in cells, and removal of stem II bases to shorten RNA synthesis times and reduce chemical requirements.

Supermirs

[0120] A supermir refers to a single stranded, double stranded or partially double stranded oligomer or polymer of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) or both or modifications thereof, which has a nucleotide sequence that is substantially identical to an miRNA and that is antisense with respect to its target. This term includes oligonucleotides composed of naturally-occurring nucleobases, sugars and covalent internucleoside (backbone) linkages and which contain at least one non-naturally-occurring portion which functions similarly. Such modified or substituted oligonucleotides are preferred over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for nucleic acid target and increased stability in the presence of nucleases. In a preferred embodiment, the supermir does not include a sense strand, and in another preferred embodiment, the supermir does not self-hybridize to a significant extent. A supermir can have secondary structure, but it is substantially single-stranded under physiological conditions. A supermir that is substantially single-stranded is single-stranded to the extent that less than about 50% (*e.g.*, less than about 40%, 30%, 20%, 10%, or 5%) of the supermir is duplexed with itself. The supermir can include a hairpin segment, *e.g.*, sequence, preferably at the 3' end can self-hybridize and form a duplex region, *e.g.*, a duplex region of at least 1, 2, 3, or 4 and preferably less than 8, 7, 6, or *n* nucleotides, *e.g.*, 5 nucleotides. The duplexed region can be connected by a linker, *e.g.*, a nucleotide linker, *e.g.*, 3, 4, 5, or 6 dTs, *e.g.*, modified dTs. The supermir is duplexed with a shorter oligo, *e.g.*, of 5, 6, 7, 8, 9, or 10 nucleotides in length, *e.g.*, at one or both of the 3' and 5' end or at one end and in the non-terminal or middle of the supermir.

Aptamers

[0121] Aptamers are nucleic acid or peptide molecules that bind to a particular molecule of interest with high affinity and specificity (Tuerk and Gold, *Science* 249:505 (1990); Ellington and Szostak, *Nature* 346:818 (1990)). DNA or RNA aptamers have been successfully produced which bind many different entities from large proteins to small organic molecules. *See* Eaton, *Curr. Opin. Chem. Biol.* 1:10-16 (1997), Famulok, *Curr. Opin. Struct. Biol.* 9:324-9 (1999), and Hermann and Patel, *Science* 287:820-5 (2000). Aptamers may be RNA or DNA based, and may include a riboswitch. Regulatory elements are known as riboswitches and are defined as mRNA elements that bind metabolites or metal ions as ligands and regulate mRNA expression by

forming alternative structures in response to this ligand binding (Figure 1; Nudler & Mironov 2004; Tucker & Breaker 2005; Winkler 2005). Although they can bind proteins like antibodies, aptamers are not immunogenic, even at doses up to 1000 times the therapeutic dose in primates.

[0122] A riboswitch is a part of an mRNA molecule that can directly bind a small target molecule, and whose binding of the target enables it to regulate its own activity, depending on the presence or absence of its target molecule. Riboswitches are most often located in the 5' untranslated region (5' UTR; a stretch of RNA that precedes the translation start site) of bacterial mRNA. There they regulate the occlusion of signals for transcription attenuation or translation initiation. Edwards, A. L. *et al.*, (2010) Riboswitches: A Common RNA Regulatory Element. *Nature Education* 3(9):9.

[0123] Generally, aptamers are engineered through repeated rounds of *in vitro* selection or equivalently, SELEX (systematic evolution of ligands by exponential enrichment) to bind to various molecular targets such as small molecules, proteins, nucleic acids, and even cells, tissues and organisms. The aptamer may be prepared by any known method, including synthetic, recombinant, and purification methods, and may be used alone or in combination with other aptamers specific for the same target. Further, as described more fully herein, the term "aptamer" specifically includes "secondary aptamers" containing a consensus sequence derived from comparing two or more known aptamers to a given target.

miRNA Mimics

[0124] miRNA mimics represent a class of molecules that can be used to imitate the gene silencing ability of one or more miRNAs. In the embodiments of the present invention, miRNA mimics are immunostimulatory agents. Thus, the term "microRNA mimic" refers to synthetic non-coding RNAs (*i.e.* the miRNA is not obtained by purification from a source of the endogenous miRNA) that are capable of entering the RNAi pathway and regulating gene expression through inhibiting targeted mRNA. miRNA mimics can be designed as mature molecules (*e.g.* single stranded) or mimic precursors (*e.g.*, pri- or pre-miRNAs) miRNA mimics can be comprised of nucleic acid (modified or modified nucleic acids) including oligonucleotides comprising, without limitation, RNA, modified RNA, DNA, modified DNA, locked nucleic

acids, or 2'-O,4'-C-ethylene-bridged nucleic acids (ENA), or any combination of the above (including DNA-RNA hybrids). In addition, miRNA mimics can comprise conjugates that can affect delivery, intracellular compartmentalization, stability, specificity, functionality, strand usage, and/or potency. In one design, miRNA mimics are double stranded molecules (*e.g.*, with a duplex region of between about 16 and about 31 nucleotides in length) and contain one or more sequences that have identity with the mature strand of a given miRNA. Modifications can comprise 2' modifications (including 2'-O methyl modifications and 2' F modifications) on one or both strands of the molecule and internucleotide modifications (*e.g.* phosphorothioate modifications) that enhance nucleic acid stability and/or specificity. In addition, miRNA mimics can include overhangs. The overhangs can consist of 1-6 nucleotides on either the 3' or 5' end of either strand and can be modified to enhance stability or functionality. In one embodiment, a miRNA mimic comprises a duplex region of between 16 and 31 nucleotides and one or more of the following chemical modification patterns: the sense strand contains 2'-O-methyl modifications of nucleotides 1 and 2 (counting from the 5' end of the sense oligonucleotide), and all of the Cs and Us; the antisense strand modifications can comprise 2' F modification of all of the Cs and Us, phosphorylation of the 5' end of the oligonucleotide, and stabilized internucleotide linkages associated with a 2 nucleotide 3' overhang.

Pharmaceutical Formulations and Administration

[0125] The present invention also includes pharmaceutical compositions and formulations of JNK1 and JNK2 inhibitors and of the PPAR agonists, PPAR γ agonists, dual PPAR α and PPAR γ agonists, or combinations thereof, JNK inhibitors, or antisense nucleic acids, siRNAs, shRNAs, microRNAs (miRNA), ribozymes, microRNA mimics, supermirs, and aptamers that increase PPAR α , PPAR α -co-activator-1 (PGC-1), estrogen-related receptor (ERR) α , or a combination thereof and other therapeutic agents for treating or preventing cardiac dysfunction, hereafter "the therapeutic agents." Preferred are pharmaceutical compositions comprising therapeutically effective amounts of one member from at least two of the following groups: (i) JNK1 or a JNK2 inhibitors including antisense nucleic acids, siRNAs, shRNAs, microRNAs (miRNA), ribozymes, microRNA mimics, supermirs, and aptamers; (ii) a PPAR agonists selected from the group comprising PPAR agonists, PPAR γ agonists, dual PPAR α and PPAR γ agonists; and (iii) PPAR α -co-activator-1 (PGC-1) and (iii) estrogen-related receptor (ERR), which amounts treat or prevent cardiac dysfunction in a subject

having heart failure or sepsis or at risk of developing them; or in amounts that increase cardiac function also in the patient having heart failure or sepsis or at risk of developing them.

Pharmaceutical compositions for use in the present methods include therapeutically effective amounts of one or more of the therapeutic agents, i.e., an amount sufficient to prevent or treat the diseases described herein in a subject, formulated for local or systemic administration. The subject is preferably a human but can be non-human as well. A suitable subject can be an individual who is suspected of having, has been diagnosed as having, or is at risk of developing one of the described diseases, including sepsis-associated cardiac dysfunction associated or other forms of heart failure.

[0126] The duration of treatment can extend over several days or longer, depending on the condition, with the treatment continuing until the symptoms of cardiac dysfunction are sufficiently reduced or eliminated. Active agents for therapeutic administration are preferably low in toxicity. Some PPAR agonists have been reported to have toxic effects when administered long term therefore in such cases, administration may be interrupted to balance treating the disease or condition with minimizing toxicity. Treatment of acute cardiac function associated with sepsis is short term, therefore drugs that have long term toxic side effects can be used at the physician's discretion. The progress of this therapy is easily monitored by conventional techniques and assays that may be used to adjust dosage to achieve a desired therapeutic effect.

[0127] A composition of the therapeutic agents can also include a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" includes solvents, dispersion media, coatings, antiviral agents, antibacterial agents, antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Supplementary active compounds can also be incorporated into the compositions. Other topical formulations are described in Sheele *et al.*, 7,151,091.

[0128] Therapeutic compositions may contain, for example, such normally employed additives as binders, fillers, carriers, preservatives, stabilizing agents, emulsifiers, buffers and excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, cellulose, magnesium carbonate, and the like. These compositions typically contain 1%-95% of active ingredient, preferably 2%-70% active ingredient.

[0129] The therapeutic agents can also be mixed with diluents or excipients which are compatible and physiologically tolerable as selected in accordance with the route of administration and standard pharmaceutical practice. Suitable diluents and excipients are, for example, water, saline, dextrose, glycerol, or the like, and combinations thereof. In addition, if desired, the compositions may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, stabilizing or pH buffering agents.

[0130] In some embodiments, the therapeutic compositions of the present invention are prepared either as liquid solutions or suspensions, or in solid forms. The formulations may include such normally employed additives such as binders, fillers, carriers, preservatives, stabilizing agents, emulsifiers, buffers and excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, cellulose, magnesium carbonate, and the like. Solutions, suspensions, or sustained release formulations typically contain 1 %-95% of active ingredient, preferably 2%-70%.

[0131] The formulations may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

[0132] Suitable examples of sustained release preparations include semipermeable matrices of solid hydrophobic polymers containing the therapeutic agents, which matrices are in the form of shaped articles, *e.g.*, films, or microcapsule. Examples of sustained release matrices include, but are not limited to, polyesters, hydro gels (for example, poly (2-hydroxyethyl-methacrylate), or poly (vinylalcohol)), polylactides, copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT (injectable micro spheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods.

[0133] The therapeutic agents of the present invention may be formulated for administration by any suitable means. For *in vivo* administration, the pharmaceutical compositions are preferably administered orally or parenterally, *i.e.*, intraarticularly, intravenously, intraperitoneally,

subcutaneously, or intramuscularly. In particular embodiments, the pharmaceutical compositions are administered intravenously or intraperitoneally by a bolus injection. Stadler, *et al.*, U.S. Pat. No. 5,286,634. For the prevention or treatment of disease, the appropriate dosage will depend on the severity of the disease, whether the drug is administered for protective or therapeutic purposes, previous therapy, the patient's clinical history and response to the drugs and the discretion of the attending physician.

[0134] The resulting pharmaceutical preparations may be sterilized by conventional, well known sterilization techniques. The aqueous solutions can then be packaged for use or filtered under aseptic conditions and lyophilized, the lyophilized preparation being combined with a sterile aqueous solution prior to administration. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, etc. Additionally, the lipidic suspension may include lipid-protective agents which protect lipids against free-radical and lipid-peroxidative damages on storage. Lipophilic free-radical quenchers, such as α -tocopherol and water-soluble iron-specific chelators, such as ferrioxamine, are suitable.

[0135] The pharmaceutical compositions of this invention may be in a variety of forms, which may be selected according to the preferred modes of administration. These include, for example, solid, semi-solid and liquid dosage forms such as tablets, pills, powders, liquid solutions or suspensions, suppositories, and injectable and infusible solutions. The preferred form depends on the intended mode of administration and therapeutic application.

[0136] The pharmaceutical compositions of this invention may, for example, be placed into sterile, isotonic formulations with or without cofactors which stimulate uptake or stability. The formulation is preferably liquid, or may be lyophilized powder. For example, the compositions of the invention may be diluted with a formulation buffer comprising 5.0 mg/ml citric acid monohydrate, 2.7 mg/ml trisodium citrate, 41 mg/ml mannitol, 1 mg/ml glycine and 1 mg/ml polysorbate 20. This solution can be lyophilized, stored under refrigeration and reconstituted prior to administration with sterile Water-For-Injection (USP).

[0137] Any of the compositions described herein may be comprised in a kit. In a non-limiting example, a PPAR α agonist is included in a kit. The kit may further include water and buffer. The kit may also include one or more transfection reagent(s) to facilitate delivery of the agonists to cells. The components of the kits may be packaged either in aqueous media or in lyophilized form. The container means of the kits will generally include at least one vial, test tube, flask, bottle, syringe or other container means, into which a component may be placed, and preferably, suitably aliquoted. Where there is more than one component in the kit (labeling reagent and label may be packaged together), the kit also will generally contain a second, third or other additional container into which the additional components may be separately placed. However, various combinations of components may be comprised in a vial. The kits of the present invention also will typically include a means for containing the agents, and any other reagent containers in close confinement for commercial sale. Such containers may include injection or blow-molded plastic containers into which the desired vials are retained.

[0138] When the components of the kit are provided in one and/or more liquid solutions, the liquid solution is an aqueous solution, with a sterile aqueous solution being particularly preferred. However, the components of the kit may be provided as dried powder(s). When reagents and/or components are provided as a dry powder, the powder can be reconstituted by the addition of a suitable solvent. It is envisioned that the solvent may also be provided in another container means.

[0139] The container means will generally include at least one vial, test tube, flask, bottle, syringe and/or other container means, into which the nucleic acid formulations are placed, preferably, suitably allocated. The kits may also comprise a second container means for containing a sterile, pharmaceutically acceptable buffer and/or other diluent.

[0140] Such kits may also include components that preserve or maintain the agonists or that protect against their degradation. Such kits generally will comprise, in suitable means, distinct containers for each individual reagent or solution. A kit will also include instructions for employing the kit components as well the use of any other reagent not included in the kit. Instructions may include variations that can be implemented. A kit may also include utensils or devices for administering the agonist by various administration routes, such as parenteral or catheter administration or coated stent.

[0141] Suitable Solvates Include Hydrates. Suitable salts include those formed with both organic and inorganic acids or bases. Pharmaceutically acceptable base salts include ammonium salts, alkali metal salts such as those of sodium and potassium, alkaline earth metal salts such as those of calcium and magnesium and salts with organic bases such as dicyclohexylamine and N-methyl-D-glucamine.

[0142] Where the PPAR- γ agonist is rosiglitazone, it may be formulated as rosiglitazone maleate. Where the PPAR- γ agonist is pioglitazone, it may be formulated as pioglitazone hydrochloride. Where the PPAR- γ agonist is farglitazar, an exemplary salt form is the sodium salt.

[0143] Formulations of use in the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

[0144] Formulations for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example saline or water-for-injection, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

[0145] Spray compositions for topical delivery to the lung by inhalation may for example be formulated as aqueous solutions or suspensions or as aerosols delivered from pressurized packs, such as a metered dose inhaler, with the use of a suitable liquefied propellant. Aerosol compositions suitable for inhalation can be either a suspension or a solution of the therapeutic and a suitable.

[0146] Medicaments for administration by inhalation desirably have a controlled particle size. The optimum particle size for inhalation into the bronchial system is usually 1-10 μm , preferably

2-5 μm . Particles having a size above 20 μm are generally too large when inhaled to reach the small airways.

[0147] It will be understood that the methods and uses of the invention may be employed prophylaxis as well as (more suitably) in the treatment of subjects suffering from sepsis or heart failure.

[0148] Therapeutic agents of the present invention may be administered simultaneously meaning the administration of medicaments such that the individual medicaments are present within a subject at the same time. In addition to the concomitant administration of medicaments (via the same or alternative routes), simultaneous administration may include the administration of the medicaments (via the same or an alternative route) at different times.

[0149] The invention has been described with reference to specific embodiments. It will, however, be evident that various modifications and changes may be made thereto without departing from the broader spirit and scope of the invention. The specification and drawings are, accordingly, to be regarded in an illustrative rather than a restrictive sense. The invention is illustrated herein by the experiments described above and by the following examples, which should not be construed as limiting. The contents of all references, pending patent applications and published patents, cited throughout this application are hereby expressly incorporated by reference. Although specific terms are employed, they are used as in the art unless otherwise indicated.

Examples

Materials and Methods

[0150] **Chemical Reagents**- All chemical reagents were obtained from SIGMA.

[0151] **Animals** - All procedures involving animals were approved by the Institutional Animal Care and Use Committee at Columbia University. Mice were maintained under appropriate barrier conditions in a 12hr light-dark cycle and received food and water *ad libitum*. The animals that were used for this study were C57BL/6 mice and mice expressing specifically in cardiomyocytes peroxisome proliferator activating receptor- γ ($\alpha\text{MHC-PPAR}\gamma$) that are also on the C57BL/6 background. All studies were performed on the different genotypes with littermates

as controls. Hearts from these mice were harvested, flash frozen and stored at -80°C until further use. All analyses involving animals were performed with at least 5 mice per experimental group.

[0152] Cells – A human ventricular cardiomyocyte-derived cell line, designated AC-16, was kindly provided by M. M. Davidson.¹²⁶ Cells were maintained in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (Ham) (DMEM:F12) (Invitrogen) supplemented with fetal bovine serum (10%) and a mixture of penicillin and streptomycin (1%). Prior to infection with recombinant adenoviruses the medium was changed to 2% heat inactivated horse serum and the cells were infected in at least duplicates with control adenovirus that expresses the Green Fluorescent Protein (Ad-GFP) or the adenovirus expressing the constitutively active form of JNK2 (Ad-JNK2 α 2) at a multiplicity of infection (MOI) of 10. Sixteen hours post-infection, cells were washed with phosphate-buffered saline, and fresh 10% fetal bovine serum containing medium was added. To assess gene expression 48 hours post incubation, cell lysates were collected and analyzed for mRNA and protein expression.

[0153] Construction of recombinant adenovirus expressing a constitutively active form of JNK2 - The pEGFP-C1-JNK2 α 2 plasmid that contained the cDNA of the constitutively active JNK2 (JNK2 α 2)¹²⁷ was kindly provided by Albert J. Wong, MD (Stanford University Medical Center, CA). The JNK2 α 2 cDNA was isolated by digestion with XhoI and BamHI and was initially cloned in the pcDNA3.1 plasmid. Double digestion with XhoI and HindIII was then applied to the pcDNA3.1-JNK2 α 2 to isolate the JNK2 α 2 cDNA and clone it in the pAdTrack-CMV plasmid. The pAdTrack-CMV-JNK2 α 2 plasmid was used to generate recombinant adenovirus as described previously.¹²⁸

[0154] LPS-mediated induction of sepsis- LPS (5mg/kg) (Sigma) was administered intraperitoneally (i.p.) in mice. Control mice were treated with equal volume of saline. Cardiac function was assessed by 2D echocardiography 5-6 hours post-LPS administration and mice were sacrificed 2-3 hours later (7-9 hours post-LPS injection).

[0155] Echocardiography - Two-dimensional echocardiography was performed on conscious 10 to 12 week-old male and female (n = 5-8 per group) mice (Sonos5500 system; Philips Medical Systems).¹²⁹ Echocardiographic images were recorded in a digital format. Images were then analyzed off-line by a single observer blinded to the murine genotype.¹³⁰

[0156] RNA purification and gene expression analysis - Total RNA was purified from cells or hearts using the TRIzol reagent according to the instructions of the manufacturer (Invitrogen). cDNA was synthesized using the SuperScript III First-Strand Synthesis SuperMix (Invitrogen). cDNA was analyzed with quantitative real-time PCR that was performed with SYBR Green PCR Core Reagents (Stratagene). Incorporation of the SYBRgreen dye into the PCR products was monitored in real time with an Mx3000 sequence detection system (Stratagene). Samples were normalized against β -actin.

[0157] Protein purification and analysis - Isolated heart tissues or cells were homogenized in PBS containing protease inhibitors and phosphatase inhibitors (Roche, Indianapolis, IN). Membrane and cytosolic fractions were separated by ultracentrifugation. 20 μ g from each fraction was applied to SDS-PAGE and transferred onto nitrocellulose membranes. Antibodies were obtained from Santa Cruz (JNK) and Cell Signaling (phospho-JNK, phospho-AMPK^{Thr 172} and total AMPK).

[0158] FA oxidation - FA oxidation was measured in pieces of hearts isolated from 10-12 week old mice. The heart pieces were incubated at 37°C for 2h in modified Krebs-Ringer buffer (MKR: 115 mM NaCl, 2.6 mM KCl, 1.2 mM KH₂PO₄, 10 mM NaHCO₃, 10 mM HEPES, pH 7.4) that contained 2% BSA, 0.2 mmol/ml palmitate and 10 μ Ci/ml 9,10(n)-[³H]-palmitate and was gassed with 95% O₂ and 5% CO₂. Water was then extracted with chloroform : methanol (2:1) extraction. Palmitate oxidation was determined by measuring the amount of ³H₂O in the aqueous phase.

[0159] Cardiac ATP measurement - Cardiac pieces of 10 mg were used to determine ATP levels. Heart pieces were dissolved in ice-cold 0.1% trichloro-acetic acid and centrifuged. Supernatant was resuspended in 50mM Tris-acetate containing 2mM EDTA, pH 7.8 and a portion was used to measure ATP. The measurement was performed with the ATP determination kit (Invitrogen) according to the instructions of the manufacturer.

[0160] Electron microscopy - Left ventricles from 3-month-old mice were fixed with 2.5% glutaraldehyde in 0.1 M Sorensen's buffer (0.2 M monobasic phosphate/0.2 M dibasic phosphate, 1:4 vol/vol; pH 7.2), postfixated in osmium tetroxide, and embedded in EPON 812 (Electron Microscopy Sciences). Ultrathin sections were stained with uranyl acetate and lead citrate and examined under a JEM-1200ExII electron microscope (JEOL).

[0161] Assessment of survival - C57BL/6 mice were challenged in groups of 10-14 with a lethal dose of LPS (15 mg/kg) by i.p. injection and were observed for survival for 72h. The effect of rosiglitazone on LPS-induced mortality was assessed by giving i.p. doses of rosiglitazone (35 mg/kg/day). Control animals received saline and DMSO instead of LPS and rosiglitazone, respectively.

[0162] Statistical analysis - Comparisons between 2 groups were performed using unpaired 2-tailed Student's *t* tests. All values are presented as mean $\pm$ SE. Differences between groups were considered statistically significant at $P < 0.05$.

[0163] PPAR agonists that can be used to prevent or treat cardiac dysfunction associated with sepsis include, but are not limited, the following:

PPAR γ agonists	PPAR α agonists	PPAR α /PPAR γ dual agonists
thiazolidinedione	Alpha WY-14643	
rosiglitazone (SKB)	GW9578	MK-767/KRP-297
pioglitazone (Takeda)	GW-590735	(Merck/Kyorin)
Mitsubishi's MCC-555 (disclosed in U.S. Pat. No. 5,594,016)	K-111	E966-E971
Glaxo-Wellcome's GL- 262570	LY-674	AZ-242
englitazone (CP-68722, Pfizer)	KRP-101	tesaglitazar; Astra-Zeneca
darglitazone (CP-86325, Pfizer)	DRF-10945	muraglitazar;
isaglitazone (MIT/J&J)	LY518674	and certain compounds described in U.S. Pat. No.
JTT-501 (JPNT/P&U)	Propanoic Acid	6,414,002.
	2-[4-[3-[2,5-dihydro-1- [(4- methylphenyl)methyl]-5- oxo-1H-1,2,4-triazol-3-- yl]propyl]phenoxy]-2-	

T-895645 (Merck)	methyl, fibrate	
R-119702 (Sankyo/WL)	fenofibrate	
N,N-2344 (Dr. Reddy/NN)	clofibrate	
YM-440 (Yamanouchi)	bezafibrat	
GI 262570		
R-483 and		
CS-011 (rivoglitazone)		

[0164] Other PPAR agonists include GW 9578NN622/ragaglitazar, BMS 298585, BRL49634, KRP-297, JTT-501, SB 213068, GW 1929, GW 7845, GW 0207, L-796449, L-165041 and GW 2433.

[0165] Heart failure that is not caused by or associated with acute sepsis can also be treated with the methods described above for increasing FAO in cardiomyocytes by administering therapeutically effective amounts of PPAR α and or PPAR γ agonists or JNK inhibition, preferably those that do not have long term adverse side effects. However agents that have unacceptably high toxicity over the long term could be used for relative brief periods to treat heart failure.

Example 1

[0166] An animal model of cardiac septic shock was reproduced by treating 10 week old C57BL6 mice with LPS (5mg/kg of body) for 6-8h. Consistently with previous studies,^{154, 165} LPS reduced fractional shortening (FIG. 3(A) and mRNA levels for cardiac PPAR α , CD36, LpL, FATP, Cpt1 β , PGC-1 α and PGC-1 β (FIG. 3(D)). These changes were associated with increased cardiac gene expression of inflammation-associated genes (IL-1 α , IL-6 and TNF α) and plasma IL-6 protein levels (FIG. 3(B)-(C)). Cardiac ATP was reduced by 44% in LPS-treated mice and FAO was reduced by 61%. Thus, the model is valid for cardiac septic shock. FIG. 2.

Example 2

[0167] The next experiment assessed whether the cardiac miR (microRNA) profiles of LPS-treated mice resemble the respective profiles in heart failure. (FIG 5(A)-(C)). MiR array analysis of cardiac RNA from LPS-treated mice showed that none of the miRs that have been associated with heart failure, [miR-1, -133, -208, -195, -24, -125b, -199a and 214¹⁴⁹⁻¹⁵¹], was affected (FIG. 5 (C)). Another indication of heart failure is the switch from the α -MHC isoform to β -MHC.^{166, 167} Cardiac mRNA analysis did not indicate such change (FIG. 6) in the model, so the mechanism of LPS-mediated reduction in FAO is distinct from that which occurs with afterload induced heart failure.

Example 3

[0168] Sepsis following Gram negative (-) bacterial infection is associated with both elevated inflammation (FIG. 1) and reduced FAO (FIG. 2). Animals that are knock-out for inflammation-related genes¹³³⁻¹³⁹ are resistant to LPS-mediated sepsis and have improved cardiac function or viability. However, anti-inflammatory drugs, although treatment of inflammation may be protective in the early stage of sepsis, has not improved mortality.¹⁴⁰⁻¹⁴⁵ LPS treatment leads to reduced expression of PPAR α and other FA metabolism related-genes¹⁴⁶⁻¹⁴⁸ (FIG. 3). LPS also suppresses glucose catabolism. FIG. 4(B) Heart failure reduces FAO and increases glucose utilization FIG. 4(A). Several miRs have been linked to heart failure¹⁴⁹⁻¹⁵¹ that are also associated with impaired FAO, however, none of these miRs are significantly altered in the hearts of LPS-treated mice. (FIG.5) Thus, mechanisms that reduce FAO exclusive of miR-induced changes are ignited by LPS treatment.

Example 4

[0169] Cardiac function in normal C57BL/6 mice was dramatically improved by administering the JNK inhibitor SP600125, which increased FAO, even though there was no decrease in inflammation. FIGs. 7-14. *In vivo* and *in vitro* experiments show that JNK-mediated activation of c-Jun is needed for cardiac PPAR α downregulation. The results in FIG. 8(A)-(E) show that constitutively active JNK2 reduced PPAR α expression *in vitro* in a human cardiomyocyte cell line. LPS did not affect cardiac function which is already reduced in JNK2^{-/-} mice. FIG. 9(A). But LPS dramatically reduced PPAR α gene expression in JNK2^{-/-} mice and in normal mice.

FIG. 9 (B)-(C). It has now been discovered that inhibiting JNK by administering JNK inhibitor SP600125 to LPS-treated septic C57BL/6 mice within 24 hours after LPS administration increased PPAR α , blocked the LPS-induced reduction of PPAR α gene expression and normalized cardiac function (measured as % of fractional shortening (FS))FIG. 11(A)-(C). That these effects were associated with increased FAO is shown by the normalization of (FIG. 12(A)) palmitate oxidation and gene expression of carnitine palmitoyl transferase type 1 alpha (CPT-1alpha) (FIG. 12(C), a key enzyme in mitochondrial fatty acid catabolism. FIG. 12 (A)-(C). This increase in FAO occurred even though inflammatory cytokine markers were elevated (FIG. 13(A)-(C)), supporting the discovery that in sepsis the inflammatory pathway and the FAO pathway that is associated with cardiac function are separate. FIG. 14.

[0170] Therefore certain embodiments of the invention are directed to a method for increasing or maintaining cardiac function in a subject who has or is at risk of developing sepsis or heart failure, by administering therapeutically effective amounts of an agent that increases fatty acid oxidation in the heart. In a preferred embodiment, the agent is a PPAR α agonist or a JNK inhibitor such as SP600125, or a combination thereof. Another embodiment is directed to treating cardiac dysfunction by administering an antisense nucleic acid, siRNA or shRNA, microRNA (miRNA), ribozyme, microRNA mimics, supermir, and aptamer that specifically hybridizes to JNK thereby reducing its expression alone or with PPAR α agonists. PPAR α agonists for use in the present invention are listed above in the Materials and Methods section.

Example 5

[0171] The LPS-mediated reduction in FAO in normal mice can be explained by a reduction of cardiac PPAR α .;^{148, 154} however, PPAR γ levels increased by ~60%. The heart expresses all three members of the PPAR family of nuclear transcription factors, each of which has been shown *in vivo* to activate pathways required for FAO.¹⁵⁵⁻¹⁵⁹ To determine the role of PPAR γ during sepsis, PPAR α knockout transgenic mice that constitutively express cardiac-specific PPAR γ (α MHC-PPAR γ /PPAR $\alpha^{-/-}$) were induced to develop sepsis by administering LPS. α MHC-PPAR γ /PPAR $\alpha^{-/-}$ mice showed elevated FAO levels compared to both α MHC-PPAR γ and PPAR $\alpha^{-/-}$ mice.¹⁵⁹ (FIG. 15). The role of PPAR γ is shown in a schematic model that explains the role of the activation of PPAR γ in the induction of fatty acid oxidation and prevention of LPS-mediated cardiac dysfunction. FIG. 27.

[0172] Unexpectedly, constitutive expression of PPAR γ in PPAR α knockout transgenic mice protected against LPS-induced cardiac dysfunction despite PPAR α downregulation in α MHC-PPAR γ mice. FIG. 16 (A)-(B). Despite a 77% reduction in PPAR α , the expression of FAO-associated genes (such as AOX, PGC-1 α , PPAR δ , Cpt1 β , UCP2 and UCP3) increased by 27.1-, 30.1-, 31.6-, 14.3-, 23.7- and 17.4-fold, respectively FIG. 24(B). Similarly, cardiac ATP content was also significantly increased. FIG. 22(B). As expected, LPS increased the inflammation-related gene expression of IL-1 α , FIG. 24(G); IL-6 FIG. 24(H); and TNF α FIG. 24(I) by 23.1-, 288- and 12.5-fold, respectively showing that cardiac function of LPS-treated α MHC-PPAR γ mice remained normal despite elevated inflammation. *See also* FIG. 17(A)-(C).

[0173] PPAR α and/or PPAR γ agonists, pharmaceutically acceptable salts, solvates, solvates of such salts or prodrugs thereof are well known in the art and many of them are FDA-approved drugs. The PPAR γ agonists rosiglitazone, troglitazone and pioglitazone are thiazolidine derivatives that are well known insulin sensitizers, prescribed to ameliorate insulin resistance (or enhance the action of insulin) and lower blood glucose without promoting secretion of insulin from the pancreas. Thiazolidine-type chemicals induce differentiation of adipocytes, and exhibit their action via the intranuclear receptor PPAR γ ¹⁹⁸ for which they act as agonists.¹⁹⁹ Unfortunately, some PPAR γ agonists particularly, rosiglitazone (that has recently been pulled from the market) and troglitazone, cause hepatic damage with long term use. Short term use of these drugs for treating acute sepsis, however, will not have toxicity issues.

Example 6

[0174] C57BL/6 mice were treated with LPS and the PPAR γ agonist, rosiglitazone intraperitoneally (i.p.) (30mg/kg) to determine whether pharmacologic activation of PPAR γ would have the same effects on normal mice that were observed with α MHC-PPAR γ mice. FIG. 18. Rosiglitazone increased various indicia of FAO in normal LPS-treated mice: PGC1- α expression was increased about 75% above control values; palmitate oxidation increased about 20%; and cardiac function measured as % FS was restored to about 80% of normal levels. FIGS. 19(A)-(D), 22(C) and 22(D). It is important to note that the LPS-mediated downregulation of PPAR α mRNA and upregulation of the inflammatory cytokines IL-1 α , IL-6 and TNF α was not reversed by rosiglitazone treatment. Thus, activation of PPAR γ in normal LPS-treated mice by rosiglitazone prevented cardiac dysfunction, increased cardiac FAO gene expression and

stabilized ATP content, even though the mice displayed concurrent cardiac inflammation and PPAR α downregulation. As expected, inflammation was not elevated by the PPAR γ agonist R treatment (FIG. 20(A)-(B)) and cardiac function was normalized in LPS-treated C57BL/6 mice. These results show that pharmacological-mediated activation of PPAR γ in septic mice compensated for PPAR α downregulation, boosted energy production and prevented cardiac dysfunction.

Example 7

[0175] Our group²⁰⁰ and others²⁰¹⁻²⁰⁴ have shown that administration of LPS in mice reduces PPAR α mRNA levels in the heart. The downregulation of PPAR α may account for the LPS-driven reduction of cardiac FAO. The suppressing effects of LPS administration were confirmed on cardiac PPAR α mRNA levels (FIG. 23A) that was reduced by 78% and on heart function (FIGs. 23(B), 23(C)), as shown by 2D-echocardiography that indicated 50% reduction in fractional shortening. Compromised cardiac function was associated with reduced cardiac FAO (Fig. 23(D)) and ATP (FIG. 23(E)) levels. Despite the energy deficiency state of the heart phosphorylation of AMP kinase (AMPK) was not stimulated (FIG. 23(F)). Mitochondrial structure analysis and intracellular arrangement by electron microscopy (at 6000x, 20,000x, and 50,000x magnification) of cardiac tissue from LPS-treated C57BL/6 mice showed that LPS treatment did not affect either the morphology of mitochondria or their intracellular arrangement as compared to control mice that were treated with saline. (FIG. 23(G))

Example 8

[0176] C57BL/6 mice were treated with combination of LPS (5 mg/kg) and the pharmacological PPAR α agonist (WY-14643, 30 mg/kg). Administration of the PPAR α agonist generated a trend for improved fractional shortening by 25% (p=0.09) in control saline-treated mice. FIG. 28.

Example 9

[0177] A previous study of our group showed that cardiac FAO and energy production are increased in *Ppara*^{-/-} mice that express PPAR γ in cardiomyocytes (α MHC-PPAR γ xPPAR α ^{-/-})²⁰⁵ In order to assess whether PPAR γ may substitute for the reduced PPAR α and rescue FAO, 5mg/kg LPS was administered to α MHC-PPAR γ mice.²⁰⁶ Treatment of α MHC-PPAR γ mice

with LPS activated JNK (Fig. 24A) and downregulated PPAR α mRNA levels by 78%. FIG. 24(B). Opposite to the inhibitory effect of LPS in C57BL/6 mice mRNA levels of genes mediating FAO were increased in the hearts of LPS-treated α MHC-PPAR γ mice. Specifically, the mRNA levels of AOX, PPAR δ , PGC-1 α and Cpt-1 β were increased by 27.1-, 31.6-, 30.1- and 14.3-fold, respectively, as compared to α MHC-PPAR γ mice that were treated with saline. FIG. 24(B). On the other hand cardiac PGC-1 β and CD36 gene expression levels were reduced by 76% and 93%, respectively. FIG. 24(B). Perilipin 5 mRNA levels were also reduced by 50%, while estrogen-related receptor (ERR) α and perilipin 2 were not modulated by treatment of the α MHC-PPAR γ mice with LPS. FIG. 24(B). PDK4 mRNA levels were increased by 5.4-fold, FIG. 24(B), indicating that LPS-treated α MHC-PPAR γ mice were unlikely to have switched to greater glucose utilization. Consistent with cardiac FAO-associated gene expression profile, the levels of FAO were increased by 2.6-fold in LPS-treated α MHC-PPAR γ mice as compared to saline treated α MHC-PPAR γ mice. FIG. 24(C). Accordingly, cardiac ATP levels of LPS-treated α MHC-PPAR γ mice were also increased by 2.2-fold. FIG. 24(D). Phosphorylated AMPK levels were reduced in the LPS-treated mice that express PPAR γ in cardiomyocytes and have increased cardiac energy production. FIG. 24(A). Therefore the α MHC-PPAR γ transgenic mice were protected from LPS-induced defective FAO and reduction in ATP levels.

[0178] The improvement of cardiac FAO was associated with prevention of LPS-mediated cardiac dysfunction but not the induction of inflammation. 2D-echocardiography (FIG. 24(E)) showed preserved fractional shortening levels in LPS-treated α MHC-PPAR γ mice. FIG. 24(F). Inflammation is a major component of sepsis and its alleviation has been proposed as a therapeutic approach in sepsis based on studies in animal models that are knock-out for inflammation-related genes.²⁰⁷⁻²¹⁰ These mice are resistant to LPS-mediated sepsis and have improved cardiac function or viability. However, PPAR γ -mediated prevention of cardiac dysfunction in LPS-treated mice was not accompanied by alleviation of inflammation, as shown by cardiac mRNA levels of IL-1 α (FIG. 24(G)); IL-6 (FIG. 24(H)); and TNF α (FIG. 24(I)) that were elevated by, 23-, 285- and 12.5-fold, respectively. Thus, the beneficial effect of PPAR γ in the prevention of cardiac dysfunction during sepsis cannot be accounted for by anti-inflammatory effect.

Example 10

Prevention

[0179] In order to assess whether pharmacologic activation of PPAR γ prevents LPS-mediated cardiac dysfunction, C57BL/6 mice were treated parenterally with the PPAR γ agonist, rosiglitazone (35 mg/kg) 30 min prior to the administration of LPS. Administration of rosiglitazone in LPS-treated mice prevented LPS-mediated cardiac dysfunction, and did not reduce left ventricular fractional shortening. FIGs. 25(A), 25(B). LPS treatment of mice induced phosphorylation of JNK and reduced phospho-AMPK in both controls and rosiglitazone-treated animals. FIG. 25(C). Despite the increased levels of cardiac pJNK, cardiac FAO was not reduced in mice receiving both LPS and rosiglitazone. FIG 25(D). The improvement in FAO of LPS-treated mice that were co-administered with rosiglitazone was associated with increased gene expression levels of PGC1- α (4-fold) and PGC-1 β (2.1-fold) levels, as well as with reduced perilipin 5 (60%) mRNA levels(FIG. 25(E), as compared to mice that were treated with LPS only. On the other hand, the LPS-mediated reduction of PPAR α , ERR α and CD36 mRNA levels was not prevented by rosiglitazone treatment. FIG. 25(E). In addition, cardiac perilipin 2 and AOX mRNA was increased by 3.3- and 42%, respectively in LPS-treated mice and remained elevated in mice treated with LPS and rosiglitazone. FIG. 25(E). PPAR γ mRNA was increased by 57% following LPS treatment and was normalized following administration of rosiglitazone in LPS-treated animals, while PPAR δ was not modulated in any of the treatment groups. FIG. 25(E). PDK4 was increased 16-fold by LPS treatment and was reduced but remained significantly higher (5.8-fold) than saline-treated mice in mice that received LPS and rosiglitazone treatment. FIG. 25(E).

Example 11

[0180] In order to test whether rosiglitazone-mediated improvement of cardiac function in LPS-treated C57BL/6 mice was due to attenuation of inflammation cardiac gene expression levels of TNF α , IL-1 α and IL-6 were assessed. Analysis of cardiac mRNA with qRT-PCR showed that TNF α , IL-1 α and IL-6 levels were increased by 26-, 90- and 1250-fold, respectively in LPS-treated mice. FIGs. 25(F)-25(H). Combined treatment with LPS and rosiglitazone did not prevent the increase of inflammatory markers (FIGs. 25(F)-25(H)), thus indicating that the improvement in cardiac function by rosiglitazone was not associated with attenuation of LPS-triggered inflammation.

Example 12

[0181] In order to assess whether rosiglitazone may improve survival during LPS-induced sepsis, C57BL/6 mice were administered with a lethal dose of LPS (15 mg/kg, i.p.) or combination of LPS and daily injections of rosiglitazone (35 mg/kg). Mice that were treated with LPS alone started dying 36hours post-injection with the vast majority of lethal events occurring between 48hours and 72hours post-injection. Table 1. I.p administration of rosiglitazone on daily basis reduced significantly the lethal events of the LPS-treated mice (FIG. 26).

<i>Strain</i>	<i>Gender</i>	<i>Treatment</i>	<i>No of mice</i>	<i>Survivors 24h</i>	<i>Survivors 36h</i>	<i>Survivors 48h</i>	<i>Survivors 60h</i>	<i>Survivors 72h</i>
C57BL/6	F	Saline + DMSO	8	8	8	8	8	8
C57BL/6	M	Saline + Rosiglitazone	8	8	8	8	8	8
C57BL/6	F	LPS + DMSO	15	14	14	10	5	2
C57BL/6	M	LPS + Rosiglitazone	17	17	16	16	15	13

Table 1

LPS: 15mg/kg

Rosiglitazone: 33 mg/kg/day

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What is claimed is

1. A method for increasing or maintaining cardiac function in a subject, comprising administering therapeutically effective amounts of an agent that increases fatty acid oxidation in the heart.
2. The method of claim 1, wherein the subject has sepsis or is at risk of developing sepsis.
3. The method of claim 1, wherein the subject has heart failure or is at risk of developing heart failure.
4. The method of claim 1, wherein the agent is selected from the group comprising PPAR α agonists, PPAR γ agonists, dual PPAR α and PPAR γ agonists, or combinations thereof.
5. The method of claim 1, wherein the agent inhibits JNK 1 or JNK 2 or both.
6. The method of claim 5 wherein the JNK inhibitor is SP600125.
7. The method of claim 5, wherein the JNK inhibitor is an inhibitory RNA selected from the group comprising an antisense nucleic acid, siRNA micro RNA (miRNA), short hairpin RNA, ribozyme, microRNA mimic, supermir, and aptamer that specifically hybridizes to JNK thereby reducing its expression.
8. The method of claim 4, wherein the agent is PPAR α -coactivator-1 (PGC-1), estrogen-related receptor (ERR) α , or a combination thereof.
9. The method of claim 4, wherein the PPAR α agonist is selected from the group comprising Alpha WY-14643 GW9578, GW-590735, K-111, LY-674, KRP-101, DRF-10945, LY518674, Propanoic Acid 2-[4-[3-[2,5-dihydro-1-[(4-methylphenyl)methyl]-5-oxo-1H-1,2,4-triazol-3-yl]propyl]phenoxy]-2-methyl, fibrate, fenofibrate, clofibrate, and bezafibrate.
10. The method of claim 4, wherein the PPAR γ agonist is selected from the group comprising thiazolidinedione, rosiglitazone, pioglitazone, MCC-555, GL-262570, englitazone, darglitazone, isaglitazone, JTT-501, T-895645, R-119702, N,N-2344, YM-440, GI 262570, R-483 and rivoglitazone.

11. A pharmaceutical composition comprising therapeutically effective amounts of one member from at least two of the following groups: (i) JNK1 or a JNK2 inhibitors including antisense nucleic acids, siRNAs, shRNAs, microRNAs (miRNA), ribozymes, microRNA mimics, supermirs, and aptamers; (ii) a PPAR agonists selected from the group comprising PPAR agonists, PPAR γ agonists, dual PPAR α and PPAR γ agonists; and (iii) PPAR α -co-activator-1 (PGC-1) and (iii) estrogen-related receptor (ERR), which amounts treat or prevent cardiac dysfunction in a subject having heart failure or sepsis or at risk of developing them; or in amounts that increase cardiac function also in the patient having heart failure or sepsis or at risk of developing them.
12. A kit comprising the pharmaceutical composition of claim 11.
13. A method for treating or preventing cardiac dysfunction in a subject having sepsis or at risk of developing sepsis, comprising administering therapeutically effective amounts of an agent that increases fatty acid oxidation in the heart.
14. The method of claim 1 wherein the agent is administered before cardiac function is diminished, or significantly reduced.
15. The method of claim 1, wherein the agent comprises at least two members selected from the group comprising JNK inhibitors, PPAR α agonists, PPAR γ agonists, dual PPAR α and PPAR γ agonists, or combinations thereof, or PPAR α -coactivator-1 (PGC-1), estrogen-related receptor (ERR) α , or a combination thereof.
16. The method of claim 1, wherein the therapeutically effective amount of the agent is administered within 24 hours of a diagnosis of sepsis or at a risk of developing sepsis.
17. The method of claim 13, wherein the therapeutically effective amount of the agent is administered within 24 hours of a diagnosis of sepsis or at a risk of developing sepsis.
18. A method for treating or preventing cardiac dysfunction in a subject having heart failure comprising administering therapeutically effective amounts of an agent that increases fatty acid oxidation in the heart.
19. The method of claim 18, wherein the therapeutically effective amount of the agent is administered within 24 hours of a diagnosis of sepsis or at a risk of developing sepsis.

FIG. 1
Sepsis is associated with increased inflammation
but anti-inflammatory treatments
do not prevent septic shock

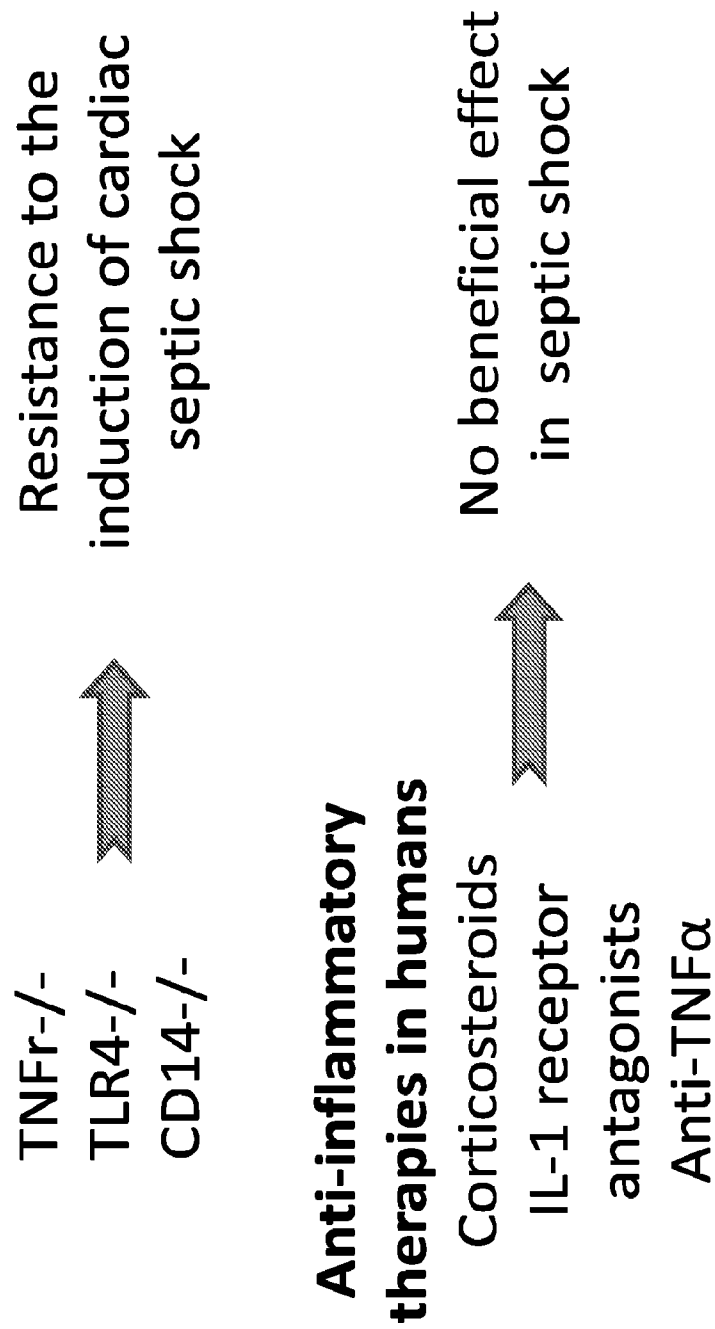
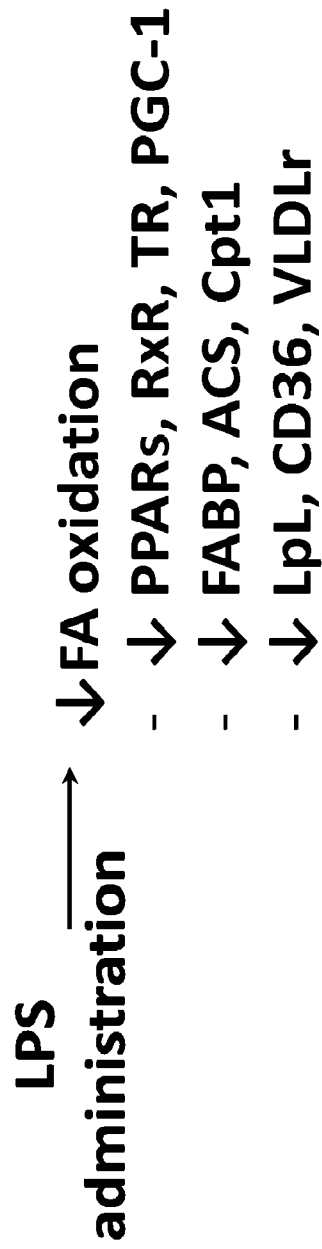


FIG. 2
Sepsis is associated with reduced fatty acid oxidation



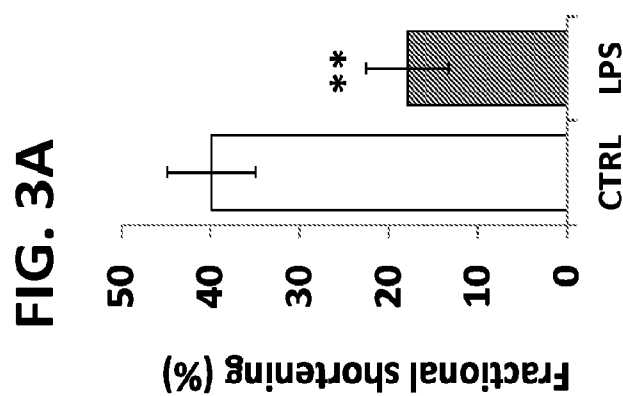
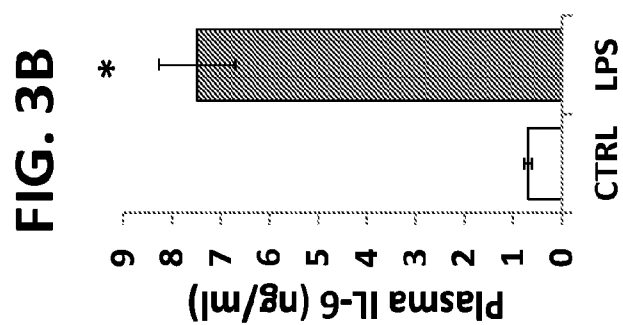


FIG. 3C

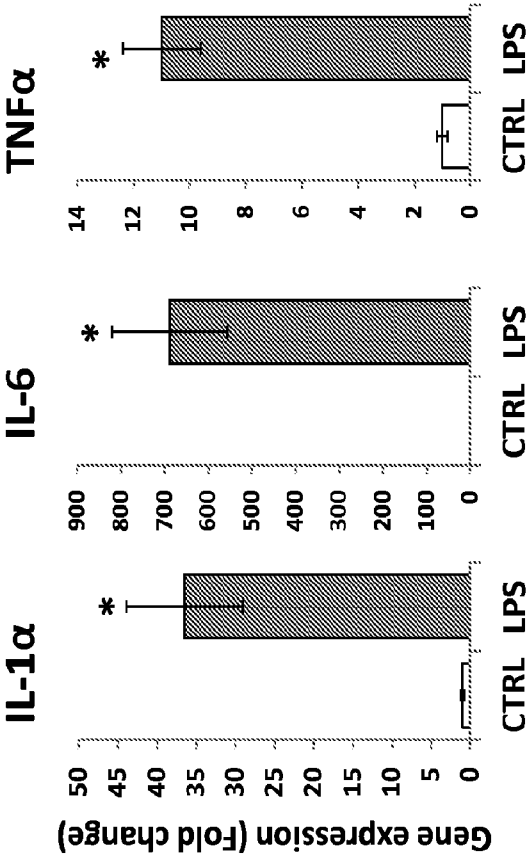


FIG. 3D

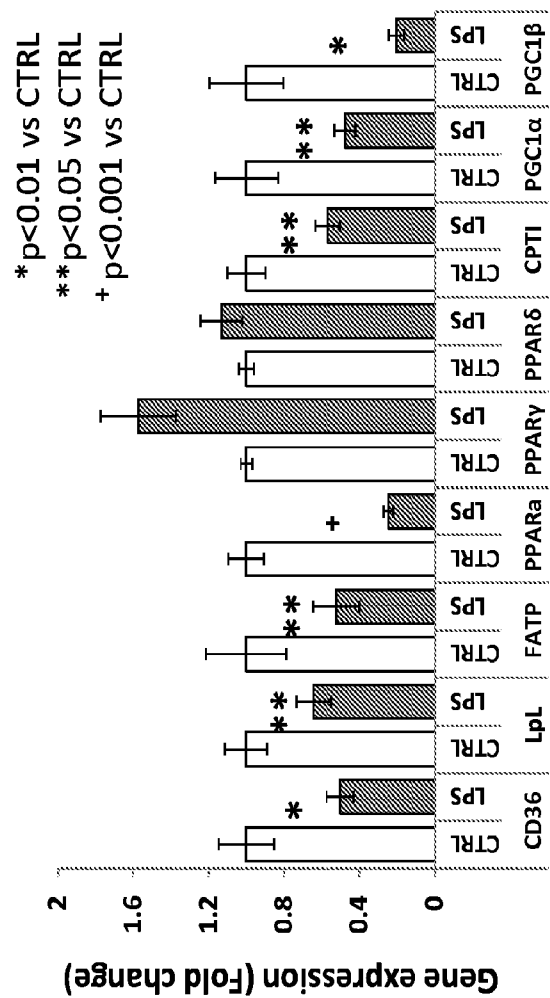
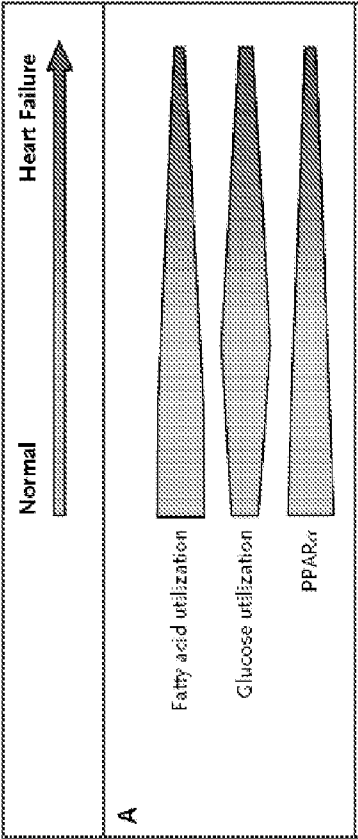
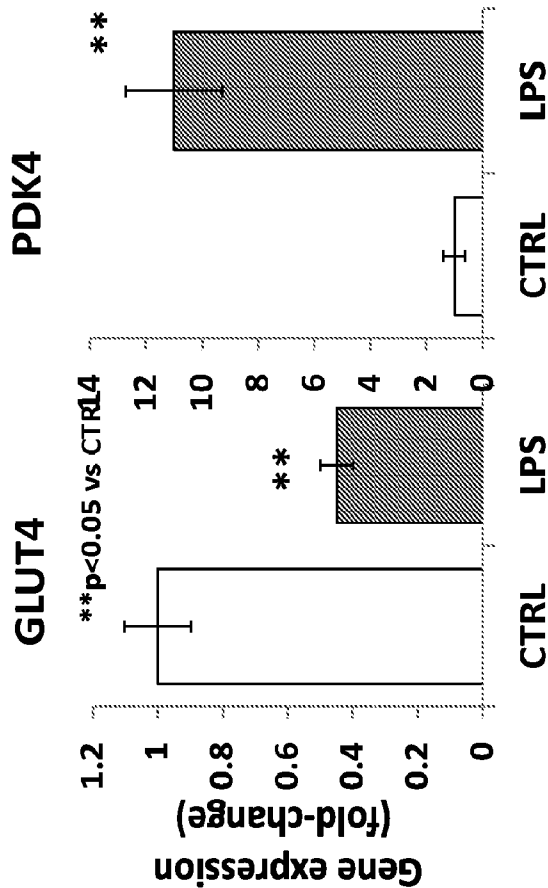


FIG. 4A
Heart failure reduces FA oxidation and
increases glucose utilization



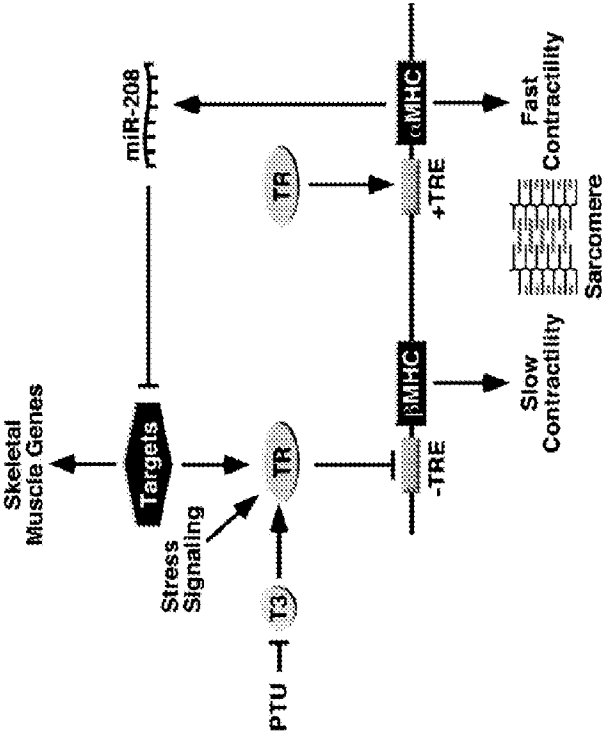
Neubauer S, NEJM, 2007

FIG. 4 B
LPS suppressed glucose catabolism
markers in C57BL/6 mice



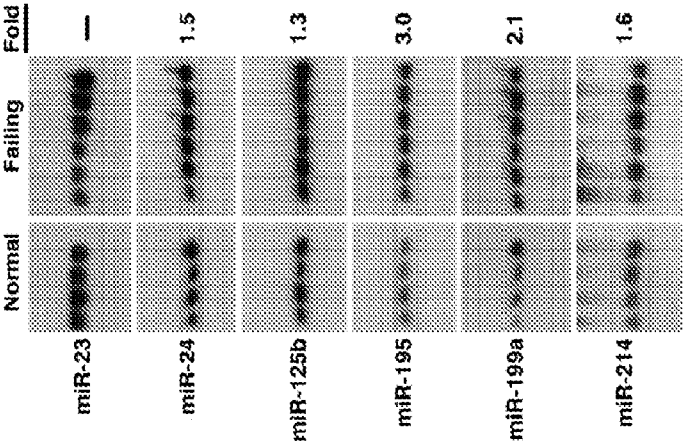
Tessier JP et. al.; Cardiovascular
Research 2003

FIG. 5A



Van Rooij E et al;
Science 2007

FIG. 5B



Van Rooij E et al;
PNAS 2006

FIG. 5 C

HF Studies	Our study
miR-1	↑ No change
miR-133	↑ No change
miR-208	↑ No change
miR-195	↑ No change
miR-24	↑ No change
miR-125b	↑ No change
miR-214	↑ No change

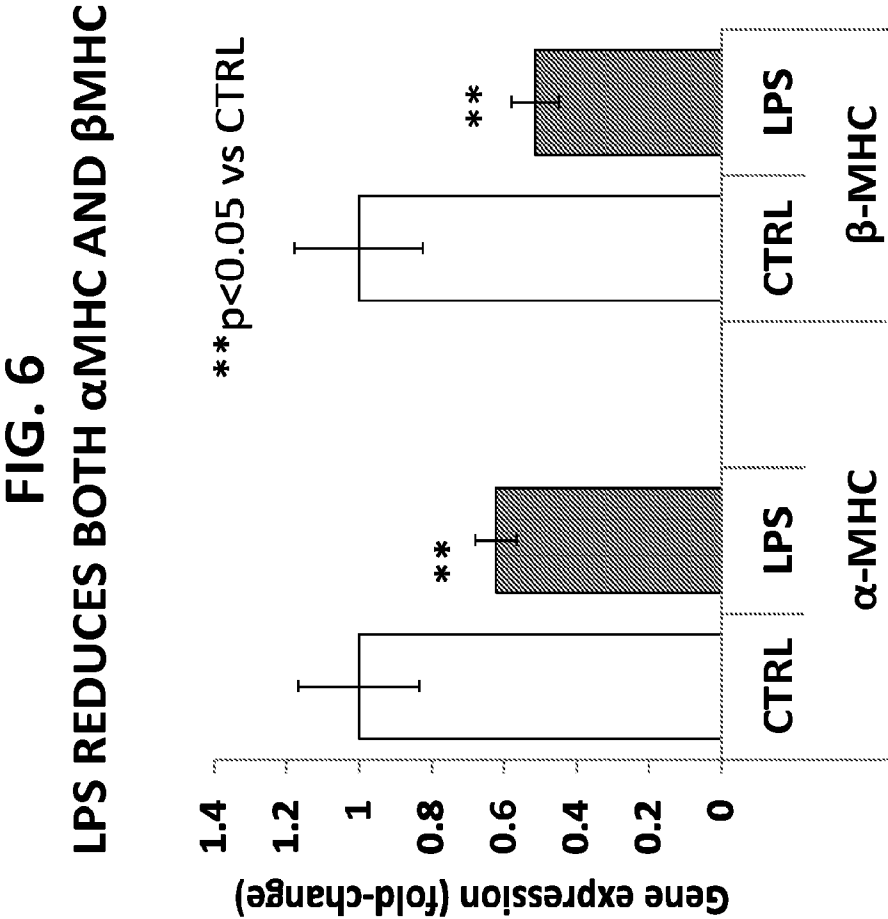


FIG. 7
JNK and c-Jun are activated in LPS-treated C57BL/6 mice

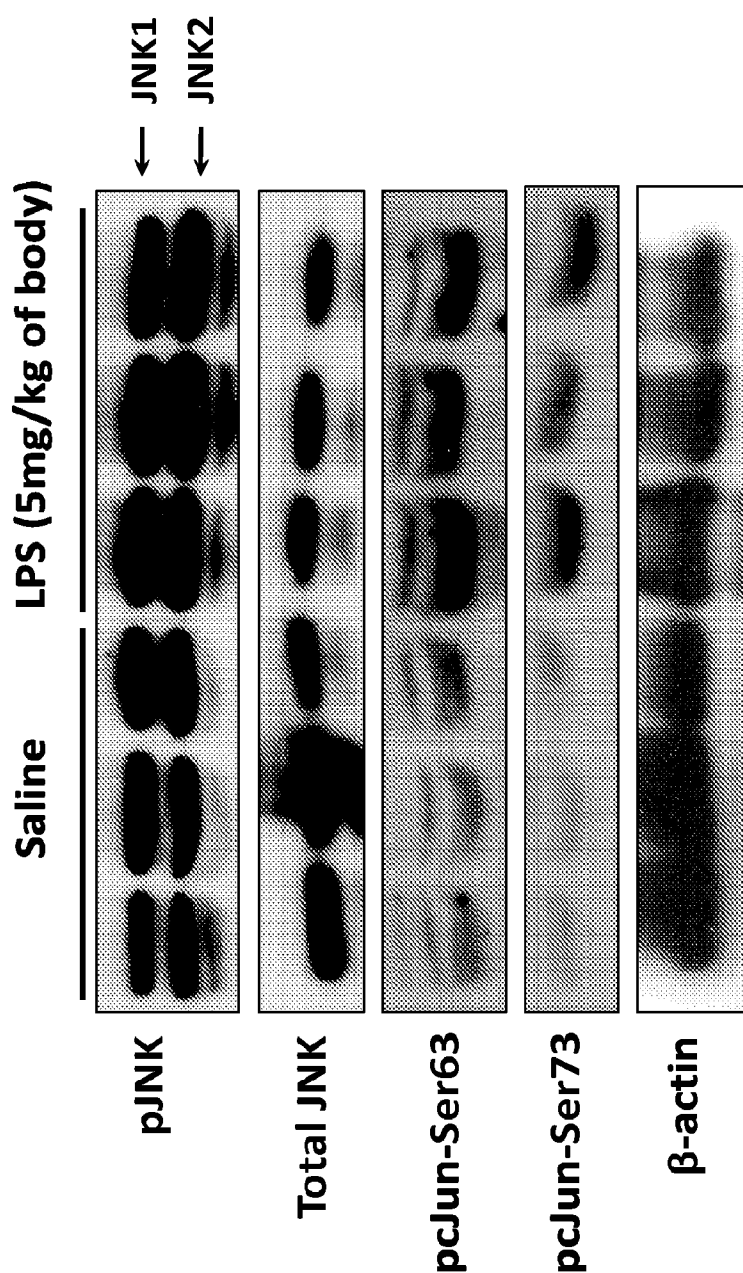


FIG. 8 A

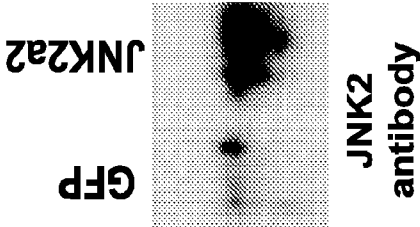


FIG. 8 B

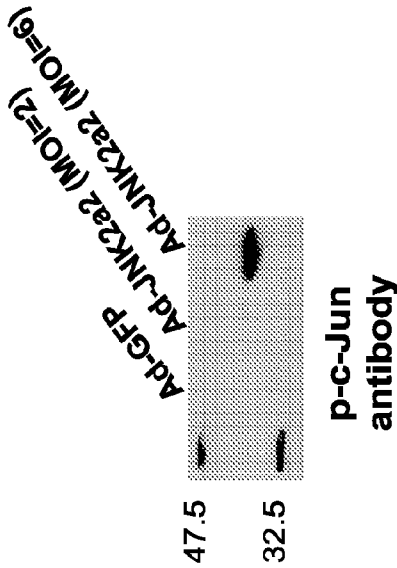


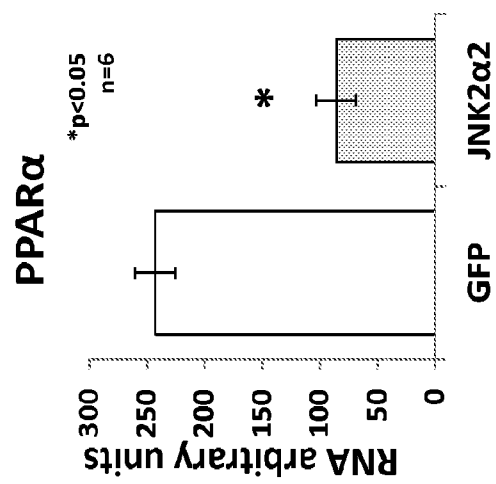
FIG. 8 C

FIG. 8 D

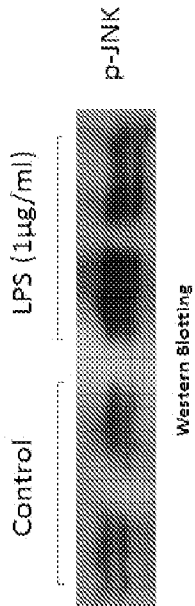


FIG. 8 E

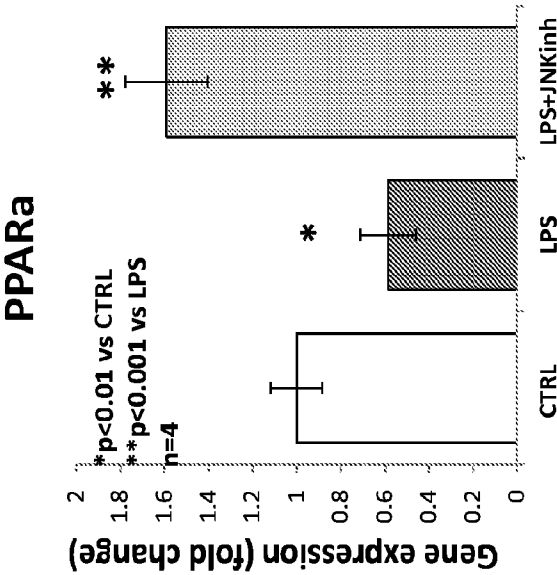


FIG. 9A

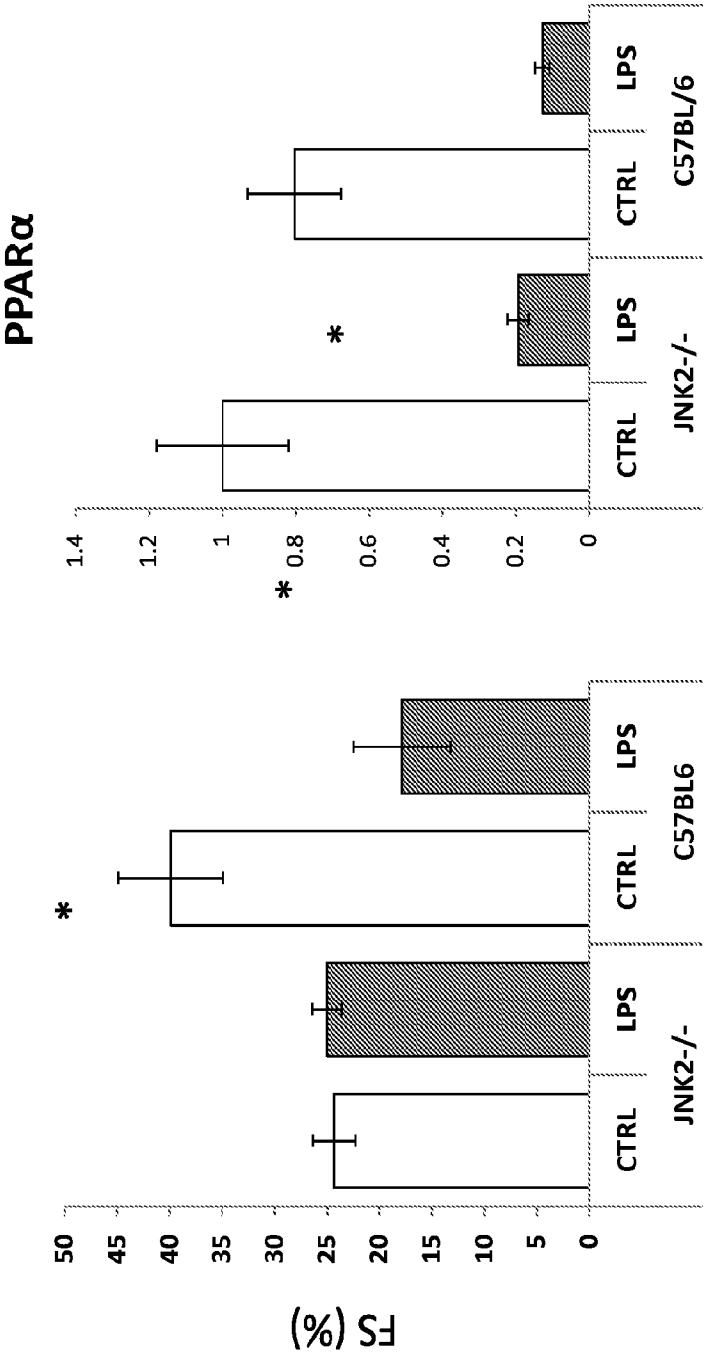


FIG. 9B

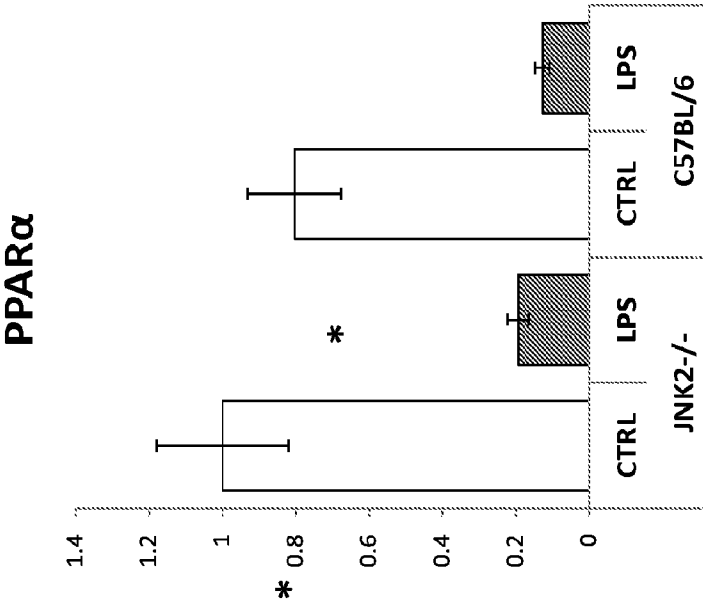
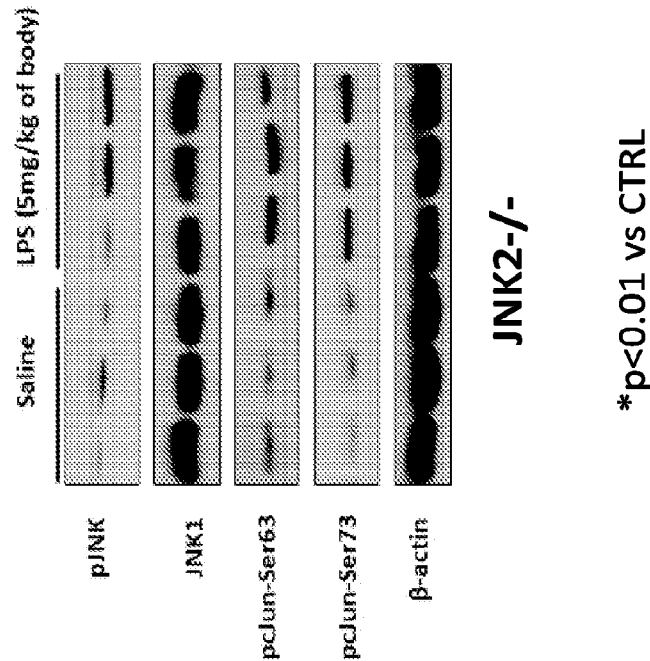


FIG. 9C



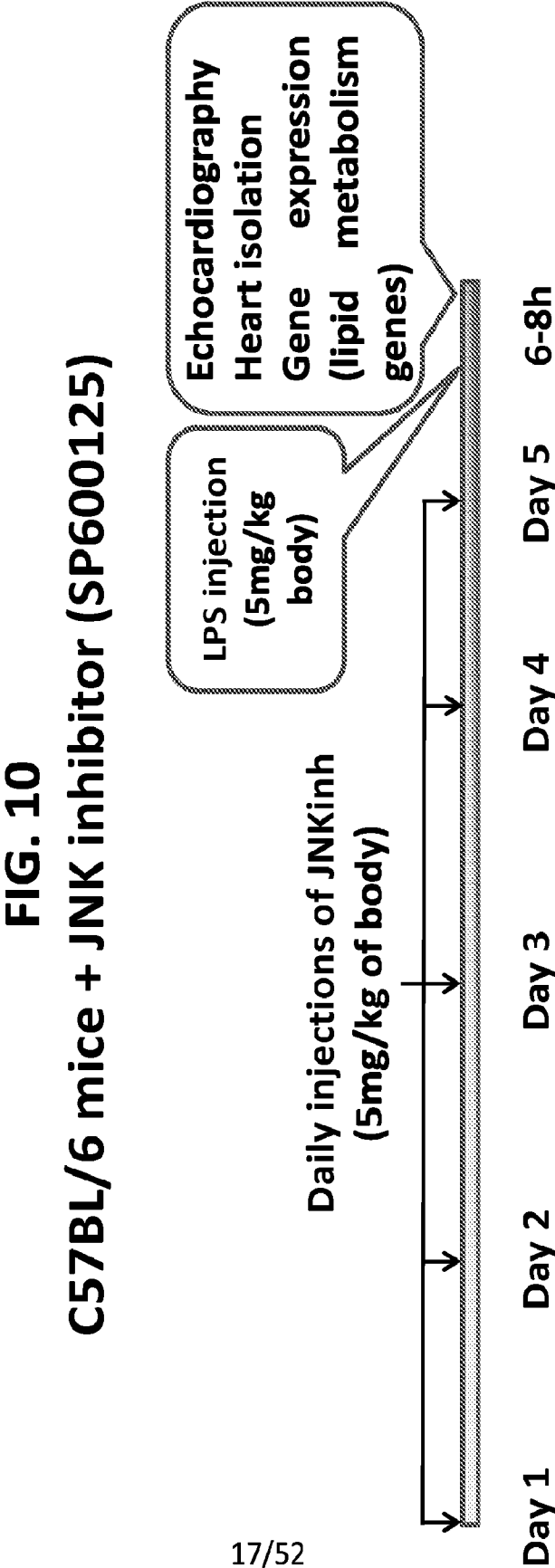


FIG. 11A

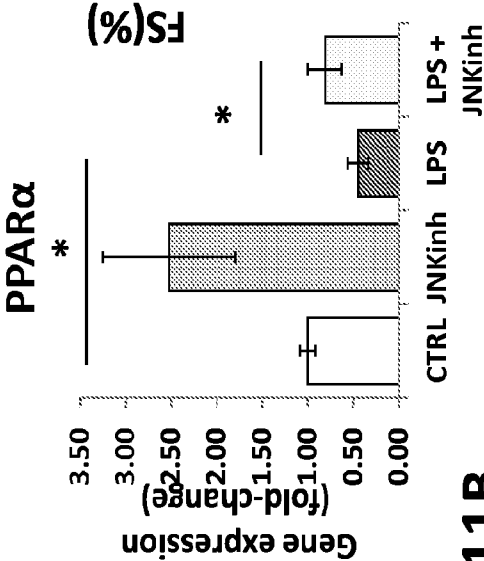
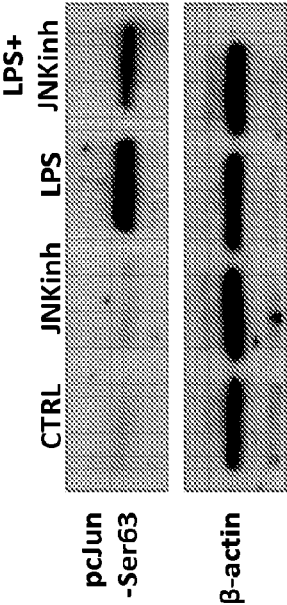


FIG. 11B

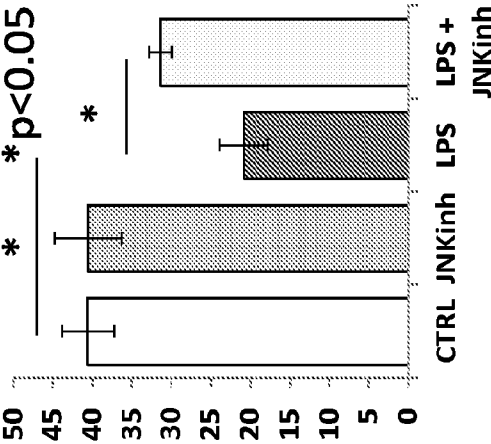


FIG. 11C

FIG. 12A

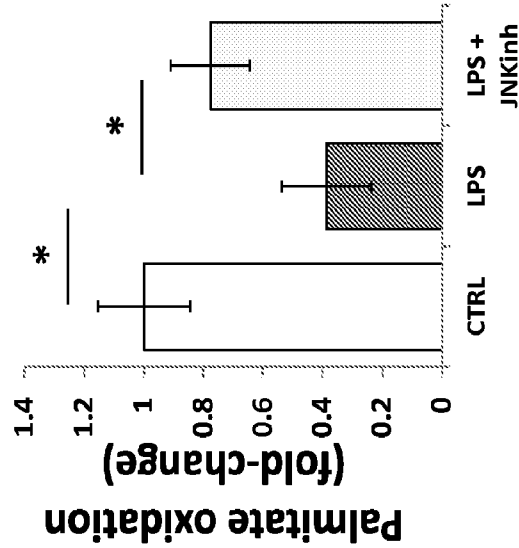
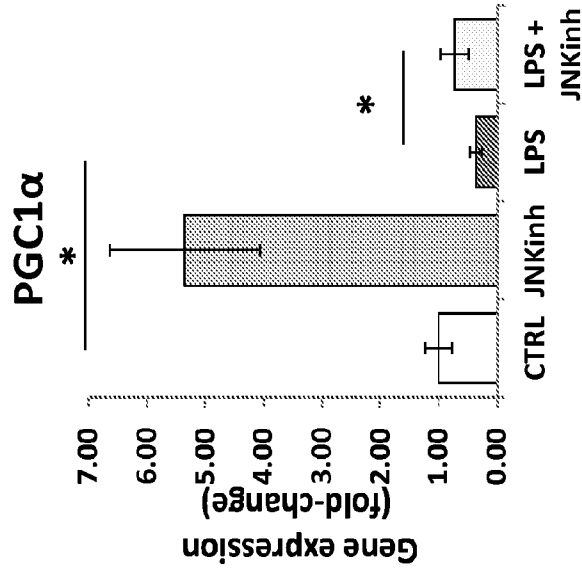


FIG. 12B



* p<0.05

FIG. 12C

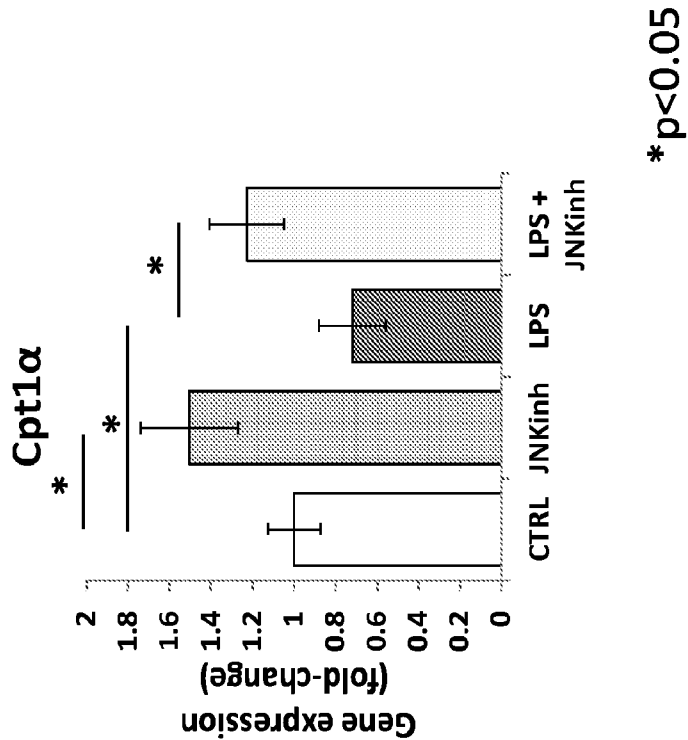


FIG. 13B

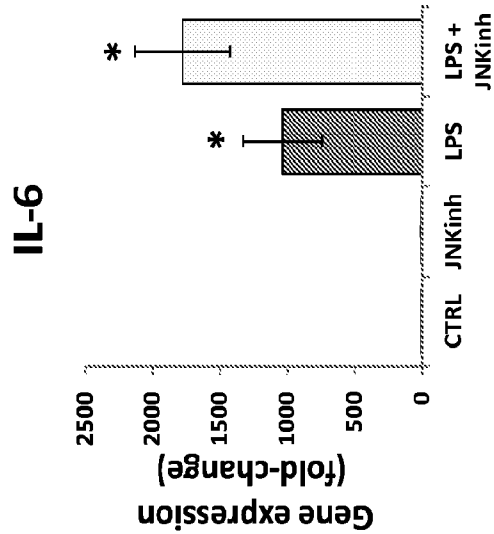
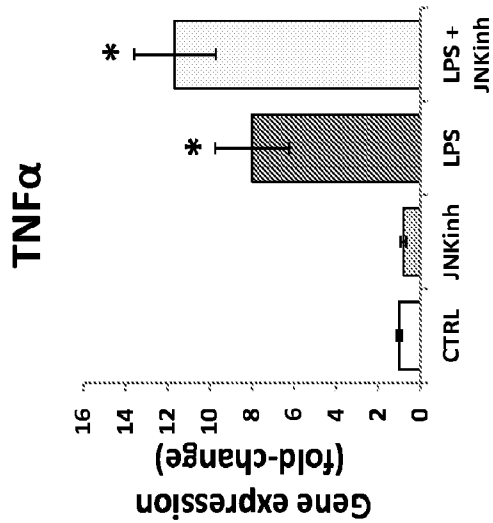
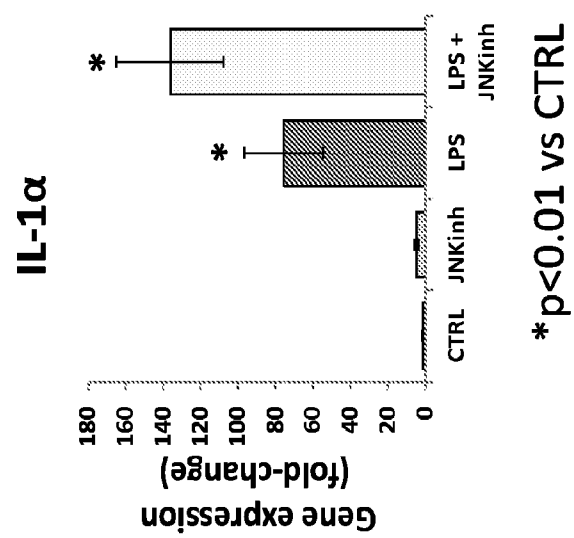


FIG. 13A



*p<0.01 vs CTRL

FIG. 13C



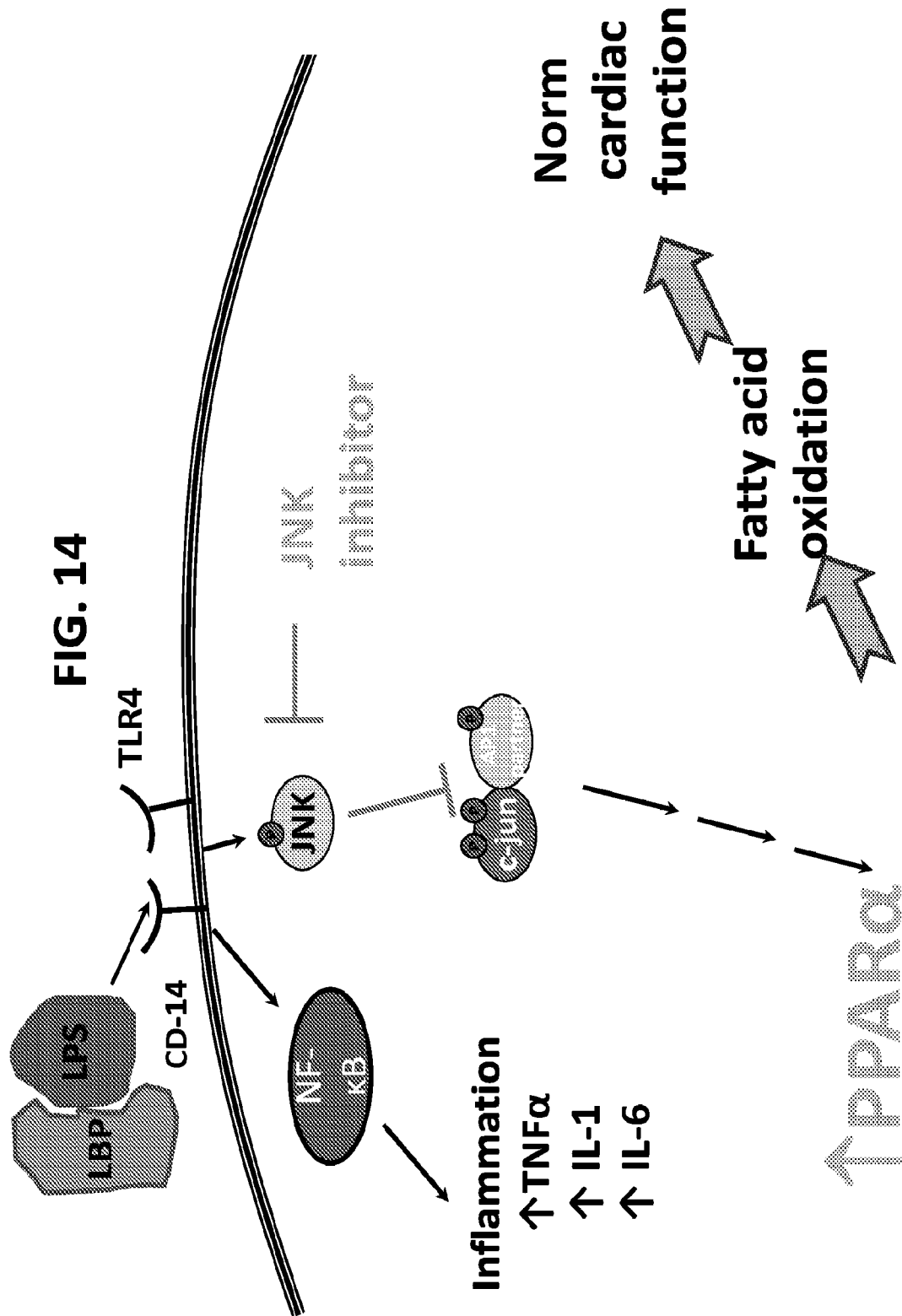


FIG. 15

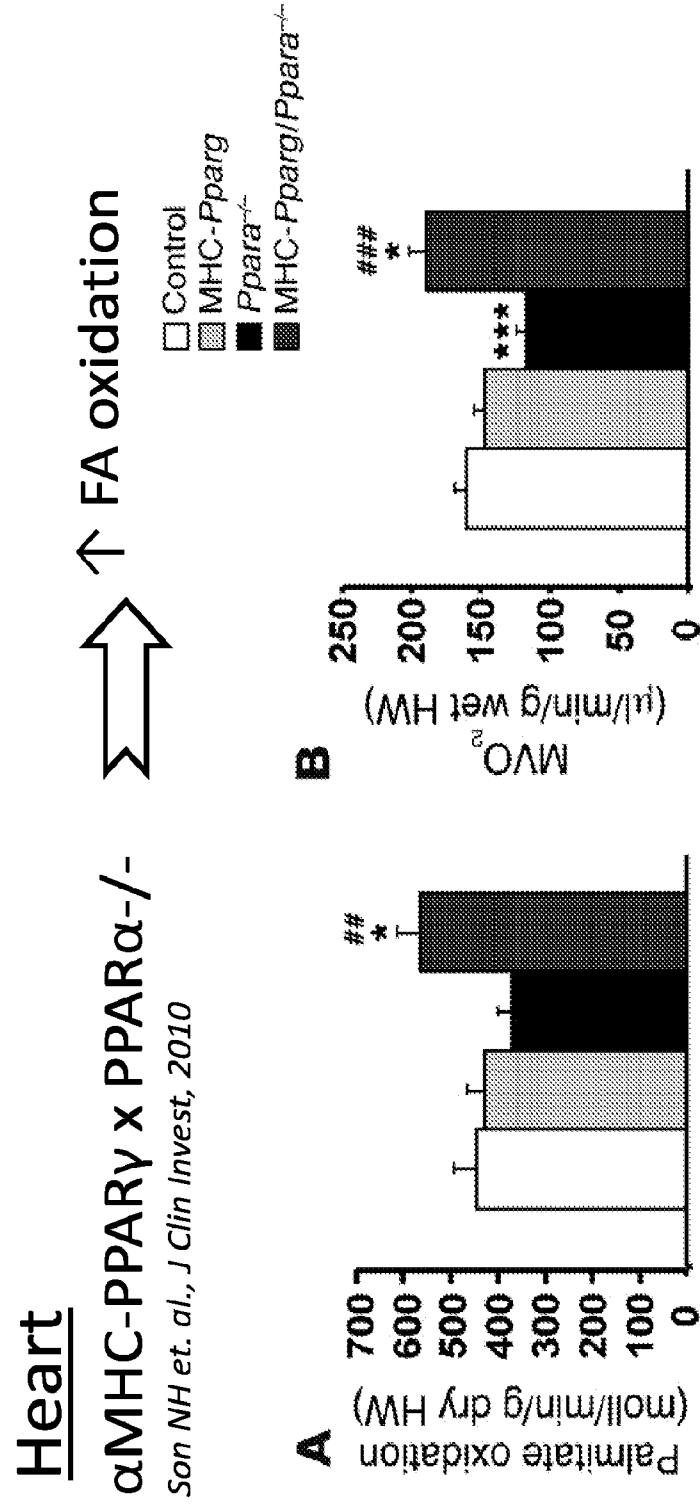


FIG 16A

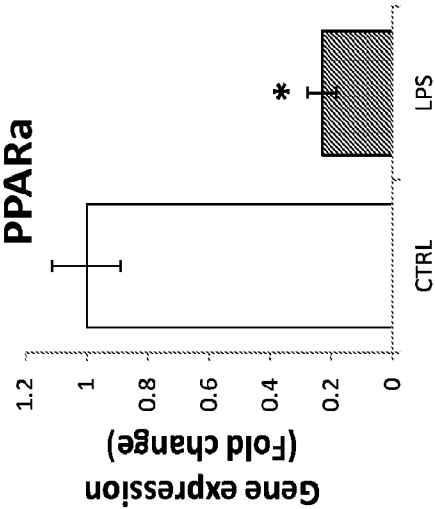


FIG 16B

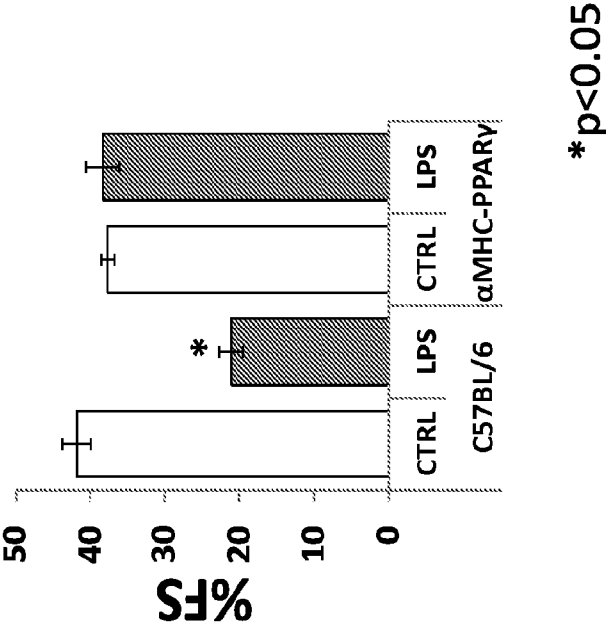


FIG. 17A

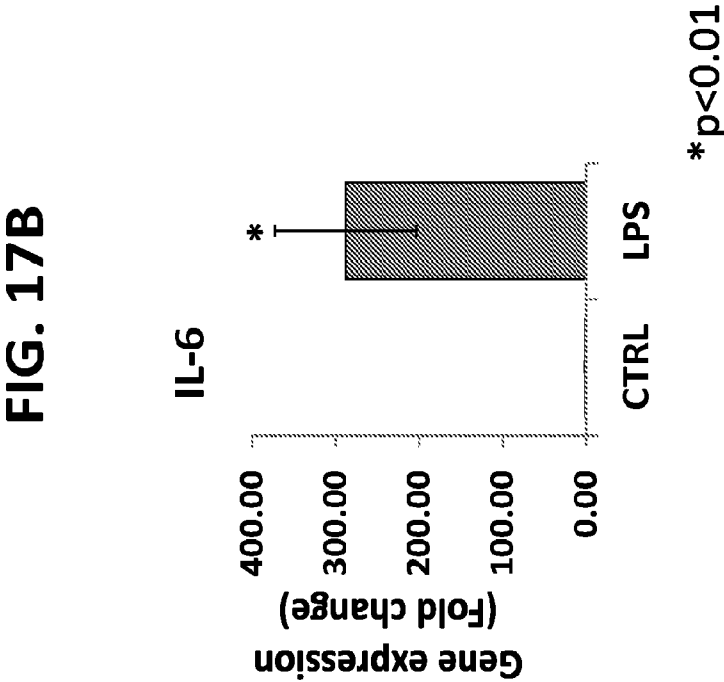
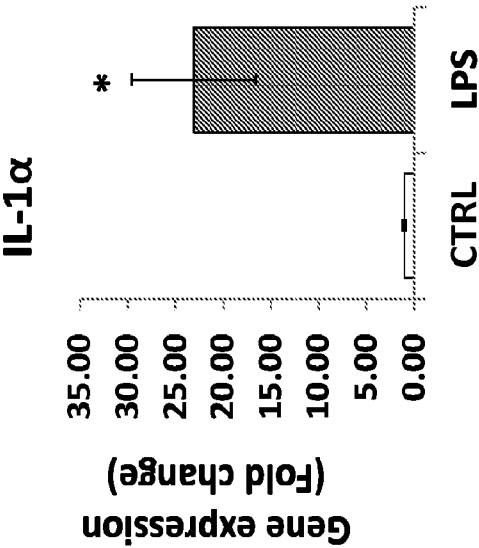


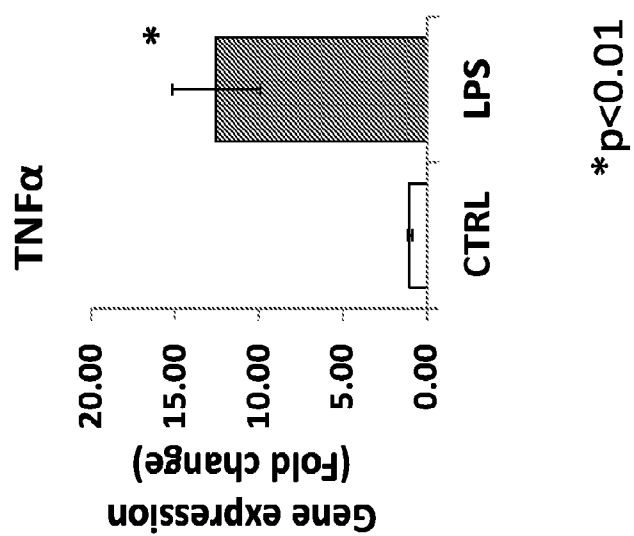
FIG. 17C

FIG. 18
C57BL/6 mice +
Rosiglitazone

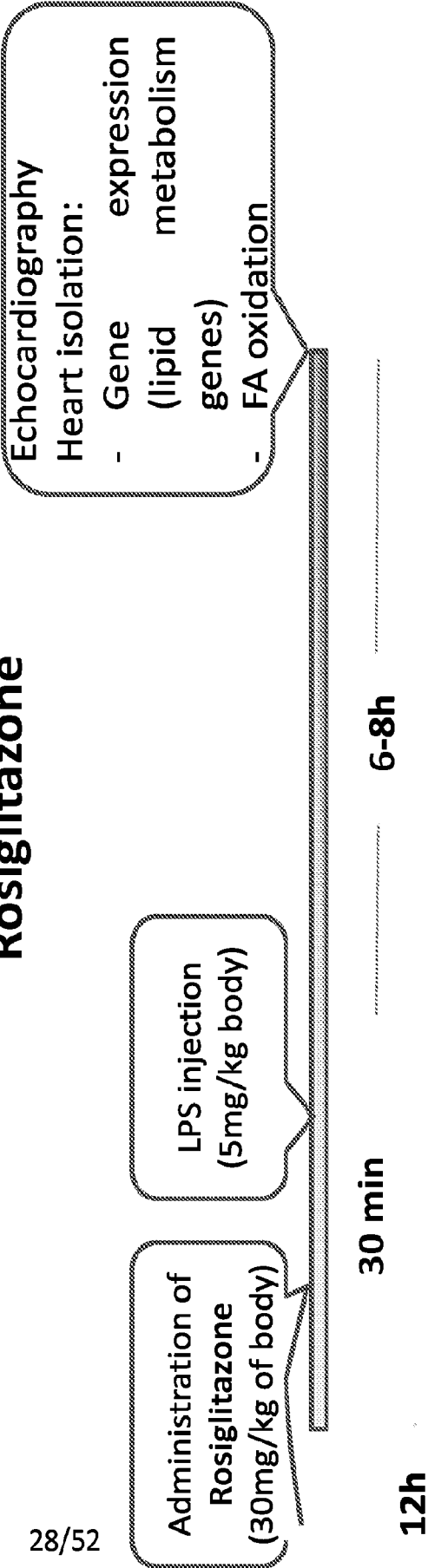


FIG.19A

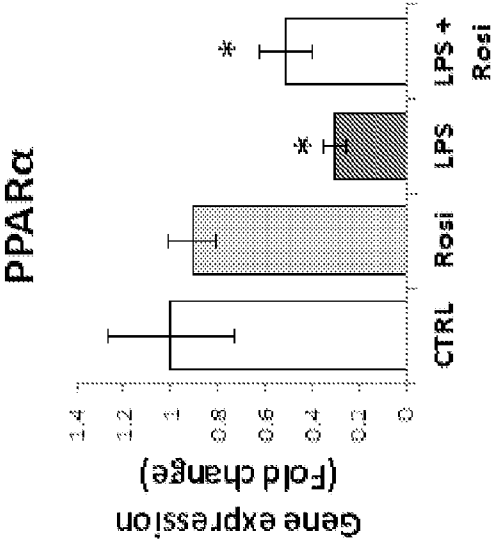


FIG.19B

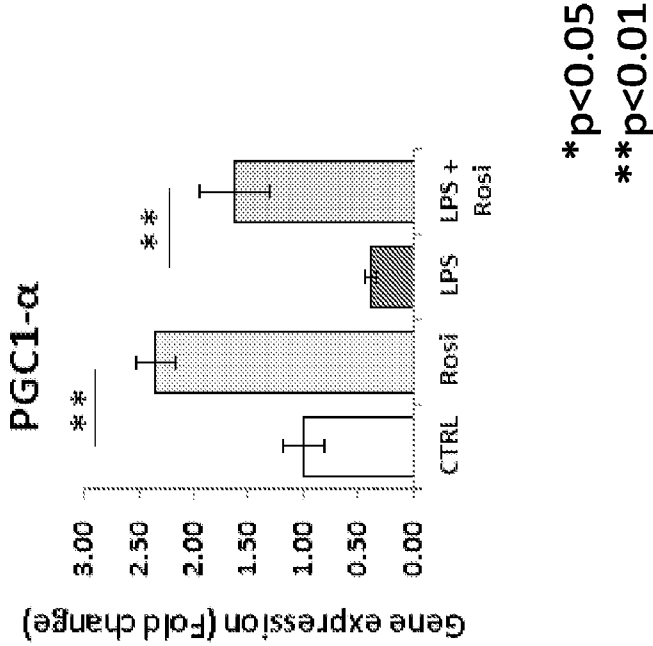


FIG.19C

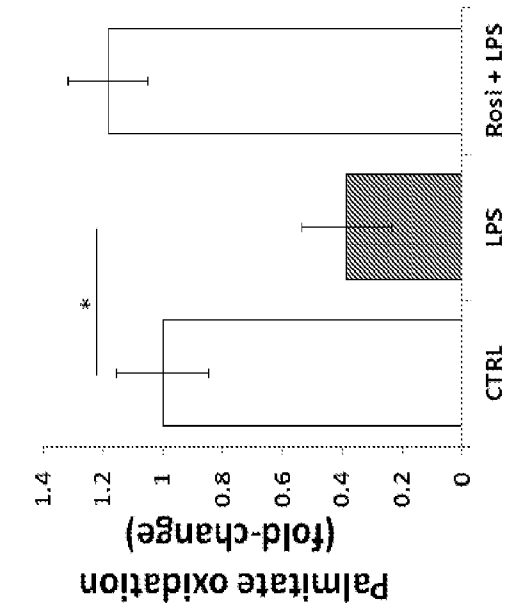
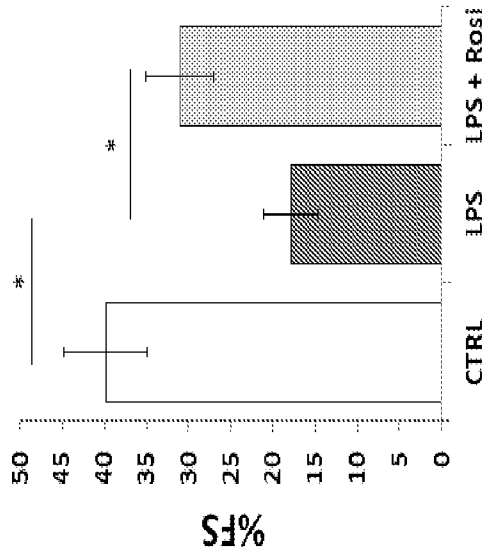


FIG.19D



* p<0.05
** p<0.01

FIG.20A

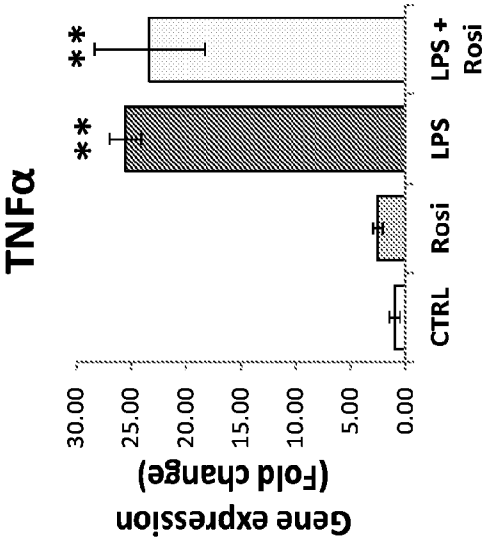
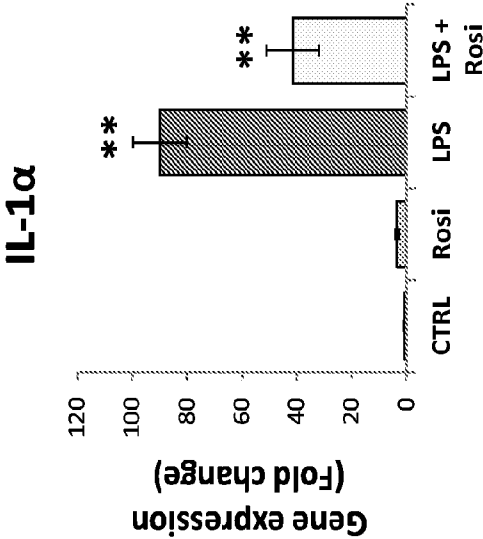


FIG.20B



** p<0.01 vs non-LPS CTRL

FIG. 21

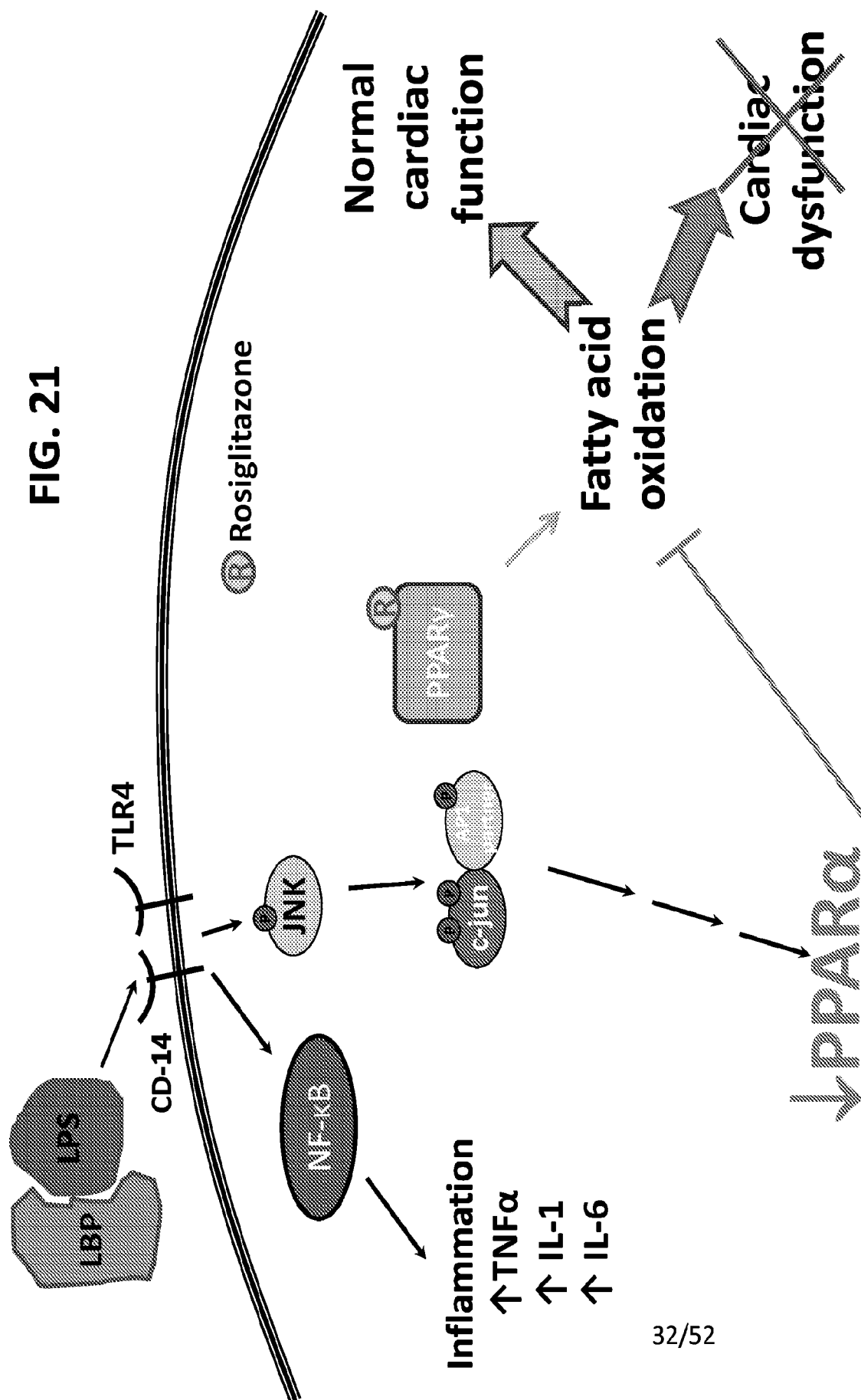


FIG. 22A

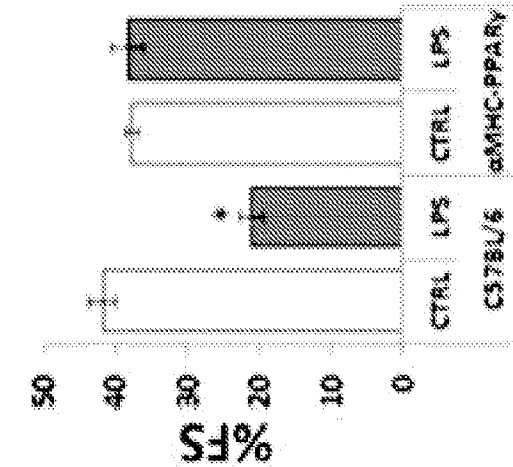


FIG. 22B

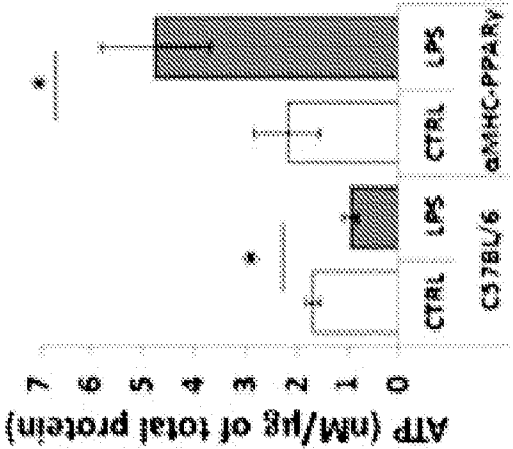


FIG. 22D

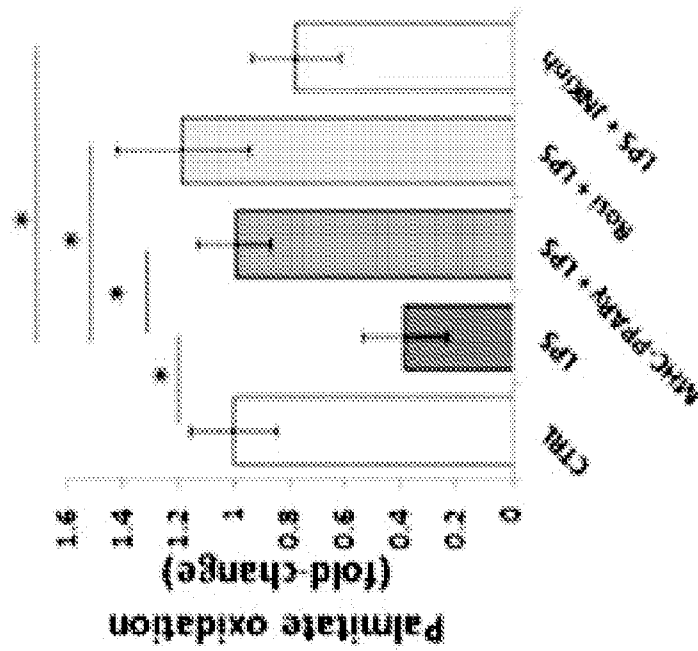


FIG. 22C

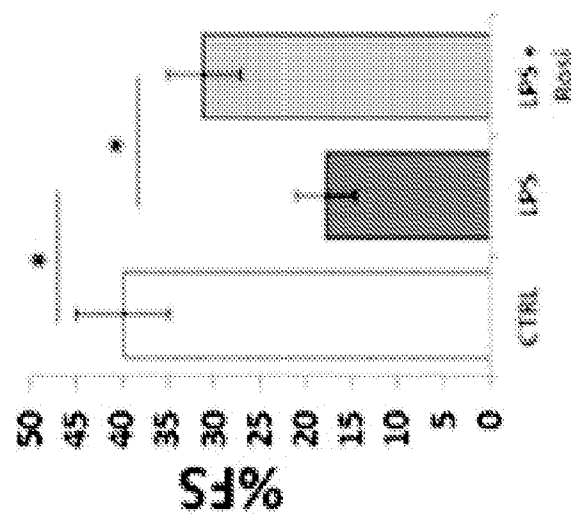


FIG. 23B

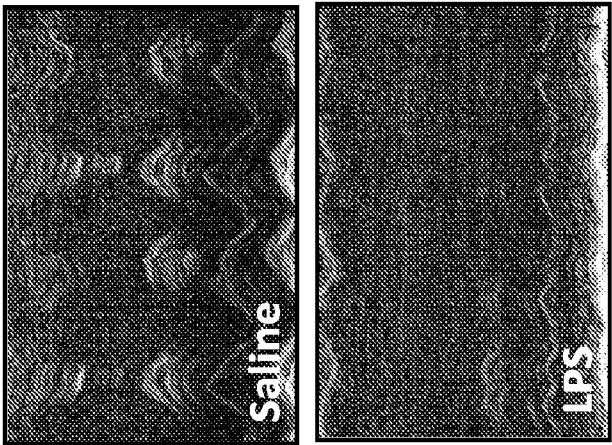
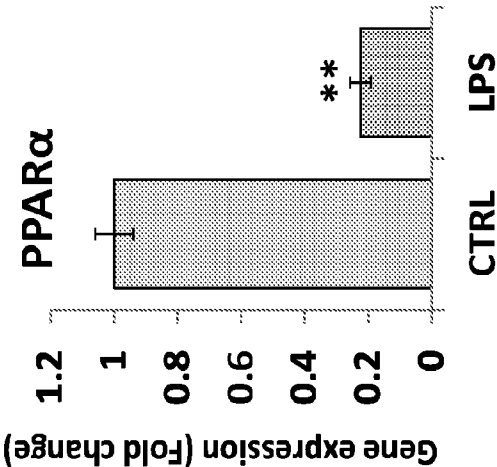
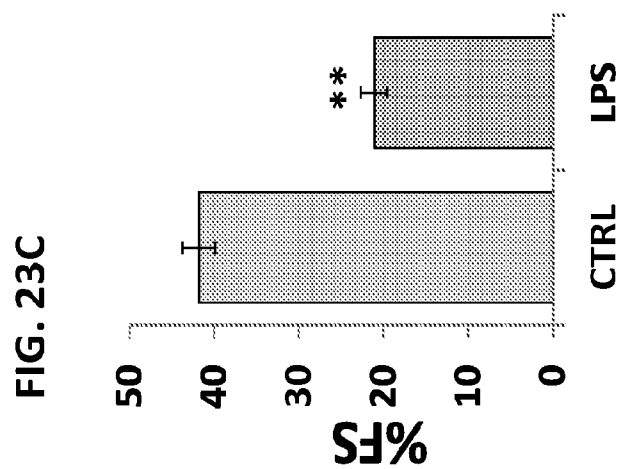
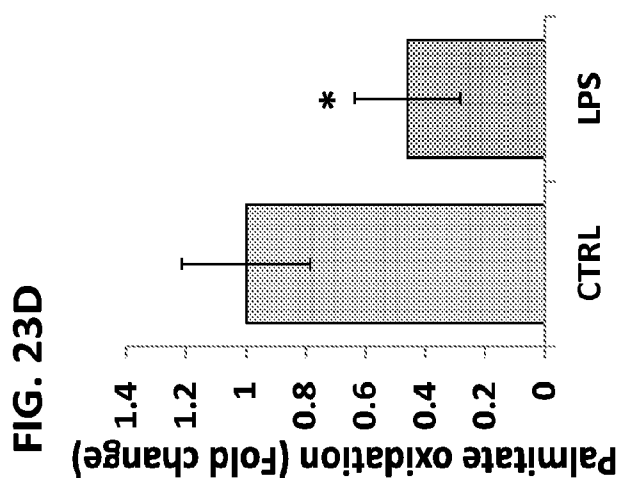


FIG. 23A





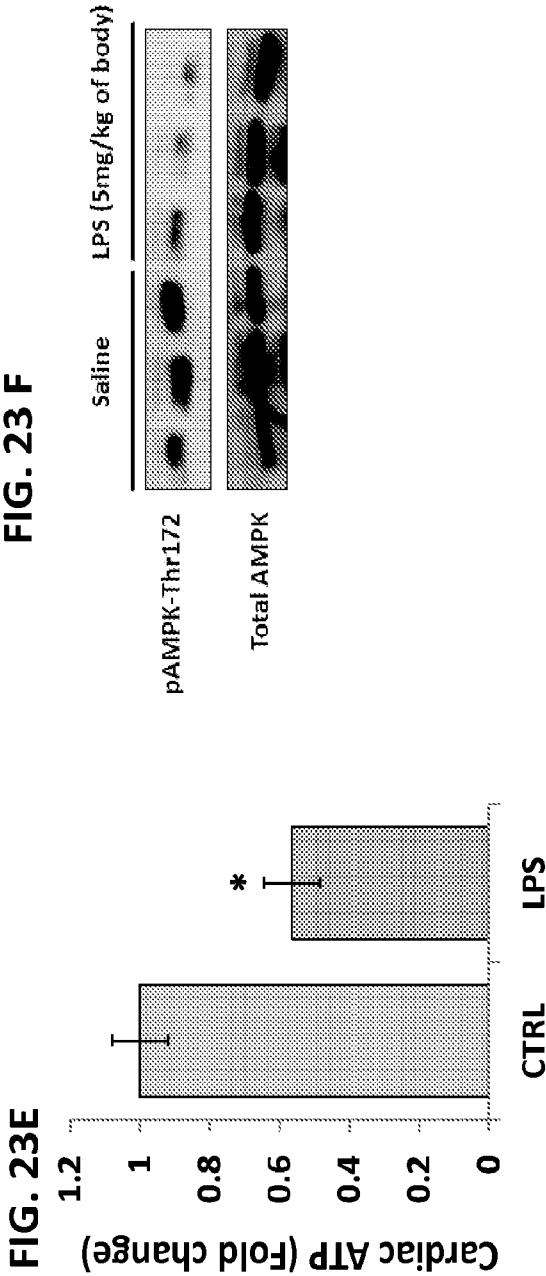
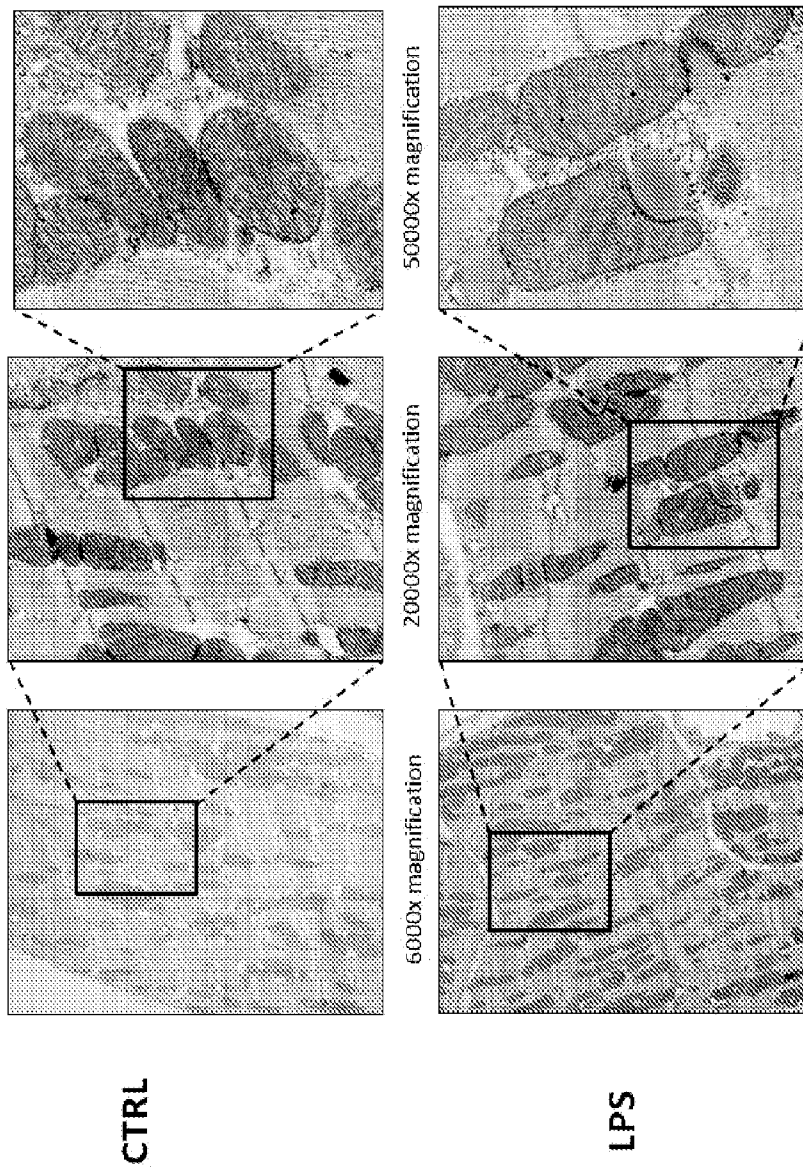


FIG. 23G



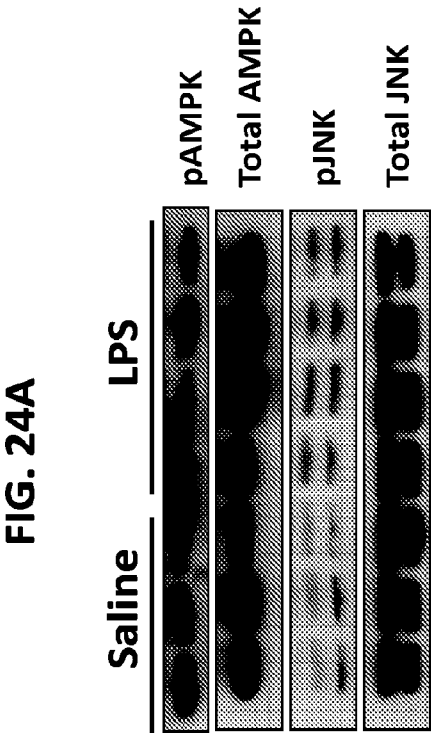


FIG. 24B

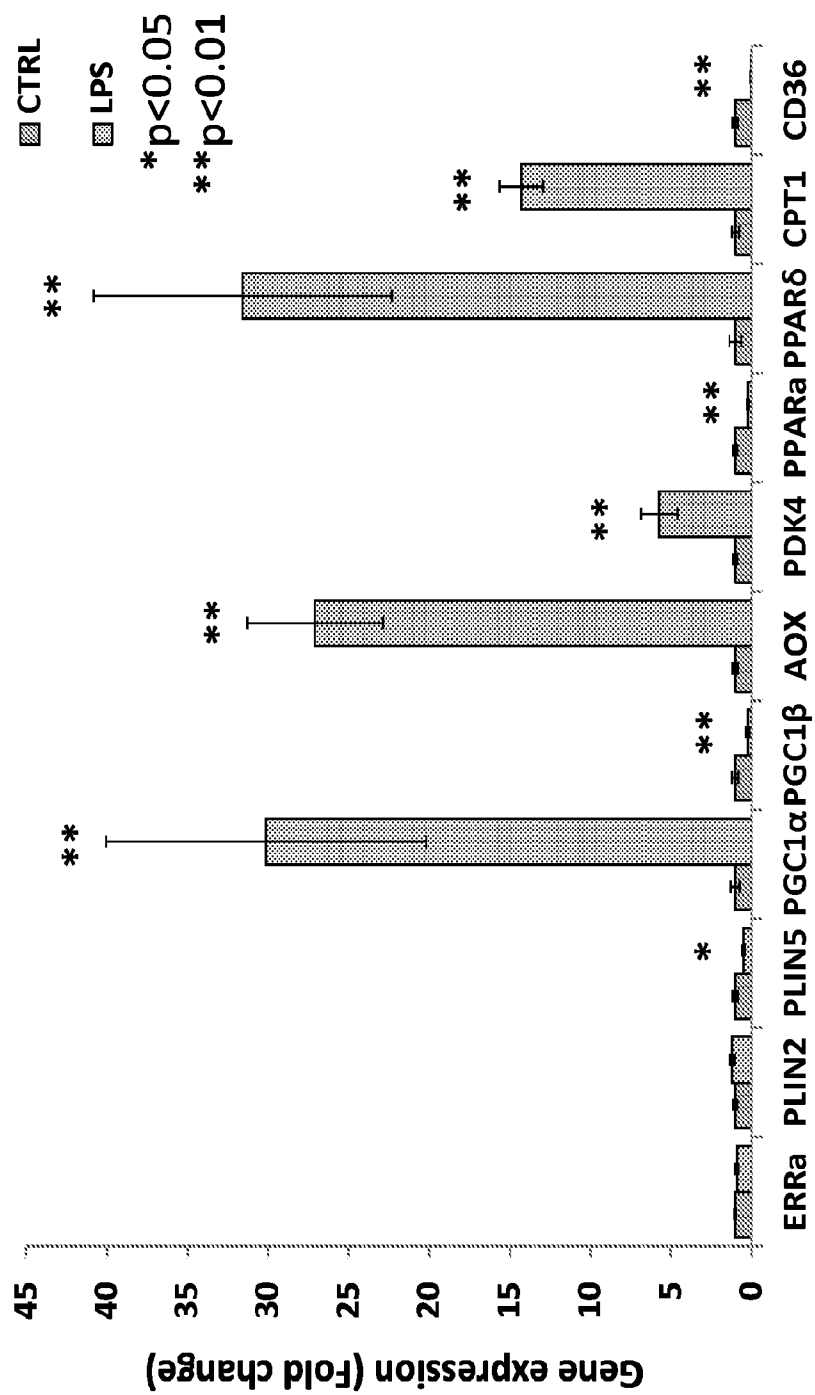


FIG. 24D

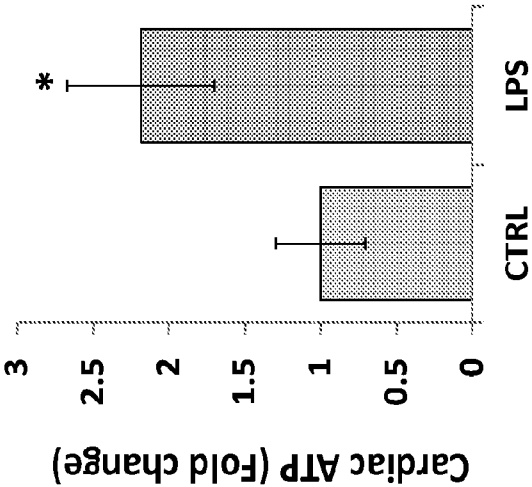


FIG. 24C

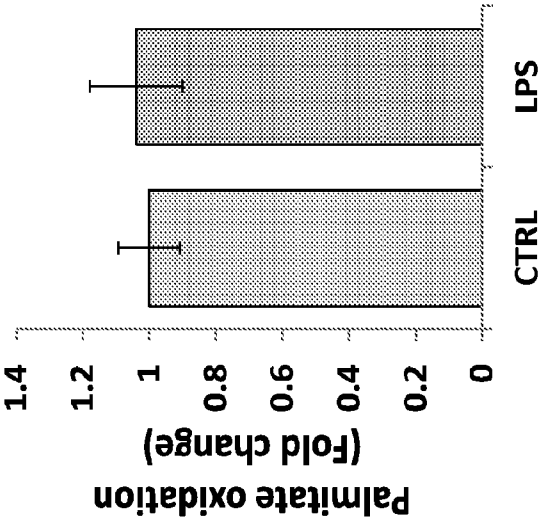


FIG. 24F

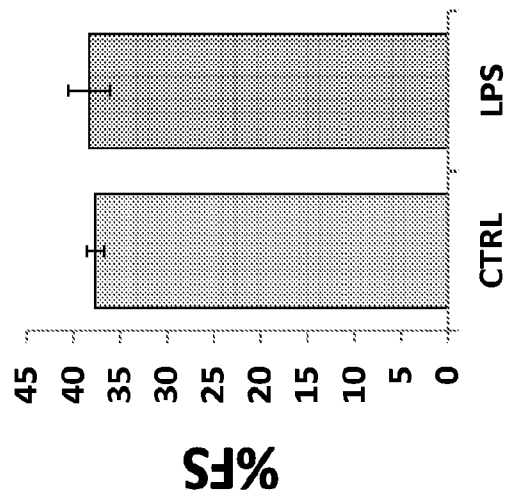


FIG. 24E

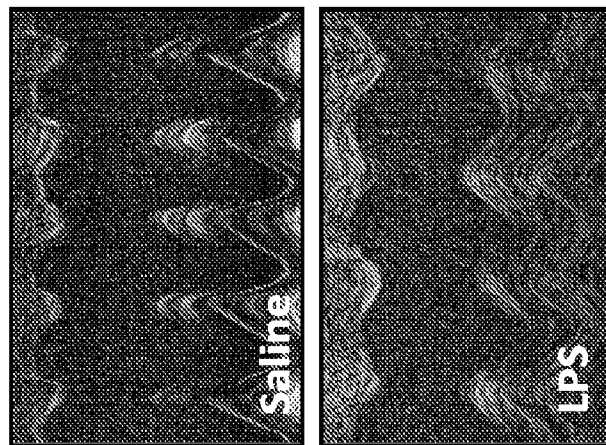
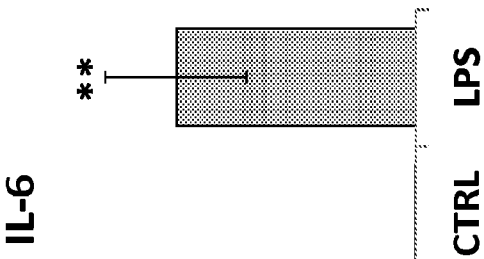
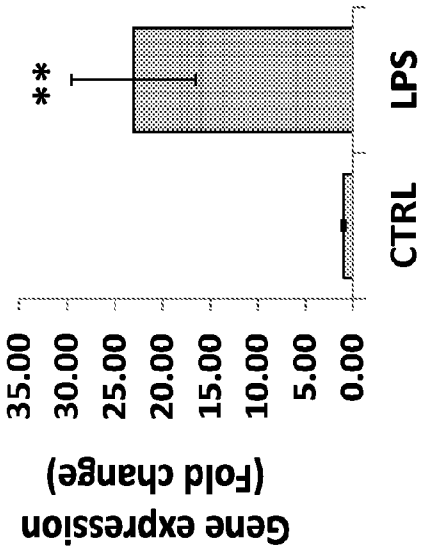


FIG. 24H



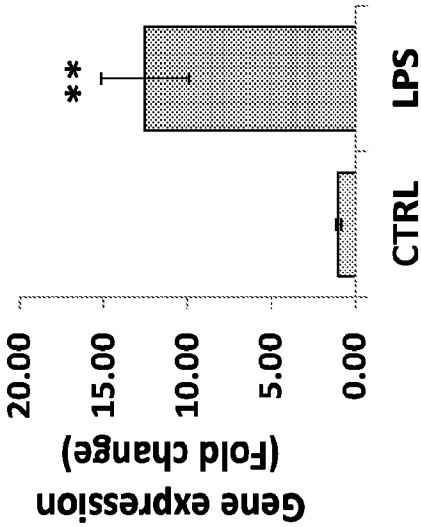
IL-1 α

FIG. 24G

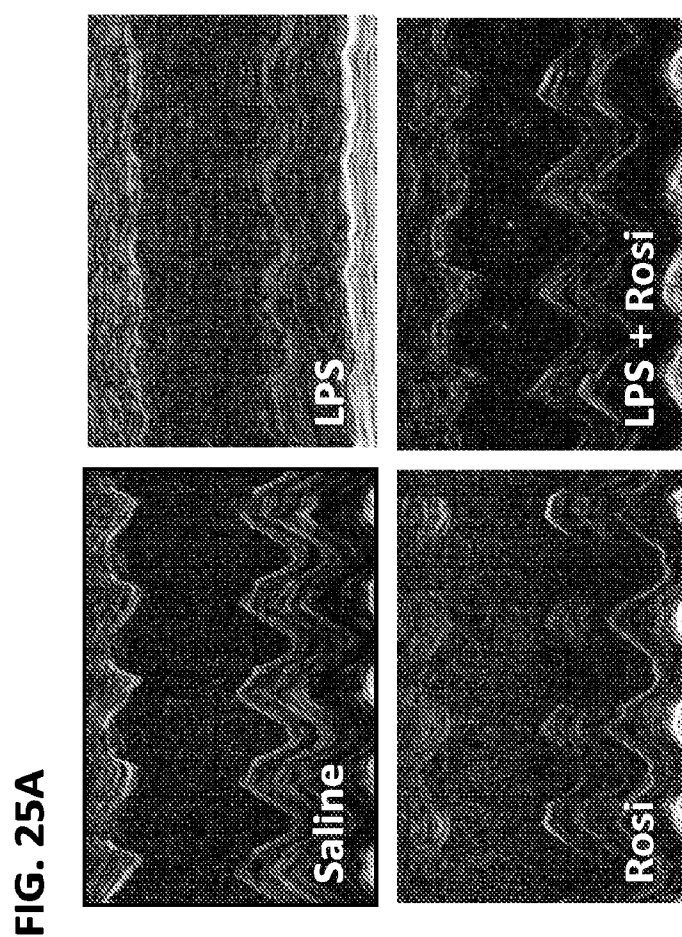


TNF α

FIG. 24I



** p<0.01



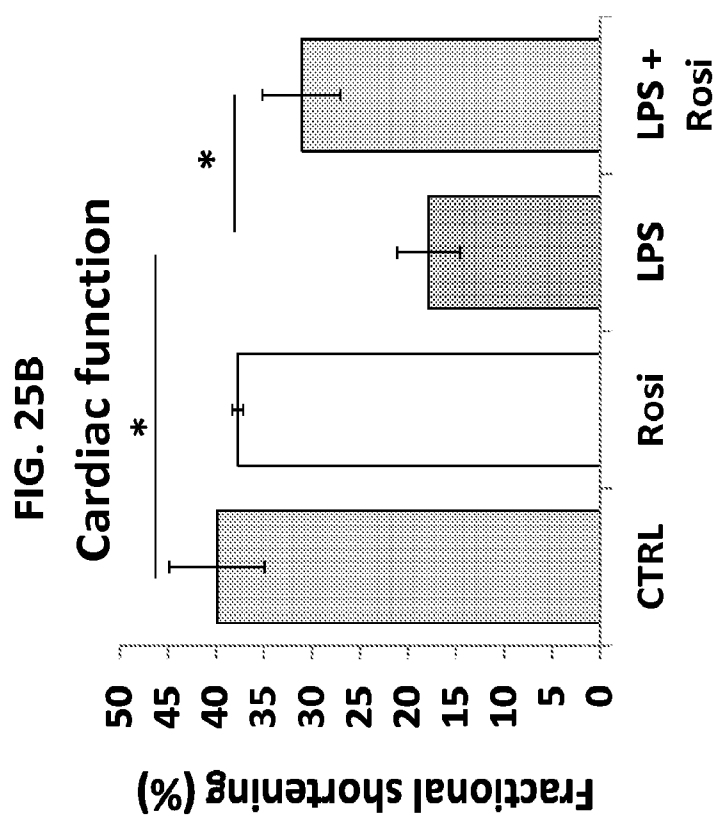


FIG. 25C

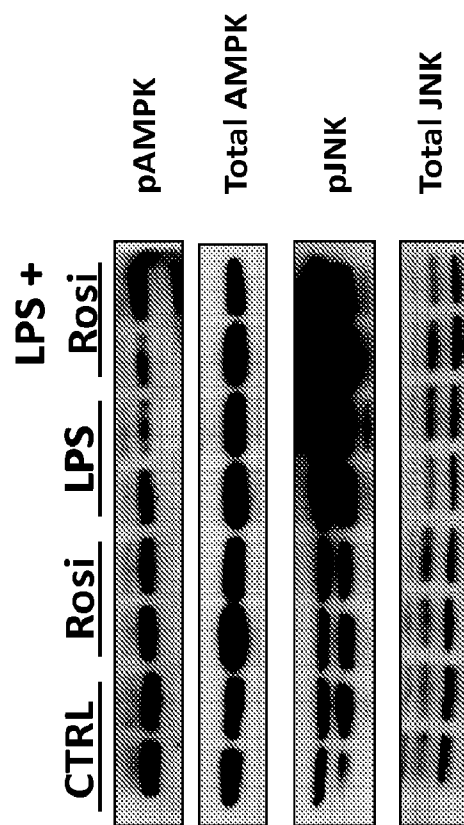


FIG. 25D

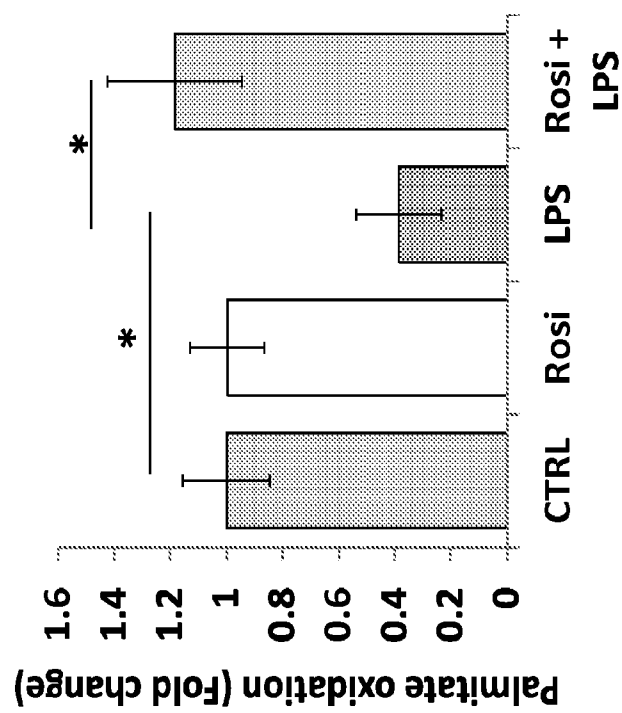


FIG. 25E



FIG. 25H

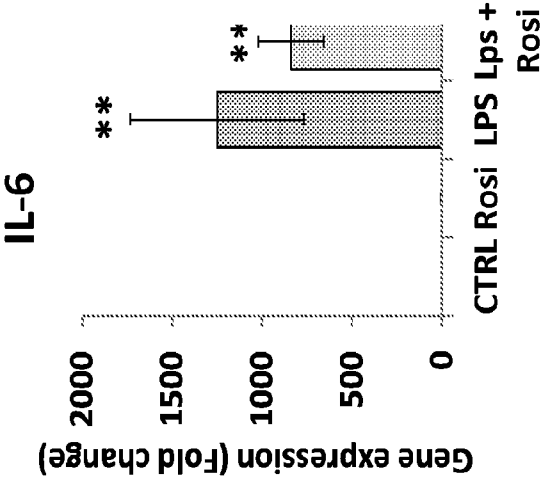


FIG. 25G

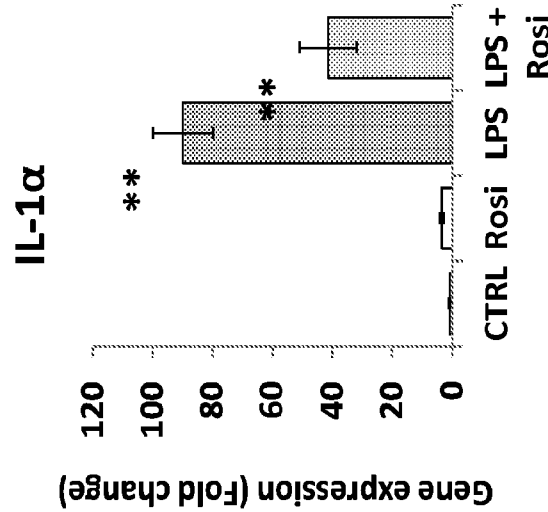
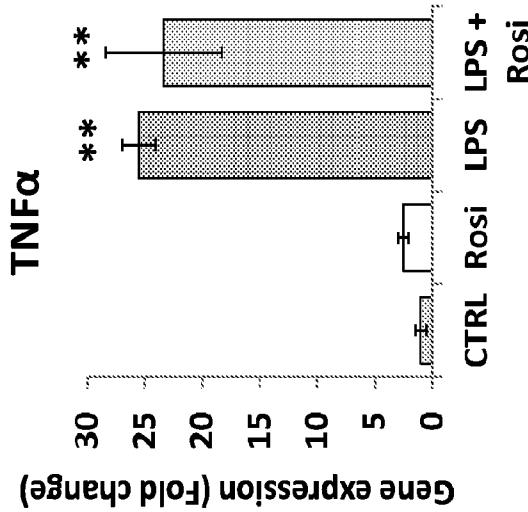
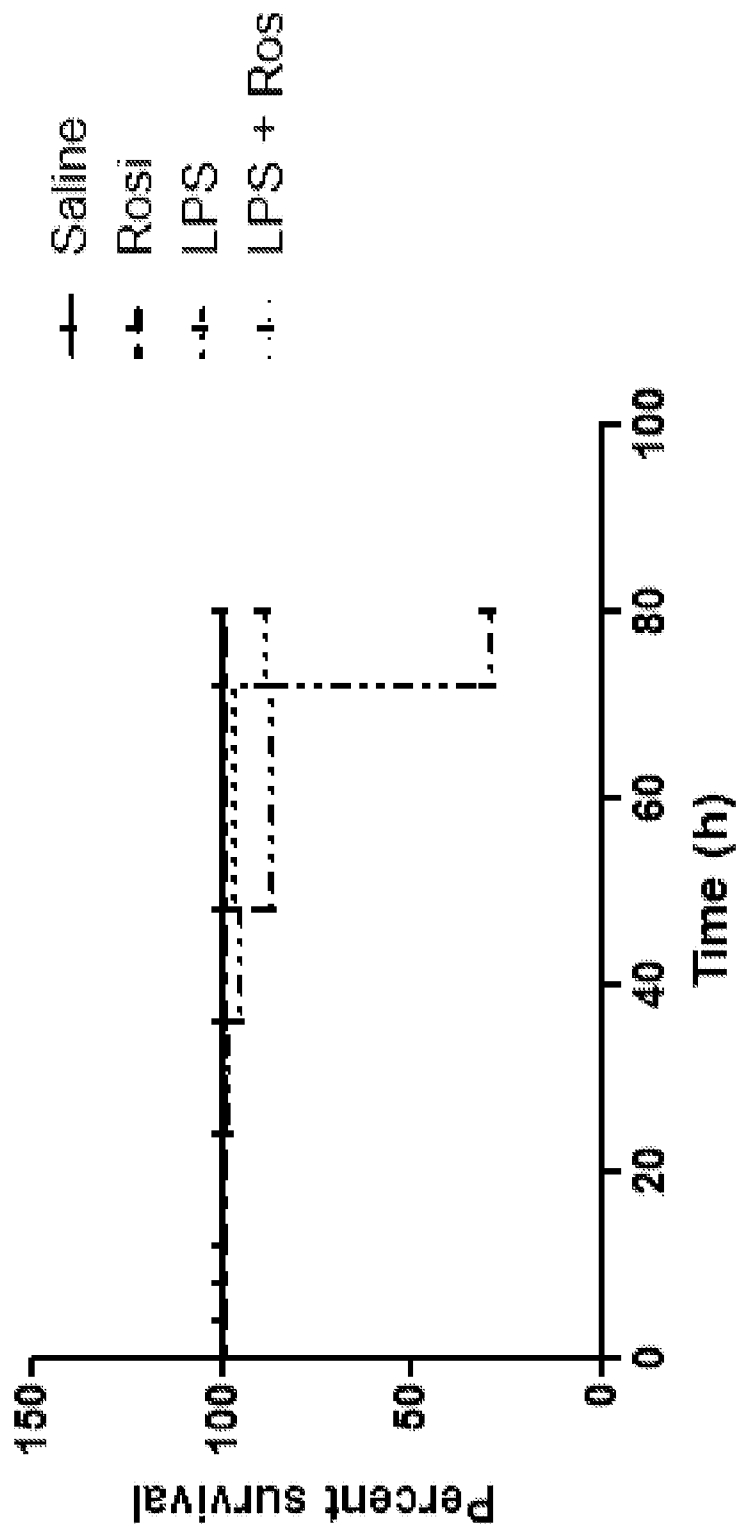


FIG. 25F



**p<0.01 vs non-LPS CTRL

FIG. 26



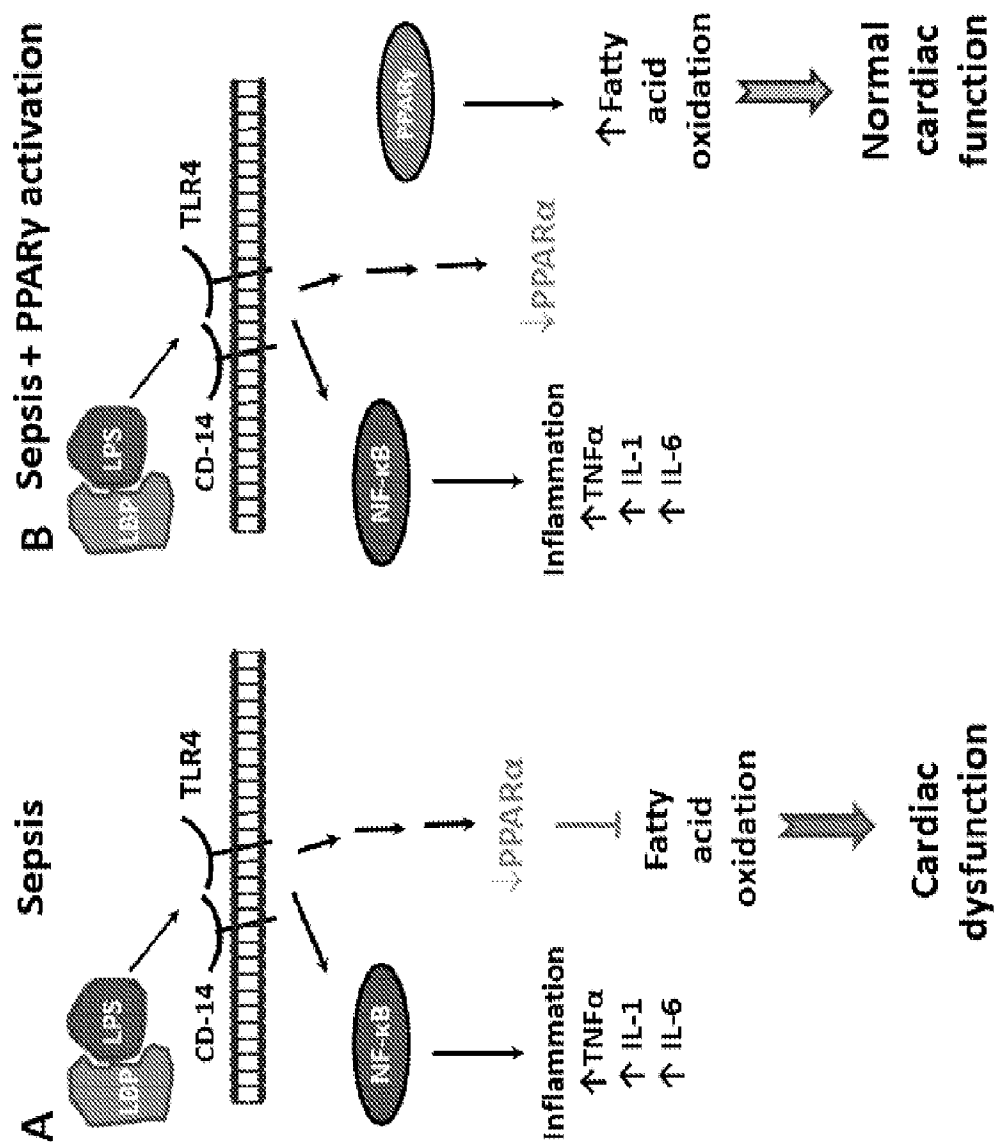


FIG. 27

