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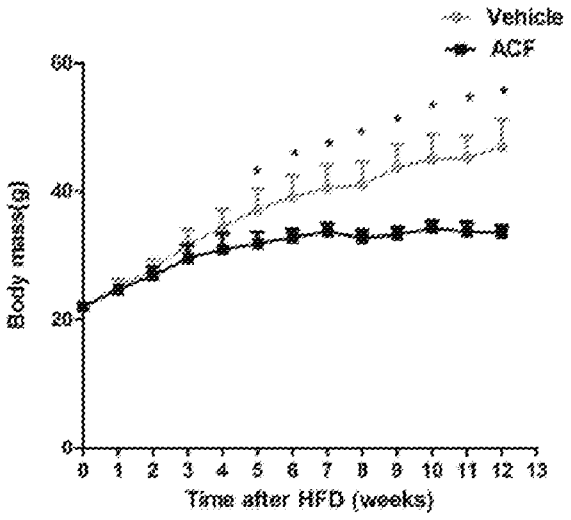
[Continued on next page]

(54) Title: METHODS FOR DECREASING BODY WEIGHT AND TREATING DIABETES

(57) Abstract: Disclosed herein are methods of treating diabetes (e.g., increasing glucose tolerance, reducing insulin resistance, and decreasing serum lipids) and/or reducing body weight (e.g., treating overweight or obesity) including administering a therapeutically effective amount of a composition including an inhibitor of hypoxia- inducible factor la (HIF1α) to a subject with diabetes or a subject in need of reduction of body weight. The HIF1α inhibitor can be administered in combination with a pharmaceutically acceptable carrier. In some examples, the HIF1α inhibitor is administered orally.

FIG. 14A



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METHODS FOR DECREASING BODY WEIGHT AND TREATING DIABETES

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 61/423,936, filed December 16, 2010, which is incorporated by reference herein in its entirety.

FIELD

This disclosure relates to methods for treating diabetes and reducing body weight, particularly using inhibitors of hypoxia-inducible factor 1α.

BACKGROUND

Obesity has been identified as a major risk factor for chronic diseases such as type 2 diabetes, cardiovascular disease, hepatosteatosis, and even cancer (Bluher, *Exp. Clin. Endocrinol. Diabetes* 117:241-250, 2009). Insulin resistance, defined as dysregulation of the insulin-signaling cascade in insulin target tissues, such as adipose tissue, skeletal muscle, and liver, is a hallmark of type 2 diabetes (Kahn and Flier, *J. Clin. Invest.* 106:473-481, 2000). Increasing evidence shows that obesity is strongly associated with insulin resistance (Kim, *Cell Metab.* 4:417-419, 2006).

During obesity, oxygen supply cannot meet the demand of expanding adipose, resulting in relative hypoxia within adipose tissue. This has been demonstrated by abundant pimonidazole expression (a marker of hypoxia), increased lactate production, and hypoperfusion in both obese human and animal models (Trayhurn *et al., Br. J. Nutr.* 100:227-235, 2008; Ye *et al., Am. J. Physiol. Endocrinol. Metab.* 293:E1118-1128, 2007). Hypoxia is observed in white adipose tissue of obese individuals (Hosogai *et al., Diabetes* 56:901-911, 2007). However, the role of hypoxia in adipose tissue during obesity and insulin resistance remains unclear.

Regulation of hypoxia-mediated responses is mainly dependent on members of the hypoxia inducible factor (HIF) family. HIFs are nuclear transcription factors and function as oxygen sensitive α and β subunit heterodimers. Three isoforms of

HIFα have been identified as HIF1α, HIF2α and HIF3α. Each requires a ubiquitously expressed subunit as an obligate heterodimerization partner, the aryl hydrocarbon nuclear translocator (ARNT; also called HIF1β) for transcriptional activation of target genes. HIF1α, HIF2α, HIF3α, and ARNT are all expressed in adipose tissue (Hatanaka *et al., Biol. Pharm. Bull.* 32:1166-1172, 2009; Shimba *et al., J. Biol. Chem.* 279:40946-40953, 2004; Ye, *Int. J. Obes. (Lond.)* 33:54-66, 2009).

SUMMARY

Disclosed herein are methods of treating diabetes (*e.g.,* increasing glucose tolerance, reducing insulin resistance, and decreasing serum lipids) and/or reducing body weight (*e.g.,* treating overweight or obesity). The methods include administering a therapeutically effective amount of a composition including an inhibitor of hypoxia-inducible factor 1α (HIF1α) to a subject with diabetes or a subject in need of reduction of body weight.

In some embodiments, administration of a therapeutically effective amount of a HIF1α inhibitor to a subject decreases body weight by decreasing total body weight, decreasing body mass index (BMI), decreasing waist circumference, decreasing total body fat, or a combination of two or more thereof, as compared to a control. In other embodiments, administration of a therapeutically effective amount of a HIF1α inhibitor to a subject treats diabetes in the subject by increasing glucose tolerance, decreasing insulin resistance, decreasing triglyceride levels, decreasing free fatty acid levels, decreasing hemoglobin A1c (HbA1c) levels, or a combination of two or more thereof, as compared to a control.

In some examples, the inhibitor of HIF1α decreases expression or half-life of HIF1α mRNA or protein (such as decreasing transcription and/or translation of HIF1α or increasing degradation of HIF1α mRNA and/or protein), activity of HIF1α protein, or a combination thereof relative to a control. In particular examples, the inhibitor is a small molecule (for example, acriflavine), a nucleic acid molecule, or an antibody. In some embodiments, the HIF1α inhibitor is targeted to

adipose tissue (such as white adipose tissue), for example using a peptide or antibody that specifically localizes to adipose tissue.

In some examples, the composition includes a HIF1α inhibitor and at least one pharmaceutically acceptable carrier. In further examples, the inhibitor of HIF1α is administered orally.

The foregoing and other features of the disclosure will become more apparent from the following detailed description, which proceeds with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A and 1B are bar graphs of qPCR analysis of *Arnt* mRNA expression in the indicated tissues or adipocytes from $Arnt^{F/F}$ and $Arnt^{\Delta Adipo}$ mice (FIG. 1A) or HIF1α mRNA expression in the indicated tissues or adipocytes from $Hif1\alpha^{F/F}$ and $Hif1\alpha^{\Delta Adipo}$ mice (FIG. 1B). WAT, white adipose tissue; BAT, brown adipose tissue. FIGS. 1C and 1D are bar graphs showing qPCR analysis of CYP1A1 mRNA induction by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) treatment in WAT (FIG. 1C) and BAT (FIG. 1D) of $Arnt^{F/F}$ or $Arnt^{\Delta Adipo}$ mice. For qPCR analysis the expression was normalized to β-actin and each bar represents the mean value ± S.D. *P<0.05, **P<0.01 compared to wild-type floxed littermates.

FIGS. 2A and 2B are graphs showing growth curves of $Arnt^{F/F}$ and $Arnt^{\Delta Adipo}$ mice (FIG. 2A) and $Hif1\alpha^{F/F}$ and $Hif1\alpha^{\Delta Adipo}$ mice (FIG. 2B) maintained on a chow diet or a high-fat diet (HFD). n=5 per group. FIGS. 2C and 2D are bar graphs showing fat mass, fat mass ratio determined using NMR, and adipocyte size in $Arnt^{F/F}$ and $Arnt^{\Delta Adipo}$ mice (FIG. 2C) or $Hif1\alpha^{F/F}$ and $Hif1\alpha^{\Delta Adipo}$ mice (FIG. 2D) after 12 weeks of HFD (n = 5 mice per group). FIG. 2E is a pair of bar graphs showing cumulative food intake adjusted to body weight and metabolic efficiency for 2 weeks in $Hif1\alpha^{F/F}$ and $Hif1\alpha^{\Delta Adipo}$ mice after 4-6 weeks of HFD. FIG. 2F is a pair of bar graphs showing resting and total O_2 consumption adjusted to body weight. FIG. 2G is a pair of bar graphs showing resting and total respiratory exchange ratio (RER; $vCO_2/vO2$). FIG. 2H is a pair of bar graphs showing ambulatory and total activity levels. Data shown in FIGS. 2E-H are from the same set of $Hif1\alpha^{F/F}$ and $Hif1\alpha^{\Delta Adipo}$ mice. Indirect calorimetry (FIGS. 2F and 2G) was

performed after 7 weeks of HFD at 24°C or 30°C (thermoneutrality) (n=6-8 mice per group). Data are mean ± SEM. *P<0.05; **P<0.01 compared to wild-type floxed littermates.

FIG. 3A-C is a series of bar graphs showing body weight (FIG. 3A), cumulative food intake (FIG. 3B), and metabolic efficiency (FIG. 3C) for 2 weeks in 6 to 8 week old male mice maintained on a chow diet. FIG. 3D is a pair of bar graphs showing body weight and resting O_2 consumption in 9-week old mice before and after a 4 day HFD. Experiments shown in FIGS. 3A-D were performed in the same set of mice (n=5 mice per group). FIG. 3E is a bar graph showing cumulative food intake for 2 weeks in mice fed HFD from 4-6 weeks. FIG. 3F is a pair of bar graphs showing resting and total O_2 consumption in mice after 7 weeks of high fat diet (same mice as data shown in FIG. 3E). Data are mean ± SEM.

FIG. 4A is a pair of graphs showing glucose tolerance test (GTT) in 18-week old $Arnt^{F/F}$ and $Arnt^{\Delta Adipo}$ mice on chow diet or mice fed HFD for 11 weeks (n=5 mice per group). FIG. 4B is a pair of graphs showing insulin tolerance test (ITT) in 18-week old $Arnt^{F/F}$ and $Arnt^{\Delta Adipo}$ mice on chow diet or mice fed HFD for 12 weeks (n=5 mice per group). Data are mean ± SD. *P<0.05 compared to $Arnt^{\Delta Adipo}$ littermates.

FIG. 5A is a graph showing GTT in $Hif1\alpha^{F/F}$ and $Hif1\alpha^{\Delta Adipo}$ mice fed HFD for 11 weeks (n=5 mice per group). FIG. 5B is a graph showing ITT in $Hif1\alpha^{F/F}$ and $Hif1\alpha^{\Delta Adipo}$ mice fed HFD for 12 weeks (n=5 mice per group). Data are mean ± SD. *P<0.05 compared to $Hif1\alpha^{\Delta Adipo}$ littermates. FIG. 5C is a graph showing time courses of blood glucose during the hyperinsulinemic-euglycemic clamp. FIG. 5D is a bar graph showing plasma glucose in the basal state and during the clamp. FIG. 5E is a bar graph showing plasma insulin levels in the basal state and during the clamp. FIG. 5F is a graph showing glucose infusion rate during the clamp. FIG. 5G is a bar graph showing basal and clamp endogenous glucose production (EGP), glucose infusion rate (GIR), whole body glucose disposal (Rd), and glucose uptake in skeletal muscle, WAT, and BAT. The hyperinsulinemic-euglycemic clamp was performed after 15 weeks of HFD (n=6/group). Data are mean ± SEM. *P<0.05 compared to floxed littermates.

FIGS. 6A and 6B are a series of graphs showing body mass, GTT, and ITT of *Hif1α*$^{F/F}$ and *Hif1α*$^{\Delta Adipo}$ mice fed HFD for 4 weeks (FIG. 6A) or 8 weeks (FIG. 6B). *P<0.05 compared to *Hif1α*$^{\Delta Adipo}$ littermates. FIG. 6C is a series of graphs showing body mass, fasted glucose, fasted serum insulin levels, and HOMA index of *Hif1α*$^{F/F}$ and *Hif1α*$^{\Delta Adipo}$ mice after 6 weeks on a HFD. FIG. 6D is a series of graphs showing serum total adiponectin levels, high molecular weight (HMW) adiponectin, and the ratio of HMW to total adiponectin of *Hif1α*$^{F/F}$ and *Hif1α*$^{\Delta Adipo}$ mice after 6 weeks on a HFD. Data are mean ± SD. *P<0.05, **P<0.01 compared to *Hif1α*$^{F/F}$ littermates. (n =5 mice per group).

FIG. 7A is a graph showing gene expression in WAT in wild-type C57BL/6 mice fed chow or HFD for 12 weeks. *P<0.05, **P<0.01 compared to chow-fed mice. FIG. 7B is a graph showing gene expression in WAT of 10-week old *Hif1α*$^{F/F}$ and *Hif1α*$^{\Delta Adipo}$ mice on a chow diet. *P<0.05, **P<0.01 compared to *Hif1α*$^{F/F}$ littermates (n=5 mice per group). For qPCR analysis, expression was normalized to β-actin. Data are mean ± SD.

FIG. 8A and B are a pair of bar graphs showing qPCR analysis of gene expression in WAT of *Arnt*$^{F/F}$ and *Arnt*$^{\Delta Adipo}$ (FIG. 8A) or *Hif1α*$^{F/F}$ and *Hif1α*$^{\Delta Adipo}$ mice (FIG. 8B). FIG. 8C is a series of bar graphs showing total adiponectin, HMW adiponectin, and HMW/total adiponectin ratio in *Arnt*$^{F/F}$ and *Arnt*$^{\Delta Adipo}$ mice (top) and *Hif1α*$^{F/F}$ and *Hif1α*$^{\Delta Adipo}$ mice (bottom) after 12 weeks of HFD. FIG. 8D is a pair of bar graphs showing qPCR analysis of fibrosis-related gene expression in WAT of *Arnt*$^{F/F}$ and *Arnt*$^{\Delta Adipo}$ mice (top) and *Hif1α*$^{F/F}$ and *Hif1α*$^{\Delta Adipo}$ mice (bottom). FIG. 8E is a pair of bar graphs showing *Socs3* expression determined by qPCR in WAT of *Arnt*$^{F/F}$ and *Arnt*$^{\Delta Adipo}$ mice (top) and *Hif1α*$^{F/F}$ and *Hif1α*$^{\Delta Adipo}$ mice (bottom). FIG. 8F is a pair of bar graphs showing quantitation of SOCS3 expression and STAT3 phosphorylation. Relative protein levels were normalized to levels for *Hif1α*$^{F/F}$ littermates. For qPCR analysis, expression was normalized to β-actin. Data are mean ± S.D. *P<0.05, **P<0.01 compared to wild-type floxed littermates.

FIG. 9A is a bar graph showing qPCR analysis of F4/80 and CD68 mRNA in adipose tissue in *Arnt*$^{F/F}$ and *Arnt*$^{\Delta Adipo}$ mice after 12 weeks of HFD. FIG. 9B a bar graph showing qPCR analysis of F4/80 and CD68 mRNA in adipose tissue in *Hif1α*$^{F/F}$ and *HIF1α*$^{\Delta Adipo}$ mice after 12 weeks of HFD. For each, the expression was

normalized to β-actin and each bar represents the mean value ± S.D. **P<0.01 compared to wild-type floxed littermates.

FIG. 10A is a bar graph showing qPCR analysis of *Arnt* mRNA expression in peritoneal macrophages (P-Mϕ) from $Arnt^{F/F}$ and $Arnt^{\Delta Adipo}$ mice. FIGS. 10B and 10C are a pair of bar graphs showing qPCR analysis of HIF1α, SOCS3, and adiponectin mRNA expression in adipocytes (FIG. 10B) and SVF-Mϕ (FIG. 10C) of $Hif1\alpha^{F/F}$ and $HIF1\alpha^{\Delta Adipo}$ mice after 12 weeks on HFD. The expression was normalized to β-actin and each bar represents the mean value ± S.D. **P<0.01 compared to wild-type floxed littermates.

FIG. 11A is a pair of bar graphs showing SOCS3 mRNA in 3T3-L1 adipocytes after hypoxia (1% O_2) and hypoxia mimicking reagent 100 μM $CoCl_2$ treatment. FIG. 11B is a series of bar graphs showing ARNT, SOCS3, and HIF1α expression. 3T3-L1 adipocytes were transfected with scrambled siRNA, ARNT siRNA, or HIF1α siRNA. After 24 hours, cells were incubated with 100 μM $CoCl_2$. For qPCR analysis, the expression was normalized to β-actin and each bar represents the mean value ± S.D. **P<0.01 compared to control, ##P<0.01 compared to $CoCl_2$ (100 μM) treatment. FIG. 11C is a bar graph showing the of 3T3-L1 adipocytes transiently transfected with the *Socs3* promoter luciferase construct and co-transfected with empty vector, HIF1α or HIF2α expression plasmids. **P<0.01 compared to control. FIG. 11D is a pair of digital images of ChIP assays of the *Socs3* promoter in 3T3-L1 cells treated with hypoxia for 8 hours using primers flanking HRE1 (left panel) or HRE2 and 3 (right panel).

FIG. 12A is a pair of bar graphs showing the effect of SOCS3 siRNA on expression of *Socs3* (left) and *adiponectin* (right) in 3T3-L1 adipocytes. FIG. 12B is a bar graph of adiponectin concentrations in medium of 3T3-L1 adipocytes transfected with scrambled siRNA or Socs3 siRNA. After 24 hours, cells were incubated for another 24 hours with 100 μM $CoCl_2$. FIG. 12C is a digital image of a Western blot showing STAT3 phosphorylation in 3T3-L1 adipocytes with the indicated treatment. FIG. 12D is a bar graph showing expression of adiponectin mRNA in 3T3-L1 cells treated with the indicated siRNA and the STAT3 inhibitor NSC 74859 (100 μM) in hypoxia conditions by 100 μM $CoCl_2$. For qPCR analysis, the expression was normalized to β-actin and each bar represents the mean value ±

S.D. **P<0.01 compared to control (scramble siRNA), #P<0.05 compared to scrambled siRNA + $CoCl_2$ (100 μM) or *Hif1α* siRNA treatment.

FIGS. 13A and B are a series of graphs showing body mass (FIG. 13A) and total fat mass and fat mass ratio (FIG. 13B) in mice treated with 12 weeks HFD and vehicle or NSC 74859 treatment for 6 weeks. FIGS. 13C and D show GTT (FIG. 13C), and ITT (FIG. 13D) in mice treated with 11 weeks HFD and vehicle or NSC 74859 treatment for 5 weeks. FIG. 13E is a series of graphs showing fasted glucose, fasted serum insulin levels, and HOMA index. FIG. 13F is a series of graphs showing serum total adiponectin, HMW adiponectin, and HMW/total adiponectin ratio. FIG. 13G is a digital image of Western blot analysis of STAT3 phosphorylation of WAT. FIGS. 13D-G show data from mice after 12 weeks HFD and NSC 74859 treatment for 6 weeks. Data are mean ± SD. *P<0.05 and ** P<0.01 compared to vehicle treated mice (n=5 mice per group).

FIG. 14A is a graph showing growth curves of vehicle and ACF treated mice on HFD. FIG. 14B is a pair of bar graphs showing fat mass and fat mass ratio of vehicle and ACF treated mice on HFD for 12 weeks. FIG. 14C is a pair of graphs showing GTT (left) of vehicle and ACF treated mice on HFD for 11 weeks and ITT (right) of vehicle and ACF treated mice on HFD for 12 weeks. *P<0.05 compared to ACF-treated mice. FIG. 14D is a series of bar graphs showing fasted glucose, fasted serum insulin levels, and HOMA index of vehicle and ACF treated mice on HFD for 12 weeks. FIG. 14E is a series of graphs showing serum total adiponectin, HMW adiponectin, and HMW/total adiponectin ratio of vehicle and ACF treated mice on HFD for 12 weeks. FIG. 14F is a bar graph showing qPCR analysis of gene expression in WAT of vehicle and ACF treated mice on HFD for 12 weeks. The expression was normalized to β-actin. Data are mean ± SD. *P<0.05, **P<0.01 compared to vehicle treated mice. n=5 mice per group.

FIG. 15A is a bar graph showing the effect of ACF (5 μM), $CoCl_2$, or ACF plus $CoCl_2$ on expression of SOCS3 mRNA in 3T3-L1 adipocytes. FIG. 15B is a pair of graphs showing GTT after 4 and 8 weeks of HFD. FIG. 15C is a pair of graphs showing ITT after 5 and 9 weeks of HFD. FIG. 15D is a series of graphs showing fasted glucose, fasted serum insulin, and HOMA index in vehicle and ACF treated mice after 6 weeks of HFD. FIG. 15E is a series of graphs showing total

adiponectin, HMW adiponectin and HMW/total adiponectin ratio in vehicle and ACF treated mice after 6 weeks (upper) or 9 weeks (lower) HFD. Data are mean ± SD. *P<0.05, **P<0.01 compared to vehicle-treated mice. (n=5/group).

FIG. 16A is a graph showing typical growth curves of ACF-treated *Hif1α*$^{F/F}$ (left panel) and *Hif1α*$^{\Delta Adipo}$ (right panel) mice maintained on high-fat diet (HFD). FIG. 16B is a pair of bar graphs showing body composition determined by NMR to show the fat mass and fat mass ratio in ACF-treated *Hif1α*$^{F/F}$ (left panel) and *Hif1α*$^{\Delta Adipo}$ (right panel) mice 16 weeks after ACF treatment (n = 5/group). *P<0.05, **P<0.01 compared to vehicle-treated mice.

FIG. 17A is a graph showing blood glucose levels in 2-hour glucose tolerance test (GTT, top) and the area under the curve (AUC; bottom) 11 weeks after ACF treatment. FIG. 17B is a graph showing insulin tolerance test (ITT) 16 weeks after ACF treatment (n =5/group). FIGS. 17C and 17D are graphs showing fasted glucose, fasted serum insulin levels and HOMA index of ACF-treated *Hif1α*$^{F/F}$ and *Hif1α*$^{\Delta Adipo}$ mice, respectively, 16 weeks after ACF treatment. (n =5/group). *P<0.05, **P<0.01 compared to vehicle-treated mice; #P<0.05 compared to ACF-treated mice at the same genotyping mice.

FIGS. 18A-B are a series of graphs showing fasted serum triglyceride, cholesterol, and FFA levels of ACF-treated *Hif1α*$^{F/F}$ and *Hif1α*$^{\Delta Adipo}$ mice, respectively, after 16 weeks of ACF treatment. *P<0.05, **P<0.01 compared to vehicle-treated mice.

FIGS. 19A-B are a series of graphs showing total adiponectin levels, HMW adiponectin, and the ratio of HMW to total adiponectin of ACF-treated *Hif1α*$^{F/F}$ and *Hif1α*$^{\Delta Adipo}$ mice, respectively, after 16 weeks of ACF treatment. *P<0.05 compared to vehicle-treated mice.

SEQUENCE LISTING

Any nucleic acid and amino acid sequences listed herein or in the accompanying Sequence Listing are shown using standard letter abbreviations for nucleotide bases, and three letter code for amino acids, as defined in 37 C.F.R. § 1.822. In at least some cases, only one strand of each nucleic acid sequence is

shown, but the complementary strand is understood as included by any reference to the displayed strand.

The Sequence Listing is submitted as an ASCII text file in the form of the file named Sequence_Listing.txt, which was created on December 1, 2011, and is 16,823 bytes, which is incorporated by reference herein.

SEQ ID NOs: 1-4 are primers for chromatin immunoprecipitation assays.

SEQ ID NOs: 5-7 are primers for SOCS3 luciferase assays.

SEQ ID NOs: 8-13 are RT-PCR primers.

SEQ ID NOs: 14-67 are qPCR primers.

SEQ ID NOs: 68-73 are siRNAs.

SEQ ID NOs: 74-76 are exemplary white adipose tissue targeting peptides.

DETAILED DESCRIPTION

I. Abbreviations

ACF	acriflavine
Arnt	aryl hydrocarbon receptor nuclear translocator
AUC	area under the curve
BAT	brown adipose tissue
BMI	body mass index
EGP	endogenous glucose production
FPG	fasting plasma glucose
FSIVGTT	frequently sampled intravenous glucose tolerance test
GIR	glucose infusion rate
GTT	glucose tolerance test
HFD	high fat diet
HIF1α	hypoxia-inducible factor 1α
HMW	high molecular weight
HOMA	homeostatic model assessment
HRE	hypoxia response element
IGT	impaired glucose tolerance
ITT	insulin tolerance test
Mϕ	macrophage
OGTT	oral glucose tolerance test
qPCR	quantitative real-time PCR
QUICKI	quantitative insulin sensitivity check index
Rd	whole body glucose disposal
RER	respiratory exchange ratio
WAT	white adipose tissue

II. Terms

Unless otherwise noted, technical terms are used according to conventional usage. Definitions of common terms in molecular biology may be found in Benjamin Lewin, *Genes V*, published by Oxford University Press, 1994 (ISBN 0-19-854287-9); Kendrew *et al.* (eds.), *The Encyclopedia of Molecular Biology*, published by Blackwell Science Ltd., 1994 (ISBN 0-632-02182-9); and Robert A. Meyers (ed.), *Molecular Biology and Biotechnology: a Comprehensive Desk Reference*, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8).

Unless otherwise explained, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The singular terms "a," "an," and "the" include plural referents unless context clearly indicates otherwise. Similarly, the word "or" is intended to include "and" unless the context clearly indicates otherwise. Hence "comprising A or B" means including A, or B, or A and B. It is further to be understood that all base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or polypeptides are approximate, and are provided for description. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. All GenBank Accession Nos. mentioned herein are incorporated by reference in their entirety as present in GenBank on December 16, 2010. In case of conflict, the present specification, including explanations of terms, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

In order to facilitate review of the various embodiments of the invention, the following explanations of specific terms are provided:

Adiponectin: Also known as adiponectin, C1Q and collagen domain containing; 30 kDa adipocyte complement-related protein; adipose specific collagen-like factor; gelatin-binding protein 28 (*e.g.,* GenBank Gene ID No. 9370). Adiponectin is expressed in adipose tissue. Receptors for adiponectin are found primarily in skeletal muscle and liver and adiponectin improves insulin sensitivity

by increasing energy expenditure, fatty acid oxidation, and expression of PPARα target genes (Rasouli and Kern, *J. Clin. Endocrinol. Metab.* 93:S64-S73, 2008).

Adiponectin nucleic acid and protein sequences are publicly available. For example, GenBank Accession Nos. NM_004797 and NM_001177800 disclose exemplary human adiponectin nucleic acid sequences and GenBank Accession Nos. NP_004788 and NP_00117127 disclose exemplary human adiponectin protein sequences, each of which is incorporated herein by reference as present in GenBank on December 16, 2010.

Body mass index (BMI): A mathematical formula for measuring body mass in humans, also sometimes called Quetelet's Index. BMI is calculated by dividing weight (in kg) by height^2 (in meters^2). The current standards for both men and women accepted as "normal" are a BMI of 20-24.9 kg/m^2. In one embodiment, a BMI of greater than 25 kg/m^2 can be used to identify an obese subject. Grade I obesity (also called "overweight") corresponds to a BMI of 25-29.9 kg/m^2. Grade II obesity corresponds to a BMI of 30-40 kg/m^2; and Grade III obesity corresponds to a BMI greater than 40 kg/m^2 (Jequier, *Am. J Clin. Nutr.,* 45:1035-47, 1987). Ideal body weight will vary among individuals based on height, body build, bone structure, and sex.

Control: A "control" refers to a sample or standard used for comparison with an experimental sample. In some embodiments, the control is a sample obtained from a healthy patient. In other embodiments, the control is a historical control or standard reference value or range of values (such as a previously tested sample, subject, or group of samples or subjects).

Diabetes mellitus: A disease caused by a relative or absolute lack of insulin leading to uncontrolled carbohydrate metabolism, commonly simplified to "diabetes," though diabetes mellitus should not be confused with diabetes insipidus. As used herein, "diabetes" refers to diabetes mellitus, unless otherwise indicated. A "diabetic condition" includes pre-diabetes and diabetes. Type 1 diabetes (sometimes referred to as "insulin-dependent diabetes" or "juvenile-onset diabetes") is an auto-immune disease characterized by destruction of the pancreatic β cells that leads to a total or near total lack of insulin. In type 2 diabetes (T2DM; sometimes referred to

as "non-insulin-dependent diabetes" or "adult-onset diabetes"), the body does not respond to insulin, though it is present.

Symptoms of diabetes include: excessive thirst (polydipsia); frequent urination (polyuria); extreme hunger or constant eating (polyphagia); unexplained weight loss; presence of glucose in the urine (glycosuria); tiredness or fatigue; changes in vision; numbness or tingling in the extremities (hands, feet); slow-healing wounds or sores; and abnormally high frequency of infection. Diabetes may be clinically diagnosed by a fasting plasma glucose (FPG) concentration of greater than or equal to 7.0 mmol/L (126 mg/dL), or a plasma glucose concentration of greater than or equal to 11.1 mmol/L (200 mg/dL) at about two hours after an oral glucose tolerance test (OGTT) with a 75 g load. A more detailed description of diabetes may be found in *Cecil Textbook of Medicine*, J.B. Wyngaarden, *et al.*, eds. (W.B. Saunders Co., Philadelphia, 1992, 19th ed.).

Hypoxia-inducible factor 1 (HIF1): A transcription factor found in mammalian cells cultured under reduced oxygen tension (hypoxia) that plays a role in cellular and systemic response to hypoxia. HIF1 is a heterodimer composed of an alpha subunit and a beta subunit. The beta subunit is the aryl hydrocarbon receptor nuclear translocator (Arnt; also known as HIF1β). Arnt is constitutively present in the cell nucleus. There are three HIF alpha subunits, HIF1α, HIF2α (also known an EPAS1), and HIF3α, which accumulate in hypoxic conditions. Under normoxic conditions, HIF alpha subunits are hydroxylated by a prolyl hydroxylase and targeted for proteasome dependent degradation. The prolyl hydroxylase is inhibited in hypoxia, leading to accumulation of HIF alpha subunits. HIF alpha subunits dimerize with Arnt to form a functional transcription factor capable of binding DNA at hypoxia response elements (HRE) and transcriptional activation.

HIF1 nucleic acid and protein sequences are publicly available. For example, GenBank Accession Nos. NM_001530 and NM_181054 disclose exemplary human HIF1α nucleic acid sequences and GenBank Accession Nos. NP_001521 and NP_851397 disclose exemplary human HIF1α protein sequences. GenBank Accession No. NM_001430 discloses an exemplary human HIF2α nucleic acid sequence and GenBank Accession No. NP_001421 discloses an exemplary human HIF2α protein sequence. GenBank Accession Nos. NM_152795,

NM_022462, and NM_152794 disclose exemplary human HIF3α nucleic acid sequences and GenBank Accession Nos. NP_690008, NP_071907, and NP_690007 disclose exemplary human HIF3α protein sequences. GenBank Accession Nos. NM_001668 and NM_178427 disclose exemplary human Arnt nucleic acid sequences and GenBank Accession Nos. NP_001659 and NP_848514 disclose exemplary human Arnt protein sequences. Each of these sequences is incorporated by reference herein, as present in GenBank on December 16, 2010.

Inhibitor: Any chemical compound, nucleic acid molecule, or peptide (such as an antibody), specific for a gene product that can reduce activity of a gene product or directly interfere with expression of a gene, such as genes that encode HIF1, such as HIF1α. An inhibitor of the disclosure, for example, can inhibit the activity of a HIF1α protein either directly or indirectly. Direct inhibition can be accomplished, for example, by binding to a HIF1α protein and thereby preventing the protein from binding an intended target, such as a dimerization partner (*e.g.,* Arnt) or a DNA sequence (such as a HRE). Indirect inhibition can be accomplished, for example, by binding to a HIF1α protein intended target, such as a receptor or binding partner, thereby blocking or reducing activity of HIF1α. Furthermore, an inhibitor of the disclosure can inhibit a HIF1α gene by reducing or inhibiting expression of the gene, inter alia by interfering with gene expression (transcription, processing, translation, post-translational modification), for example, by interfering with the mRNA and blocking translation of the HIF1α gene product or by altering post-translational modification of a HIF1α gene product (such as prolyl hydroxylation), or by causing changes in intracellular localization.

Insulin resistance: A state in which the cells of a subject do not respond appropriately to insulin, and increased amounts of insulin are required for glucose to be taken up by the cells. In some examples, insulin resistance is defined as a state where 200 units of insulin per day or more are required to attain glycemic control and prevent ketosis. Subjects with insulin resistance often have increased plasma glucose levels, increased plasma insulin levels, or both, as compared with a subject without insulin resistance or standard normal ranges.

In some examples, insulin resistance is determined by measuring blood glucose (such as fasting plasma glucose) and/or blood insulin (such as fasting plasma insulin) levels. In other examples, insulin resistance is determined by oral glucose tolerance test, glucose clamp (such as hyperinsulinemic euglycemic clamp), modified insulin suppression test, homeostatic model assessment, or quantitative insulin sensitivity check index (QUICKI).

Obesity: A condition in which excess body fat may put a person at health risk (see Barlow and Dietz, *Pediatrics* 102: E29, 1998; National Institutes of Health, *Obes. Res.* 6 (suppl. 2):51S-209S, 1998). Excess body fat is a result of an imbalance of energy intake and energy expenditure. In one embodiment in humans, the Body Mass Index (BMI) is used to assess obesity. In one embodiment, a BMI of 25.0 kg/m^2 to 29.9 kg/m^2 is overweight (also called grade I obesity), while a BMI of 30 kg/m^2 or more is truly obese (also called grade II obesity). In another embodiment in humans, waist circumference is used to assess obesity. In this embodiment, in men a waist circumference of 102 cm or more is considered obese, while in women a waist circumference of 89 cm or more is considered obese.

Strong evidence shows that obesity affects both the morbidity and mortality of individuals. For example, an obese individual is at increased risk for heart disease, non-insulin dependent (type 2) diabetes, hypertension, stroke, cancer (*e.g.* endometrial, breast, prostate, and colon cancer), dyslipidemia, gall bladder disease, sleep apnea, reduced fertility, and osteoarthritis, amongst others (see Lyznicki *et al., Am. Fam. Phys.* 63:2185, 2001).

Pharmaceutically acceptable carrier: The pharmaceutically acceptable carriers useful in this disclosure are conventional. *Remington: The Science and Practice of Pharmacy*, The University of the Sciences in Philadelphia, Editor, Lippincott, Williams, & Wilkins, Philadelphia, PA, 21st Edition (2005), describes compositions and formulations suitable for pharmaceutical delivery of compounds, such as an inhibitor of HIF1α (for example, acriflavine).

In general, the nature of the carrier will depend on the particular mode of administration employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose,

glycerol, or the like as a vehicle. For solid compositions (*e.g.*, powder, pill, tablet, or capsule forms), conventional non-toxic solid carriers can include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition to biologically-neutral carriers, pharmaceutical compositions to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, pH buffering agents, or the like, for example sodium acetate or sorbitan monolaurate.

Purified: The term purified does not require absolute purity; rather, it is intended as a relative term. Thus, for example, a purified preparation of a HIF1α inhibitor is one in which the HIF1α inhibitor is more enriched than in its environment within a cell or other preparation, such as the environment in which it is synthesized. Preferably, a preparation is purified such that the HIF1α inhibitor represents at least 50% of the total content of the preparation, for example, at least 50% by weight. In one embodiment, the HIF1α inhibitor is at least 50%, for example at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or more free of proteins, lipids, carbohydrates or other materials with which it is originally associated.

RNA interference (RNAi): Refers to a cellular process that inhibits expression of genes, including cellular and viral genes. RNAi is a form of antisense-mediated gene silencing involving the introduction of double stranded RNA-like oligonucleotides leading to the sequence-specific reduction of RNA transcripts. Double-stranded RNA molecules that inhibit gene expression through the RNAi pathway include siRNAs, miRNAs, and shRNAs.

Short (or Small) Interfering Nucleotide Sequence (siRNA): A nucleotide sequence capable of interfering with gene expression, for instance by inducing gene-specific inhibition of expression. Typically, the sequence of a siRNA is substantially identical to a portion of a transcript of a target gene (mRNA) for which interference or inhibition of expression is desired. For example, small, double stranded RNAs of about 15 to about 40 nucleotides in length (the length of each of the individual strands of the dsRNA), such as about 15 to about 25 nucleotides in length (for example, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides), that interfere with, or inhibit, expression of a target sequence.

The RNA backbone and/or component nucleotides can be unmodified or modified. For instance, the dsRNA can contain one or more deoxynucleic acids. Synthetic small dsRNAs may be used to induce gene-specific inhibition of expression. The dsRNAs can be formed from complementary single stranded RNAs ("ssRNAs") or from a ssRNA that forms a hairpin or from expression from a DNA vector. In certain examples, these small interfering nucleotide sequences have 3′ and/or 5′ overhangs on each strand of the duplex. These overhangs can be 0 nucleotides (that is, blunt ends) to 5 nucleotides in length.

Such siRNA molecules can be used as reverse genetic and therapeutic tools in mammalian cells, including human cells, both *in vitro* and *in vivo*. These small interfering nucleotide sequences are suitable for interference or inhibition of expression of a target gene wherein the sequence of the small interfering nucleotide sequence is substantially identical to a portion of an mRNA or transcript of the target gene for which interference or inhibition of expression is desired.

In addition to native nucleotide molecules, nucleotides suitable for inhibiting or interfering with the expression of a target sequence include nucleotide derivatives and analogs. For example, a non-natural linkage between nucleotide residues can be used, such as a phosphorothioate linkage. The nucleotide strand can be derivatized with a reactive functional group or a reporter group, such as a fluorophore. For example, the 2'-hydroxyl at the 3' terminus can be readily and selectively derivatized with a variety of groups. Other useful nucleotide derivatives incorporate nucleotides having modified carbohydrate moieties, such as 2'-O-alkylated residues or 2'-deoxy-2'-halogenated derivatives. Particular examples of such carbohydrate moieties include 2'-O-methyl ribosyl derivatives and 2'-O-fluoro ribosyl derivatives.

The nucleotide bases can be modified. Any modified base useful for inhibiting or interfering with the expression of a target sequence can be used. For example, halogenated bases, such as 5-bromouracil and 5-iodouracil can be incorporated. The bases may also be alkylated, for example, 7-methylguanosine may be incorporated in place of a guanosine residue. Non-natural bases that yield successful inhibition can also be incorporated.

Subject: A living multi-cellular vertebrate organism, a category that includes both human and non-human mammals.

Therapeutically effective amount: An amount or dose sufficient to prevent advancement, or to cause regression of a disease or syndrome or is capable of relieving symptoms of a disease or syndrome (such as diabetes, obesity, or insulin resistance).

Treatment: Refers to both prophylactic inhibition of initial disease or syndrome, and therapeutic interventions to alter the natural course of a disease process or syndrome, such as diabetes, obesity, or insulin resistance.

III. Methods of Reducing Body Weight and Treating Diabetes

Disclosed herein are methods for decreasing body weight of a subject (for example an overweight or obese subject) utilizing an inhibitor of HIF1α. Also disclosed are methods for treating diabetes in a subject (for example, type 2 diabetes) utilizing an inhibitor of HIF1α. In some examples, the methods include administering a therapeutically effective amount of a composition including a HIF1α inhibitor (such as acriflavine) to a subject, thereby decreasing body weight of the subject. In other examples, the methods include administering a therapeutically effective amount of a composition including a HIF1α inhibitor (such as acriflavine) to a subject having diabetes, thereby treating diabetes in the subject.

In particular examples, administering an inhibitor of HIF1α to a subject increases levels of total adiponectin, high molecular weight (HMW) adiponectin, and/or the ratio of HMW to total adiponectin, for example in a serum sample from the subject. Adiponectin is a bioactive peptide secreted from adipocytes that is considered an adipokine. Receptors for adiponectin are found primarily in skeletal muscle and liver and adiponectin improves insulin sensitivity by increasing energy expenditure, fatty acid oxidation, and expression of PPARα target genes (Rasouli and Kern, *J. Clin. Endocrinol. Metab.* 93:S64-S73, 2008). Thus, in some examples, administration of a HIF1α inhibitor reduces body weight and/or treats diabetes by increasing circulating levels of total adiponectin, HMW adiponectin, HMW:total adiponectin ratio, or a combination of two or more thereof. In some examples, one or more of total adiponectin, HMW adiponectin, or HMW:total adiponectin ratio is increased by at least 5% (such as at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, or more) as compared with a control. In some examples, blood adiponectin

levels are increased to more than about 8 μg/mL (such as about 8-13.9 μg/mL). In some embodiments, the disclosed methods include measuring adiponectin (such as total adiponectin, HMW adiponectin, and/or HMW:total adiponectin ratio) in a sample from a subject.

A. Decreasing Body Weight

In some embodiments, the disclosure includes decreasing body weight of a subject by administering a therapeutically effective amount of a composition including an inhibitor of HIF1α to a subject. In some examples, the method includes selecting a subject in need of decreasing body weight (such as an overweight or obese subject). A subject may be considered overweight or obese if their BMI is greater than 25 kg/m2, their waist circumference is greater than 35 inches (female) or 40 inches (male) or body fat percentage is greater than 25% (male) or 32% (female). In some examples, decreasing body weight includes one or more of decreasing total body weight, decreasing BMI, decreasing waist circumference, and decreasing body fat (such as total body fat, subcutaneous body fat, or visceral body fat). In some embodiments, the disclosed methods include measuring total body weight, BMI, waist circumference, and/or body fat amount in a subject.

In some examples, decreasing body weight of a subject includes reducing total body weight of the subject by at least about 1% (such as at least about 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, or more). In particular examples, reduction in total body weight is determined relative to the starting total body weight of the subject (for example, prior to treatment with a HIF1α inhibitor).

In other examples, decreasing body weight of a subject includes decreasing BMI of the subject by at least about 1% (such as at least about 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, or more). BMI is calculated by dividing weight (in kg) by height^2 (in meters^2). The current standards for both men and women accepted as "normal" are a BMI of 20-24.9 kg/m^2. In one embodiment, a BMI of greater than 25 kg/m^2 can be used to identify an obese subject. Grade I obesity (also called "overweight") corresponds to a BMI of 25-29.9 kg/m^2. Grade II obesity corresponds to a BMI of 30-40 kg/m^2; and Grade III obesity corresponds to a BMI greater than 40 kg/m^2. In particular examples, reduction in BMI is determined

relative to the starting BMI of the subject (for example, prior to treatment with a HIF1α inhibitor). In other examples, decreasing BMI of a subject includes reduction of BMI from a starting point (for example BMI greater than 30 kg/m^2) to a target level (for example, BMI less than 30 kg/m^2, 29 kg/m^2, 28 kg/m^2, 27 kg/m^2, 26 kg/m^2, or 25 kg/m^2).

In further examples, decreasing body weight of a subject includes decreasing waist circumference by at least 1% (such as at least about 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, or more). In particular examples, reduction in waist circumference is determined relative to the starting waist circumference of the subject (for example, prior to treatment with a HIF1α inhibitor). In other examples, decreasing waist circumference of a subject includes reduction of waist circumference from a starting point (for example greater than 40 inches for men or greater than 35 inches for women) to a target level (for example, waist circumference less than 40 inches for men or less than 35 inches for women).

In additional examples, decreasing body weight of a subject includes decreasing body fat (such as total body fat, subcutaneous body fat, or visceral body fat) of the subject by at least 1% (such as at least about 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, or more). Methods of determining body fat (such as body fat percentage) are known to one of skill in the art. Such methods include near-infrared interactance, dual energy X-ray absorptiometry, body average density measurement, bioelectrical impedance analysis, skinfold tests (for example, Durnin-Womersley skinfold method or Jackson-Pollock skinfold method), and U.S. Navy circumference method. In particular examples, reduction in body fat is determined relative to the starting body fat of the subject (for example, prior to treatment with a HIF1α inhibitor). In other examples, decreasing body fat of a subject includes reduction of body fat from a starting point (for example greater than about 25% body fat for men or greater than about 32% body fat for women) to a target level (for example, body fat of less than about 25% for men or less than about 32% for women). In some examples, a target body fat level may be about 14-24% body fat for men or about 21-31% body fat for women.

B. Treating Diabetes

In some embodiments, the disclosure includes treating diabetes or pre-diabetes in a subject by administering a therapeutically effective amount of a composition including an inhibitor of HIF1α to the subject. In some examples, the method includes selecting a subject with diabetes or at risk for diabetes (such as a subject with pre-diabetes). In some examples, a subject with diabetes may be clinically diagnosed by a fasting plasma glucose (FPG) concentration of greater than or equal to 7.0 mmol/L (126 mg/dL), or a plasma glucose concentration of greater than or equal to 11.1 mmol/L (200 mg/dL) at about two hours after an oral glucose tolerance test (OGTT) with a 75 g load, or in a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose concentration of greater than or equal to 11.1 mmol/L (200 mg/dL), or HbA1c levels of greater than or equal to 6.5%. In other examples, a subject with pre-diabetes may be diagnosed by impaired glucose tolerance (IGT). An OGTT two-hour plasma glucose of greater than or equal to 140 mg/dL and less than 200 mg/dL (7.8-11.0 mM), or a fasting plasma glucose (FPG) concentration of greater than or equal to 100 mg/dL and less than 125 mg/dL (5.6–6.9 mmol/L), or HbA1c levels of greater than or equal to 5.7% and less than 6.4% (5.7–6.4%) is considered to be IGT, and indicates that a subject has pre-diabetes. A more detailed description of diabetes may be found in *Standards of Medical Care in Diabetes—2010* (American Diabetes Association, *Diabetes Care* 33:S11-61, 2010). In some examples, treating diabetes includes one or more of increasing glucose tolerance, decreasing insulin resistance (for example, decreasing plasma glucose levels, decreasing plasma insulin levels, or a combination thereof), decreasing serum triglycerides, decreasing free fatty acid levels, and decreasing HbA1c levels in the subject. In some embodiments, the disclosed methods include measuring glucose tolerance, insulin resistance, plasma glucose levels, plasma insulin levels, serum triglycerides, free fatty acids, and/or HbA1c levels in a subject.

In some examples, administration of a HIF1α inhibitor treats diabetes by increasing glucose tolerance, for example, by decreasing blood glucose levels (such as two-hour plasma glucose in an OGTT or FPG) in a subject. In some examples, the method includes decreasing blood glucose by at least 5% (such as at least 10%,

15%, 20%, 25%, 30%, 35%, or more) as compared with a control. In particular examples, a decrease in blood glucose level is determined relative to the starting blood glucose level of the subject (for example, prior to treatment with a HIF1α inhibitor). In other examples, decreasing blood glucose levels of a subject includes reduction of blood glucose from a starting point (for example greater than about 126 mg/dL FPG or greater than about 200 mg/dL OGTT two-hour plasma glucose) to a target level (for example, FPG of less than 126 mg/dL or OGTT two-hour plasma glucose of less than 200 mg/dL). In some examples, a target FPG may be less than 100 mg/dL. In other examples, a target OGTT two-hour plasma glucose may be less than 140 mg/dL. Methods to measure blood glucose levels in a subject (for example, in a blood sample from a subject) are routine.

In some embodiments, the disclosed methods include treating a subject with diabetes by decreasing insulin resistance in the subject. In some examples, a subject with insulin resistance is a subject with diabetes, while in other examples, a subject with insulin resistance does not have diabetes, but may, for example, be pre-diabetic. Insulin resistance is a decreased sensitivity or responsiveness to the metabolic actions of insulin. In some examples, insulin resistance results in increased blood glucose and/or increased blood insulin levels (such as fasting blood glucose or fasting blood insulin levels).

In some examples, insulin resistance is determined by hyperinsulinemic euglycemic clamp (glucose clamp), which measures the amount of glucose necessary to compensate for increased insulin levels without causing hypoglycemia (see, *e.g.,* DeFronzo *et al., Am. J. Physiol.* 237:E214-E223, 1979). In one example, the glucose clamp method includes infusing insulin in a subject at 10-120 $mU/m^2/min$ and infusing 20% glucose to maintain blood glucose levels between about 90-100 mg/dL. If low levels of glucose (such as ≤4 mg/min) are required to maintain blood glucose levels, then the subject is considered insulin resistant. High levels of glucose (such as ≥7.5 mg/min) indicate that the subject is insulin sensitive, while between 4-7.5 mg/min of glucose is considered to indicate impaired glucose tolerance (IGT), which is an early sign of insulin resistance.

In some examples of the disclosed method, administration of an inhibitor of HIF1α decreases insulin resistance by increasing the amount of glucose required to

maintain blood glucose levels in a glucose clamp in a subject, for example, by at least 5% (such as at least 10%, 15%, 20%, 25%, 30%, 35%, or more) as compared with a control. In some examples, the method includes increasing the amount of glucose required to maintain blood glucose levels in a glucose clamp to >4 mg/min glucose. In other examples, the method includes increasing the amount of glucose required to maintain blood glucose levels in a glucose clamp to ≥7.5 mg/min glucose.

In another example, insulin resistance is determined by the frequently sampled intravenous glucose tolerance test (FSIVGTT; Bergman, *Diabetes* 38:1512-1527, 1989). FSIVGTT is performed by administering intravenous glucose with frequent blood sampling to determine glucose and insulin levels. Insulin is injected 20 minutes after the start of glucose administration. The insulin sensitivity index (SI), reflecting increase in fractional glucose disappearance per unit of insulin increase, is calculated. In some examples, an SI value of ≤2 μU/min/mL indicates insulin resistance. In some examples of the disclosed method, administration of a HIF1α inhibitor decreases insulin resistance by increasing the insulin sensitivity index of a subject, for example, by at least 5% (such as at least 10%, 15%, 20%, 25%, 30%, 35%, or more) as compared with a control (such as the subject prior to administration of the HIF1α inhibitor). In some examples, the method includes increasing the insulin sensitivity index to >2 μU/min/mL.

In other examples, insulin resistance is determined by QUICKI (Katz *et al.*, *J. Clin. Endocrinol. Metab.* 85:2402-2410, 2000). QUICKI is calculated from fasting glucose and fasting insulin levels:

$$QUICKI = 1/[(\log(I_0) + (\log(G_0)]$$

wherein I_0 is the fasting plasma insulin level (μU/mL) and G_0 is the fasting blood glucose level (mg/dL). In some examples of the disclosed method, administration of an inhibitor of HIF1α decreases insulin resistance by increasing the QUICKI value in a subject by at least 5% (such as at least 10%, 15%, 20%, 25%, 30%, 35%, or more) as compared with a control (such as the subject prior to administration of the

HIF1α inhibitor). In some examples, the method includes increasing the subject's QUICKI to >0.350.

In other examples, insulin resistance is determined by the homeostasis model assessment (HOMA-IR; Matthews *et al., Diabetologia* 28:412-429, 1985). HOMA-IR is calculated from fasting glucose and fasting insulin levels:

HOMA-IR = [fasting plasma insulin × fasting plasma glucose]/22.5

wherein fasting plasma insulin is expressed as μU/mL and fasting plasma glucose is expressed as mM. In some examples of the disclosed method, administration of an inhibitor of HIF1α decreases insulin resistance by decreasing the HOMA-IR value in a subject by at least 5% (such as at least 10%, 15%, 20%, 25%, 30%, 35%, or more) as compared with a control (such as the subject prior to administration of the HIF1α inhibitor). In some examples, the method includes decreasing HOMA-IR to ≤4.

In additional examples, administration of an inhibitor of HIF1α decreases insulin resistance by decreasing plasma insulin levels (such as fasting plasma insulin or 2-hour insulin levels following OGTT) in a subject, for example, decreasing plasma insulin levels by at least 5% (such as at least 10%, 15%, 20%, 25%, 30%, 35%, or more) as compared with a control (such as the subject prior to administration of the HIF1α inhibitor). In some examples, the method includes decreasing fasting plasma insulin levels to <15 μU/mL. Methods to measure plasma insulin in a subject (for example, in a blood sample from a subject), such as immunoassays, are routine.

One of skill in the art will recognize that because of a lack of standardized assays, interassay variability in insulin measurements can confound defining universal ranges for insulin resistance and insulin sensitivity. Therefore, in some examples, insulin sensitive subjects include the top 25th percentile of insulin sensitive subjects in a given cohort where insulin levels are measured in the same central reference laboratory. Similarly, in some examples, insulin resistant subjects include the bottom 25th percentile of insulin sensitive subjects in a given cohort where insulin levels are measured in the same central reference laboratory. In

additional examples, impaired glucose tolerance can be defined according the results of an oral glucose tolerance test using guidelines that are published by the American Diabetes Association. See, *e.g., Diabetes Care* 33:S62-S69, 2010.

In some embodiments, the disclosed methods include treating a subject with diabetes by decreasing triglyceride or free fatty acid levels in the subject. In some examples, administration of an inhibitor of HIF1α treats diabetes by decreasing blood triglyceride levels in a subject, for example decreasing triglyceride levels by at least 5% (such as at least 10%, 15%, 20%, 25%, 30%, 35%, or more) as compared with a control (such as the subject prior to administration of the HIF1α inhibitor). In some examples, the method includes decreasing triglyceride levels in a subject to <150 mg/dL. Methods of determining triglyceride levels in a subject (for example in a blood sample from a subject) are routine.

In further examples, administration of a HIF1α inhibitor treats diabetes by decreasing blood free fatty acid levels in a subject, for example, decreasing free fatty acid levels by at least 5% (such as at least 10%, 15%, 20%, 25%, 30%, 35%, or more) as compared with a control (such as the subject prior to administration of the HIF1α inhibitor). In some examples, the method includes decreasing free fatty acid levels below 0.6 mmol/L (such as about 0.1-0.6 mmol/L). Methods of determining free fatty acid levels in a subject (for example, in a blood sample from a subject) are routine.

In additional examples, the disclosed methods include treating a subject with diabetes by decreasing HbA1c levels in the subject. HbA1c (glycated hemoglobin) is a form of hemoglobin formed by non-enzymatic glycation by exposure of hemoglobin to plasma glucose. As the average amount of plasma glucose increases, the fraction of glycated hemoglobin increases. Thus HbA1c level is a marker for average blood glucose levels over time (such as weeks or months) and is an indicator of glycemic control in a subject. In some examples, administration of an inhibitor of HIF1α treats diabetes by decreasing HbA1c levels in a subject, for example decreasing HbA1c levels by at least 5% (such as at least 10%, 15%, 20%, 25%, 30%, 35%, or more) as compared with a control (such as the subject prior to administration of the HIF1α inhibitor). In some examples, the method includes decreasing HbA1c levels in a subject to less 7% (such as 6.5%, 6%, 5.5%, 5%, or

less). Methods of determining HbA1c levels in a subject (for example in a blood sample from a subject) are routine, for example, by HPLC or immunoassay.

C. Controls

In some embodiments, the disclosed methods include comparing one or more indicators of body weight or obesity (such as body weight, body mass index, waist circumference, or body fat) to a control, wherein a decrease in the particular indicator relative to the control indicates effective reduction of body weight or treatment of obesity.

The control can be any suitable control against which to compare the indicator of body weight or obesity in a subject. In some examples, the control is a historical control or standard reference value or range of values (such as a previously tested control sample, such as a group of subjects which are overweight or obese, or group of samples from subjects which are not overweight or obese). In further examples, the control is a reference value, such as a standard value obtained from a population of individuals that is used by those of skill in the art. Similar to a control population, the value of the sample from the subject can be compared to the mean reference value or to a range of reference values (such as the high and low values in the reference group or the 95% confidence interval). In other examples, the control is the subject (or group of subjects) treated with placebo compared to the same subject (or group of subjects) treated with the therapeutic compound in a cross-over study. In further examples, the control is the subject (or group of subjects) prior to treatment with the HIF1α inhibitor.

In other embodiments, the disclosed methods include comparing one or more indicator of diabetes (such as glucose tolerance, insulin resistance, triglyceride levels, free fatty acid levels, or HbA1c levels) to a control, wherein an increase or decrease in the particular indicator relative to the control (as discussed above) indicates effective treatment of diabetes. In particular examples, the disclosed methods include comparing one or more indicator of insulin resistance (such as blood glucose levels, blood insulin levels, insulin sensitivity index, HOMA-IR, or QUICKI) to a control, wherein a decrease in the particular indicator relative to the control (as discussed above) indicates effective treatment of insulin resistance. In further embodiments, the disclosed methods include comparing adiponectin levels to

a control, wherein an increase in adiponectin levels (such as total adiponectin, HMW adiponectin, and/or the ratio of HMW to total adiponectin) indicates an effective treatment of diabetes or obesity.

The control can be any suitable control against which to compare the indicator of diabetes in a subject. In some embodiments, the control is a sample obtained from a healthy subject (such as a subject without diabetes). In some embodiments, the control is a historical control or standard reference value or range of values (such as a previously tested control sample, such as a group of subjects with diabetes, or group of samples from subjects that do not have diabetes). In further examples, the control is a reference value, such as a standard value obtained from a population of normal individuals that is used by those of skill in the art. Similar to a control population, the value of the sample from the subject can be compared to the mean reference value or to a range of reference values (such as the high and low values in the reference group or the 95% confidence interval). In other examples, the control is the subject (or group of subjects) treated with placebo compared to the same subject (or group of subjects) treated with the therapeutic compound in a cross-over study. In further examples, the control is the subject (or group of subjects) prior to treatment with the HIF1α inhibitor.

IV. HIF1α Inhibitors

The disclosed methods include administering a therapeutically effective amount of an inhibitor of HIF1α to a subject. HIF1α inhibitors include compounds that decrease the expression, longevity (*e.g.,* half-life) or activity of HIF1α (directly or indirectly), for example, relative to a control. Direct inhibition can be accomplished, for example, by binding to a HIF1α protein and thereby preventing the protein from binding an intended target, such as a dimerization partner (*e.g.,* Arnt) or a DNA sequence (such as a HRE). Indirect inhibition can be accomplished, for example, by binding to a HIF1α protein intended target, such as a receptor or binding partner, thereby blocking or reducing activity of the protein. Furthermore, an inhibitor of the disclosure can inhibit a HIF1α gene by reducing or inhibiting expression of the gene, inter alia by interfering with gene expression (transcription, processing, translation, post-translational modification, or stability), for example, by

interfering with the mRNA and blocking translation of the HIF1α gene product or by altering post-translational modification of a HIF1α gene product (such as prolyl hydroxylation), or by causing changes in intracellular localization.

HIF1α inhibitors can include small organic molecules. Some small molecule inhibitors may inhibit multiple HIF alpha subunits (such as HIF1α, HIF2α, and/or HIF3α), while others may be specific for HIF1α. In some examples, a small molecule inhibitor inhibits more than one HIF alpha subunit, including HIF1α. In particular examples, a small molecule inhibitor specifically inhibits HIF1α expression or activity (such as dimerization, DNA binding, and/or transcriptional activity).

In some examples, the small molecule inhibitor of HIF1α is a previously identified HIF1α inhibitor. In one non-limiting examples, the inhibitor is acriflavine (ACF; see, *e.g.,* Lee *et al., Proc. Natl. Acad. Sci. USA* 106:17910-17915, 2009). ACF is a mixture of 3,6-diamino-10-methylacridinium chloride (trypaflavin) and 3,6-diaminoacridine (proflavine), such as about a 1:1 mixture. In other examples, a HIF1α small molecule inhibitor is a cardiac glycoside, such as digoxin, ouabain, proscillaridin A, digitoxin, acetydigitoxin, convallatoxin, peruvoside, strophanthin K, nerifolin, cymarin, or periplocymarin (see *e.g.,* Zhang *et al., Proc. Natl. Acad. Sci. USA* 105:19579-19586, 2008), rapamycin or an analog thereof (such as rapamycin, everolimus, temsirolimus, or tacrolimus), an anthracycline or analog thereof (such as doxorubicin or daunorubicin), a proteasome inhibitor (such as bortezomib (PS-341)), or camptothecin or an analog thereof (such as CRLX-101, SN-38, EZN-2208, irinotecan, or topotecan). In additional examples, a HIF1α small molecule inhibitor includes echinomycin (see, *e.g.,* Kong *et al., Cancer Res.* 65:9047-9055, 2005), 17-allylamino-17-demethoxygeldanamycin (see, *e.g.,* Liu *et al., Mol. Cell* 25:207-217, 2007), 17-dimethylaminoethylamino-17-demethoxygeldanamycin (*e.g.,* WO 02/079167), NSC 644221 (see, *e.g.,* Creighton-Gutteridge *et al., Clin. Cancer Res.* 13:1010-1018, 2007), YC-1 (*e.g.,* Yeo *et al., J. Natl. Cancer Inst.* 95:498-499, 2003), PX-478 (see, *e.g.,* U.S. Pat. 7,399,785), 2-methoxyestradiol or derivatives thereof (*e.g.,* ENMD-1198 or ENMD-2076), wondonin (*e.g.,* Jun *et al., FEBS Lett.* 581:4977-4982, 2007), Palomid-529 (Paloma

Pharmaceuticals), CLT-003 (Charlesson), cyclopentabenzofuranes (*e.g.,* IMD-026260; WO 2010/063471), furoquinoline-based molecules (*e.g.,* Lohar *et al., Bioorg. Med. Chem. Lett.* 18:3603-3606, 2008), BAY 87-2243, BTG-6228 (BTG), or KC7F2 (*e.g.,* Naria *et al., Clin. Cancer Res.* 15:6128-6136, 2009); alpha-ketoglutarates (*e.g.,* WO 06/016143); P3155 or P2630 (*e.g.,* Kumar *et al., Bioorg. Med. Chem. Lett.* 20:6426-6429, 2010); EL-102 (McLaughlin *et al.,* American Association for Cancer Research, Abstract LB-385, 2011); CX-4715 or CX-3800 series compounds (Cylene Pharmaceuticals). In another example, a small molecule inhibitor of HIF1α is aminoflavone (*e.g.,* Terzuoli *et al., Cancer Res.* 20:6837-6848, 2010). It is to be understood that HIF1α inhibitors for use in the present disclosure also include novel HIF1α small molecule inhibitors developed in the future.

HIF1α inhibitors also include nucleic acid molecules, including, but not limited to antisense molecules. Some HIF1α nucleic acid inhibitors may inhibit multiple HIF alpha subunits (such as HIF1α, HIF2α, and/or HIF3α), while others may be specific for HIF1α. In some examples, a nucleic acid inhibitor inhibits more than one HIF alpha subunit, including HIF1α. In particular examples, a nucleic acid inhibitor specifically inhibits HIF1α expression or activity (such as dimerization, DNA binding, and/or transcriptional activity).

In some examples, the nucleic acid inhibitor of HIF1α decreases expression of HIF1α, such as an antisense molecule or an RNAi molecule (such as siRNA, miRNA, or shRNA). In a particular non-limiting example, a HIF1α siRNA includes SEQ ID NOs: 70 or 71. In other examples, the inhibitor is RX-0047 or RX-0149 (see, *e.g.,* U.S. Pat. No. 7,205,283), double-stranded HIF decoy oligonucleotides (*e.g.,* WO 2005/056795), ACU-HHY-011 (Opko Health), or EZN-2968 (see, *e.g.,* U.S. Pat. Nos. 7,589,190 and 7,737,264). In further examples, the nucleic acid inhibitor of HIF1α decreases activity of HIF1α, such as a G-rich oligodeoxynucleotide (for example, a G-quartet structure). In particular, non-limiting examples, the inhibitor is JG243 or JG244 (see, *e.g.,* Guan *et al., Mol. Ther.* 18:188-197, 2010). It is to be understood that HIF1α inhibitors for use in the present disclosure also include novel HIF1α nucleic acid molecule inhibitors developed in the future.

In additional examples, inhibitors of HIF1α can include HIF1α-specific binding agents, such as polyclonal or monoclonal antibodies. Specific examples of HIF1α-specific binding agents include HIF1α-specific antibodies or functional fragments thereof, for instance monoclonal antibodies or fragments of monoclonal antibodies, including humanized monoclonal antibodies. Methods for producing antibodies (including monoclonal antibodies) using standard procedures are described in a number of texts, including Harlow and Lane (*Antibodies, A Laboratory Manual*, CSHL, New York, 1988). Techniques for producing humanized monoclonal antibodies are described, for example, by Jones *et al.*, *Nature* 321:522, 1986; Riechmann *et al.*, *Nature* 332:323, 1988; Verhoeyen *et al.*, *Science* 239:1534, 1988; Carter *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 89:4285, 1992; Sandhu, *Crit. Rev. Biotech.*12:437, 1992; and Singer *et al.*, *J. Immunol.*150:2844, 1993.

Monoclonal or polyclonal antibodies may be produced to either the normal HIF1α protein or mutant forms of this protein, for instance particular portions that contain a mutation and therefore may provide a distinguishing epitope. Optimally, antibodies raised against these proteins or peptides specifically detect the protein or peptide with which the antibodies are generated. That is, an antibody generated to the HIF1α protein or a fragment thereof would recognize and bind the HIF1α protein and would not substantially recognize or bind to other proteins found in mammalian cells (for example, human cells).

Methods of making antibodies that can be used clinically are known in the art. Antibodies for HIF1α can also be obtained from commercially available sources, including from Santa Cruz Biotechnology (Santa Cruz, CA), Abcam (Cambridge, MA), and Millipore (Billerica, MA). It is to be understood that HIF1α antibodies for use in the present disclosure also include novel anti-HIF1α antibodies developed in the future.

In some embodiments, the HIF1α inhibitor is targeted to adipose tissue (such as white adipose tissue). WAT targeting motifs include peptides that preferentially bind to WAT cells or WAT vasculature. In some examples, the targeting peptide includes CKGGRAKDC (SEQ ID NO: 74), CMLAGWIPC (SEQ ID NO: 75), and CWLGEWLGC (SEQ ID NO: 76). See, *e.g.,* Kolonin *et al., Nat. Med.* 10:625-632,

2004 and Nie *et al., Stem Cells* 26:2735-2745, 2008; U.S. Pat. No. 7,951,362; each of which is incorporated herein by reference. In other examples, an adipose tissue targeting molecule includes an antibody that preferentially binds to adipose tissue (such as WAT), for example an antibody that preferentially binds to resistin. In some examples, a HIF1α inhibitor is coupled to an adipose tissue targeting molecule (such as a peptide or antibody). Methods of coupling molecules are well known to one of skill in the art. This includes, but is not limited to, covalently bonding one molecule to another molecule (for example, directly or via a linker molecule), noncovalently bonding one molecule to another (*e.g.* electrostatically bonding) (see, for example, U.S. Patent No. 6,921,496, which discloses methods for electrostatic conjugation), non-covalently bonding one molecule to another molecule by hydrogen bonding, non-covalently bonding one molecule to another molecule by van der Waals forces, and any and all combinations of such couplings.

V. Pharmaceutical Compositions and Administration

Pharmaceutical compositions that include an inhibitor of HIF1α can be formulated with an appropriate pharmaceutically acceptable carrier, depending upon the particular mode of administration chosen. In one example, the pharmaceutical composition includes a HIF1α inhibitor and a pharmaceutically acceptable carrier. In some examples, the pharmaceutical composition consists essentially of an inhibitor of HIF1α and a pharmaceutically acceptable carrier.

The pharmaceutically acceptable carriers and excipients useful in this disclosure are conventional. See, *e.g., Remington: The Science and Practice of Pharmacy*, The University of the Sciences in Philadelphia, Editor, Lippincott, Williams, & Wilkins, Philadelphia, PA, 21st Edition (2005). For instance, parenteral formulations usually comprise injectable fluids that are pharmaceutically and physiologically acceptable fluid vehicles such as water, physiological saline, other balanced salt solutions, aqueous dextrose, glycerol or the like. For solid compositions (*e.g.*, powder, pill, tablet, or capsule forms), conventional non-toxic solid carriers can include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition to biologically-neutral carriers, pharmaceutical compositions to be administered can contain minor amounts of non-

toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, pH buffering agents, or the like, for example sodium acetate or sorbitan monolaurate. Excipients that can be included are, for instance, other proteins, such as human serum albumin or plasma preparations.

In some embodiments, the HIF1α inhibitor is included in a controlled release formulation, for example, a microencapsulated formulation. Various types of biodegradable and biocompatible polymers, methods can be used, and methods of encapsulating a variety of synthetic compounds, proteins and nucleic acids, have been well described in the art (see, for example, U.S. Pat. Publication Nos. 2007/0148074; 2007/0092575; and 2006/0246139; U.S. Patent Nos. 4,522, 811; 5,753,234; and 7,081,489; PCT Publication No. WO/2006/052285; Benita, *Microencapsulation: Methods and Industrial Applications*, 2nd ed., CRC Press, 2006).

In other examples, the HIF1α inhibitor is included in a nanodispersion system. Nanodispersion systems and methods for producing such nanodispersions are well known to one of skill in the art. See, *e.g.,* U.S. Pat. No. 6,780,324; U.S. Pat. Publication No. 2009/0175953. For example, a nanodispersion system includes a biologically active agent and a dispersing agent (such as a polymer, copolymer, or low molecular weight surfactant). Exemplary polymers or copolymers include polyvinylpyrrolidone (PVP), poly(D,L-lactic acid) (PLA), poly(D,L-lactic-co-glycolic acid (PLGA), poly(ethylene glycol). Exemplary low molecular weight surfactants include sodium dodecyl sulfate, hexadecyl pyridinium chloride, polysorbates, sorbitans, poly(oxyethylene) alkyl ethers, poly(oxyethylene) alkyl esters, and combinations thereof. In one example, the nanodispersion system includes PVP and a HIF1α inhibitor (such as 80/20 w/w). In some examples, the nanodispersion is prepared using the solvent evaporation method. See, *e.g.,* Kanaze *et al., Drug Dev. Indus. Pharm.* 36:292-301, 2010; Kanaze *et al., J. Appl. Polymer Sci.* 102:460-471, 2006.

In some examples, the HIF1α inhibitor includes pharmaceutically acceptable salts of such compounds. "Pharmaceutically acceptable salts" of the presently disclosed compounds include those formed from cations such as sodium, potassium, aluminum, calcium, lithium, magnesium, zinc, and from bases such as ammonia,

ethylenediamine, N-methyl-glutamine, lysine, arginine, ornithine, choline, N,N'-dibenzylethylenediamine, chloroprocaine, diethanolamine, procaine, N-benzylphenethylamine, diethylamine, piperazine, tris(hydroxymethyl)aminomethane, and tetramethylammonium hydroxide. These salts may be prepared by standard procedures, for example by reacting the free acid with a suitable organic or inorganic base. Any chemical compound recited in this specification may alternatively be administered as a pharmaceutically acceptable salt thereof. "Pharmaceutically acceptable salts" are also inclusive of the free acid, base, and zwitterionic forms. Description of suitable pharmaceutically acceptable salts can be found in *Handbook of Pharmaceutical Salts, Properties, Selection and Use,* Wiley VCH (2002).

In some examples, the pharmaceutical compositions disclosed herein comprise a HIF1α inhibitor and at least one pharmaceutically acceptable carrier. In other examples, the composition consists essentially of a HIF1α inhibitor and at least one pharmaceutically acceptable carrier. In the present disclosure, "consists essentially of" indicates that additional active compounds (for example additional inhibitors of HIF1α) are not included in the composition, but that other inert agents (such as fillers, wetting agents, or the like) can be included, and "consists of" indicates that additional agents are not included in the composition.

The dosage form of the pharmaceutical composition will be determined by the mode of administration chosen. For instance, in addition to injectable fluids, topical, inhalation, oral and suppository formulations can be employed. Topical preparations can include eye drops, ointments, sprays, patches and the like. Inhalation preparations can be liquid (*e.g.,* solutions or suspensions) and include mists, sprays and the like. Oral formulations can be liquid (*e.g.,* syrups, solutions or suspensions), or solid (*e.g.*, powders, pills, tablets, or capsules). Suppository preparations can also be solid, gel, or in a suspension form. For solid compositions, conventional non-toxic solid carriers can include pharmaceutical grades of mannitol, lactose, cellulose, starch, or magnesium stearate. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in the art.

The pharmaceutical compositions that include an inhibitor of HIF1α can be formulated in unit dosage form, suitable for individual administration of precise

dosages. In one specific, non-limiting example, a unit dosage contains from about 1 mg to about 1 g of an HIF1α inhibitor (such as about 10 mg to about 100 mg, about 50 mg to about 500 mg, about 100 mg to about 900 mg, about 250 mg to about 750 mg, or about 400 mg to about 600 mg HIF1α inhibitor). The amount of active compound(s) administered will be dependent on the subject being treated, the severity of the affliction, and the manner of administration, and is best left to the judgment of the prescribing clinician. Within these bounds, the formulation to be administered will contain a quantity of the active component(s) in amounts effective to achieve the desired effect in the subject being treated.

The compositions of this disclosure including an inhibitor of HIF1α can be administered to humans or other animals on whose tissues they are effective in various manners such as orally, intravenously, intramuscularly, intraperitoneally, intranasally, intradermally, intrathecally, subcutaneously, via inhalation or via suppository. In one non-limiting example, the composition is administered orally. In further examples, site-specific administration of the composition can be used, for example by administering a HIF1α inhibitor to adipose tissue (for example by injection in adipose tissue, such as visceral adipose tissue). The particular mode of administration and the dosage regimen will be selected by the attending clinician, taking into account the particulars of the case (*e.g.* the subject, the disease, the disease state involved, the particular treatment, and whether the treatment is prophylactic). Treatment can involve daily or multi-daily doses of compound(s) over a period of a few days to months, or even years. In a particular non-limiting example, treatment involves once daily dose of a HIF1α inhibitor (such as acriflavine).

In some examples, a therapeutically effective amount of a HIF1α inhibitor is about 0.01 mg/kg to about 50 mg/kg (for example, about 0.5 mg/kg to about 25 mg/kg or about 1 mg/kg to about 10 mg/kg). In a specific example, a therapeutically effective amount of a HIF1α inhibitor is about 1 mg/kg to about 5 mg/kg, for example about 2 mg/kg. In a particular example, a therapeutically effective amount of a HIF1α inhibitor includes about 1 mg/kg to about 10 mg/kg acriflavine, such as about 2 mg/kg acriflavine.

A therapeutically effective amount of a HIF1α inhibitor can be the amount of a HIF1α inhibitor necessary to treat diabetes or reduce body weight in a subject. A therapeutically effective amount of an inhibitor of HIF1α can be administered in a single dose, or in several doses, for example daily, during a course of treatment. However, the therapeutically effective amount will be dependent on the subject being treated, the severity and type of the affliction, and the manner of administration of the therapeutic(s).

The present disclosure also includes combinations of a HIF1α inhibitor with one or more other agents useful in the treatment of diabetes or insulin resistance or in the reduction of body weight. For example, the compounds of this disclosure can be administered in combination with effective doses of anti-diabetic agents (such as biguanides, thiazolidinediones, or incretins) and/or lipid lowering compounds (such as statins or fibrates). The term "administration in combination" or "co-administration" refers to both concurrent and sequential administration of the active agents. Administration of a HIF1α inhibitor may also be in combination with lifestyle modifications, such as increased physical activity, low fat diet, and smoking cessation.

A subject that has diabetes or a subject with insulin resistance (for example, a fasting plasma glucose of >100 mg/dL) is a candidate for treatment using the therapeutic methods disclosed herein. A subject in need of a reduction in body weight, for example, a subject with overweight or obesity (for example, a subject with a body mass index of 25 kg/m^2 or more) is a candidate for treatment using the therapeutic methods herein.

The following examples are provided to illustrate certain particular features and/or embodiments. These examples should not be construed to limit the invention to the particular features or embodiments described.

EXAMPLES

At least some of the subject matter of the examples is included in Jiang *et al.* (*Diabetes* 60:2484-2495, 2011 and supplementary data; doi:10.233377db11-0174), which is incorporated herein by reference in its entirety.

Example 1

Materials and Methods

Animals and Diets: Arnt-floxed ($Arnt^{F/F}$) (Tomita *et al., Mol. Endocrinol.* 14:1674-1681, 2000) and *Hif1α*-floxed ($Hif1\alpha^{F/F}$) (Tomita *et al., Mol. Cell Biol.* 23:6739-6749, 2003) mice containing loxP sites flanking exons 6 and 13-15, of the *Arnt* and *Hif1α* genes, respectively, were crossed with mice harboring the Cre recombinase under control of the aP2 promoter (aP2-Cre mice) (Bluher *et al., Dev. Cell* 3:25-38, 2002). All mice were on the C57BL/6 background. Only male mice were used for experiments. The adipocyte-specific knockout mice of the *Arnt* and *Hif1α* genes were designated $Arnt^{\Delta Adipo}$ and $Hif1\alpha^{\Delta Adipo}$ mice, respectively. Mice were housed in temperature- and light-controlled rooms and were supplied with water and pelleted NIH-31 standard chow diet consisting of 10 kcal% fat ad libitum. In the high-fat diet (HFD) study, 6-week-old male mice were given HFD consisting of 60 kcal% fat (BioServ, Frenchtown, NJ) for 12 weeks. All animal studies were performed in accordance with Institute of Laboratory Animal Resources guidelines and approved by the National Cancer Institute Animal Care and Use Committee.

Recombination Efficiency and Genotype Determination: To assess the efficiency of aP2-Cre-mediated recombination in mice, adipocytes and all other tissues were harvested and kept in liquid nitrogen. DNA was isolated using the DNeasy® kit (Qiagen, Valencia, CA). The primers used to assess recombination and routine genotyping for the *Arnt* and *Hif1α* allele are listed in Table 1.

Metabolic Assays: For glucose tolerance tests (GTT), mice were fasted for 16 hours, blood was drawn, and mice were injected intraperitoneally (i.p.) with 1g/kg glucose. For insulin tolerance tests (ITT), mice were fasted for 4 hours, blood was drawn, and then mice were injected i.p. with insulin (Humulin® R, Eli Lilly, Indianapolis, IN), 1U/kg body weight. Blood samples were taken from the tail vein at 15, 30, 60, 90, and 120 minutes after injection and glucose was measured using a Glucometer (Elite, Bayer Health Care, Tarrytown, NY). Hyperinsulinemic-euglycemic clamps were performed in awake mice fasted for 12 hours as previously described (Toyoshima *et al., Endocrinology* 146:4024-4035, 2005) with modifications. Primed-continuous infusion of [3-^{3}H] glucose was used; 2.5 μCi bolus, 0.05 μCi/minute during the basal state, and 0.1 μCi/minute during the clamp

period. Insulin (Humulin® R, Eli Lilly, Indianapolis, IN) was infused as a bolus of 18 mU/kg over a period of 3 minutes, followed by continuous insulin infusion at the rate of 3.5 mU/kg lean mass/minute (in *Hif1α*$^{F/F}$ mice) and 9.4 m/kg lean mass/minute (in *Hif1α*$^{\Delta Adipo}$) to raise plasma insulin concentration to 4 ng/ml.

***In vivo* Insulin Stimulation and Analysis of Insulin Signaling:** Mice were fasted overnight and injected i.p. with 5 U/kg body weight of insulin (Humulin®, Eli Lilly). Mice were killed 5 minutes after insulin stimulation. Liver, white adipose tissue and quadriceps were removed, snap-frozen in liquid nitrogen, and stored at -80°C until use.

Measurement of Body Composition and Food Intake: Body composition was measured in non-anesthetized mice using an Echo 3-in-1 NMR analyzer (Echo Medical Systems, Houston, TX). Daily food intake was measured in individually caged mice using glass metabolic chambers for 24 hours (Jencons, Leighton Buzzard, UK). Cumulative food intake was measured for 2 weeks in 6-8 week old male mice maintained on regular chow and 10-12 week old mice fed HFD from 4-6 weeks. Mice were individually housed in their home cages for a week prior to recording food intake. Metabolic efficiency was calculated as the ratio of weight gain to energy consumed during a 2-week period. Total and resting metabolic rates were measured by indirect calorimetry using the Oxymax system (Columbus Instruments, Columbus, OH) as previously described (Gavrilova *et al., Diabetes* 49:1910-1916, 2000). Mice had free access to food and water during the measurements and were allowed to adapt to metabolic cage for 24 hours prior to data collection. Following adaptation period, data were recorded for 24 hours at 24°C and at 30°C for an additional 24 hours. Four mutant and four control mice were tested at the same time and each mouse was tested every 20 minutes. Motor activities were measured by infrared beam interruption (Opto-Varimex mini, Columbus Instruments, Columbus, OH) and resting was defined as time points with ambulation equal to zero. Diet induced thermogenesis was measured as described (Chen *et al., Cell Metab.* 11:320-330, 2010).

Biochemical Assays: Fasted serum insulin was measured by use of an ELISA kit (Crystal Chem Inc., Downers Grove, IL). Fasted serum cholesterol, free fatty acids, and triglycerides were measured using reagents from Wako (Richmond,

VA). Adiponectin serum levels were measured with a mouse adiponectin ELISA kit (ALPCO, Salem, NH). The amounts of adiponectin in culture medium for 3T3-L1 adipocytes were measured using Quantikine® Mouse Adiponectin/Acrp30 Immunoassay kit (R&D Systems, Minneapolis, MN).

RNA Analysis: Total RNA was extracted from adipose tissue, liver, and skeletal muscle using TRIzol reagents (Invitrogen, Carlsbad, CA). Quantitative real-time PCR (qPCR) was performed using cDNA generated from 1 μg of total RNA with the SuperScript® II Reverse Transcriptase kit (Invitrogen). qPCR reactions were carried out by use of SYBR® green PCR master mix (Applied Biosystems, Carlsbad, CA) in an ABI Prism 7900HT sequence detection system (Applied Biosystems). Values were quantified by comparative CT methods and normalized to β-actin. Primer sequences are listed in Table 1.

Western Blot Analysis: Tissues were lysed by use of RIPA for whole cell extract. The membranes were incubated with antibodies against IR, IRS-1, total Akt, phospho-Akt, SOCS3, total STAT3, phospho-STAT3 (Cell Signaling Technologies, Danvers, MA), and phosphotyrosine (Upstate Biotechnology, Lake Placid, NY). The signals obtained were normalized to β-actin (Millipore Corp, Temecula, CA) for whole cell extracts.

Microarray Analysis: Dye-coupled cDNAs were purified with a MiniElute PCR purification kit (Qiagen) and hybridized to an Agilent 44 K mouse 60-mer oligo microarray (Agilent Technologies, Santa Clara, CA). The procedures were repeated for replicate experiments with independent hybridization and processing, and the data processed and analyzed by Genespring GX software (Agilent Technologies).

Cell Culture and siRNA Transfection in 3T3-L1 Adipocytes: 3T3-L1 fibroblasts were grown in Dulbecco's modified Eagle's medium supplemented with 10% Calf Serum and differentiated into adipocytes as described previously (Jiang *et al., J. Clin. Invest.* 106:473-481, 2003). 3T3-L1 adipocytes were transfected with siRNA duplexes by electroporation using Amaxa® Cell Line Nucleofector® Kit L (Amaxa Biosystems, Cologne, Germany). Sequences corresponding to the siRNA of ARNT, HIF1α, and SOCS3 are listed in Table 1. A scrambled Stealth RNAi

duplex (catalog nos. 12935200 and 12935300, Invitrogen) served as a negative control.

Isolation of Adipocytes and Macrophage of Stromal Vascular Fraction (SVF-Mϕ): Adipocytes and SVF-Mϕ were isolated from epididymal WAT as previously described (Sugii *et al., Proc. Natl. Acad. Sci. USA* 106:22504-22509, 2009).

Hypoxia Treatment: 3T3-L1 adipocytes were incubated for the indicated time in a water-jacketed CO_2 incubator containing 1% O_2, 5% CO_2, and 94% nitrogen gas.

Luciferase Assay: The mouse *Socs3* luciferase reporter plasmids were constructed by cloning the upstream regions using the primers listed in Table 1. The PCR fragments were cloned into the MluI and XhoI restriction sites in the pGL3-basic vector (Promega, Madison WI). Oxygen stable mouse HIF1α or HIF2α were generated by mutating the prolines in the degradation domain to alanines by site-directed mutagenesis (Stratagene, La Jolla, CA). These luciferase reporters were co-transfected with the mammalian expression plasmid for an oxygen stable HIF1α or oxygen stable HIF2α into differentiated 3T3-L1 adipocytes by using Amaxa® Cell Line Nucleofector® Kit L (Amaxa Biosystems, Cologne, Germany). Cells were incubated for 24 hours. Standard dual luciferase assay was used and normalized to a co-transfected control reporter (Promega).

ChIP Assays: Chromatin immunoprecipitation (ChIP) assays were performed using the SimpleChIP™ Enzymatic Chromatin IP Kit (Cell Signaling Technology, Danvers, MA). Briefly, 1×10^7 differentiated 3T3L1 cells were exposed to normoxia or hypoxia (1% oxygen) for 8 hours, crosslinked in 1% formaldehyde and lysed. Chromatin was fragmented by partial digestion with micrococcal nuclease, sheared in a chilled Bioruptor (Diagenode, Liege, Belgium) and the nuclear lysate was cleared by centrifugation at 16,000 *g* for 5 minutes. Soluble chromatin was immunoprecipitated with primary antibody for HIF1α (Santa Cruz Biotechnology, Santa Cruz, CA). The de-crosslinked samples were incubated with RNase A and proteinase K. DNA was purified using DNA purification spin columns, and 2 μL of samples was amplified by PCR by using primers listed in Table 1.

STAT3 Inhibitor NSC 74859 Treatment Mice: NSC 74859 (also known as S3I-201) was purchased from Calbiochem (La Jolla, CA, USA). Six-week-old *Hif1α*$^{\Delta Adipo}$ mice were fed a HFD for 6 weeks and then administered the STAT3 inhibitor NSC 74859 at 5 mg/kg daily via i.p. injection for an additional 6 weeks while maintained on a HFD.

HIF1α Inhibitor Acriflavine (ACF) Treatment Mice: ACF was purchased from Sigma-Aldrich (St. Louis, MO). Male C57BL/6 mice were administrated vehicle (saline) or ACF (2 mg/kg) daily via i.p. injection and fed a HFD for 12 weeks from 6 weeks of age.

Data Analysis: Results are expressed as mean ± SD. Differences between groups were examined for statistical significance with Student's t test. A P value of less than 0.05 was considered statistically significant.

Table 1. Primer sequences

Primer	Sequence	SEQ ID NO:
ChIP		
SOCS3 HRE1 FWD	5'-CCAGGAGTAAACAACCTTTCACCTC-3'	1
SOCS3 HRE1 REV	5'-AATGGACCCTCTCTTGCCTCTACG-3'	2
SOCS3 HRE2-3 FWD	5'-CACAGTTCGCCACGCAGGTTG-3'	3
SOCS3 HRE2-3 REV	5'-GAAGCAGCTACCTGGCCTCAG-3'	4
Luciferase		
SOCS3 FWD1	5'-CGACGCGTCGGACAGATGGGCACCCATCCCT-3'	5
SOCS3 FWD2	5'-CGACGCGTCGCCACTTCCTAGGTCCCCAGTG-3'	6
SOCS3 REV	5'-CCGCTCGAGCGGAGCAGGCGAGTGTAGAGTCAG-3'	7
RT-PCR		
ARNT FWD1	5'-ACGCACTACAACACCTGAGCTAA-3'	8
ARNT FWD2	5'-TGCCAACATGTGCCACCATGT-3'	9
ARNT REV	5'-GCATGCTGGCACATGCCTGTCT-3'	10
HIF-1α FWD1	5'-CTGTCTTCCCTGCTTAGGTCTTTCTAAC-3'	11
HIF-1α FWD2	5'-GAGATGGAGAAGGAGGTTAGTGTATCC-3'	12
HIF-1α REV	5'-ACGTTGGCTCATGGTGTACTTTG-3'	13
qPCR		
HIF-1α FWD	5'-ATAGCTTCGCAGAATGCTCAGA-3'	14
HIF-1α REV	5'-CAGTCACCTGGTTGCTGCAA-3'	15
ARNT FWD	5'-CAAGCCATCTTTCCTCACTGATC-3'	16
ARNT REV	5'-ACACCACCCGTCCAGTCTCA-3'	17
SOCS3 FWD	5'-GCGGGCACCTTTCTTATCC-3'	18
SOCS3 REV	5'-TCCCCGACTGGGTCTTGAC-3'	19
VEGF-c FWD	5'-AGACGGACACACATGGAGGT-3'	20
VEGF-c REV	5'-AAAGACTCAATGCATGCCAC-3'	21

Primer	Sequence	SEQ ID NO:
ADIPOQ FWD	5'-CTCTAAAGATTGTCAGTGGATCTG-3'	22
ADIPOQ REV	5'-ACGTCATCTTCGGCATGACT-3'	23
GLUT4 FWD	5'-TTTTAAAACAAGATGCCGTCG-3'	24
GLUT4 REV	5'-CAGTGTTCCAGTCACTCGCT-3'	25
Leptin FWD	5'-TCAAGACCATTGTCACCAGG-3'	26
Leptin REV	5'-TGAAGCCCAGGAATGAAGTC-3'	27
GLUT-1 FWD	5'-CCAGCTGGGAATCGTCGTT-3'	28
GLUT-1 REV	5'-CAAGTCTGCATTGCCCATGAT-3'	29
β-actin FWD	5'-TATTGGCAACGAGCGGTTCC-3'	30
β-actin REV	5'-GGCATAGAGGTCTTTACGGATGTC-3'	31
PGC1α FWD	5'-TGTAGCGACCAATCGGAAAT-3'	32
PGC1α REV	5'-TGAGGACCGCTAGCAAGTTT-3'	33
PGC1β FWD	5'-GCTCTCGTCCTTCTTCCTCA-3'	34
PGC1β REV	5'-GAGGTCAAGCTCTGGCAAGT-3'	35
Resistin FWD	5'-TGAAGCCATCGACAAGAAGA-3'	36
Resistin REV	5'-CTTCCCTCTGGAGGAGACTG-3'	37
CFD FWD	5'-GCTGTCAGAATGCACAGCTC-3'	38
CFD REV	5'-CTCCTGGCCACCCAGAAT-3'	39
PPARG FWD	5'-TCTGGGAGATTCTCCTGTTGA-3'	40
PPARG REV	5'-GGTGGGCCAGAATGGCATCT-3'	41
C/EBPα FWD	5'-CCAAGAAGTCGGTGGACAAG-3'	42
C/EBPα REV	5'-TTGTTTGGCTTTATCTCGGC-3'	43
ATGL FWD	5'-CCACTCACATCTACGGAGCC-3'	44
ATGL REV	5'-TAATGTCACCTGCTTCA-3'	45
HSL FWD	5'-CCTGCAAGAGTATGTCACGC-3'	46
HSL REV	5'-GGAGAGAGTCTGCAGGAACG-3'	47
TNFα FWD	5'-CCACCACGCTCTTCTGTCTAC-3'	48
TNFα REV	5'-AGGGTCTGGGCCATAGAACT-3'	49
MCP-1 FWD	5'-CCTGCTGTTCACAGTTGCC-3'	50
MCP-1 REV	5'-ATTGGGATCATCTTGCTGGT-3'	51
Lox FWD	5'-GGAGGACACGTCCTGTGACT-3'	52
Lox REV	5'-CTATGTCTGCCGCATAGGTG-3'	53
Col1a1 FWD	5'-ACATGTTCAGCTTTGTGGACC-3'	54
Col1a1 REV	5'-TAGGCCATTGTGTATGCAGC-3'	55
Col3a1 FWD	5'-GGAACCTGGTTTCTTCTCACC-3'	56
Col3a1 REV	5'-TAGGACTGACCAAGGTGGCT-3'	57
Loxl FWD	5'-GGGTAGTGTGTACCGACCCA-3'	58
Loxl REV	5'-GATGGGCTCTCTGCACGTAT-3'	59
CD68 FWD	5'-ATCCCCACCTGTCTCTCTCA-3'	60
CD68 REV	5'-ACCGCCATGTAGTCCAGGTA-3'	61
F4/80 FWD	5'-GGATGTACAGATGGGGGATG-3'	62
F4/80 REV	5'-CATAAGCTGGGCAAGTGGTA-3'	63
PAI-1 FWD	5'-GCCTCCTCATCCTGCCTAA-3'	64
PAI-1 REV	5'-GCCAGGGTTGCACTAAACAT-3'	65
HIF-2α FWD	5'-TGAGTTGGCTCATGAGTTGC-3'	66
HIF-2α REV	5'-TATGTGTCCGAAGGAAGCTG-3'	67
siRNA		
ARNT sense	5'-CCGUCAUGUUCCGAUUCCGAUCUAA-3'	68
ARNT antisense	5'-UUAGAUCGGAAUCGGAACAUGACGG-3'	69

Primer	Sequence	SEQ ID NO:
HIF-1α sense	5'-UACUCAGAGCUUUGGAUCAAGUUAA-3'	70
HIF-1α antisense	5'-UUAACUUGAUCCAAAGCUCUGAGUA-3'	71
SOCS3 sense	5'-GCCACUUCUUCACGUUGAGCGUCAA-3'	72
SOCS3 antisense	5'-UUGACGCUCAACGUGAAGAAGUGGC-3'	73

Example 2

Generation and Characterization of $Arnt^{\Delta Adipo}$ and $Hif1\alpha^{\Delta Adipo}$ Mice

This example describes generation of transgenic mice with adipocyte-specific knockout of *Arnt* or *HIF1α* genes and their initial characterization.

To examine the role of HIF transcription factors in obesity and insulin resistance, *Arnt* and *Hif1α* genes were disrupted in adipocytes. $Arnt^{F/F}$ and $Hif1\alpha^{F/F}$ mice were bred with a transgenic mouse line expressing Cre recombinase under the control of the murine aP2 promoter. To estimate the extent of cell-specific disruption of the *Arnt* and *Hif1α* loci, qPCR analysis was used. A PCR amplicon for the *Arnt* null allele amplified as a 340 base pair product, and was detected in genomic DNA isolated from adipocytes or adipose tissue of $Arnt^{\Delta Adipo}$ mice, and not in adipose DNA isolated from $Arnt^{F/F}$ mice. In contrast, the floxed allele was the only band detected in adipose tissue from $Arnt^{F/F}$ mice, and from all non-adipose tissues in $Arnt^{\Delta Adipo}$ mice. The *Hif1α* null amplicon amplified as a 355 base pair product, and was detected in genomic DNA isolated from adipose tissue of $Hif1\alpha^{\Delta Adipo}$ mice and was not detected in adipose tissue DNA isolated from $Hif1\alpha^{F/F}$ mice. The null allele was only detected in adipose tissue and not in liver, skeletal muscle, spleen, and kidney from $Hif1\alpha^{\Delta Adipo}$ mice. The expression of Arnt mRNA was specifically decreased by 50% in white adipose tissue (WAT) and brown adipose tissue (BAT) in the $Arnt^{\Delta Adipo}$ mice compared to $Arnt^{F/F}$ mice; no decrease was evident from liver or skeletal muscle. In addition, qPCR showed nearly absent expression of ARNT mRNA in the adipocytes of $Arnt^{\Delta Adipo}$ mice (FIG. 1A). Similar results were obtained from tissues of $Hif1\alpha^{\Delta Adipo}$ mice and an approximately 88% decrease in *Hif1α* mRNA from adipocytes was observed (FIG. 1B).

To confirm that loss of ARNT was of functional significance, the extent of activation of the ARNT-dependent aryl hydrocarbon receptor (AHR) pathway upon 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) challenge was determined. Induction

of the AHR target gene *Cyp1a1* was markedly attenuated in WAT and BAT of $Arnt^{\Delta Adipo}$ mice (FIGS. 1C and 1D), however, no significant difference in the extent of induction of CYP1A1 mRNA was noted in liver or skeletal muscle as compared to $Arnt^{F/F}$ mice. These results demonstrate adipocyte-specific knockout of the *Arnt* and *Hif1α* genes in mice.

Example 3

Response of $Arnt^{\Delta Adipo}$ or $Hif1\alpha^{\Delta Adipo}$ Mice to High Fat Diet

This example describes characterization of $Arnt^{\Delta Adipo}$ or $Hif1\alpha^{\Delta Adipo}$ mice fed a high fat diet (HFD).

To explore the role of HIF1 in fat metabolism and glucose homeostasis, male mice were fed either a chow diet or HFD. When fed a chow diet, $Arnt^{\Delta Adipo}$ and $Hif1\alpha^{\Delta Adipo}$ mice grew at a rate similar to that of $Arnt^{F/F}$ and $Hif1\alpha^{F/F}$ mice, respectively. However, 12 weeks of HFD led to weight gain in $Arnt^{F/F}$ and $Hif1\alpha^{F/F}$ mice while $Arnt^{\Delta Adipo}$ and $Hif1\alpha^{\Delta Adipo}$ mice were resistant to the HFD-induced weight gain (FIGS. 2A and 2B). $Arnt^{\Delta Adipo}$ and $Hif1\alpha^{\Delta Adipo}$ mice maintained on a HFD for 12 weeks exhibited a significant reduction in body mass compared to littermate controls. NMR measurements confirmed that the body fat mass and the ratio of fat and body mass of $Arnt^{\Delta Adipo}$ and $Hif1\alpha^{\Delta Adipo}$ mice fed a HFD were decreased compared to $Arnt^{F/F}$ and $Hif1\alpha^{F/F}$ mice, respectively (FIGS. 2C and 2D). The adipocyte size in $Arnt^{\Delta Adipo}$ and $Hif1\alpha^{\Delta Adipo}$ mice was significantly decreased compared to $Arnt^{F/F}$ and $Hif1\alpha^{F/F}$ mice respectively, after 12 weeks of HFD (FIGS. 2C and 2D).

To explore the mechanism of reduced adiposity in $Arnt^{\Delta Adipo}$ and $Hif1\alpha^{\Delta Adipo}$ mice, cumulative food intake, metabolic efficiency, and metabolic rates were measured in young mice maintained on chow diet as well as in mice fed HFD for 4 to 7 weeks, which is before the difference between control and mutant mice became apparent. There were no significant differences in weight, cumulative food intake, or metabolic efficiency for 2 weeks between 6 to 8 week old $Hif1\alpha^{F/F}$ and $Hif1\alpha^{\Delta Adipo}$ mice maintained on chow diet (FIGS. 3A-C). Similarly, indirect calorimetry performed at 24°C and thermoneutrality (30°C) on the same set of mice did not reveal significant differences in metabolic rate, respiratory exchange ratio (RER),or

activity between *Hif1α*$^{F/F}$ and *Hif1α*$^{\Delta Adipo}$ mice fed chow diet. A short 4-day exposure to HFD caused comparable increases in body weight and resting metabolic rate in *Hif1α*$^{F/F}$ and *Hif1α*$^{\Delta Adipo}$ mice indicating that adipose specific inactivation of *Hif1α* did not alter the acute thermogenic response to HFD (FIGS. 3D-F). On 4-6 weeks of HFD, cumulative food intake remained similar between *Hif1α*$^{F/F}$ and *Hif1α*$^{\Delta Adipo}$ mice, however, the metabolic efficiency was decreased in the Hif1α$^{\Delta Adipo}$ mice (FIG. 2E), suggesting an increase in the metabolic rate. The indirect calorimetry performed on week 7 of HFD showed that resting and total O_2 consumption adjusted to body weight were significantly increased in *Hif1α*$^{\Delta Adipo}$ mice at both 24°C and 30°C (FIG. 2F). *Hif1α*$^{\Delta Adipo}$ mice had reduced resting and total RER at 30°C, suggesting increased fatty acid oxidation (FIG. 2G) and a tendency towards increased activity (FIG. 2H). All of these changes in *Hif1α*$^{\Delta Adipo}$ mice could contribute to the decrease in weight gain on a HFD as compared to *Hif1α*$^{F/F}$ mice.

To explore the role of adipocyte HIF1 deficiency in obesity-induced insulin resistance, glucose and insulin tolerance tests (GTT and ITT) were performed. When fed a chow diet, there were no significant differences in GTT and ITT between *Arnt*$^{F/F}$ and *Arnt*$^{\Delta Adipo}$ mice (FIGS. 4A and 4B). However, GTT revealed that after 11 weeks of HFD challenge, *Arnt*$^{\Delta Adipo}$ and *Hif1α*$^{\Delta Adipo}$ mice displayed significantly reduced blood glucose compared to *Arnt*$^{F/F}$ and *Hif1α*$^{F/F}$ mice, respectively, at 30, 60, 90, and 120 minutes after glucose loading, suggesting that disruption of HIF1 in adipocytes could markedly improve the HFD-induced glucose intolerance (FIGS. 4A and 5A). Insulin sensitivity was further determined by performing ITT. Sixty minutes after insulin challenge, glucose levels in *Arnt*$^{\Delta Adipo}$ mice decreased to approximately 50% of baseline with only a 15% decline noted in *Arnt*$^{F/F}$ mice; similar results were obtained from the *Hif1α*$^{\Delta Adipo}$ mice, suggesting a significant improvement in insulin sensitivity by adipose HIF1 disruption (FIGS. 4B and 5B). Moreover, fed glucose, fasted glucose, and fasted serum insulin levels were significantly lower in *Arnt*$^{\Delta Adipo}$ and *Hif1α*$^{\Delta Adipo}$ mice compared to *Arnt*$^{F/F}$ and *Hif1α*$^{F/F}$ mice, respectively, after 12 weeks of HFD. The calculated homeostasis model assessment (HOMA) measure of insulin resistance was significantly decreased in *Arnt*$^{\Delta Adipo}$ and *Hif1α*$^{\Delta Adipo}$ mice (Table 2). *Arnt*$^{\Delta Adipo}$ and *Hif1α*$^{\Delta Adipo}$

mice also had reduced fasted serum triglycerides and free fatty acid levels consistent with improved glucose tolerance and insulin sensitivity in these mice (Table 2).

Table 2. Metabolic parameters of mice after 12 weeks of HFD

Parameter	*Arnt*$^{F/F}$	*Arnt*$^{\Delta Adipo}$	*Hif1α*$^{F/F}$	*Hif1α*$^{\Delta Adipo}$
Fasted glucose (mg/dl)	150 ±21.7	106±21.5*	172 ±32.5	131±19.8*
Fed glucose (mg/dl)	269±28.1	139±27.9**	206±23.5	149±30.4**
Fasted serum insulin (ng/ml)	3.6±1.4	2.1±0.8*	2.6±0.6	1.1±0.2**
HOMA index	31.7±8.6	13.8±7.6	27.9±10.4	8.4±1.8**
Fasted serum triglyceride (mg/dl)	124±42.1	78.4±7.5*	162±38.8	99.4±19.8**
Fasted serum cholesterol (mg/dl)	182±37.6	169±43.6	196±45.8	160±24.1
Fasted serum FFA (mmol/l)	1.88±0.42	1.07±0.39*	1.63±0.35	1.09±0.30*

Mean value ± S.D. *P<0.05, **P<0.01 compared to control.
HOMA-IR, homeostasis model assessment of insulin resistance

Insulin sensitivity at earlier time points on the HFD prior to the change in body weight between *Arnt*$^{\Delta Adipo}$ and *Hif1α*$^{\Delta Adipo}$ mice was investigated to determine whether improved glucose metabolism was due to the reduced adiposity in these mice. Although body mass was similar between *Hif1α*$^{F/F}$ and *Hif1α*$^{\Delta Adipo}$ mice after 4 weeks, 6 weeks and 8 weeks of HFD, GTT and ITT revealed that glucose tolerance and insulin sensitivity began to be improved in *Hif1α*$^{\Delta Adipo}$ mice from 4 weeks on the HFD (FIGS. 6A and 6B). After 6 weeks of HFD challenge, fasted glucose was similar while fasted insulin levels and HOMA index in *Hif1α*$^{\Delta Adipo}$ mice were significantly decreased (FIG. 6C). These results indicated HIF1 disruption in adipocytes improved HFD-induced glucose tolerance and insulin resistance before the onset of the decrease in body mass.

To characterize *in vivo* insulin action further, a hyperinsulinemic-euglycemic clamp was performed in *Hif1α*$^{F/F}$ and *Hif1α*$^{\Delta Adipo}$ mice after 15 weeks of HFD. In the basal state, *Hif1α*$^{\Delta Adipo}$ mice had significantly reduced plasma glucose and insulin levels; basal endogenous glucose production (EGP) was similar between genotypes (FIG. 5C-E and G). During the clamp, insulin was infused to maintain

plasma insulin levels at about 4 ng/ml (FIG. 5E), and the glucose infusion rate (GIR) was adjusted in order to maintain blood glucose levels in *Hif1α*$^{F/F}$ and *Hif1α*$^{\Delta Adipo}$ mice at similar levels (FIGS. 5C, D, and F). The GIR was significantly increased in *Hif1α*$^{\Delta Adipo}$ mice (FIGS. 5F and G), which confirmed improved whole-body insulin sensitivity in *Hif1α*$^{\Delta Adipo}$ mice. During the clamp, insulin induced a more marked suppression of EGP in *Hif1α*$^{\Delta Adipo}$ mice, suggesting increased insulin sensitivity in the liver. Whole body glucose disposal (Rd) and glucose uptake into skeletal muscle and adipose tissue were significantly increased in *Hif1α*$^{\Delta Adipo}$ mice (FIG. 5G). Without being bound by any particular theory, these data suggest that adipose selective inactivation of HIF1 caused increased insulin sensitivity in major insulin target tissues, liver, skeletal muscle, and fat.

Since insulin resistance is triggered by dysregulation of the insulin signaling cascade, insulin action was investigated in white adipose tissue (WAT), liver and skeletal muscle (Saltiel and Kahn, *Nature* 414:799-806, 2001). When fed a HFD, insulin-stimulated tyrosine-phosphorylation of insulin receptor (IR), IRS-1, and phosphorylation of Akt in the WAT, liver and skeletal muscle of *Arnt*$^{F/F}$ and *Hif1α*$^{F/F}$ mice were weakly induced, in contrast to a robust induction in *Arnt*$^{\Delta Adipo}$ and *Hif1α*$^{\Delta Adipo}$ mice after insulin administration. *Arnt* deficiency in adipocytes improved the HFD-impaired insulin signaling pathway in WAT, liver and skeletal muscle, and targeted disruption of *Hif1α* in adipocytes completely mimicked the protective effects of adipose *Arnt*-deficiency on HFD-induced insulin resistance. These findings indicated that HIF1 deficiency in adipocytes improved HFD-induced glucose tolerance and insulin resistance.

Example 4

Expression of Lipid and Glucose Metabolism-Related Genes in ARNT- and HIF1-Deficient Adipose Tissue

This example describes the effect of HIF1 disruption on expression of lipid and glucose metabolism-related genes.

After 12 weeks of HFD, *Hif1α* expression was significantly induced, resulting in activation of HIF1 target genes such as *Glut1* and *Vegf* (FIG. 7A). On a chow diet, these target genes and adiponectin expression were similar between

Hif1α$^{F/F}$ and *Hif1α*$^{\Delta Adipo}$ mice (FIG. 7B). In addition, there was no significant Hif1α protein expression in WAT from chow-fed mice, while it was induced in HFD-fed mice. These data explain why the phenotype of *Hif1α*$^{\Delta Adipo}$ mice is similar with *Hif1a*$^{F/F}$ mice on a chow diet while different on a HFD.

Gene expression profiling of WAT was performed after 12 weeks of HFD in *Arnt*$^{F/F}$ and *Arnt*$^{\Delta Adipo}$ mice or *Hif1α*$^{F/F}$ and *Hif1α*$^{\Delta Adipo}$ mice. Microarray and qPCR analysis of gene expression in WAT showed that in the *Arnt*$^{\Delta Adipo}$ and *Hif1α*$^{\Delta Adipo}$ mice, the expression of *Glut1* and *Vegf-c*, well-characterized HIF1 target genes, were decreased (FIGS. 8A and 8B). Expression of genes involved in adipogenesis and glucose metabolism, *Pparg*, *C/EBPα*, *Cfd*, and *Glut4*, were upregulated in WAT of *Arnt*$^{\Delta Adipo}$ and *Hif1α*$^{\Delta Adipo}$ mice compared to *Arnt*$^{F/F}$ and *Hif1α*$^{F/F}$ mice, respectively, on a HFD. Expression of the lipolysis related genes *Atgl* and *Hsl,* and the mitochondrial biogenesis related genes *Pgc1α* and *Pgc1β,* were increased, and expression of inflammation related genes *Tnfα* and *Pai-1,* were downregulated in *Arnt*$^{\Delta Adipo}$ and *Hif1α*$^{\Delta Adipo}$ mice (FIGS. 8A and 8B). Importantly, expression of *adiponectin* was upregulated in *Arnt*$^{\Delta Adipo}$ and *Hif1α*$^{\Delta Adipo}$ mice. Total serum adiponectin, the HMW form of adiponectin, and the ratio of HMW to total adiponectin were all increased in *Arnt*$^{\Delta Adipo}$ and *Hif1α*$^{\Delta Adipo}$ mice on a 12 week HFD (FIG. 8C). HMW adiponectin and ratio of HMW to total adiponectin began to be increased on a 6 week HFD before the decrease in body weight (FIG. 6D). Expression of the fibrosis related genes *Lox*, *Col1a1*, *Col3a1* and *Loxl1* were also decreased in *Arnt*$^{\Delta Adipo}$ or *Hif1α*$^{\Delta Adipo}$ mice (FIG. 8D).

To assess the mechanism of the observed improved glucose metabolism, the inflammatory status of adipose tissue was investigated and found to be reduced in *Arnt*$^{\Delta Adipo}$ and *Hif1α*$^{\Delta Adipo}$ mice. Macrophage marker F4/80 staining of adipose tissue sections revealed that macrophage infiltration to WAT decreased significantly in *Arnt*$^{\Delta Adipo}$ and *Hif1α*$^{\Delta Adipo}$ mice. Expression of mRNAs encoding the macrophage markers F4/80 and CD68 were also decreased in *Arnt*$^{\Delta Adipo}$ mice and *Hif1α*$^{\Delta Adipo}$ mice (FIGS. 9A and 9B). Since it was reported that aP2 is expressed in macrophage (Makowski *et al., Nat. Med* 7:699-705, 2001), the possibility that HIF1 was disrupted in macrophages was investigated by determining *Hif1α* gene disruption in peritoneal macrophages (P-Mϕ) from *Arnt*$^{\Delta Adipo}$ mice. The knockout efficiency of

Arnt in peritoneal macrophages of $Arnt^{\Delta Adipo}$ mice was marginal (FIG. 10A), whereas qPCR showed little detectable expression of ARNT mRNA in the adipocytes of $Arnt^{\Delta Adipo}$ mice.

Socs3 expression was significantly upregulated in parallel with increased *Hif1α* expression in WAT from HFD wild-type mice (FIG. 7A). Further, haploinsufficiency of *Socs3* significantly protected mice against the development of diet-induced obesity and associated metabolic complications (Howard *et al., Nat. Med.* 10:734-738, 2004). Consistent with these findings, SOCS3 mRNA was also found to be downregulated in $Arnt^{\Delta Adipo}$ and $Hif1\alpha^{\Delta Adipo}$ mice as revealed by microarray analysis. qPCR confirmed that expression of SOCS3 mRNA in $Arnt^{\Delta Adipo}$ and $Hif1\alpha^{\Delta Adipo}$ mice was significantly lower than that in $Arnt^{F/F}$ and $Hif1\alpha^{F/F}$ mice, respectively (FIG. 8E). The protein level of SOCS3 was also significantly decreased in $Hif1\alpha^{\Delta Adipo}$ mice (FIG. 8F). Adipocytes and stromal vascular fractions (SVF) were prepared from WAT of $Hif1\alpha^{\Delta Adipo}$ and $Hif1\alpha^{F/F}$ mice after 12 weeks of HFD, and the macrophage enriched in SVF (SVF-Mϕ). In adipocytes, no significant expression of HIF1α mRNA in $Hif1\alpha^{\Delta Adipo}$ mice was found. The expression of *Socs3* in $Hif1\alpha^{\Delta Adipo}$ mice was about 80% decreased as compared to that in $Hif1\alpha^{F/F}$ mice. *Adiponectin* was upregulated in $Hif1\alpha^{\Delta Adipo}$ mice (FIG. 10B). Consistent with the data obtained in P-Mϕ, in SVF-Mϕ, the expression of HIF1α, SOCS3 and adiponectin (the adiponectin expression is very low and almost undetectable in SVF-Mϕ) were not significantly different between $Hif1\alpha^{\Delta Adipo}$ and $Hif1\alpha^{F/F}$ mice (FIG. 10C).

Since inactivation of STAT3 may be involved in the inhibition of adiponectin production by SOCS3, tyrosine-phosphorylation of STAT3 was assessed in WAT from $Hif1\alpha^{F/F}$ and $Hif1\alpha^{\Delta Adipo}$ mice. Tyrosine-phosphorylation of STAT3 was significantly induced in parallel with increased adiponectin level in $Hif1\alpha^{\Delta Adipo}$ mice (FIG. 8F).

There was no striking change in BAT histological appearance between $Hif1\alpha^{\Delta Adipo}$ mice and $Hif1\alpha^{F/F}$ mice after 12 weeks of HFD. HIF1α protein expression was not significantly induced in BAT after HFD treatment. qPCR analysis revealed that HIF1α and expression of its target genes (*Glut1, Vegf*, and *Socs3*) were not significantly different in BAT between chow diet and HFD

treatment, while *Adiponectin*, *Ucp-1* and *Pgc1α* expression were decreased after HFD treatment. *Ucp-1* expression was upregulated in *Hif1α*$^{\Delta Adipo}$ mice compared to *Hif1α*$^{F/F}$ mice after HFD.

Example 5

HIF1 Directly Regulates *Socs3* in Adipocytes

This example describes analysis of regulation of Socs3 expression by HIF1 in adipocytes.

SOCS3 mRNA and protein levels were decreased in WAT of *Arnt*$^{\Delta Adipo}$ mice and *Hif1α*$^{\Delta Adipo}$ mice after HFD. To determine whether *Socs3* was a direct target gene of HIF1, experiments were conducted *in vitro* with 3T3-L1 adipocytes. Expression of SOCS3 mRNA was induced after a 12 hour treatment by hypoxia (1% O_2) or the hypoxia mimic reagent $CoCl_2$ (100 μM) (FIG. 11A). To further confirm the role of HIF1 in SOCS3 expression, ARNT and HIF1α expression were knocked down with specific siRNAs. The knockdown efficiency of ARNT and HIF1α expression in 3T3-L1 adipocytes was 90%. ARNT and HIF1α siRNAs completely reversed the upregulation of SOCS3 mRNA by $CoCl_2$ (100 μM) in 3T3-L1 adipocytes (FIG. 11B).

Three potential HIF response elements (HRE) were found in the promoter region of *Socs3*. To determine whether *Socs3* was a direct target gene of HIF1α, the *Socs3* promoter including three HREs was transiently co-transfected with empty vector, constitutively activated HIF1α or HIF2α expression plasmids into 3T3-L1 adipocytes. After 24 hours, the cells were assayed for luciferase activity. Co-transfection with HIF1α increased the *Socs3*-luciferase expression about six-fold, while no increase of the *Socs3*-luciferase expression compared to the constructs in which the HREs were deleted or by co-transfection with HIF2α (FIG. 11C), thus demonstrating HIF1α as the major isoform regulating the *Socs3* promoter. Next, ChIP assays were performed using cross-linked soluble chromatin isolated from normoxic or hypoxic 3T3-L1 adipocytes. Primers flanking HRE1 or both HRE2 and HRE3 specifically amplified DNA sequences immunoprecipitated by HIF1α antibody in hypoxic samples, demonstrating that HIF-1α is able to bind the *Socs3*

regulatory region at the HREs after hypoxia (FIG. 11D). These results indicated that *Socs3* is a direct target gene of HIF1.

To determine whether *Socs3* is involved in HIF1 suppression of *Adiponectin* expression, studies were carried out in 3T3-L1 adipocytes. The knockdown efficiency of *Socs3* expression in 3T3-L1 adipocytes was 90% (FIG. 12A). SOCS3 siRNA reversed the downregulation of adiponectin mRNA by $CoCl_2$ (FIG. 12B). Moreover, SOCS3 siRNA blocked the inhibition of adiponectin secretion by the HIF1α inducer $CoCl_2$. $CoCl_2$ inhibited activation of STAT3 and HIF1α siRNA increased activation of STAT3 (FIG. 12C). Next, the STAT3 inhibitor NSC 74859 (100 μM) was found to attenuate induction of *Adiponectin* by HIF1α siRNA under $CoCl_2$ treatment (FIG. 12D). Thus, mechanistic studies in cultured cells revealed that HIF1α suppressed expression of *Adiponectin*, an insulin-sensitizing adipokine, might be through a SOCS3-STAT3 pathway. In $Hif1\alpha^{\Delta Adipo}$ mice, *Socs3* is not induced and *Adiponectin* is overexpressed as a result of activation of STAT3 by phosphorylation, resulting in increased insulin sensitivity.

In order to demonstrate the role of the SOCS3-STAT3-Adiponectin pathway in insulin resistance *in vivo*, $Hif1\alpha^{\Delta Adipo}$ mice were fed a HFD for 6 weeks and then administered the STAT3 inhibitor NSC 74859 (5 mg/kg) while the mice were maintained on the HFD. The body weight, fat mass, and fat mass ratio were increased significantly after 6 weeks of NSC 74859 treatment (FIGS. 13A-B). Mice fed the inhibitor also exhibited exacerbated insulin resistance (FIGS. 13C-E). The activation of STAT3 in WAT was significantly reduced, coincident with the decrease of total adiponectin levels, HMW adiponectin, and the ratio of HMW to total adiponectin (FIGS. 13F and G). These results suggest that the SOCS3 and adiponectin pathway may be involved, at least in part, in the improvement of HFD-induced insulin resistance in $Hif1\alpha^{\Delta Adipo}$ mice.

Example 6

Treatment of Wild-Type Mice with a HIF1α Inhibitor

This example describes the effect of treating wild-type mice fed a HFD with a HIF1α inhibitor.

Since adipocyte-specific disruption of HIF1α or its heterodimerization partner ARNT could protect mice against HFD-induced obesity and insulin resistance, it was important to determine whether chemical inhibition of HIF1α could produce the same phenotype. To further investigate the role of HIF1 during the pathogenesis of obesity and insulin resistance, HFD-fed mice were administered acriflavine (ACF), a specific inhibitor of HIF1α. ACF was identified as a drug that binds directly to HIF1α and inhibits HIF1 dimerization and transcriptional activity (Lee *et al., Proc. Natl. Acad. Sci. USA* 106:17910-17915, 2009).

ACF (5 μM) completely reversed the upregulation of SOCS3 mRNA by $CoCl_2$ (100 μM) in 3T3-L1 adipocytes (FIG. 15A). ACF (2 mg/kg) prevented mice from HFD-induced weight gain after 5 weeks of HFD (FIG. 14A). Fat mass and the ratio of fat and body mass in ACF-treated mice were decreased significantly after 12 weeks of HFD (FIG. 14B). GTT and ITT revealed that insulin sensitivity began to be improved in ACF-treated mice as early as 4 weeks on HFD, and the improvement became more significant on 8- and 12-weeks HFD (FIGS. 14C and 15B-C). After 6 weeks and 12 weeks of HFD, fasting glucose (12 week HFD), fasting insulin levels and the HOMA index in ACF-treated mice were significantly decreased (FIGS. 14D and 15D). ACF-treated mice exhibited higher serum total adiponectin levels (9- and 12-week HFD), HMW adiponectin and the ratio of HMW to total adiponectin (FIGS. 14E and 15E). ACF-treated mice exhibited higher serum total adiponectin levels, HMW adiponectin and the ratio of HMW to total adiponectin after 6, 9, and 12 weeks of HFD (FIG. 14E). qPCR analysis of HIF1α target gene (*Glut1, Vegf-c*, and *Socs3*) expression were significantly reduced, and *Adiponectin* expression was increased in WAT after ACF treatment (FIG. 14F).

Example 7

Chemical Inhibition of HIF1α Mimics Genetic Inhibition

This example describes the effect of treating wild-type and *Hif1α*$^{\Delta Adipo}$ mice with a specific inhibitor of HIF1α.

Hif1α$^{F/F}$ and *Hif1α*$^{\Delta Adipo}$ mice were fed a HFD for 7 weeks, and then administered 2 mg/kg ACF i.p. while the mice were maintained on a HFD.

Body mass of ACF-treated *Hif1α*$^{F/F}$ mice was decreased significantly beginning after 4 weeks of ACF treatment, and in *Hif1α*$^{\Delta Adipo}$ mice body mass began to decrease after 11 weeks of ACF treatment (Figure 16A). NMR measurements showed that fat mass and the ratio were significantly decreased after 16 weeks of ACF treatment (Figure 16B). GTT revealed that 11 week-ACF-treated *Hif1α*$^{F/F}$ mice displayed significantly reduced blood glucose compared to vehicle-treated *Hif1α*$^{F/F}$ mice at 15, 30, 60, 90, and 120 minutes after glucose loading, while in *Hif1α*$^{\Delta Adipo}$ mice blood glucose was lower at 90, and 120 minutes after ACF treatment (FIG. 17A). The areas under the curve (AUC) of GTT were significantly lower after ACF treatment (Figure 17A). Insulin sensitivity was further determined by performing ITT. In *Hif1α*$^{F/F}$ mice, ACF treatment decreased blood glucose significantly at 30, 60 and 120 minutes, however, in *Hif1α*$^{\Delta Adipo}$ mice blood glucose was decreased at 60, and 90 minutes after 16 weeks of ACF treatment (Figure 17B). Fasted glucose, fasted serum insulin levels and HOMA were significantly lower in 16 weeks of ACF-treated *Hif1α*$^{F/F}$ mice (Figure 17C). HOMA was also decreased in ACF-treated *Hif1α*$^{\Delta Adipo}$ mice (Figure 17D). ACF-treated *Hif1α*$^{F/F}$ mice and *Hif1α*$^{\Delta Adipo}$ mice had reduced fasted serum free fatty acid levels (Figure 18A and B). In *Hif1α*$^{F/F}$ mice, ACF treatment increased HMW adiponectin level. However adiponectin levels remained similar in *Hif1α*$^{\Delta Adipo}$ mice after 16 weeks of ACF treatment (Figure 19A and B). These results reveal that ACF has effect on obesity and type 2 diabetes. Without being bound by theory, it is believe that this effect might be partly due to the inhibition of Hif1α in adipose tissue and the improvement of adiponectin.

Example 8

Effects of HIF1α Inhibitors *In Vivo*

This example describes methods that can be used to assess the effect of treating an animal model of obesity and/or diabetes with a HIF1α inhibitor. However, one skilled in the art will appreciate based on the teachings herein that methods that deviate from these specific methods can also be used to successfully assess the effects of HIF1α inhibitors *in vivo*.

Animal models of obesity and/or diabetes (including animal models that become overweight or obese only when fed a HFD) are used to assess the *in vivo* effects of HIF1α inhibitors. In some examples, the animal model is the ob/ob mouse (Zhang *et al.*, *Nature* 372:425-432, 1994), or db/db mouse (Chen *et al., Cell* 84:491-495, 1996) which carry mutations in leptin and the leptin receptor, respectively. In another example, the animal model is the KK-A^y mouse. Heterozygous KK-A^y males develop hyperglycemia, hyperinsulinemia, glucose intolerance, and obesity by about eight weeks of age (*e.g.,* Reddi and Camerini-Davalos, *Adv. Exp. Med. Biol.* 246:7-15, 1988). In other examples, the animal model is the Hif1$\alpha^{\Delta Adipo}$ mouse (described herein). One of skill in the art can identify additional animal models of obesity and/or diabetes, for example available from The Jackson Laboratory (jax.org/jaxmice). Suitable control mice are also utilized, such as wild type mice of the same strain as the test animal (*e.g.,* C57BL/6 of Sv129 mice) or a parental strain (*e.g.,* Hif1$\alpha^{F/F}$ in the case of Hif1$\alpha^{\Delta Adipo}$ mice).

In some examples, mice are maintained on a HFD for a period of time (such as about 4-18 weeks) prior to administration of a HIF1α inhibitor. Mice are then administered a HIF1α inhibitor (for example, orally or by i.p. injection) while continuing to be fed HFD. Measurements of parameters related to obesity and diabetes, such as body mass, fat mass, food intake, metabolic efficiency, activity, oxygen consumption, blood glucose (for example in a GTT or ITT), fasting plasma glucose, fasted serum insulin, HOMA index, serum triglycerides, serum free fatty acids, and serum adiponectin (such as total, HMW, and/or HMW/total adiponectin) are measured at various time points.

One of skill in the art can select HIF1α inhibitors (such as those disclosed herein) and appropriate dosages and routes of administration. In some examples, the

mice are administered ACF (such as about 0.1 mg/kg to about 10 mg/kg) by i.p. injection daily. In other examples, a *Hif1α* shRNA is administered to the mice, for example, under the control of an adipose-specific promoter (such as the ap2 promoter). In one example, a *Hif1α* shRNA may be included in a viral vector (such as a lentiviral vector), which is administered i.p. at a dose of about 10^9-10^{12} particles.

In some examples, a decrease in body weight, for example, decreased body mass, decreased fat mass, or a combination of two or more thereof, for example, compared with mice on HFD but not treated with the HIF1α indicates the effectiveness of the treatment. In some examples, increased glucose tolerance, decreased insulin resistance (*e.g.,* decreased fasting plasma glucose or decreased fasting plasma insulin or decreased HOMA score), decreased triglycerides, decreased free fatty acids, increased adiponectin, or a combination of two or more thereof, indicates the effectiveness of the treatment.

Example 9

Method for Decreasing Body Weight

This example describes methods that can be used to decrease body in a subject. However, one skilled in the art will appreciate based on the teachings herein that methods that deviate from these specific methods can also be used to successfully decrease body weight.

In an example, a subject who is in need of a reduction in body weight (for example, an overweight or obese subject, such as a subject with a BMI of more than 25 kg/m^2) is selected. Following subject selection, a therapeutically effective dose of a composition including an inhibitor of HIF1α is administered to the subject. The amount of the composition administered to decrease body weight of the subject depends on the subject being treated, the severity of the disorder, and the manner of administration of the therapeutic composition. Ideally, a therapeutically effective amount of an agent is the amount sufficient to decrease body weight in a subject without causing a substantial cytotoxic effect in the subject.

A decrease in body weight, for example, decreased total body weight, decreased BMI, decreased waist circumference, decreased body fat, or a combination of two or more thereof, indicates the effectiveness of the treatment.

Example 10

Method for Treating Diabetes

This example describes methods that can be used to treat diabetes in a subject. However, one skilled in the art will appreciate based on the teachings herein that methods that deviate from these specific methods can also be used to successfully treat diabetes.

In an example, a subject who has been diagnosed with diabetes or pre-diabetes is identified. Following subject selection, a therapeutically effective dose of a composition including an inhibitor of HIF1α is administered to the subject. The amount of the composition administered to prevent, reduce, inhibit, and/or treat diabetes depends on the subject being treated, the severity of the disorder, and the manner of administration of the therapeutic composition. Ideally, a therapeutically effective amount of an agent is the amount sufficient to prevent, reduce, and/or inhibit, and/or treat the condition (*e.g.*, diabetes) in a subject without causing a substantial cytotoxic effect in the subject.

A reduction in the clinical symptoms associated with diabetes, for example, increased glucose tolerance, decreased insulin resistance (*e.g.,* decreased fasting plasma glucose or decreased fasting plasma insulin or decreased QUICKI score), decreased triglycerides, decreased free fatty acids, decreased HbA1c, or a combination of two or more thereof, indicates the effectiveness of the treatment.

In view of the many possible embodiments to which the principles of the disclosure may be applied, it should be recognized that the illustrated embodiments are only examples and should not be taken as limiting the scope of the invention. Rather, the scope of the invention is defined by the following claims. We therefore claim as our invention all that comes within the scope and spirit of these claims.

We claim:

1. A method for decreasing body weight of a subject, comprising administering to the subject a therapeutically effective amount of a composition comprising an inhibitor of hypoxia-inducible factor 1α (HIF1α), thereby decreasing body weight of the subject.

2. The method of claim 1, wherein decreasing body weight comprises one or more of decreasing total body weight, decreasing body mass index, decreasing waist circumference, or decreasing total body fat as compared to a control.

3. A method for treating diabetes in a subject, comprising administering to a subject having diabetes a therapeutically effective amount of a composition comprising an inhibitor of hypoxia-inducible factor 1α (HIF1α), thereby treating diabetes in the subject.

4. The method of claim 3, wherein treating diabetes in the subject comprises increasing glucose tolerance, decreasing insulin resistance, decreasing serum triglycerides, decreasing serum free fatty acid levels, decreasing hemoglobin A1c levels, or a combination of two or more thereof in the subject as compared to a control.

5. The method of claim 4, wherein decreasing insulin resistance comprises decreasing plasma glucose levels, decreasing plasma insulin levels, or a combination thereof in the subject as compared to a control.

6. The method of claim 5, wherein increasing glucose tolerance comprises decreasing blood glucose levels in a glucose tolerance test in the subject as compared to a control.

7. The method of any one of claims 1 to 6, wherein the subject is overweight or obese.

8. The method of claim 7, wherein the subject has a body mass index of 25 or more.

9. The method of any one of claims 1 to 8, wherein the HIF1α inhibitor decreases HIF1α expression or activity relative to a control.

10. The method of any one of claims 1 to 9, wherein the HIF1α inhibitor comprises a small molecule, antisense compound, or antibody.

11. The method of any one of claims 1 to 10, wherein the HIF1α inhibitor is administered parenterally or orally.

12. The method of claim 10 or claim 11, wherein the small molecule HIF1α inhibitor comprises acriflavine.

13. The method of claim 11 or claim 12, wherein the acriflavine is administered at a dose of about 2 mg/kg.

14. The method of claim 10, wherein the antisense compound is a HIF1α siRNA.

15. The method of claim 14, wherein the HIF1α siRNA comprises a nucleic acid sequence set forth as SEQ ID NO: 70 or SEQ ID NO: 71.

16. The method of any one of claims 1 to 15, wherein the subject is a mammal.

17. The method of claim 16, wherein the subject is a human.

18. The method of any one of claims 1 to 17, wherein the composition further comprises a pharmaceutically acceptable carrier.

19. The method of any one of claims 1 to 18, further comprising providing to the subject a second therapy for modulating body weight or treating diabetes that is not an inhibitor of HIF1α.

5

20. The method of claim 19, wherein the second therapy comprises a lifestyle modification, an antihyperglycemic agent, or insulin.

FIG. 1A

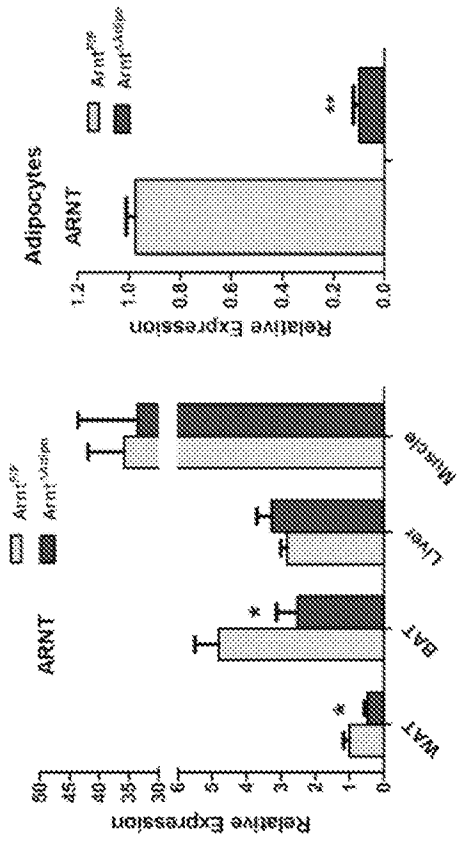


FIG. 1B

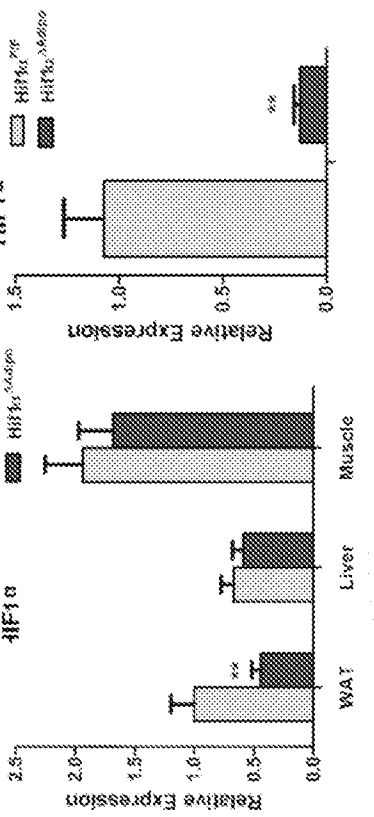


FIG. 1C

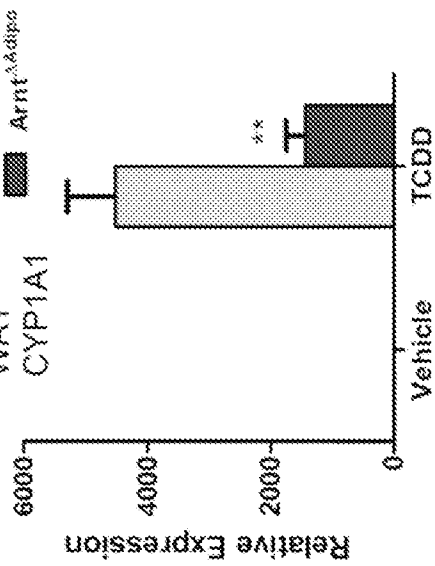


FIG. 1D

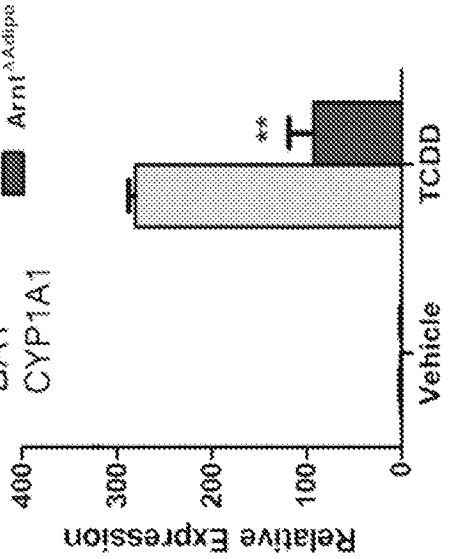


FIG. 2A

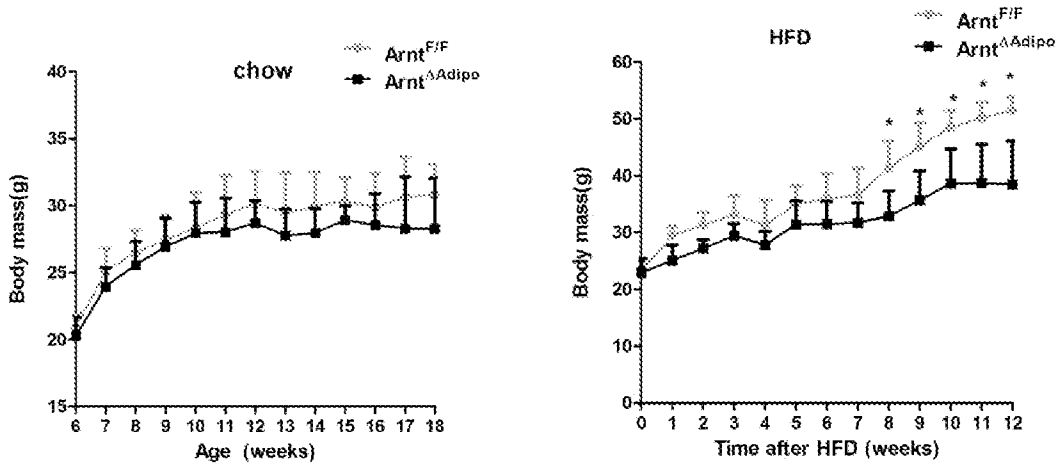
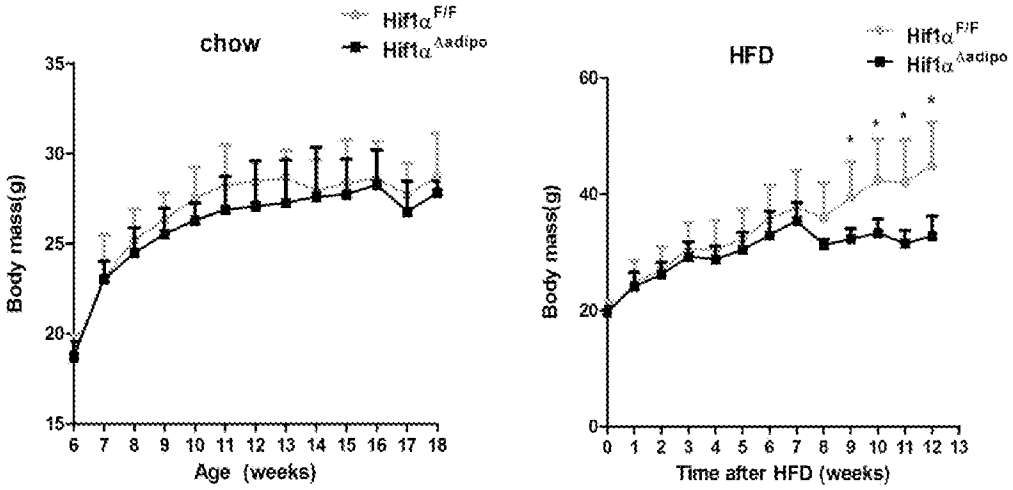
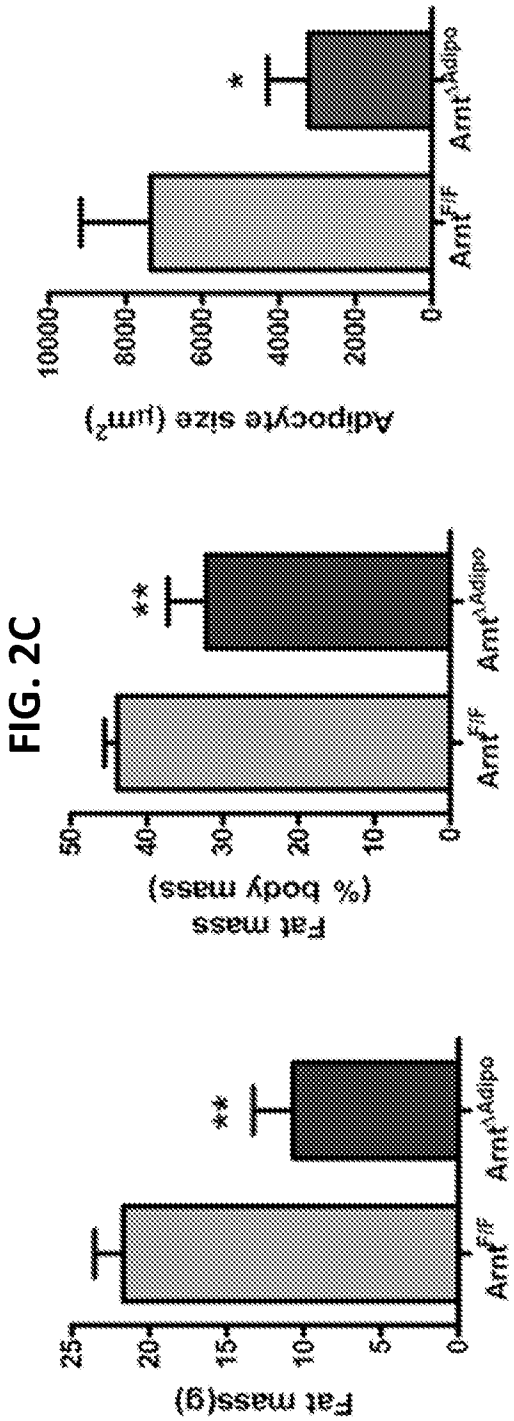

chow
Arnt$^{F/F}$
Arnt$^{\Delta Adipo}$
Body mass(g)
Age (weeks)
HFD
Time after HFD (weeks)

FIG. 2B

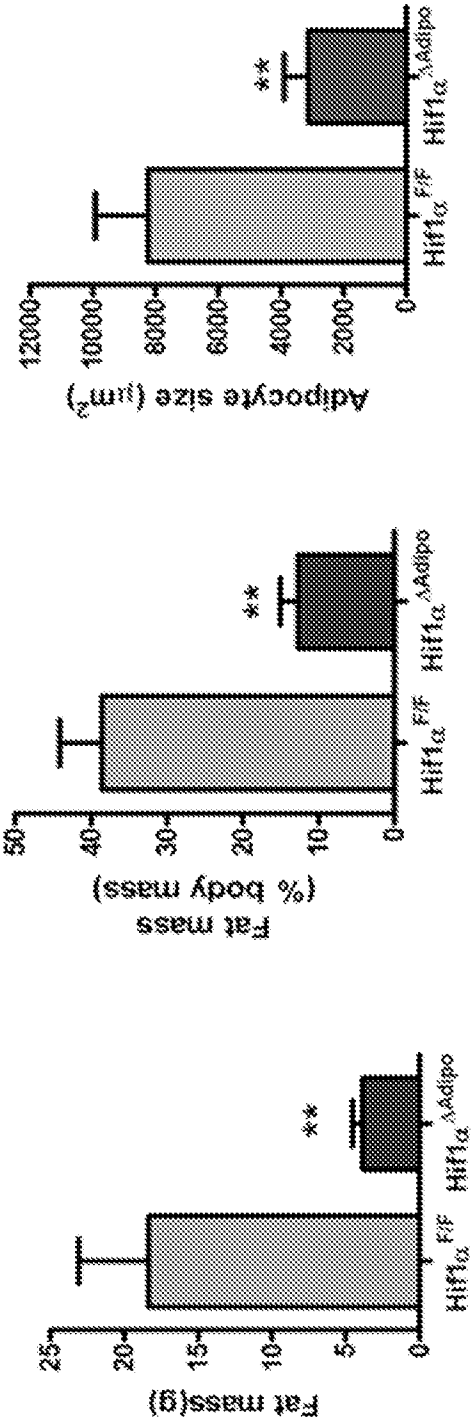

chow
Hif1α$^{F/F}$
Hif1α$^{\Delta adipo}$
Body mass(g)
Age (weeks)
HFD
Time after HFD (weeks)


Fat mass(g)
25
20
15
10
5
0
**
Arnt F/F
Arnt ΔAdipo

Fat mass
(% body mass)
50
40
30
20
10
0
**
Arnt F/F
Arnt ΔAdipo

FIG. 2C

Adipocyte size (μm²)
10000
8000
6000
4000
2000
0
*
Arnt F/F
Arnt ΔAdipo


Fat mass(g)
25
20
15
10
5
0
**
Hif1α F/F
Hif1α ΔAdipo

Fat mass
(% body mass)
50
40
30
20
10
0
**
Hif1α F/F
Hif1α ΔAdipo

FIG. 2D

Adipocyte size (μm²)
12000
10000
8000
6000
4000
2000
0
**
Hif1α F/F
Hif1α ΔAdipo

FIG. 2E

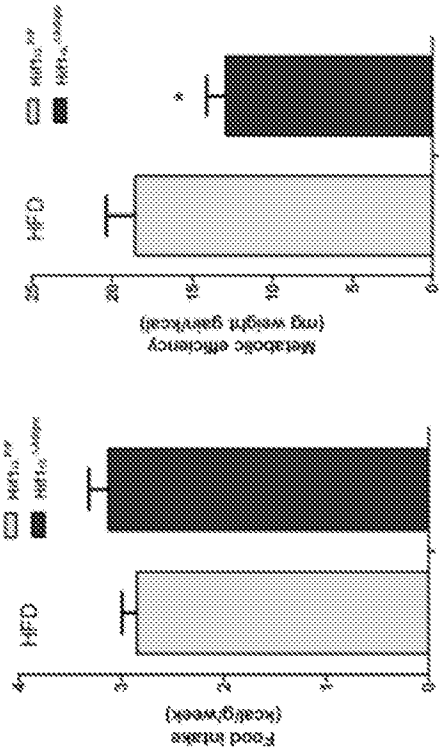


FIG. 2F

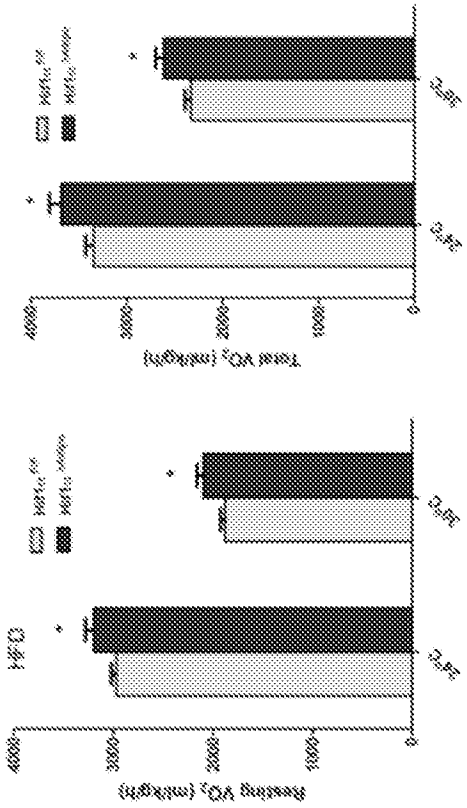


FIG. 2G

FIG. 2H

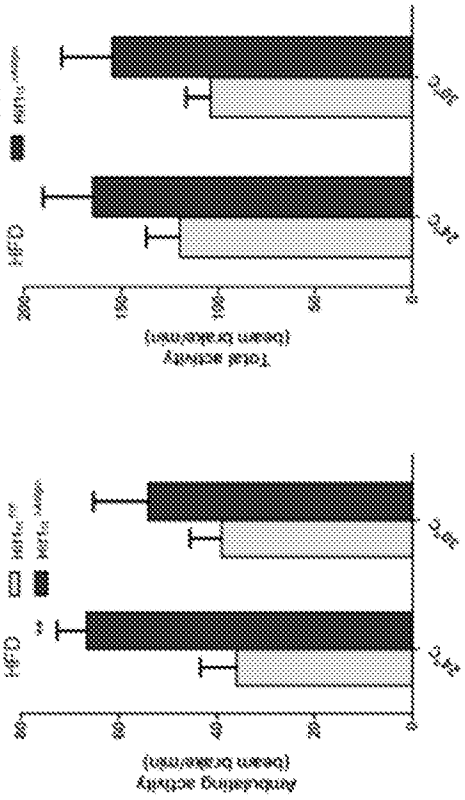


FIG. 3A

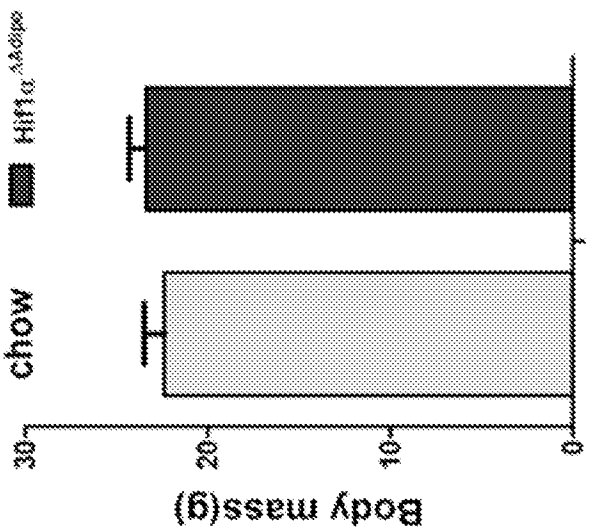


FIG. 3B

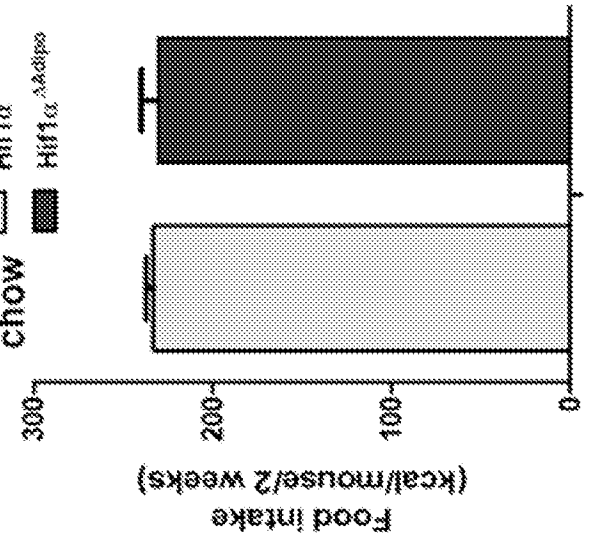
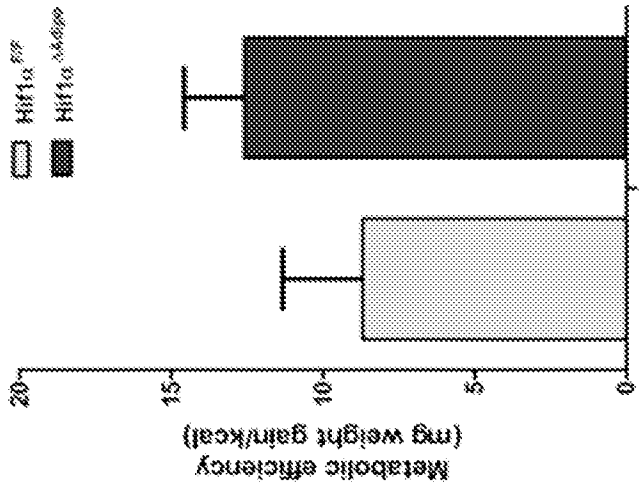


FIG. 3C



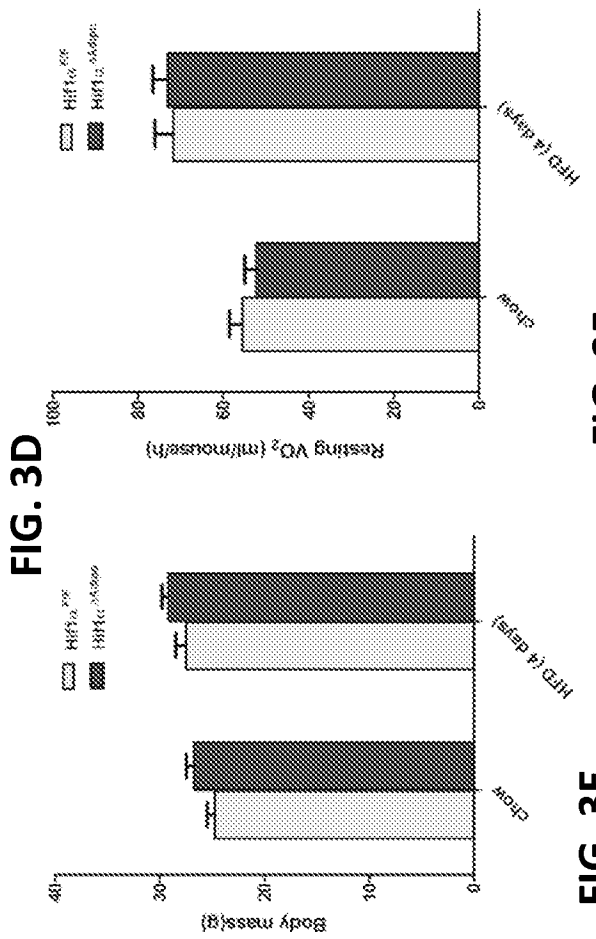
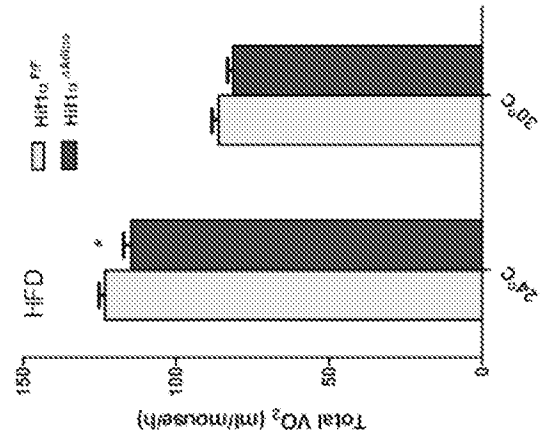


FIG. 3D



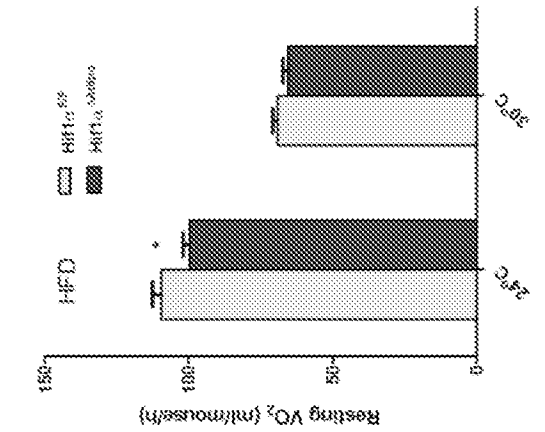


FIG. 3F

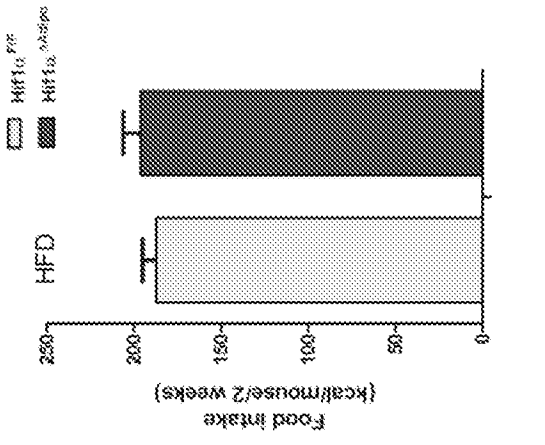


FIG. 3E

FIG. 4A

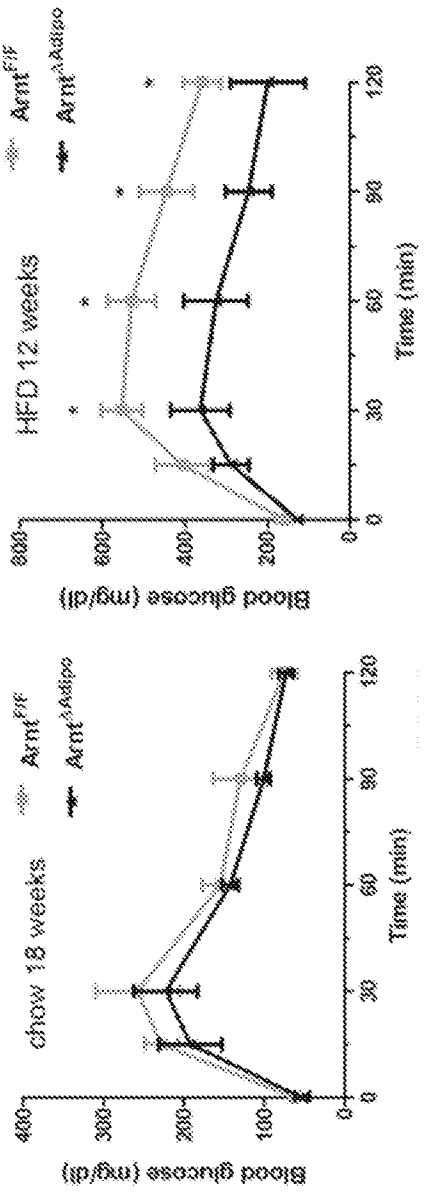

HFD 12 weeks
chow 18 weeks
Blood glucose (mg/dl)
Time (min)
Arm^F/F
Arm^ΔAdipo
0
30
60
90
120
200
400
600
800
100
300

FIG. 4B

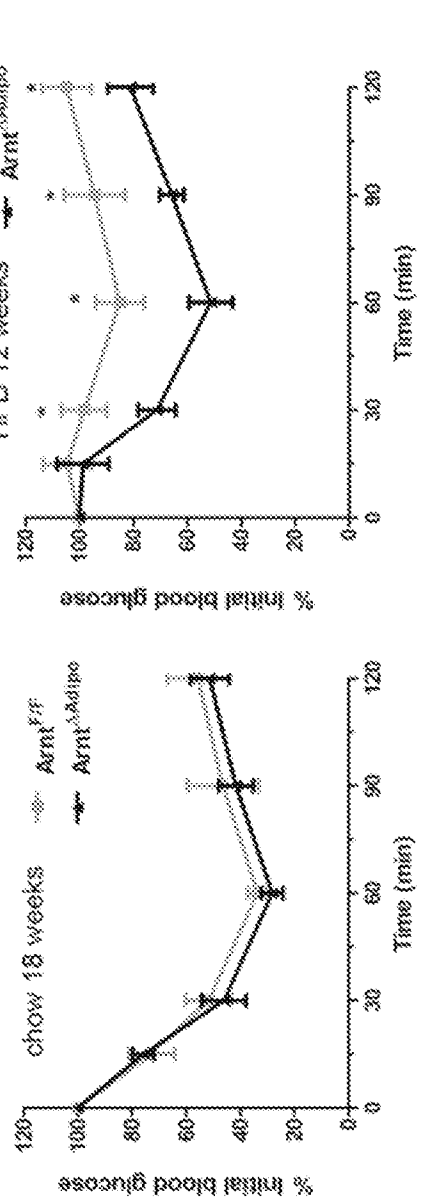

HFD 12 weeks
chow 18 weeks
% initial blood glucose
Time (min)
Arm^F/F
Arm^ΔAdipo
0
30
60
90
120
20
40
60
80
100

FIG. 5A

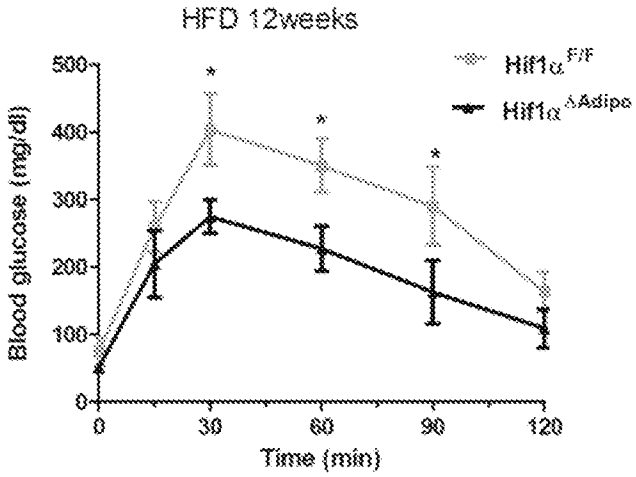

HFD 12weeks
Hif1α F/F
Hif1α ΔAdipo
Blood glucose (mg/dl)
500
400
300
200
100
0
0
30
60
90
120
Time (min)
*
*
*

FIG. 5B

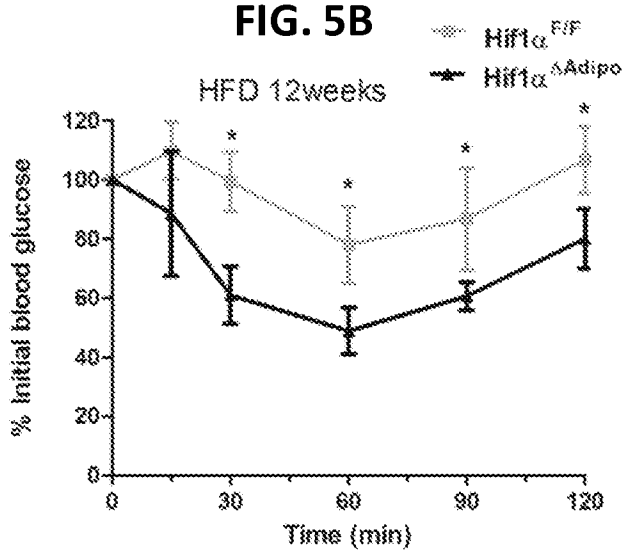

HFD 12weeks
Hif1α F/F
Hif1α ΔAdipo
% initial blood glucose
120
100
80
60
40
20
0
0
30
60
90
120
Time (min)
*
*
*
*

FIG. 5C

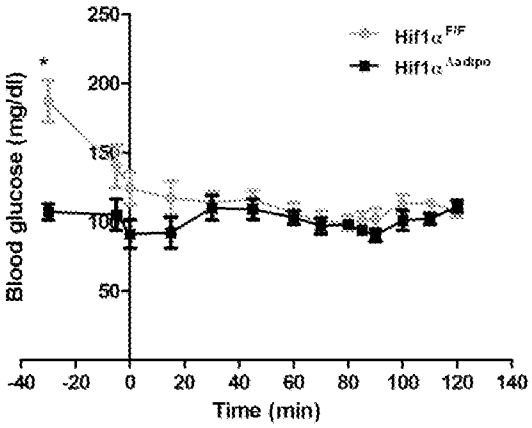


FIG. 5D

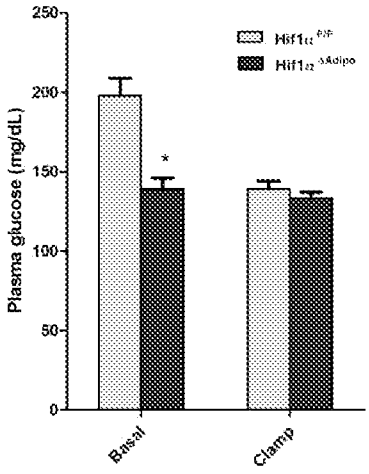


FIG. 5E

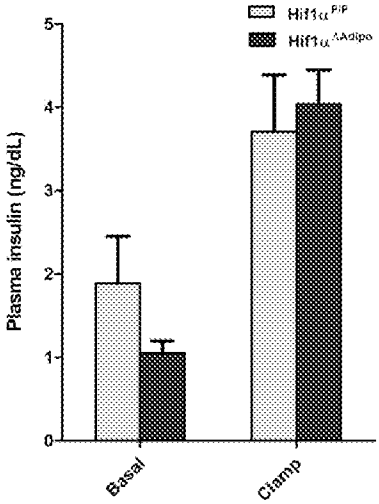


FIG. 5F

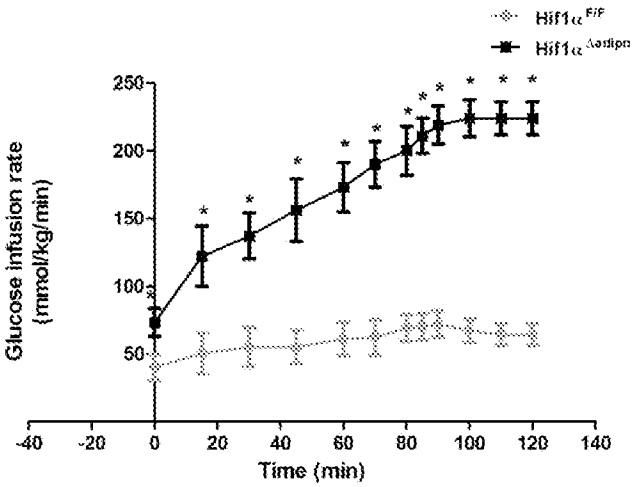
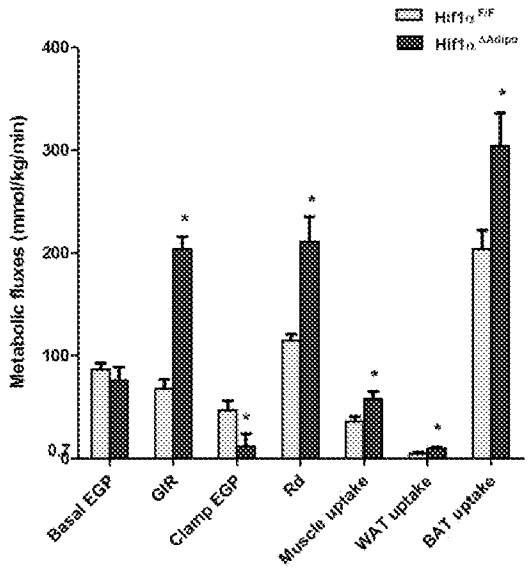


FIG. 5G



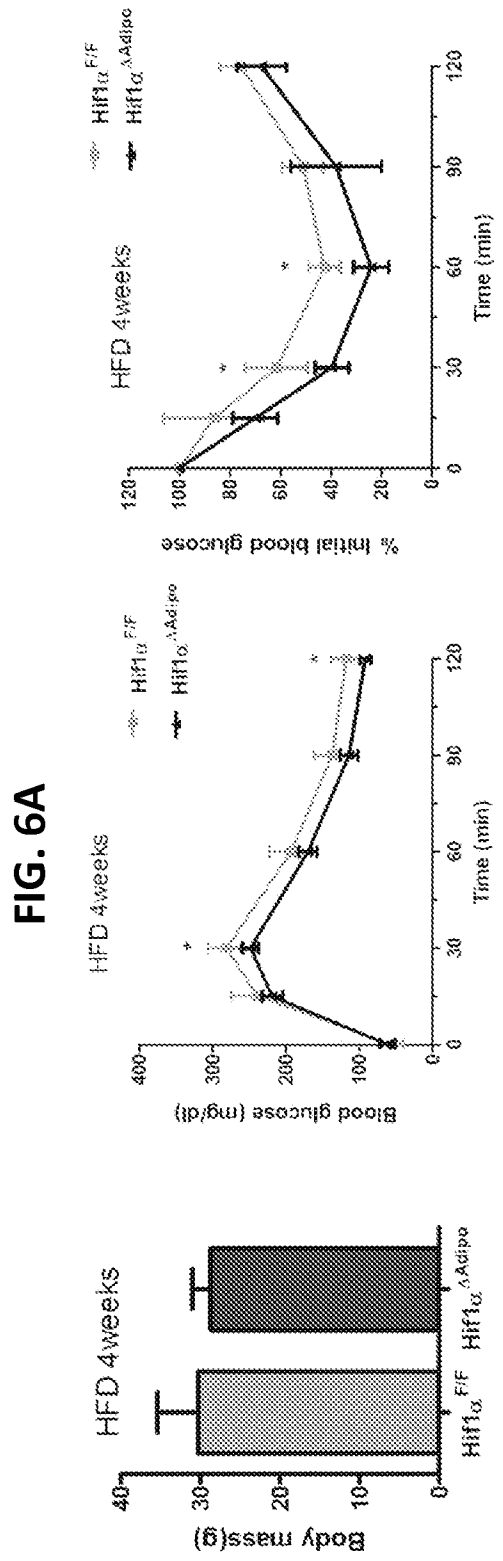

HFD 4weeks
Body mass(g)
Hif1α F/F
Hif1α ΔAdipo
HFD 4weeks
Blood glucose (mg/dl)
Time (min)
HFD 4weeks
% initial blood glucose
Time (min)

FIG. 6A

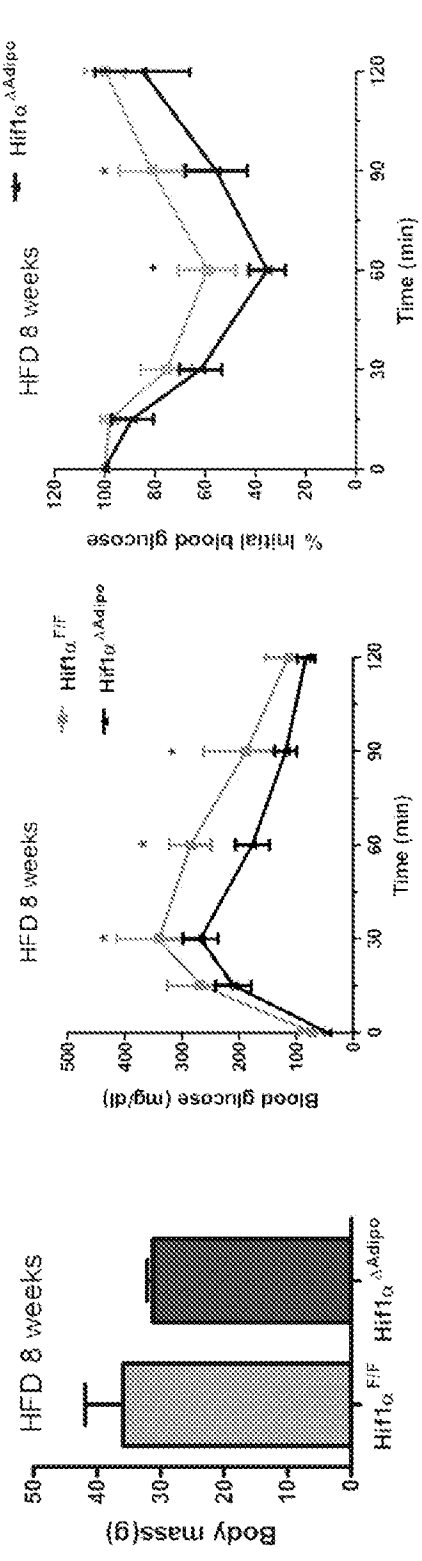

HFD 8 weeks
Body mass(g)
Hif1α F/F
Hif1α ΔAdipo
HFD 8 weeks
Blood glucose (mg/dl)
Time (min)
HFD 8 weeks
% initial blood glucose
Time (min)

FIG. 6B

FIG. 6C

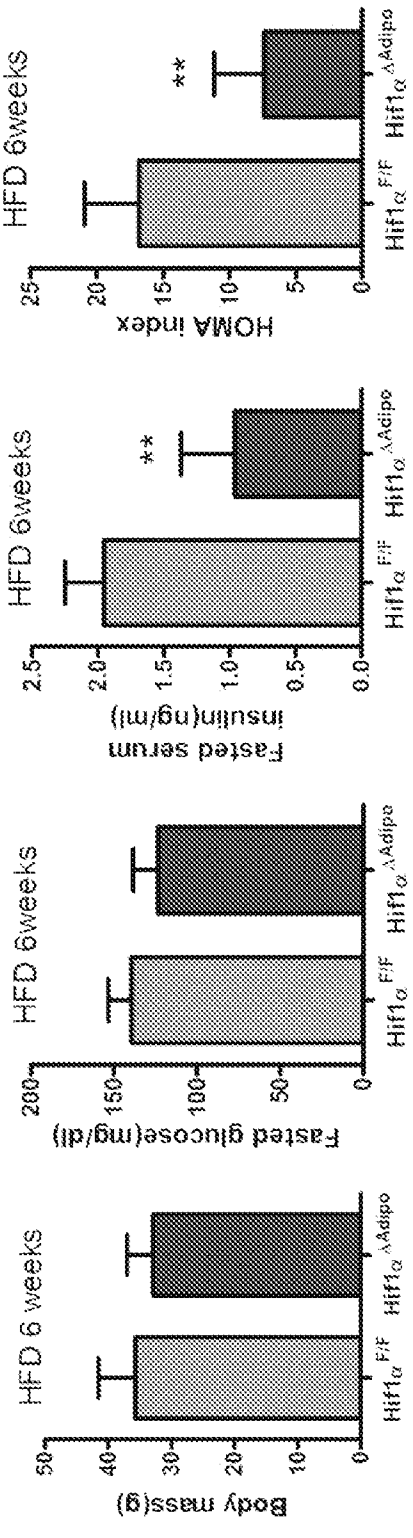

HFD 6 weeks
Body mass(g)
HFD 6weeks
Fasted glucose(mg/dl)
HFD 6weeks
Fasted serum insulin(ng/ml)
HFD 6weeks
HOMA index
Hif1α F/F
Hif1α ΔAdipo
**

FIG. 6D

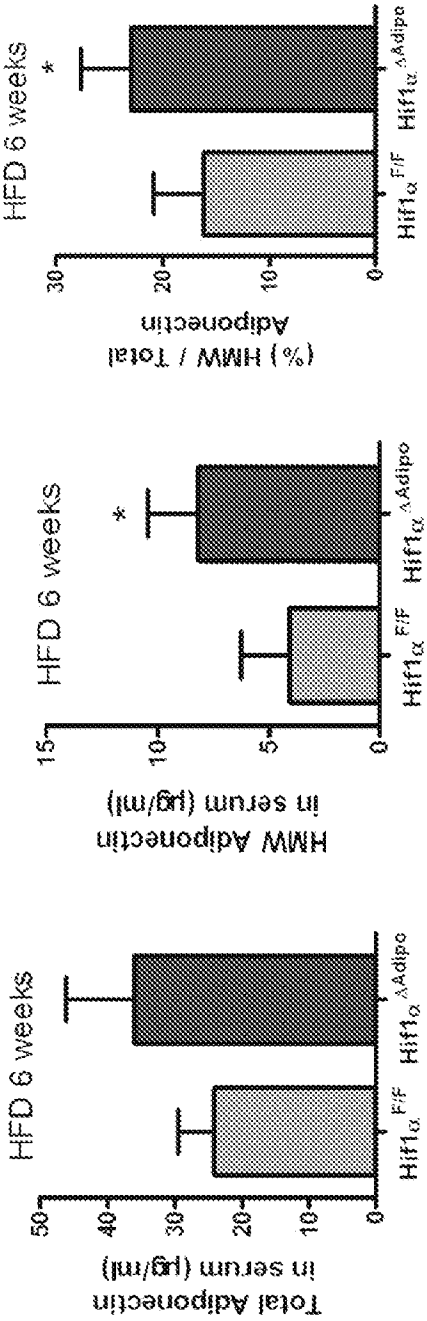

HFD 6 weeks
Total Adiponectin in serum (μg/ml)
HFD 6 weeks
HMW Adiponectin in serum (μg/ml)
HFD 6 weeks
(%) HMW / Total Adiponectin
Hif1α F/F
Hif1α ΔAdipo
*

FIG. 7B

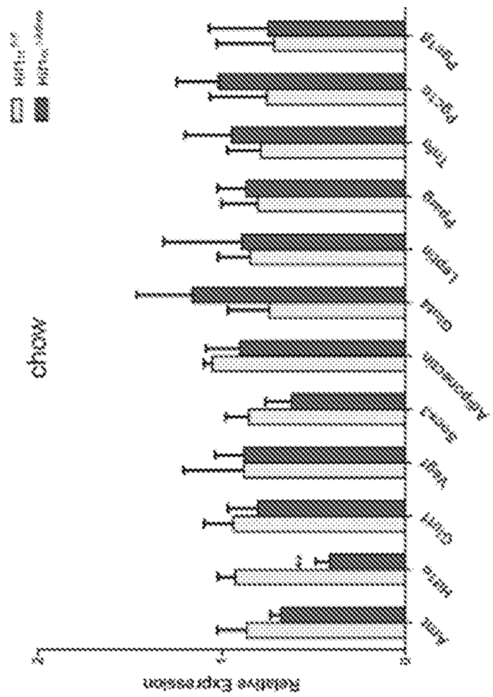

chow
Relative Expression
Adiponectin
Leptin

FIG. 7A

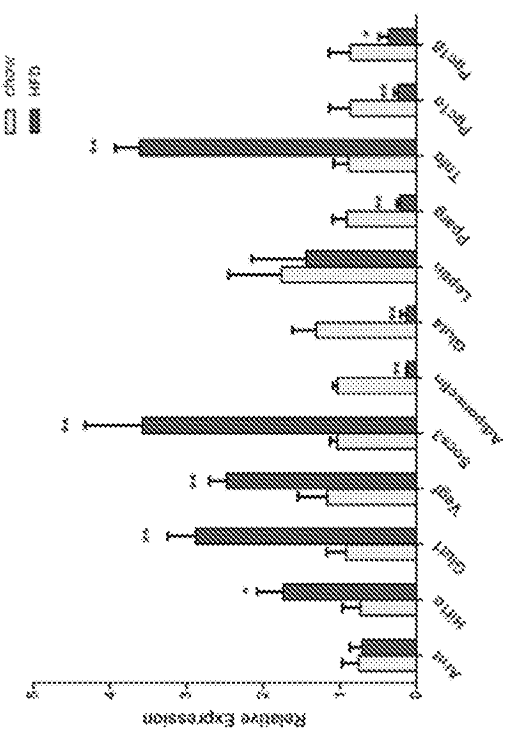

chow
HFD
Relative Expression
Adiponectin
Leptin

FIG. 8A

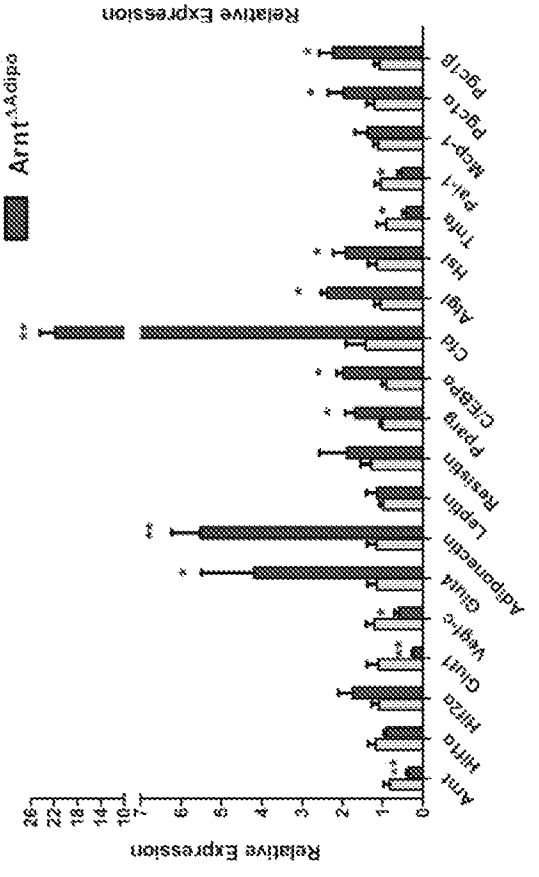

Arnt^F/F
Arnt^ΔAdipo
Relative Expression
Arnt
Hif1a
Hif2a
Glut1
Vegfc
Glut4
Adiponectin
Leptin
Resistin
Pparg
C/Ebpa
Cd
Agt
Hsl
Tnfa
Pai-1
Mcp-1
Pgc1a
Pgc1b

FIG. 8B

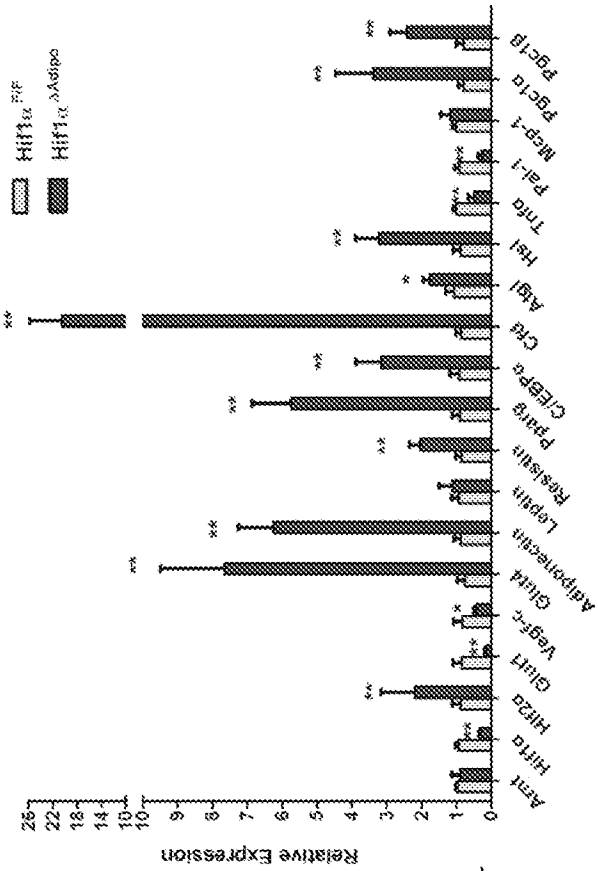

Hif1α^F/F
Hif1α^ΔAdipo
Relative Expression
Arnt
Hif1a
Hif2a
Glut1
Vegfc
Glut4
Adiponectin
Leptin
Resistin
Pparg
C/Ebpa
Cd
Agt
Hsl
Tnfa
Pai-1
Mcp-1
Pgc1a
Pgc1b

FIG. 8D

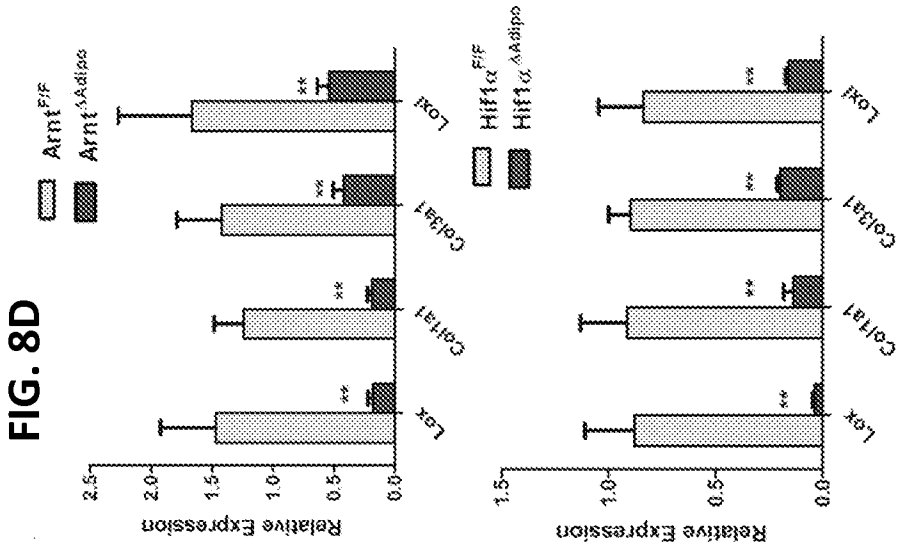

Arnt F/F
Arnt ΔAdipo
Hif1α F/F
Hif1α ΔAdipo
Relative Expression
Lox
Col7a1
Col3a1
Loxl
**

FIG. 8C

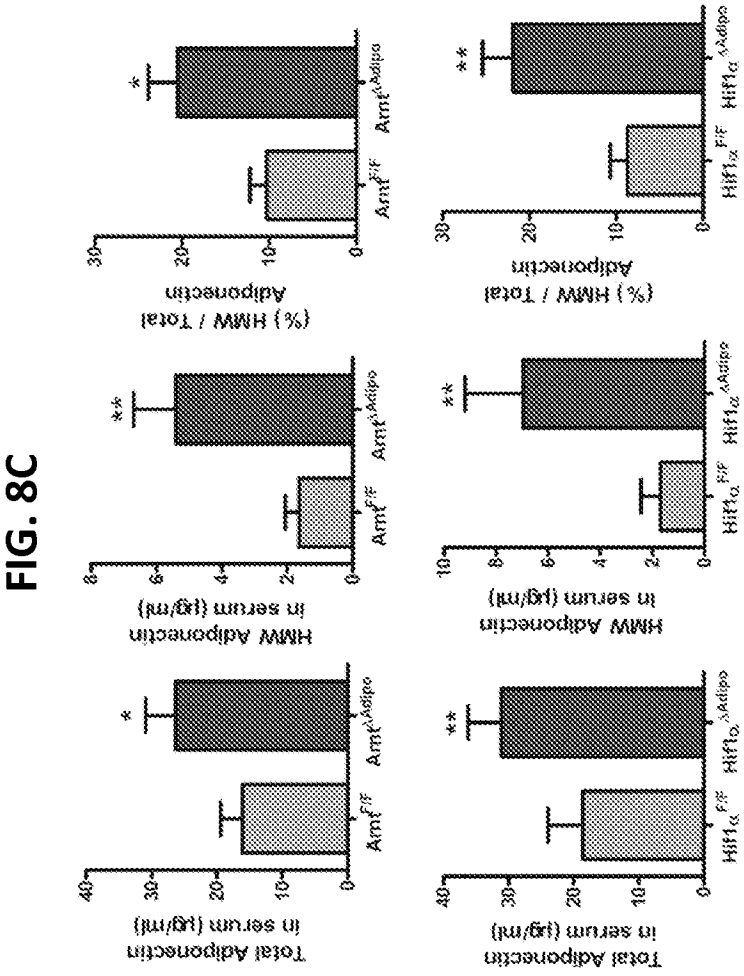
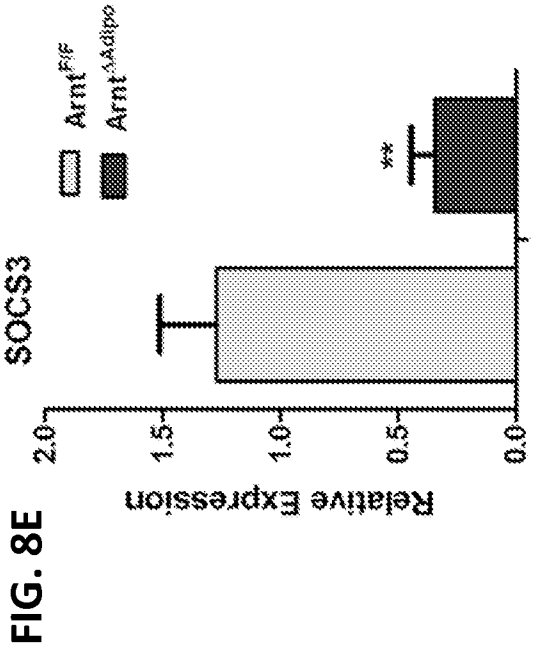

Total Adiponectin in serum (µg/ml)
HMW Adiponectin in serum (µg/ml)
(%) HMW / Total Adiponectin
Arnt F/F
Arnt ΔAdipo
Hif1α F/F
Hif1α ΔAdipo
*
**

FIG. 8E


SOCS3
Relative Expression
2.0
1.5
1.0
0.5
0.0
**
Arnt F/F
Arnt ΔAdipo

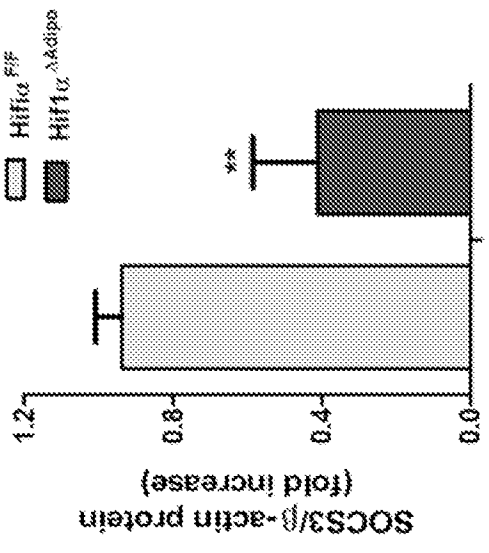
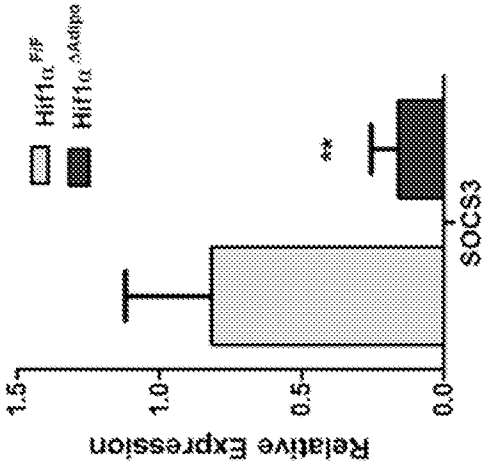

SOCS3/β-actin protein
(fold increase)
1.2
0.8
0.4
0.0
**
Hif1α F/F
Hif1α ΔAdipo

FIG. 8F


Relative Expression
1.5
1.0
0.5
0.0
**
SOCS3
Hif1α F/F
Hif1α ΔAdipo

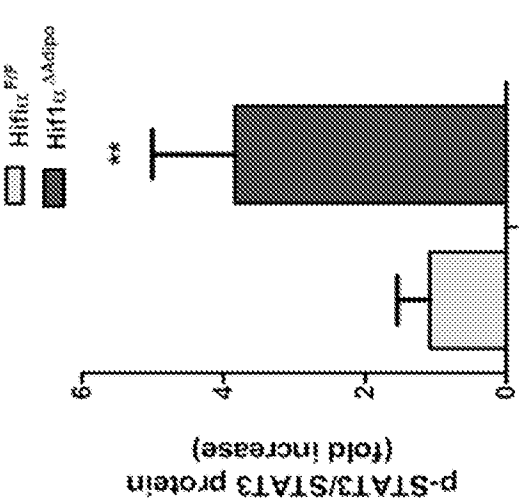

p-STAT3/STAT3 protein
(fold increase)
6
4
2
0
**
Hif1α F/F
Hif1α ΔAdipo

FIG. 9A

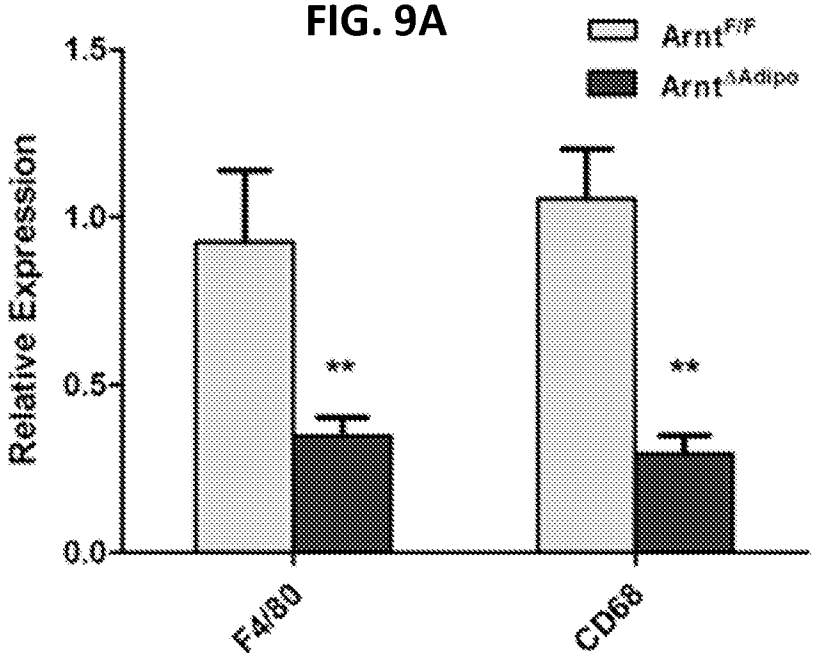

Arnt F/F
Arnt ΔAdipo
Relative Expression
1.5
1.0
0.5
0.0
**
**
F4/80
CD68

FIG. 9B

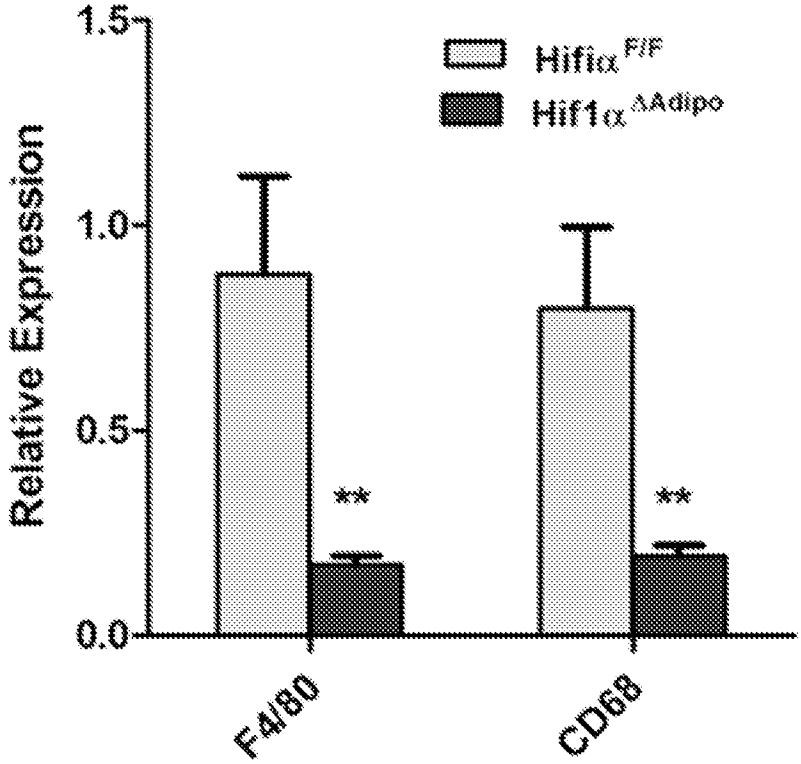

Hifiα F/F
Hif1α ΔAdipo
Relative Expression
1.5
1.0
0.5
0.0
**
**
F4/80
CD68

FIG. 10A

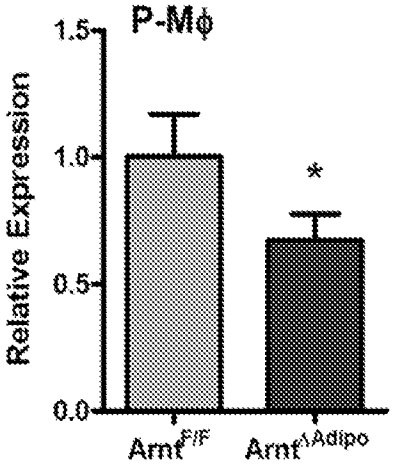


FIG. 10B

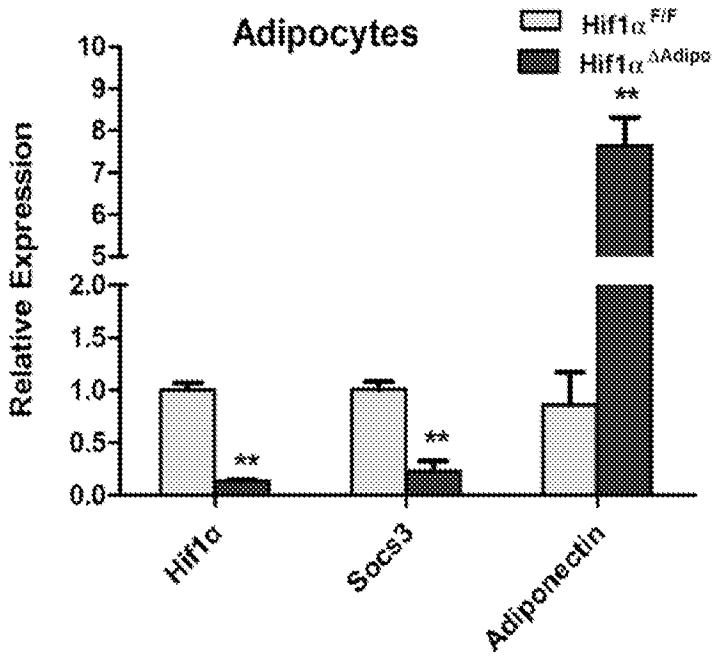


FIG. 10C

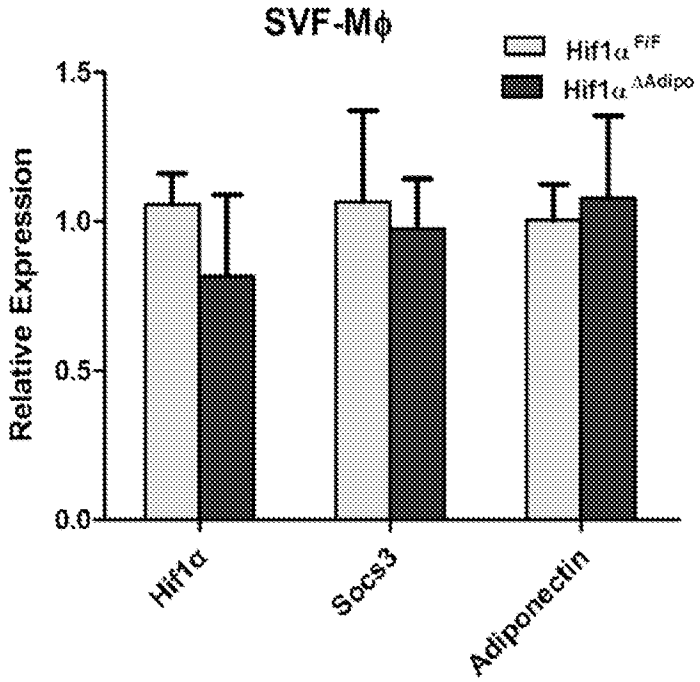


FIG. 11A

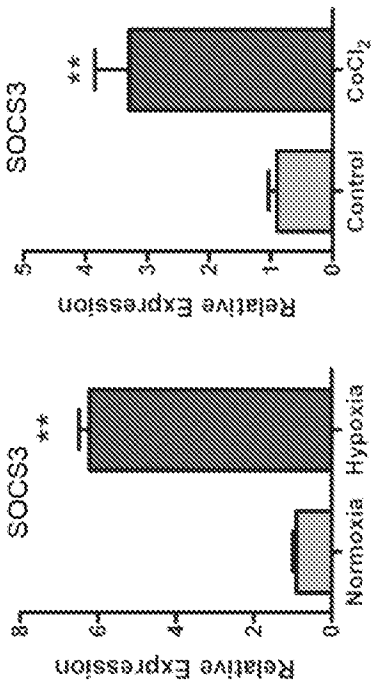


FIG. 11B

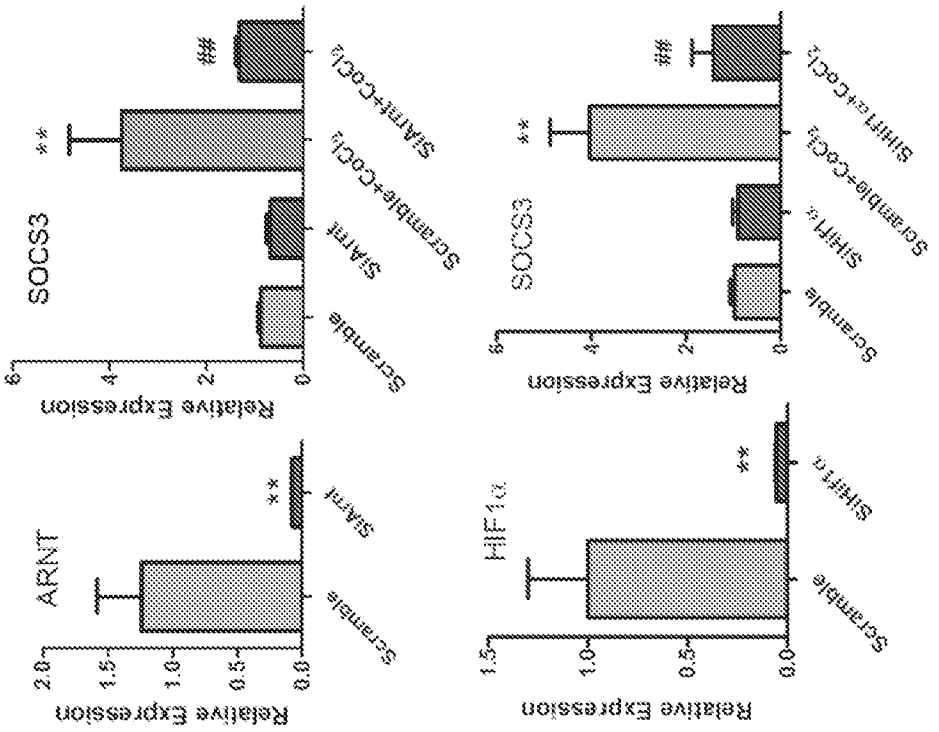


FIG. 11C

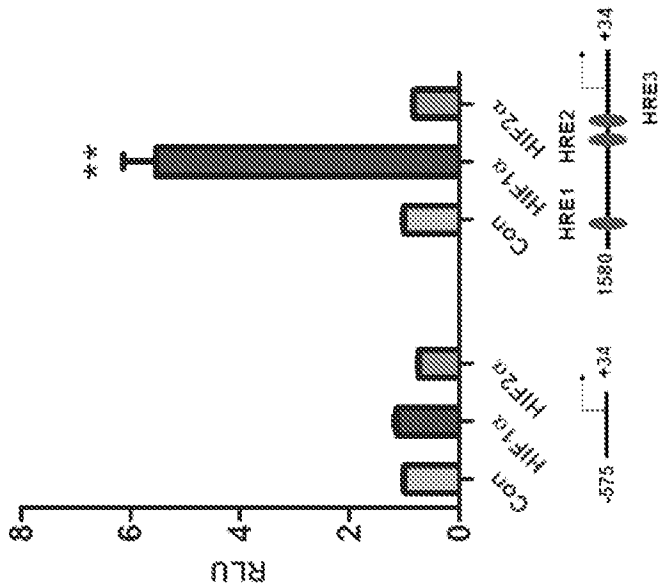


FIG. 11D

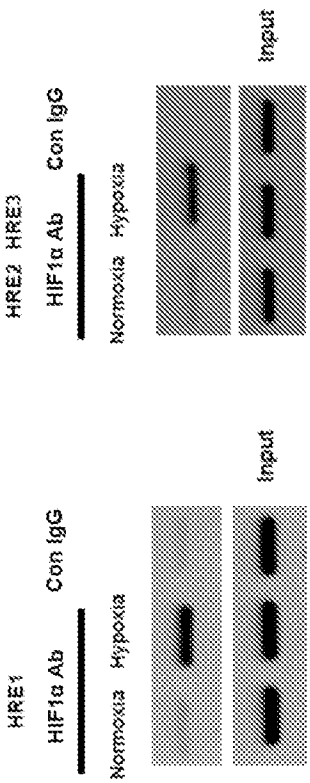


FIG. 12A

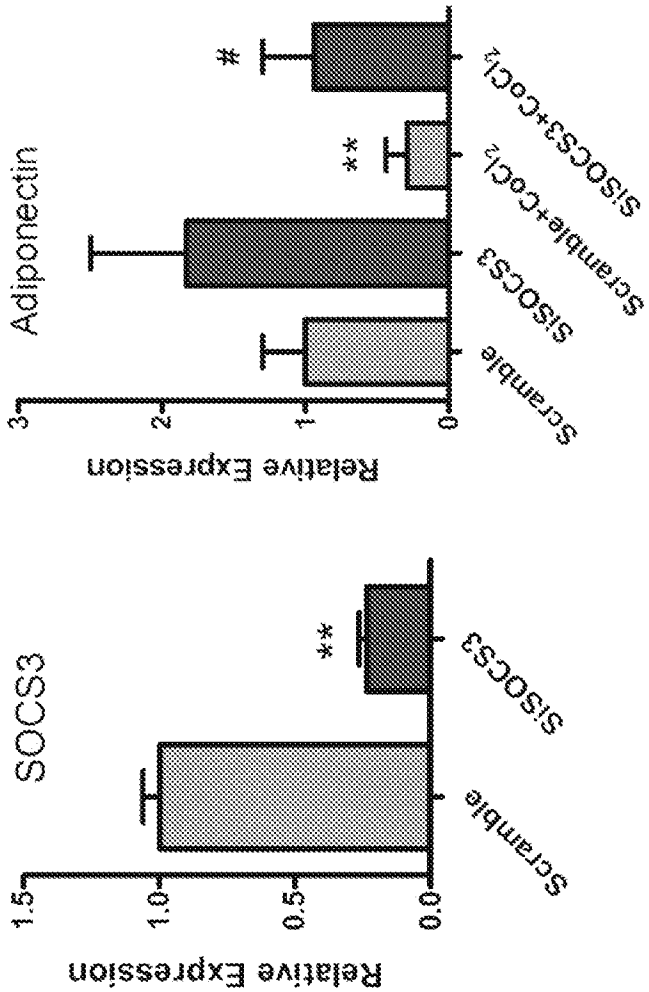


FIG. 12B

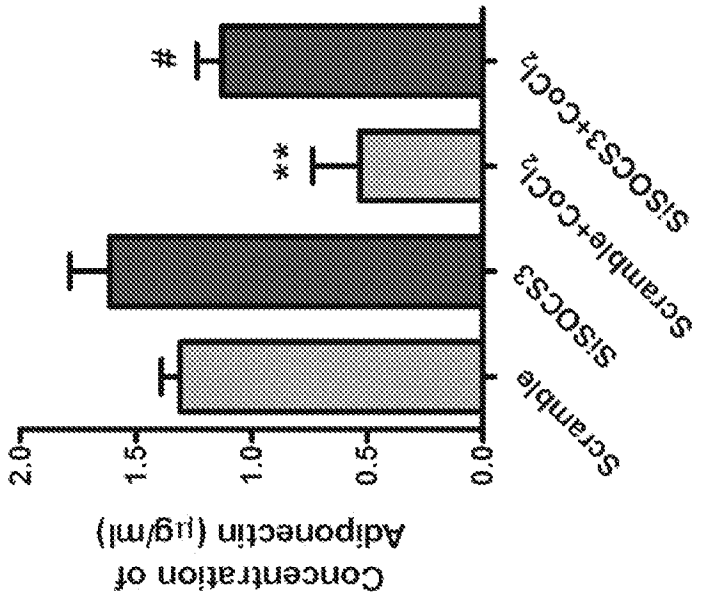


FIG. 12D

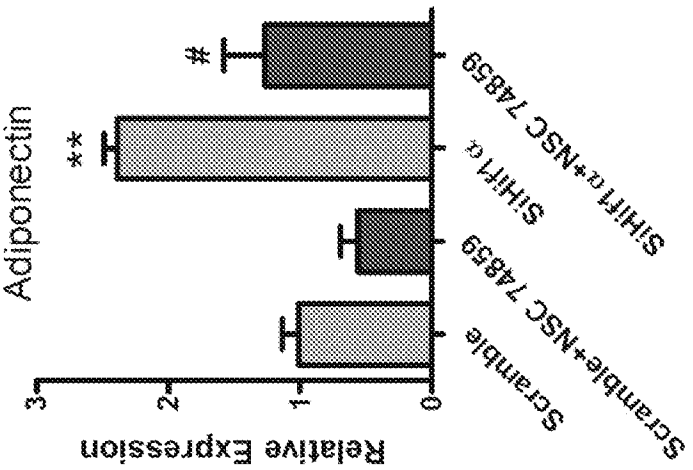


FIG. 12C

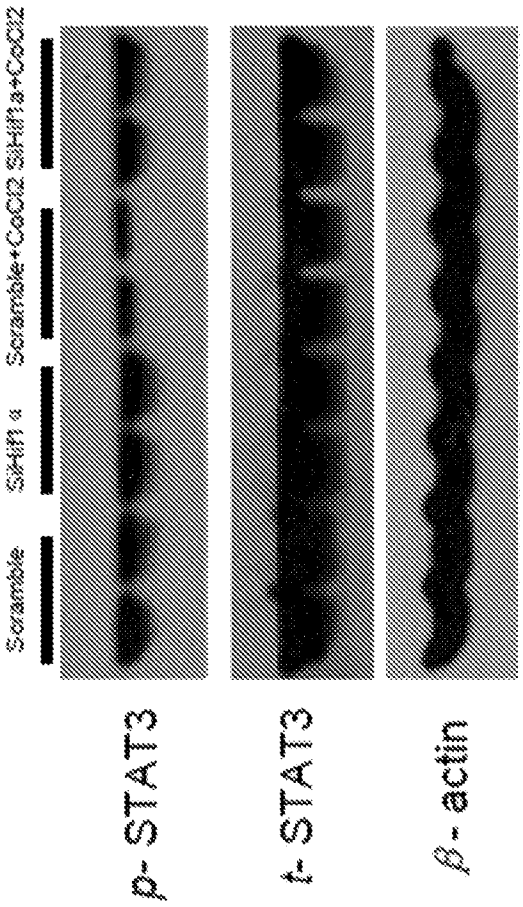


FIG. 13A

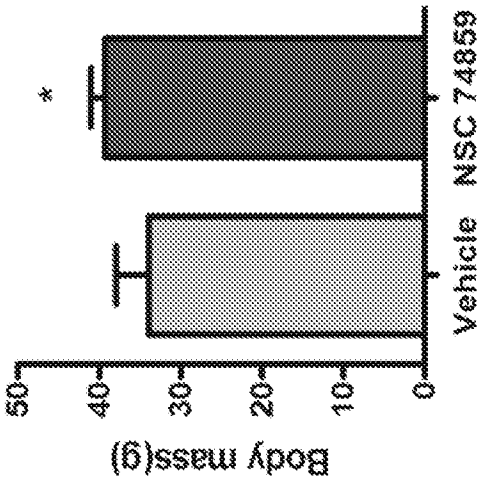


FIG. 13B

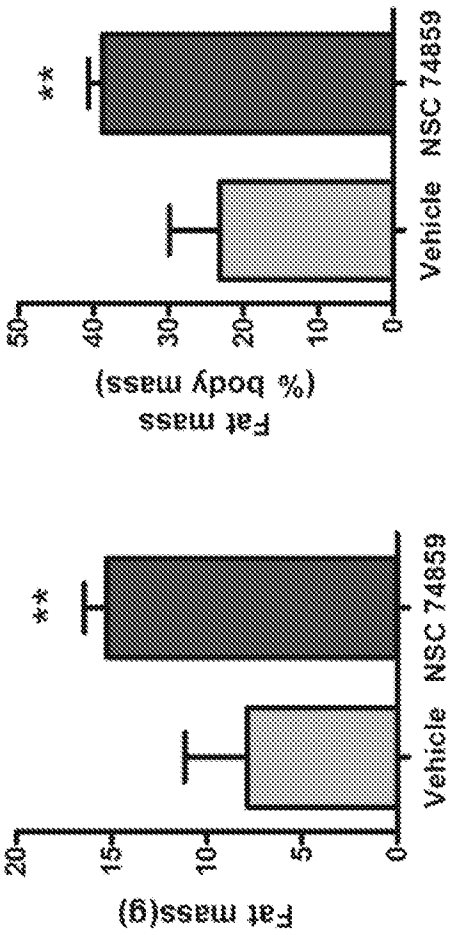


FIG. 13C

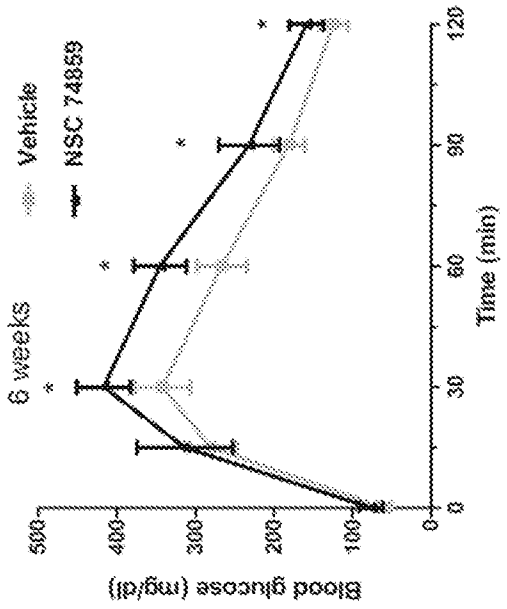
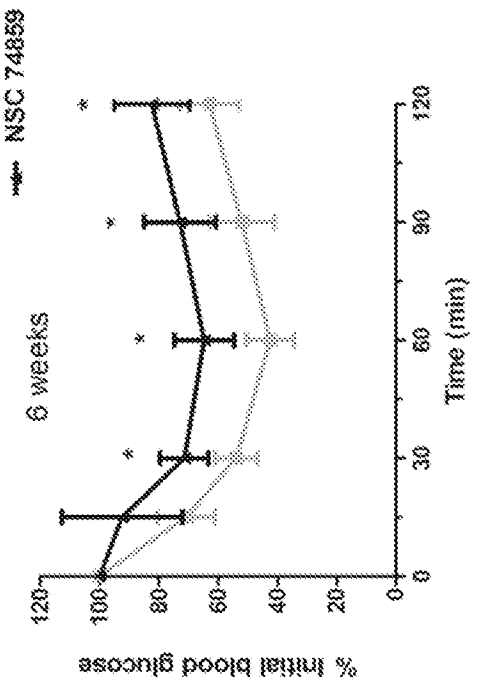


FIG. 13D



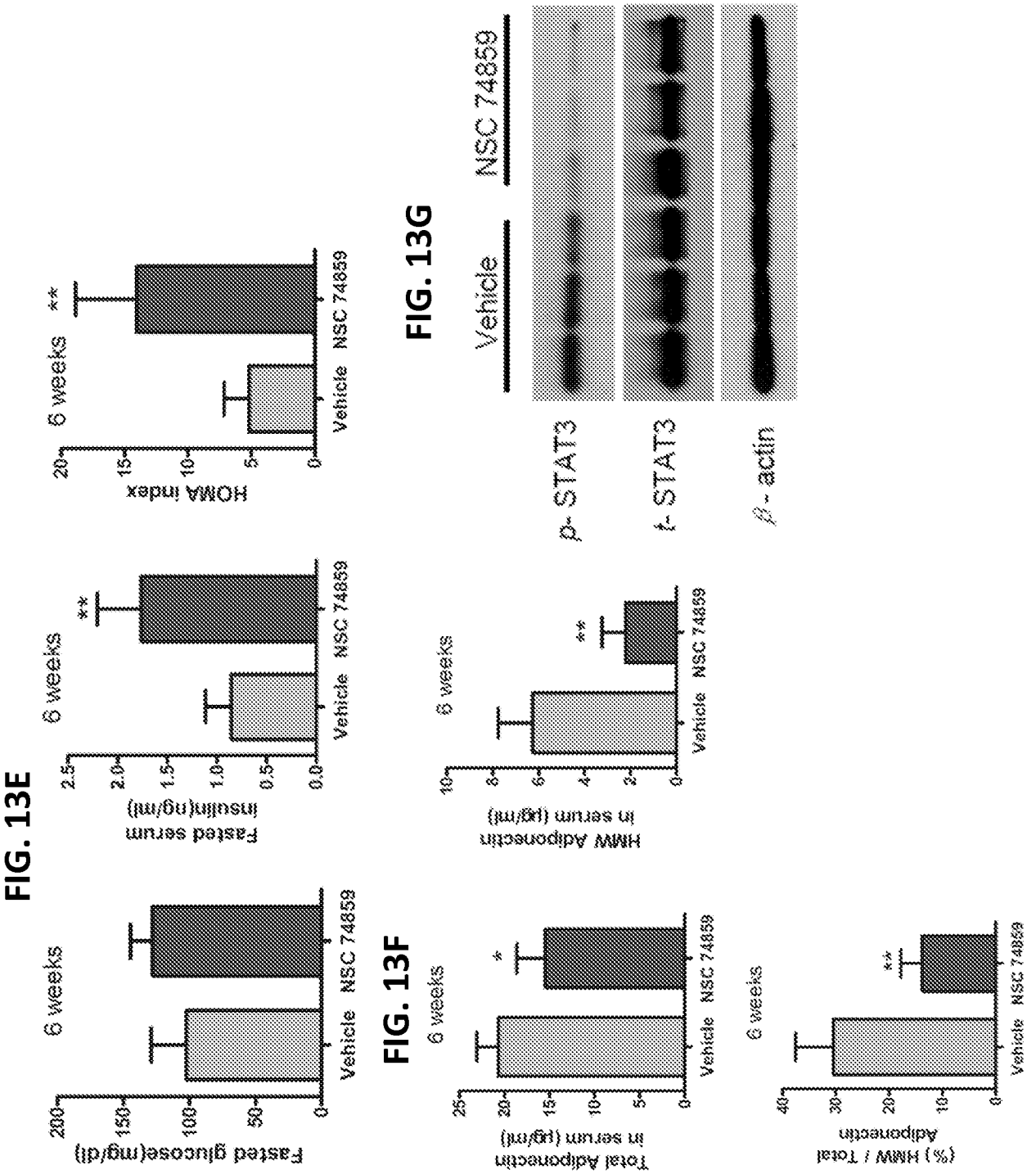


FIG. 13E
Fasted glucose(mg/dl)
6 weeks
Vehicle
NSC 74859
Fasted serum insulin(ng/ml)
HOMA index
FIG. 13F
Total Adiponectin in serum (ug/ml)
HMW Adiponectin in serum (ug/ml)
(%) HMW / Total Adiponectin
FIG. 13G
Vehicle
NSC 74859
p- STAT3
t- STAT3
β - actin

FIG. 14A

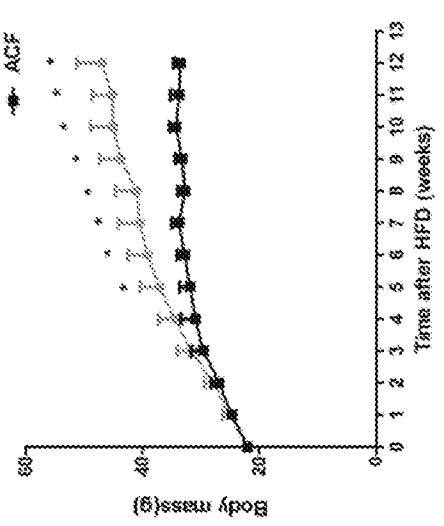


FIG. 14B

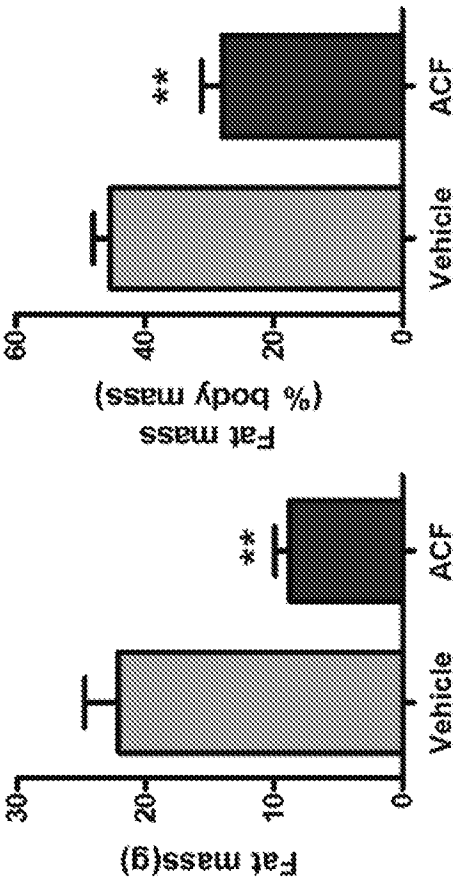
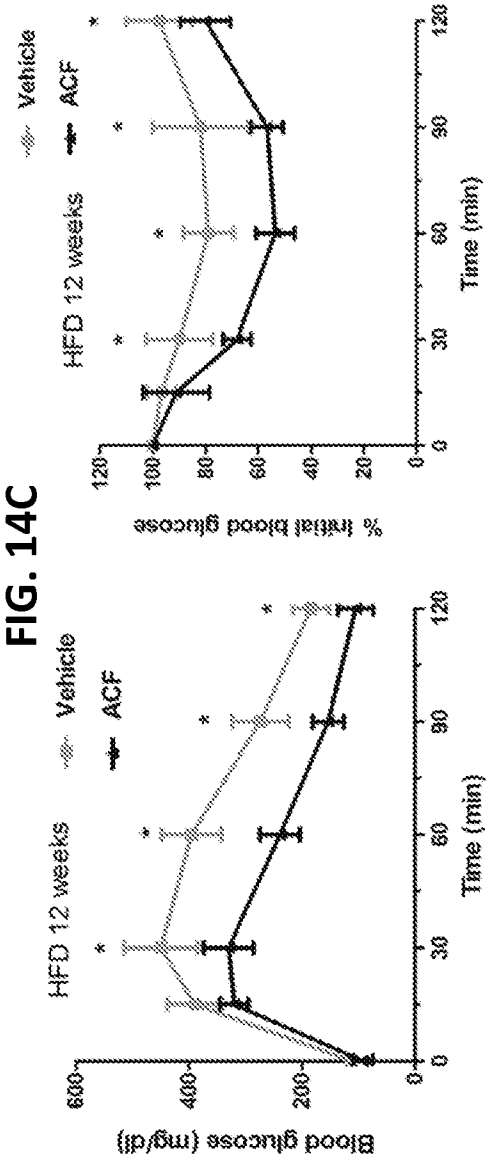
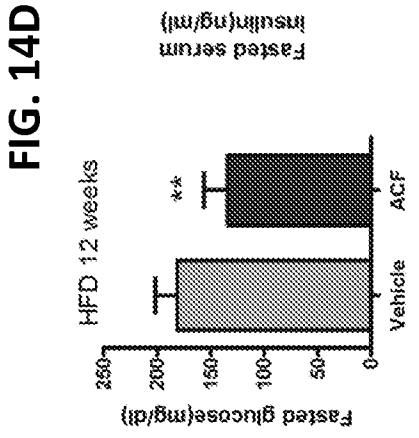


FIG. 14C





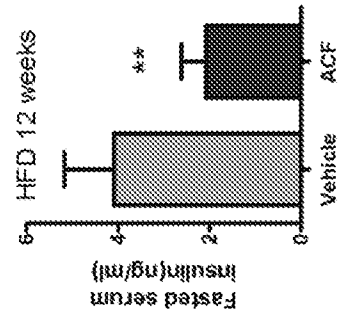
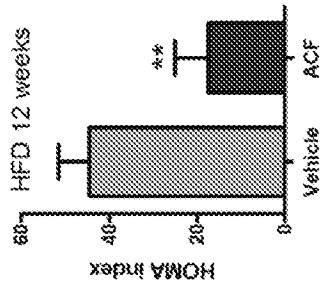
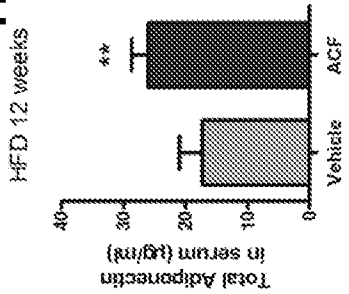


FIG. 14D





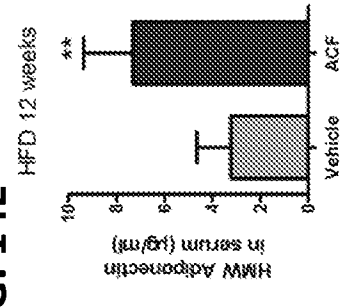
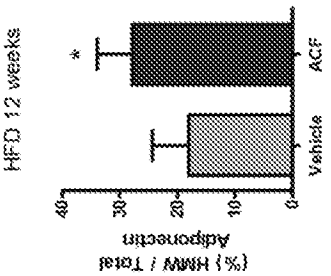


FIG. 14E



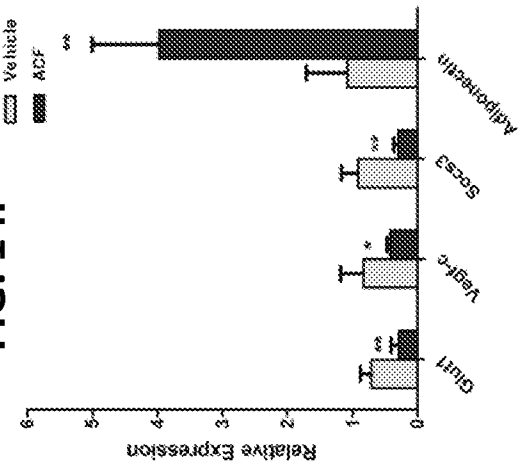


FIG. 14F

FIG. 15A

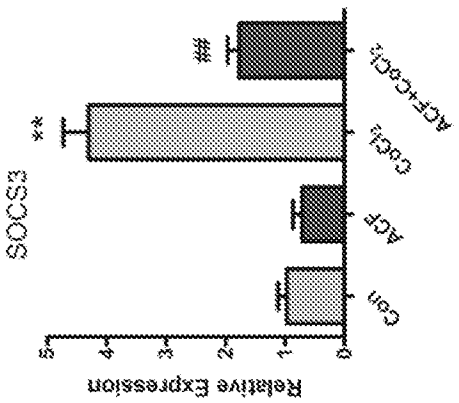


FIG. 15B

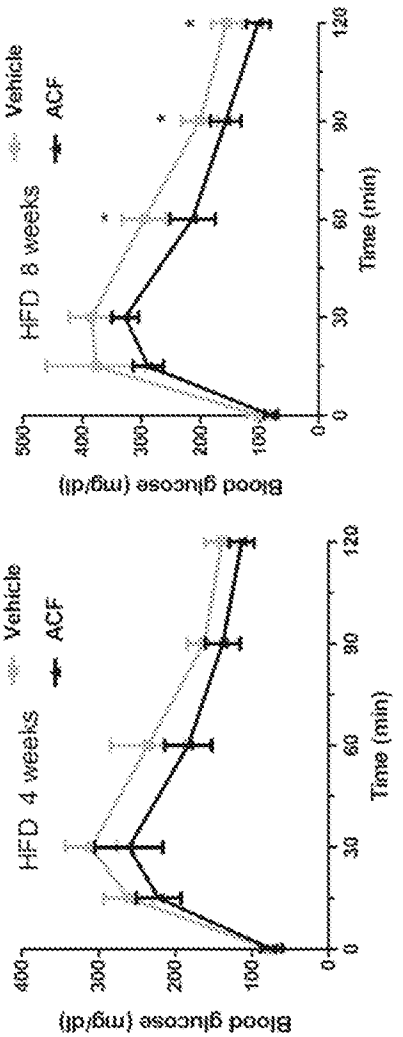


FIG. 15C

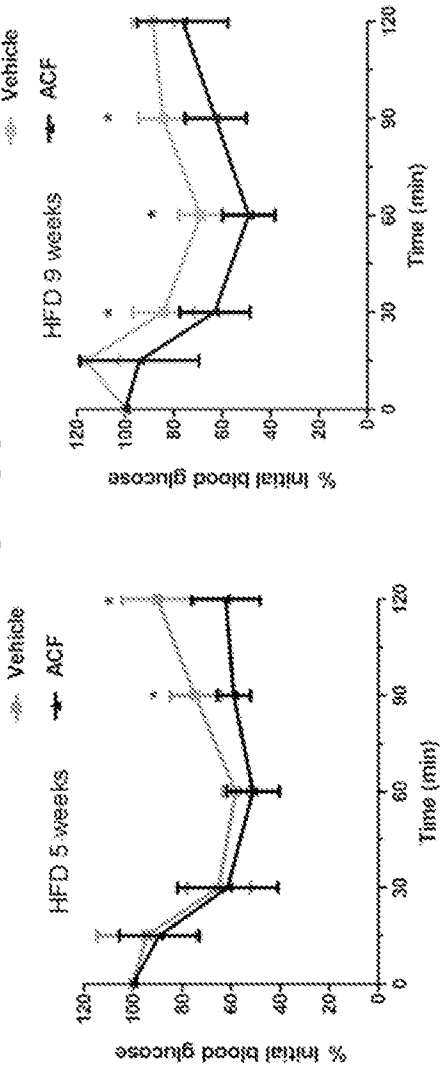


FIG. 15D

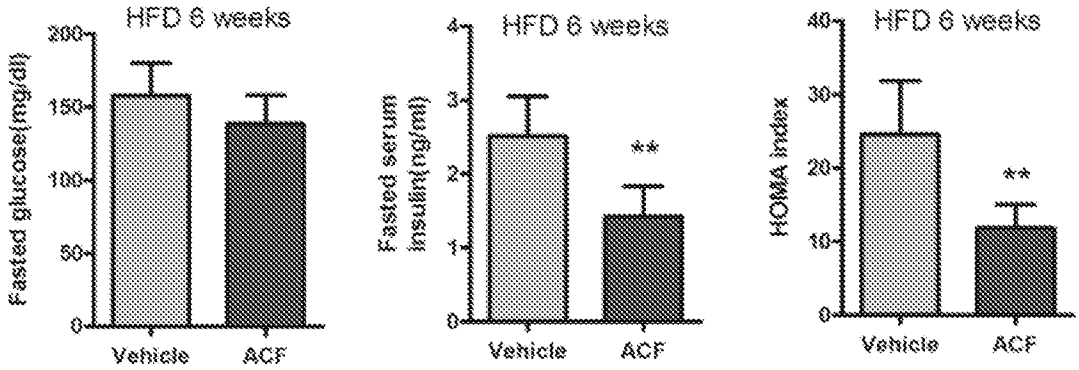


FIG. 15E

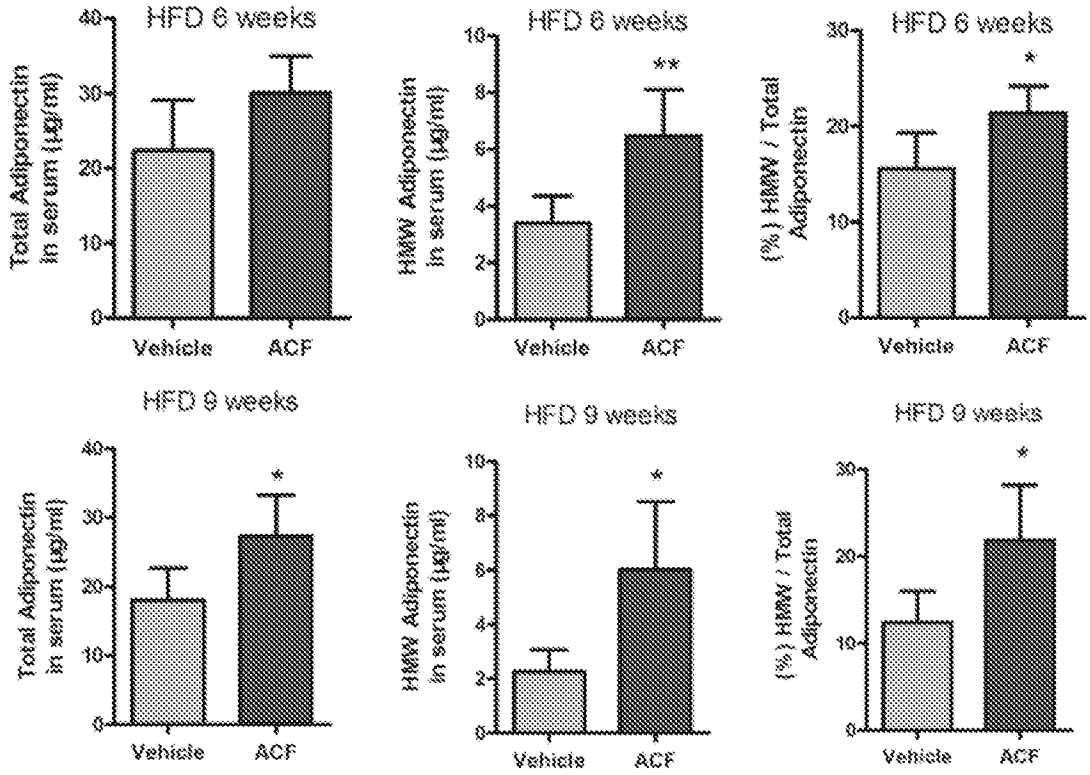


FIG. 16A

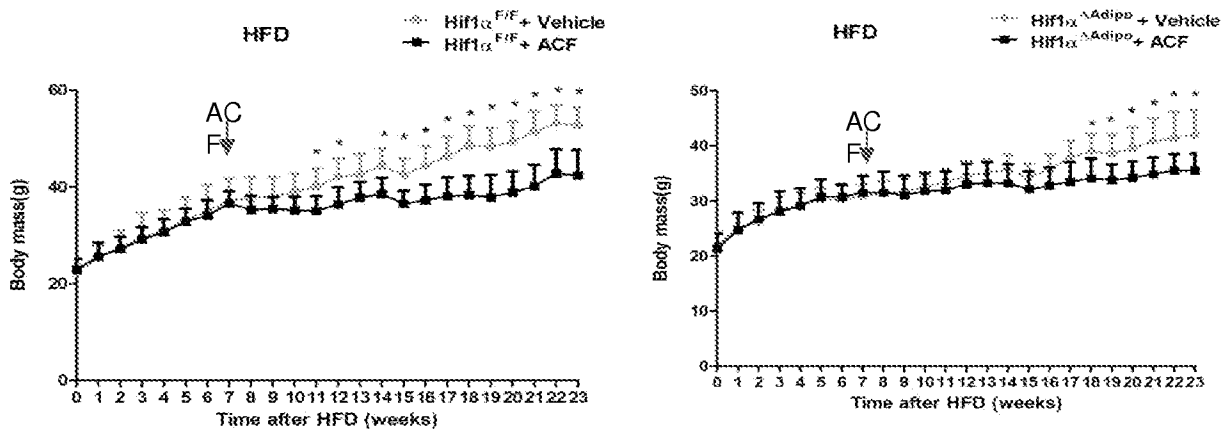


FIG. 16B

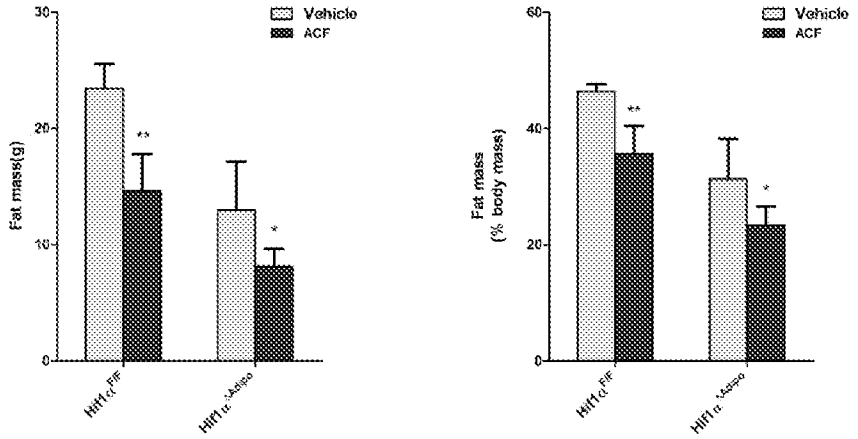
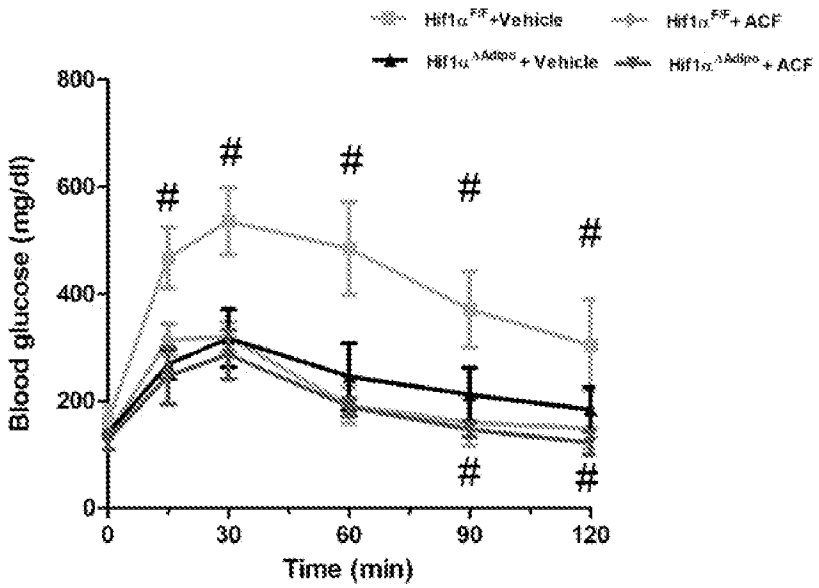


FIG. 17A


Hif1α F/F+Vehicle
Hif1α F/F+ ACF
Hif1α ΔAdipo + Vehicle
Hif1α ΔAdipo + ACF
Blood glucose (mg/dl)
800
600
400
200
0
0
30
60
90
120
Time (min)
#

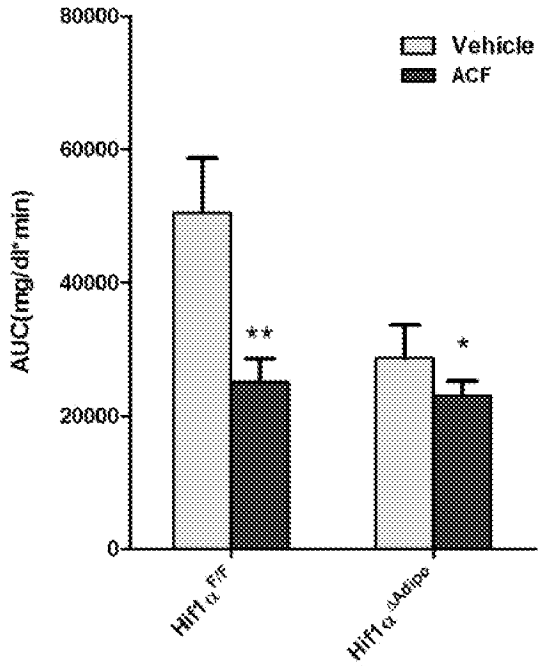

Vehicle
ACF
AUC(mg/dl*min)
80000
60000
40000
20000
0
**
*
Hif1α F/F
Hif1α ΔAdipo

FIG. 17B

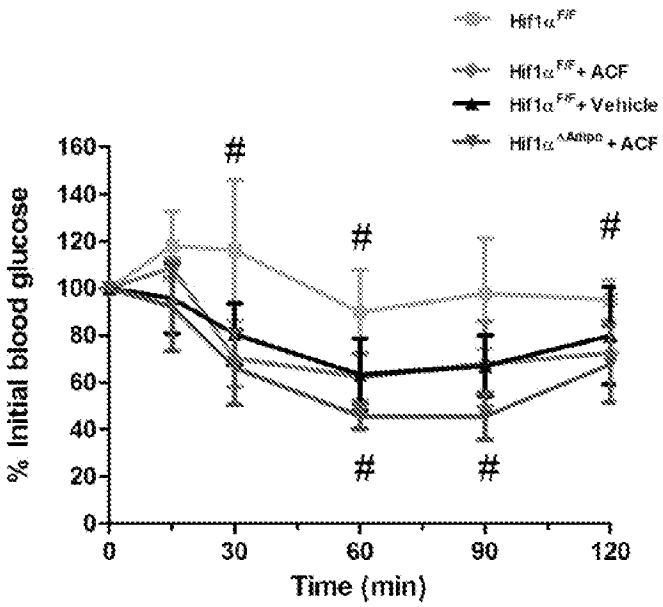


FIG. 17C

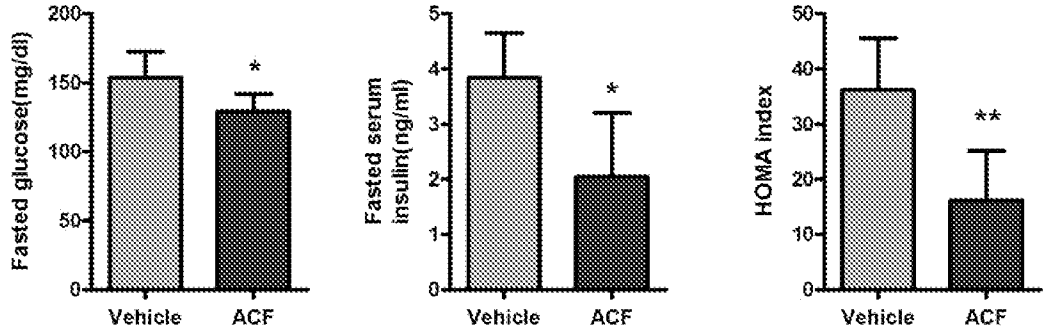


FIG. 17D

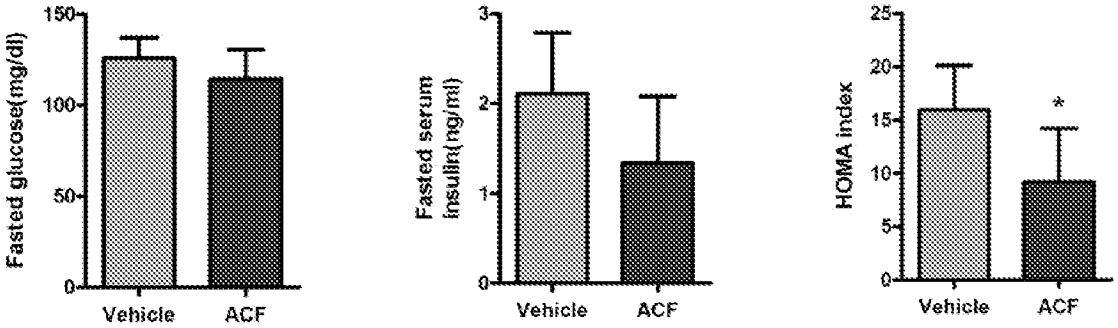


FIG. 18A

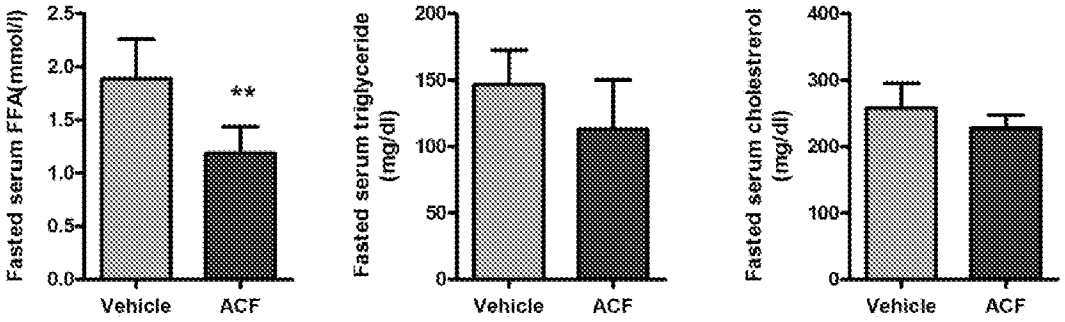


FIG. 18B

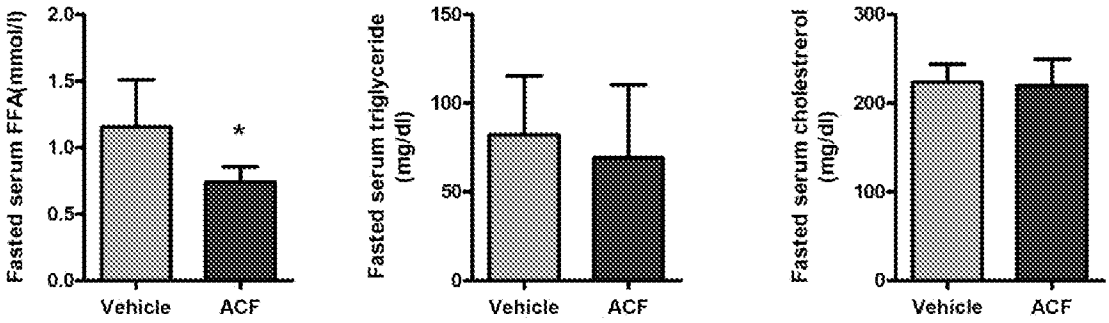


FIG. 19A

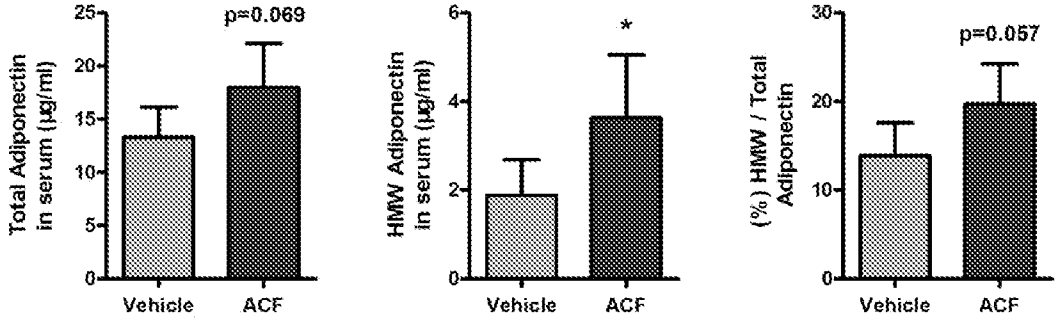


FIG. 19B