(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
13 October 2011 (13.10.2011)

PCT

(10) International Publication Number
WO 2011/127304 A2

(51) International Patent Classification:
A61K 48/00 (2006.01)

(21) International Application Number:
PCT/US2011/031608

(22) International Filing Date:
7 April 2011 (07.04.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/321,600 7 April 2010 (07.04.2010) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

WO 2011/127304 A2

(54) Title: METHODS OF TREATING AN OVERWEIGHT SUBJECT

(57) Abstract: The invention generally relates in part to methods of effecting weight loss and/or improving glucose tolerance in a patient in need thereof, comprising administering a MetAP2 inhibitor.

METHODS OF TREATING AN OVERWEIGHT SUBJECT

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to and the benefit of U.S.S.N. 61/321,600, filed April 7, 2010, which is incorporated by reference in its entirety.

BACKGROUND

[0002] Type 2 diabetes is a growing health problem affecting more than 20 million people. Type 2 diabetes is often associated with obesity or with weight gain, although patients with normal weight can also suffer from this disease. The pre-diabetic states of impaired glucose tolerance and/or impaired fasting glucose are common indicators of future development of this disease.

[0003] Impaired glucose tolerance is typically defined as two hour glucose levels of 140 to 199 mg/dL (7.8 to 11 mmol) on a 75 g oral glucose tolerance test. The criteria for impaired fasting glucose is typically either of: a fasting plasma glucose level form 6.1 mmol/l (110 mg/dL) to 6.9 mmol/l (125 mg/dL, or a fasting plasma glucose level from 5.6 mmol/L (100 mg/dL) to 6.9 mmol/L (125 mg/dL). Impairments of glucose tolerance persist and often worsen in individuals who progress to develop type 2 diabetes, such that elevated glucose levels in the fed condition are a hallmark of both impaired glucose tolerance and type 2 diabetes.

[0004] Treatment modalities typically include lifestyle management such as weight loss, limiting carbohydrate intakes, and regular exercise. Reducing excess weight, in e.g. an overweight patient, can be challenging, however, at least in part because most diet or pharmaceutical interventions result in a regain of most weight once discontinued. For example, most anti-obesity or weight loss medications fail to produce durable weight loss.

[0005] MetAP2 encodes a protein that functions at least in part by enzymatically removing the amino terminal methionine residue from certain newly translated proteins such as glyceraldehyde-3- phosphate dehydrogenase (Warder *et al.* (2008) J Proteome Res 7:4807). Increased expression of the MetAP2 gene has been historically associated with various forms

of cancer. Molecules inhibiting the enzymatic activity of MetAP2 have been identified and have been explored for their utility in the treatment of various tumor types (Wang et al. (2003) Cancer Res. 63:7861) and infectious diseases such as microsporidiosis, leishmaniasis, and malaria (Zhang et al. (2002) J. Biomed. Sci. 9:34). However, such MetAP2 inhibitors may be useful as well for patients with excess adiposity and conditions related to adiposity including type 2 diabetes, hepatic steatosis, and cardiovascular disease (*via* e.g. ameliorating insulin resistance, reducing hepatic lipid content, and reducing cardiac workload). Methods of treating overweight subjects that are more effective than e.g. dieting alone are clearly needed, as well as methods of treating patient suffering from diabetes or glucose intolerance.

SUMMARY

[0006] This disclosure generally relates to methods of effecting weight loss in a subject or patient that comprises administering a pharmaceutically effective amount of a MetAP2 inhibitor to a patient in need thereof, e.g., a human or a companion animal such as a cat or a dog.

[0007] In one embodiment, a method of effecting weight loss in a patient in need thereof is provided (e.g. an overweight or obese patient), comprising administering a pharmaceutically effective amount of a MetAP2 inhibitor to said patient. Such methods may result in, for example, durable weight loss maintained for at least about 1 month, 2 months, 6 months, 1 year, 5 years or more.

[0008] Contemplated MetAP2 inhibitors include substantially irreversible inhibitors, such as e.g., fumagillin, fumagillol or fumagillin ketone derivative, siRNA, shRNA, an antibody, or a antisense compound, or, e.g., O-(4-dimethylaminoethoxycinnamoyl)fumagillol and pharmaceutically acceptable salts thereof. In another embodiment, a contemplated MetAP2 inhibitor may be substantially reversible inhibitor.

[0009] A method of reducing weight of a patient in a patient in need thereof is contemplated herein that comprises administering a pharmaceutically effective amount of a MetAP2 inhibitor to said patient wherein the metabolic rate of the patient is not substantially reduced as compared to the metabolic rate of a diet only patient on an energy restricted diet alone. Also provided herein is a method of restoring normal metabolic action in an overweight

or obese patient in need thereof, comprising administering a pharmaceutically effective amount of a MetAP2 inhibitor to said patient.

[0010] In an embodiment, a method of improving, increasing, and/or glucose tolerance in patient (e.g. an overweight, obese or diabetic patient), in need thereof is provided that comprises administering a pharmaceutically effective amount of a MetAP2 inhibitor to said patient. Such a patient may be suffering from type 2 diabetes.

[0011] MetAP2 inhibitors may be administered parenterally, non-parenterally, orally. buccally or sublingually, topically, rectally, or transdermally in at least some disclosed methods. In some disclosed methods, a MetAP2 inhibitor may be administered subcutaneously or intravenously. In some embodiments, administration of a MetAP2 inhibitor may occur at least daily, or every other day, or at least weekly.

[0012] Also contemplated herein is a method for reducing the amount or frequency of administering supplemental insulin in a patient suffering from type 2 diabetes, comprising a pharmaceutically effective amount of a MetAP2 inhibitor.

[0013] Disclosed methods may further comprise co-administering an additional weight loss agent and/or hypoglycemic agent, and/or may further comprise administering a calorie or joule restricted diet to a patient.

BRIEF DESCRIPTION OF FIGURES

[0014] FIG. 1 is a bar graph showing TXN(104) in B16F10 cell lysate after chymotryptic digest.

[0015] FIG. 2 is a bar graph showing N-acetyl TXN(1-4) in B16F10 cell lysate after chymotriptic digest.

[0016] FIG. 3 is a photograph of a gel showing that fumagillin significantly increased TXN in both Cos-1 and 293T cells.

[0017] FIG. 4 is a photograph of a gel showing that EC50 of fumagillin to increase TXN levels is < 10 nM.

[0018] FIG. 5A is a bar graph showing the concentration of beta-hydroxybutyrate in mice fed a high fat diet (vehicle) or mice fed a high-fat diet plus oral administration of fumagillin for ten days at the concentrations indicated. FIG. 5B is a bar graph showing the concentration of nonesterified fatty acid concentrations under the conditions described for FIG. 5A. FIG. 5C is a bar graph showing the concentration of beta-hydroxybutyrate in mice fed a high fat diet (vehicle) or mice fed a high-fat diet plus oral administration of fumagillin for 250 days at the concentrations indicated.

[0019] FIG. 6 is a line graph showing the body weight over time of high-fat diet-fed C57BL/6 mice treated with 1 mg/kg fumagillin (squares) or vehicle (diamonds) for 250 days. Age-matched lean C57BL/6 mice maintained on normal mouse chow diet are shown for comparison (triangles).

[0020] FIG. 7 shows age-matched lean mice maintained on normal mouse chow diet (left) or mice fed a high fat diet-fed and treated with 1 mg/kg fumagillin (right) or vehicle (middle) for 250 days.

[0021] FIG. 8 contains two bar graphs showing a comparison of retroperitoneal fat pad weight in grams (left graph) and as a percentage of total body weight (right graph) for mice fed a high-fat diet (diet-induced obesity (DIO), left bar), mice fed a high-fat diet plus 1 mg/kg fumagillin (DIO + ZGN-201, middle bar), and age-matched lean mice (right bar).

[0022] FIG. 9 contains three bar graphs showing concentrations of glucose (top left graph), insulin (top right graph) and glucose-insulin complex (bottom graph) in mice fed a high-fat diet (diet-induced obesity (DIO), left bar), mice fed a high-fat diet plus 1 mg/kg fumagillin (DIO + ZGN-201, middle bar), and age-matched lean mice (right bar).

[0023] FIG. 10 shows two line graphs displaying body weight (left graph) and food intake (right graph) of mice fed a high fat diet-fed C57BL/6 mice and treated with 1 mg/kg of the MetAP2 inhibitor fumagillin (squares) or vehicle (diamonds) for 14 days.

[0024] FIG. 11 is a bar graph showing the percentage change in fat tissue and lean tissue in mice conditioned on a high fat (HF) diet, mice conditioned on a high-fat diet and treated with fumagillin (ZGN-201) administered by oral gavage at doses of 0.1 or 0.3 mg/kg

daily, or mice fed on a low-fat diet for 28 days. For each of "Fat Tissue Change" and "Lean Tissue Change," bars from left to right are: LF, HF, HF + 0.3, and HF + 0.1.

[0025] FIG. 12 contains two histological images showing a comparison of retroperitoneal fat pad brown adipose tissue content for mice fed a high-fat diet (diet-induced obesity (DIO)) (left image), and mice fed a high-fat diet plus 1 mg/kg fumagillin (DIO + ZGN-201 (right image).

[0026] FIG. 13 depicts the effect of 7 day administration of various MetAP2 inhibitors on body weight in DIO mice.

[0027] FIG. 14 depicts the effect of an 8 week treatment with fumagillin (ZGN-201) administered once daily by oral gavage on body weight in overweight dogs.

[0028] FIG. 15 depicts the effect of an 8 week treatment with fumagillin (ZGN-201) administered once daily by oral gavage on oral glucose tolerance in overweight dogs.

DETAILED DESCRIPTION

Overview

[0029] Obesity and being overweight refer to an excess of fat in proportion to lean body mass. Excess fat accumulation is associated with increase in size (hypertrophy) as well as number (hyperplasia) of adipose tissue cells. Obesity is variously measured in terms of absolute weight, weight:height ratio, degree of excess body fat, distribution of subcutaneous fat, and societal and esthetic norms. A common measure of body fat is Body Mass Index (BMI). The BMI refers to the ratio of body weight (expressed in kilograms) to the square of height (expressed in meters). Body mass index may be accurately calculated using the formulas: SI units: BMI=weight(kg)/(height^2(m^2), or US units: BMI= (weight(lb)*703)/(height^2(in^2).

[0030] In accordance with the U.S. Centers for Disease Control and Prevention (CDC), an overweight adult has a BMI of 25 kg/m^2 to 29.9 kg/m^2, and an obese adult has a BMI of 30 kg/m^2 or greater. A BMI of 40 kg/m^2 or greater is indicative of morbid obesity or extreme

obesity. For children, the definitions of overweight and obese take into account age and gender effects on body fat.

[0031] BMI does not account for the fact that excess adipose can occur selectively in different parts of the body, and development of adipose tissue can be more dangerous to health in some parts of the body rather than in other parts of the body. For example, "central obesity", typically associated with an "apple-shaped" body, results from excess adiposity especially in the abdominal region, including belly fat and visceral fat, and carries higher risk of co-morbidity than "peripheral obesity", which is typically associated with a "pear-shaped" body resulting from excess adiposity especially on the hips. Measurement of waist/hip circumference ratio (WHR) can be used as an indicator of central obesity. A minimum WHR indicative of central obesity has been variously set, and a centrally obese adult typically has a WHR of about 0.85 or greater if female and about 0.9 or greater if male.

[0032] Methods of determining whether a subject is overweight or obese that account for the ratio of excess adipose tissue to lean body mass may involve obtaining a body composition of the subject. Body composition can be obtained by measuring the thickness of subcutaneous fat in multiple places on the body, such as the abdominal area, the subscapular region, arms, buttocks and thighs. These measurements are then used to estimate total body fat with a margin of error of approximately four percentage points. Another method is bioelectrical impedance analysis (BIA), which uses the resistance of electrical flow through the body to estimate body fat. Another method is using a large tank of water to measure body buoyancy. Increased body fat will result in greater buoyancy, while greater muscle mass will result in a tendency to sink. Another method is fan-beam dual energy X-ray absorptiometry (DEXA). DEXA allows body composition, particularly total body fat and/or regional fat mass, to be determined non-invasively.

[0033] Without being limited by any particular theory or mechanism of action, it is believed that fat oxidation and lipolysis are stimulated through treatment with inhibitors of MetAP2 that enhance the level and function of thioredoxin and/or over-rides the inhibitory effects of hyperinsulinemia related at least in part to insulin-stimulation and/or over-rides the inhibitory effects of high fat diet induced NADPH oxidase activity. A coordinated action can be induced which leads to a physiological reduction in body adiposity through increased loss of

fat tissue-associated triglyceride, increased metabolism of free fatty acids by the liver with increased ketone body formation, and reduced food intake. These effects are evident at doses of a MetAP2 inhibitor that do not substantially modulate angiogenesis.

[0034] In obese and/or hyperinsulinemic patients, liver PKA function may be suppressed secondary to elevated NADPH oxidase expression. Ketone body production and utilization are typically suppressed in an obese patient, potentially reducing hepatic satiety signals and increasing food consumption. However, administration of a MetAP2 inhibitor, without being limited by an theory, leads to activation of adipose tissue lipase activity and/or stimulating production and/or activity of the rate-limiting enzyme of beta-hydroxybutyrate production (3-hydroxymethyl glutaryl CoA synthase), leading to elevated ketone body production, as illustrated in e.g. Fig. 5.

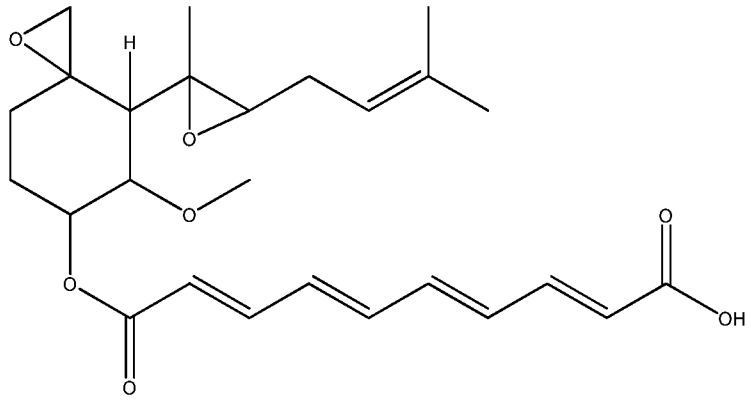
[0035] The coordinated and physiologic induction of anti-obesity activities mediated by the methods of the present invention may lead to a healthy reduction in tissue levels of triglyceride, diacylglycerol, and other fat-related mediators and oxidants, and can result in a new steady state situation that favors lean body composition and increased whole body energy metabolism. Without being bound by any theory, it is believed that the mechanistic cascade activated by MetAP2 inhibitors leads to fat tissue being converted to ketone bodies and burned as fuel, unlike existing therapies (including e.g., calorie or energy restricted diets) that target central control of food intake and that may carry adverse side effects (e.g. adverse neurological side effects). Partly as a consequence of improved metabolic function of the liver, glucose uptake by the liver is improved, reducing glucose concentrations in circulation and reducing the demand for insulin secretion and improving pancreatic beta cell function. Further, therapeutically effective doses contemplated herein will not typically induce any anti-angiogenic action.

[0036] Methods provided herein may substantially increase or stabilize glucose tolerance in a patient, for example, may increase glucose deposition as glycogen in a patient such as an overweight, obese, and/or diabetic patient or a patient that has reduced glucose tolerance.

MetAP2 inhibitors

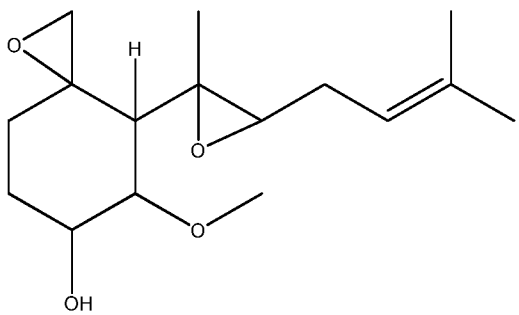
[0037] MetAP2 inhibitors refer to a class of molecules that inhibit the activity of MetAP2, e.g., the ability of MetAP2 to cleave the N-terminal methionine residue of newly synthesized proteins to produce the active form of the protein, or the ability of MetAP2 to regulate protein synthesis by protecting the subunit of eukaryotic initiation factor-2 (eIF2) from phosphorylation.

[0038] Exemplary MetAP2 inhibitors may include irreversible inhibitors that covalently bind to MetAP2. For example, such irreversible inhibitors include fumagillin, fumagillol, fumagillin ketone, Fumagillin refers to all stereoisomers, and can be represented by the following structure:



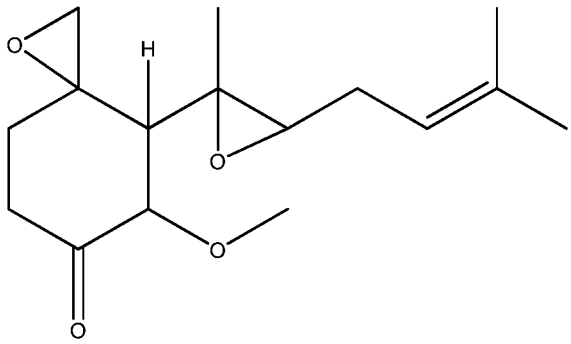
.

[0039] Fumagillol refers to all stereoisomers of the following structure:



.

[0040] Fumagillin ketone refers to all stereoisomers of the following structure, and can be represented by the following structure:



[0041] Derivatives and analogs of fumagillin, and pharmaceutically acceptable salts thereof are contemplated herein as irreversible MetAP2 inhibitors, such as O-(4-dimethylaminoethoxycinnamoyl)fumagillol (CKD-732, also referred to herein as Compound A), O-(3,4,5- trimethoxycinnamoyl)fumagillol, O-(4-chlorocinnamoyl)fumagillol; O-(4-aminocinnamoyl)fumagillol; O-(4-dimethylaminoethoxycinnamoyl)fumagillol; O-(4-methoxycinnamoyl)fumagillol; O-(4-dimethylaminocinnamoyl)fumagillol; O-(4-hydroxycinnamoyl)fumagillol; O-(3,4-dimethoxycinnamoyl)fumagillol; O-(3,4-methylenedioxycinnamoyl)fumagillol; O-(3,4,5-trimethoxycinnamoyl)fumagillol; O-(4-nitrocinnamoyl)fumagillol; O-(3,4-dimethoxy-6-aminocinnamoyl)fumagillol; O-(4-acetoxy-3,5-dimethoxycinnamoyl)fumagillol; O-(4-ethylaminocinnamoyl)fumagillol; O-(4-ethylaminoethoxycinnamoyl)fumagillol; O-(3-dimethylaminomethyl-4-methoxycinnamoyl)fumagillol; O-(4-trifluoromethylcinnamoyl)fumagillol; O-(3,4-dimethoxy-6-nitrocinnamoyl)fumagillol; O-(4-acetoxycinnamoyl)fumagillol; O-(4-cyanocinnamoyl)fumagillol; 4-(4-methoxycinnamoyl)oxy-2-(1,2-epoxy-1,5-dimethyl-4-hexenyl)-3-methoxy-- 1-chloromethyl-1-cyclohexanol; O-(3,4,5-trimethoxycinnamoyl)fumagillol; O-(4-dimethylaminocinnamoyl)fumagillol; O-(3,4,5-trimethoxycinnamoyl)oxy-2-(1,2-epoxy-1,5-dimethyl-4-hexenyl)-3-m- ethoxy-1-chloromethyl-1-cyclohexanol; O-(4-dimethylaminocinnamoyl)oxy-2-(1,2-epoxy-1,5-dimethyl-4-hexenyl)-3-me- thoxy-1-chloromethyl-1-cyclohexanol; O-(3,5-dimethoxy-4-hydroxycinnamoyl)fumagillol or O-(chloracetyl-carbamoyl) fumagillol(TNP-470).

[0042] Fumagillin, and some derivatives thereof, have a carboxylic acid moiety and can be administered in the form of the free acid. Alternatively, contemplated herein are pharmaceutically acceptable salts of fumagillin, fumagillol, and derivatives thereof. Pharmaceutically acceptable salts illustratively include those that can be made using the

following bases: ammonia, L-arginine, benethamine, benzathene, betaine, bismuth, calcium hydroxide, choline, deanol, diethanolamine, diethylarnine, 2-(diethylamino)ethanol, ethylenediamine, N-methylglucarnine, hydrabamine, 1 H-imidazole, lysine, magnesium hydroxide, 4-(2-hydroxyethyl)morpholine, piperazine, potassium hydroxide, 1-(2-hydroxyethyl)pyrrolidine, sodium hydroxide, triethanolamine, zinc hydroxide, diclyclohexlamine, or any other electron pair donor (as described in Handbook of Pharmaceutical Salts, Stan & Wermuth, VHCA and Wiley, Uchsenfurt- Hohestadt Germany, 2002). Contemplated pharmaceutically acceptable salts may include hydrochloric acid, bromic acid, sulfuric acid, phosphoric acid, nitric acid, formic acid, acetic acid, trifluoroacetic acid, oxalic acid, fumaric acid, tartaric acid, maleic acid, methanesulfonic acid, benzenesulfonic acid or para-toluenesulfonic acid.

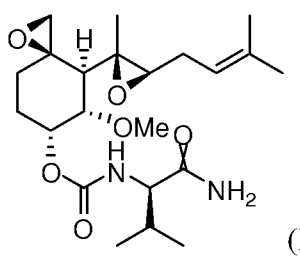
[0043] Esters of the present invention may be prepared by reacting fumagillin or fumagillol with the appropriate acid under standard esterification conditions described in the literature (Houben-Weyl 4th Ed. 1952, Methods of Organic Synthesis). Suitable fumagillin esters include ethyl methanoate, ethyl ethanoate, ethyl propanoate, propyl methanoate, propyl ethanoate, and methyl butanoate.

[0044] In another embodiment, contemplated irreversible inhibitors of MetAP2 may include a siRNA, shRNA, an antibody or an antisense compound of MetAP2.

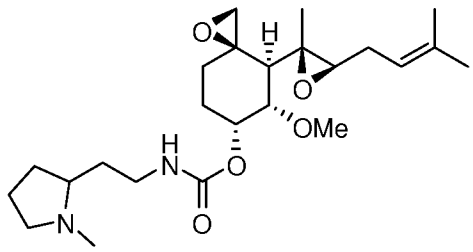
[0045] Further examples of MetAP2 inhibitors, are provided in the following references, each of which is hereby incorporated by reference: Olson et al. (US 7,084,108 and WO 2002/042295), Olson et al. (US 6,548,477; US 7,037,890; US 7,084,108; US 7,268,111; and WO 2002/042295), Olson et al. (WO 2005/066197), Hong et al. (US 6,040,337)., Hong et al. (US 6,063,812 and WO 1999/059986), Lee et al. (WO 2006/080591), Kishimoto et al. (US 5,166,172; US 5,698,586; US 5,164,410; and 5,180,738), Kishimoto et al. (US 5,180,735), Kishimoto et al. (US 5,288,722), Kishimoto et al. (US 5,204,345), Kishimoto et al. (US 5,422,363), Liu et al. (US 6,207,704; US 6,566,541; and WO 1998/056372), Craig et al. (WO 1999/057097), Craig et al. (US 6,242,494), BaMaung et al. (US 7,030,262), Comess et al. (WO 2004/033419), Comess et al. (US 2004/0157836), Comess et al. (US 2004/0167128), Henkin et al. (WO 2002/083065), Craig et al. (US 6,887,863), Craig et al. (US 2002/0002152), Sheppard et al. (2004, Bioorganic & Medicinal Chemistry Letters 14:865–868), Wang et al. (2003,

Cancer Research 63:7861–7869), Wang et al. (2007, Bioorganic & Medicinal Chemistry Letters 17:2817–2822), Kawai et al. (2006, Bioorganic & Medicinal Chemistry Letters 16:3574–3577), Henkin et al. (WO 2002/026782), Nan et al. (US 2005/0113420), Luo et al. (2003, J. Med. Chem., 46:2632-2640), Vedantham et al. (2008, J. Comb. Chem., 10:195-203), Wang et al. (2008, J. Med. Chem., XXXX, Vol. xxx, No. xx), Ma et al. (2007, BMC Structural Biology, 7:84) and Huang et al. (2007, J. Med. Chem., 50:5735-5742), Evdokimov et al. (2007, PROTEINS: Structure, Function, and Bioinformatics, 66:538-546), Garrabrant et al. (2004, Angiogenesis 7:91-96), Kim et al. (2004, Cancer Research, 64:2984-2987), Towbin et al. (2003, The Journal of Biological Chemistry, 278(52):52964-52971), Marino Jr. (U.S. patent number 7,304,082), Kallender et al. (U.S. patent application number 2004/0192914), and Kallender et al. (U.S. patent application numbers 2003/0220371 and 2005/0004116). In some embodiments, contemplated MetAP2 inhibitors do not include fumagillin, fumagillol, fumagillin ketone, CKD-732/Compound A, and/or TNP-470.

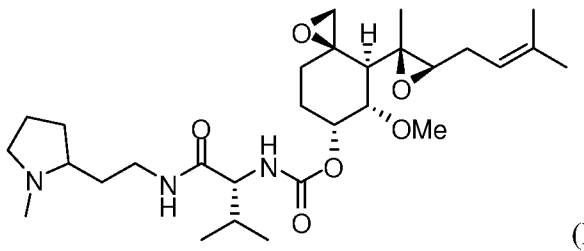
[0046] For example, contemplated MetAP2 inhibitors may include:



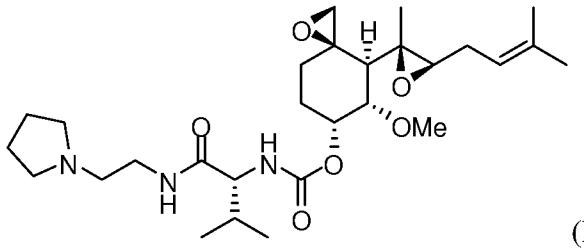
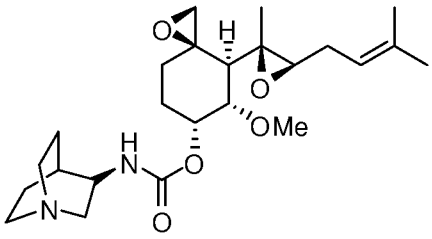
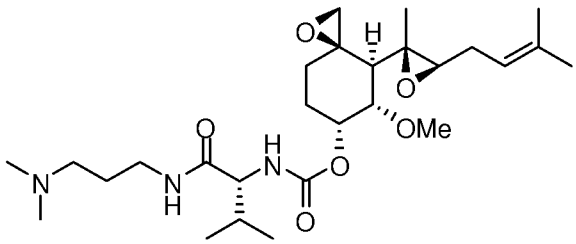
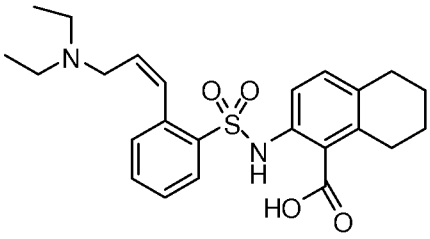
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Methods

[0047] A method of treating obesity in a patient in need thereof is provided herein, comprising non-parenterally administering a pharmaceutically effective amount of a MetAP2 inhibitor to said patient. In some embodiments, a contemplated pharmaceutically effective amount of a MetAP2 as described below, does not substantially modulate or suppress angiogenesis, but is still effective as MetAP2 inhibitor. The term "angiogenesis" is known to persons skilled in the art, and refers to the process of new blood vessel formation, and is essential for the exponential growth of solid tumors and tumor metastasis. For example, provided herein is a method of treating obesity in a patient in need thereof, comprising administering a pharmaceutically effective amount of a MetAP2 inhibitor to said patient, wherein substantially no loss of new blood vessels in fat deposits or other tissue compartments occur as compared to a patient being treated for obesity using an energy restricted diet alone.

[0048] For example, fumagillin-class molecules irreversibly inhibit enzymatic activity of MetAP2, leading to N-terminal acetylation and stabilization of these proteins at doses considerably lower than those required to suppress angiogenesis or tumor growth in vivo. Without being limited to any theory, the long-lasting covalent inhibition of MetAP2 enzymatic activity driven by such MetAP2 inhibitors may be responsible for the segregation of angiogenic effects from metabolic responses mediated by increased thioredoxin and/or glyceraldehyde-3-phosphate levels in vivo. Alternatively, anti-tumor effects driven by angiogenesis inhibition

may require a more thorough starvation of the tumor by heavily restricting blood supply, which requires high doses. Metabolic effects, however, may require a minor and incomplete perturbation of the system which occurs at lower doses and without any obvious direct effect on blood vessels. Metabolic effects that are largely dependent upon MetAP2 inhibition in the liver also may be elicited by treatment by e.g. administration of one or more MetAP2 inhibitors, such as those disclosed herein, that for example may effectively inactivate MetAP2 functions in the liver without providing sufficient exposure of the compounds to peripheral tissues to inhibit MetAP2.

[0049] Provided herein is a method of effecting weight loss in a patient in need thereof (e.g. an obese or overweight patient), comprising administering a pharmaceutically effective amount of a MetAP2 inhibitor to said patient, wherein, upon discontinuation of the MetAP2 inhibitor, the patient maintains a significant weight loss for a least about 1 month, 2 months 3 month, 6 months or a year or more. Such a patient may retain substantially more muscle mass as compared to body fat reduction in a patient using an energy restricted diet alone.

[0050] In some embodiments, disclosed methods, upon administration of said MetAP2 inhibitor e.g. daily or weekly, for about 3, 4, 5 or 6 months or more may result in at least a 5%, 10%, 20%, or 30%, or more weight loss based on the patient's original weight, which is maintained in the patient for e.g. 3 months, 6 months, 1 year, or even 5 years or more. In an embodiment, there may be a weight gain following treatment with therapeutically effective doses of MetAP2 inhibitors, but a patient durably maintains a lower body mass index as compared to a patient's body mass index before treatment.

[0051] In an embodiment, provided herein is a method of maintaining a specified weight in a formerly obese or overweight patient, comprising administering a pharmaceutically effective amount of a MetAP2 inhibitor to said patient. In another embodiment, provided herein is a method of inhibiting further weight gain in an overweight or obese patient, comprising administering a pharmaceutically effective amount of a MetAP2 inhibitor to said patient..

[0052] Also provided herein is a method for controlling or increasing glucose tolerance in a patient, such as an overweight, obese and/or diabetic patient, comprising administering a pharmaceutically effective amount of a MetAP2 inhibitor to said patient. Such methods may

result in lowering a patient's glucose levels in a 75g oral glucose tolerance 2 hour test to a normal range, e.g. about 70-140 mg/dL or about 5.5mmmol/l or less. In another embodiment, disclosed methods may reduce levels of circulating glycated hemoglobin (HbA1c), a key and accepted biochemical indicator of long-term glycemic control.

[0053] For example, a method of restoring normal metabolic action in an obese or overweight patient in need thereof is provided, comprising administering a pharmaceutically effective amount of a MetAP2 inhibitor to said patient. In an embodiment, a method of reducing weight of a patient in a patient in need thereof is provided comprising administering a pharmaceutically effective amount of a MetAP2 inhibitor to said patient wherein the metabolic rate of the patient is not substantially reduced as compared to the metabolic rate of a diet only patient on an energy restricted diet alone.

[0054] Contemplated herein is a method of reducing the amount or frequency of administering supplemental insulin in a patient suffering from type 2 diabetes, comprising administering a pharmaceutically effective amount of a MetAP2 inhibitor to said patient. Such treatment may be directed to an obese or non-obese (e.g. an overweight patient (either diabetic or non-diabetic, including pre-diabetic), or a normal weight diabetic) patient.

[0055] In addition to being overweight or obese, a subject can further have an overweight- or obesity-related co-morbidities, i.e., diseases and other adverse health conditions associated with, exacerbated by, or precipitated by being overweight or obese. Because being overweight or obese is associated with other adverse health conditions or co-morbidities, for example diabetes, administering MetAP2 inhibitors brings a benefit in ameliorating, arresting development of or, in some cases, even eliminating, these overweight- or obesity-related conditions or co-morbidities. In some embodiments, methods provided herein may further include administering at least one other agent that is directed to treatment of these overweight- or obesity-related conditions.

[0056] Contemplated other agents include those administered to treat type 2 diabetes such as sulfonylureas (e.g., chlorpropamide, glipizide, glyburide, glimepiride); meglitinides (e.g., repaglinide and nateglinide); biguanides (e.g., metformin); thiazolidinediones (rosiglitazone, troglitazone, and pioglitazone); glucagon-like 1 peptide mimetics (e.g. exenatide and liraglutide); sodium-glucose cotransporter inhibitors (e.g., dapagliflozin),

dipeptidylpeptidase 4 inhibitors such as vildagliptin, sitagliptin, saxagliptin, and alpha-glucosidase inhibitors (e.g., acarbose and meglitol), and/or those administered to treat cardiac disorders and conditions, such hypertension, dyslipidemia, ischemic heart disease, cardiomyopathy, cardiac infarction, stroke, venous thromboembolic disease and pulmonary hypertension, which have been linked to overweight or obesity, for example, chlorthalidone; hydrochlorothiazide; indapamide, metolazone; loop diuretics (e.g., bumetanide, ethacrynic acid, furosemide, lasix, torsemide); potassium-sparing agents (e.g., amiloride hydrochloride, spironolactone, and triamterene); peripheral agents (e.g., reserpine); central alpha-agonists (e.g., clonidine hydrochloride, guanabenz acetate, guanfacine hydrochloride, and methyldopa); alpha-blockers (e.g., doxazosin mesylate, prazosin hydrochloride, and terazosin hydrochloride); beta-blockers (e.g., acebutolol, atenolol, betaxolol, nisoprolol fumarate, carteolol hydrochloride, metoprolol tartrate, metoprolol succinate, Nadolol, penbutolol sulfate, pindolol, propranolol hydrochloride, and timolol maleate); combined alpha- and beta-blockers (e.g., carvedilol and labetalol hydrochloride); direct vasodilators (e.g., hydralazine hydrochloride and minoxidil); calcium antagonists (e.g., diltiazem hydrochloride and verapamil hydrochloride); dihydropyridines (e.g., amlodipine besylate, felodipine, isradipine, nicardipine, nifedipine, and nisoldipine); rennin inhibitors (e.g., aliskiren), ACE inhibitors (benazepril hydrochloride, captopril, enalapril maleate, fosinopril sodium, lisinopril, moexipril, quinapril hydrochloride, ramipril, trandolapril); angiotensin II receptor blockers (e.g., losartan potassium, valsartan, and Irbesartan); and combinations thereof, as well as statins such as mevastatin, lovastatin, pravastatin, simvastatin, velostatin, dihydrocompactin, fluvastatin, atorvastatin, dalvastatin, carvastatin, crilvastatin, bevastatin, cefvastatin, rosuvastatin, pitavastatin, and glenvastatin., typically for treatment of dyslipidemia.

[0057] Other agents that may be co-administered (e.g. sequentially or simultaneously) include agents administered to treat ischemic heart disease including statins, nitrates (e.g., Isosorbide Dinitrate and Isosorbide Mononitrate), beta-blockers, and calcium channel antagonists, agents administered to treat cardiomyopathy including inotropic agents (e.g., Digoxin), diuretics (e.g., Furosemide), ACE inhibitors, calcium antagonists, anti-arrhythmic agents (e.g., Sotolol, Amiodarone and Disopyramide), and beta-blockers, agents administered to treat cardiac infarction including ACE inhibitors, Angiotensin II receptor blockers, renin inhibitors, direct vasodilators, beta blockers, anti-arrhythmic agents and thrombolytic agents (e.g., Alteplase, Retaplase, Tenecteplase, Anistreplase, and Urokinase), agents administered to

treat strokes including anti-platelet agents (e.g., Aspirin, Clopidogrel, Dipyridamole, and Ticlopidine), anticoagulant agents (e.g., Heparin), and thrombolytic agents, agents administered to treat venous thromboembolic disease including anti-platelet agents, anticoagulant agents, and thrombolytic agents, agents administered to treat pulmonary hypertension include inotropic agents, anticoagulant agents, diuretics, potassium (e.g., K-dur), vasodilators (e.g., Nifedipine and Diltiazem), Bosentan, Epoprostenol, and Sildenafil, agents administered to treat asthma include bronchodilators, anti-inflammatory agents, leukotriene blockers, and anti-Ige agents. Particular asthma agents include Zafirlukast, Flunisolide, Triamcinolone, Beclomethasone, Terbutaline, Fluticasone, Formoterol, Beclomethasone, Salmeterol, Theophylline, and Xopenex, agents administered to treat sleep apnea include Modafinil and amphetamines, agents administered to treat nonalcoholic fatty liver disease include antioxidants (e.g., Vitamins E and C), insulin sensitizers (Metformin, Pioglitazone, Rosiglitazone, and Betaine), hepatoprotectants, and lipid-lowering agents, agentsadministered to treat osteoarthritis of weight-bearing joints include Acetaminophen, non-steroidal anti-inflammatory agents (e.g., Ibuprofen, Etodolac, Oxaprozin, Naproxen, Diclofenac, and Nabumetone), COX-2 inhibitors (e.g., Celecoxib), steroids, supplements (e.g. glucosamine and chondroitin sulfate), and artificial joint fluid, agents administered to treat Prader-Willi Syndrome include human growth hormone (HGH), somatropin, and weight loss agents (e.g., Orlistat, Sibutramine, Methamphetamine, Ionamin, Phentermine, Bupropion, Diethylpropion, Phendimetrazine, Benzphetermine, and Topamax), agents administered to treat polycystic ovary syndrome include insulin-sensitizers, combinations of synthetic estrogen and progesterone, Spironolactone, Eflornithine, and Clomiphene, agents administered to treat erectile dysfunction include phosphodiesterase inhibitors (e.g., Tadalafil, Sildenafil citrate, and Vardenafil), prostaglandin E analogs (e.g., Alprostadil), alkaloids (e.g., Yohimbine), and testosterone, agents administered to treat infertility include Clomiphene, Clomiphene citrate, Bromocriptine, Gonadotropin-releasing Hormone (GnRH), GnRH agonist, GnRH antagonist, Tamoxifen/nolvadex, gonadotropins, Human Chorionic Gonadotropin (HCG), Human Menopausal Gonadotropin (HmG), progesterone, recombinant follicle stimulating hormone (FSH), Urofollitropin, Heparin, Follitropin alfa, and Follitropin beta, agents administered to treat obstetric complications include Bupivacaine hydrochloride, Dinoprostone PGE2, Meperidine HCl, Ferro-folic-500/iberet-folic-500, Meperidine, Methylergonovine maleate, Ropivacaine HCl, Nalbuphine HCl, Oxymorphone HCl, Oxytocin, Dinoprostone, Ritodrine,

Scopolamine hydrobromide, Sufentanil citrate, and Oxytocic, agents administered to treat depression include serotonin reuptake inhibitors (e.g., Fluoxetine, Escitalopram, Citalopram, Paroxetine, Sertraline, and Venlafaxine); tricyclic antidepressants (e.g., Amitriptyline, Amoxapine, Clomipramine, Desipramine, Dosulepin hydrochloride, Doxepin, Imipramine, Iprindole, Lofepramine, Nortriptyline, Opipramol, Protriptyline, and Trimipramine); monoamine oxidase inhibitors (e.g., Isocarboxazid, Moclobemide, Phenelzine, Tranylcypromine, Selegiline, Rasagiline, Nialamide, Iproniazid, Iproclozide, Toloxatone, Linezolid, Dienolide kavapyrone desmethoxyyangonin, and Dextroamphetamine); psychostimulants (e.g., Amphetamine, Methamphetamine, Methylphenidate, and Arecoline); antipsychotics (e.g., Butyrophenones, Phenothiazines, Thioxanthenes, Clozapine, Olanzapine, Risperidone, Quetiapine, Ziprasidone, Amisulpride, Paliperidone, Symbyax, Tetrabenazine, and Cannabidiol); and mood stabilizers (e.g., Lithium carbonate, Valproic acid, Divalproex sodium, Sodium valproate, Lamotrigine, Carbamazepine, Gabapentin, Oxcarbazepine, and Topiramate), agents administered to treat anxiety include serotonin reuptake inhibitors, mood stabilizers, benzodiazepines (e.g., Alprazolam, Clonazepam, Diazepam, and Lorazepam), tricyclic antidepressants, monoamine oxidase inhibitors, and beta-blockers, and other weight loss agents, including serotonin and noradrenergic re-uptake inhibitors; noradrenergic re-uptake inhibitors; selective serotonin re-uptake inhibitors; and intestinal lipase inhibitors. Particular weight loss agents include orlistat, sibutramine, methamphetamine, ionamin, phentermine, bupropion, diethylpropion, phendimetrazine, benzphetermine, and topamax.

[0058] In some embodiments, contemplated methods may further comprising assessing one or more indices of on-going weight loss, e.g. the ketone body production level in a patient; and optionally adjusting the amount administered; thereby optimizing the therapeutic efficacy of said MetAP2 inhibitor.

Administration and Formulation

[0059] Contemplated herein are formulations suitable for non-parenteral and parenteral administration of MetAP2 inhibitors.

[0060] Contemplated non-parenteral administration includes oral, buccal, transdermal (e.g. by a dermal patch), topical, inhalation, or sublingual administration, or e.g., ocular, pulmonary, nasal, rectal or vaginal administration. Parenteral administration includes

subcutaneous and intravenous administration.

[0061] In another embodiment, provided herein are effective dosages, e.g. a daily dosage of a MetAP2 inhibitor, that may not substantially modulate or suppress angiogenesis. For example, provided here are methods that include administering doses of MetAP2 inhibitors that are effective for weight loss, but are significantly smaller doses than that necessary to modulate and/or suppress angiogenesis (which may typically require about 12.5mg/kg to about 50mg/kg or more). For example, contemplated dosage of a MetAP2 inhibitor in the methods described herein may include administering about 25 mg/day, about 10 mg/day, about 5 mg/day, about 3 mg/day, about 2 mg/day, about 1 mg/day, about 0.75 mg/day, about 0.5 mg/day, about 0.1 mg/day, about 0.05 mg/day, or about 0.01 mg/day.

[0062] For example, an effective amount of the drug for weight loss in a patient may be about 0.0001 mg/kg to about 25 mg/kg of body weight per day. For example, a contemplated dosage may from about 0.001 to 10 mg/kg of body weight per day, about 0.001 mg/kg to 1 mg/kg of body weight per day, about 0.001 mg/kg to 0.1 mg/kg of body weight per day or about 0.005 to about 0.04 mg/kg or about 0.005 to about 0.049 mg/kg of body weight a day. In an embodiment a MetAP2 inhibitor such as disclosed herein (e.g. O-(4-dimethlyaminoethoxycinnamoyl)fumagillol), may be administered about 0.005 to about 0.04 mg/kg of a patient.

[0063] For example, provided herein is a method for treating obesity in a patient in need thereof, comprising administering, parenterally (e.g. intravenously) or non-parenterally, about 0.005 to about 0.04 mg/kg of a MetAP2 inhibitor selected from O-(4-dimethylaminoethoxycinnamoyl)fumagillol and pharmaceutically acceptable salts thereof (for example, an oxalate salt), to said patient. Such a method, upon administration of said MetAP2 inhibitor e.g. daily or weekly, for about 3, 4 , 5 or 6 months or more may result in at least a 10%, 20%, 30%, or 40% or more weight loss based on the patient's original weight.

[0064] Contemplated methods may include administration of a composition comprising a MetAP2 inhibitor, for example, hourly, twice hourly, every three to four hours, daily, twice daily, 1, 2, 3 or 4 times a week, every three to four days, every week, or once every two weeks depending on half-life and clearance rate of the particular composition or inhibitor.

[0065] Treatment can be continued for as long or as short a period as desired. The compositions may be administered on a regimen of, for example, one to four or more times per day. A suitable treatment period can be, for example, at least about one week, at least about two weeks, at least about one month, at least about six months, at least about 1 year, or indefinitely. A treatment period can terminate when a desired result, for example a weight loss target, is achieved. For example, when about loss of about 20% body weight, about 30% body weight or more has been achieved. A treatment regimen can include a corrective phase, during which a MetAP2 inhibitor dose sufficient to provide reduction of excess adiposity is administered, followed by a maintenance phase, during which a lower MetAP2 inhibitor dose sufficient to prevent re-development of excess adiposity is administered.

[0066] For pulmonary (e.g., intrabronchial) administration, MetAP2 inhibitors can be formulated with conventional excipients to prepare an inhalable composition in the form of a fine powder or atomizable liquid. For ocular administration, MetAP2 inhibitors can be formulated with conventional excipients, for example, in the form of eye drops or an ocular implant. Among excipients useful in eye drops are viscosifying or gelling agents, to minimize loss by lacrimation through improved retention in the eye.

[0067] Liquid dosage forms for oral or other administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active agent(s), the liquid dosage forms may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the ocular, oral, or other systemically-delivered compositions can also include adjuvants such as wetting agents, and emulsifying and suspending agents.

[0068] Dosage forms for topical or transdermal administration of an inventive pharmaceutical composition may include ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants, or patches. The active agent is admixed under sterile conditions with a pharmaceutically acceptable carrier and any needed preservatives or buffers as may be

required. For example, cutaneous routes of administration are achieved with aqueous drops, a mist, an emulsion, or a cream.

[0069] Transdermal patches may have the added advantage of providing controlled delivery of the active ingredients to the body. Such dosage forms can be made by dissolving or dispensing the compound in the proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate can be controlled by either providing a rate controlling membrane or by dispersing the compound in a polymer matrix or gel.

[0070] When administered in lower doses, injectable preparations are also contemplated herein, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents.

[0071] Compositions for rectal or vaginal administration may be suppositories which can be prepared by mixing a MetAP2 inhibitor with suitable non-irritating excipients or carriers such as cocoa butter, polyethylene glycol or a suppository wax which are solid at ambient temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the active agent(s). Alternatively, contemplated formulations can be administered by release from a lumen of an endoscope after the endoscope has been inserted into a rectum of a subject.

[0072] Oral dosage forms, such as capsules, tablets, pills, powders, and granules, may be prepared using any suitable process known to the art. For example, a MetAP2 inhibitor may be mixed with enteric materials and compressed into tablets.

[0073] Alternatively, formulations of the invention are incorporated into chewable tablets, crushable tablets, tablets that dissolve rapidly within the mouth, or mouth wash..

EXAMPLES

[0074] The examples which follow are intended in no way to limit the scope of this invention but are provided to illustrate aspects of the disclosed methods. Many other embodiments of this invention will be apparent to one skilled in the art.

Example 1: Fumagillin increases levels of Thioredoxin-1 (TXN) in a dose-dependent manner

[0075] B16F10 cells were treated with increasing concentrations of fumagillin. N-terminal chymotryptic fragments of TXN were measured by liquid chromatography/mass spectrometry (LC/MS). Fumagillin significantly increased the cellular level of TXN containing an intact N-terminal methionine [TXN(1-4)] and caused significant accumulation of N-acetyl TXN(1-4), consistent with a role for fumagillin as a MetAP2 inhibitor. See FIGs. 1-2. The EC50 of fumagillin to increase Met-TXN acetylated Met-TXN levels is < 10 nM.

[0076] Similar results were seen in Cos-1 and 293T cells that were transfected with FLAG-tagged TXN in the absence (TXN) and presence (TXN + F) of fumagillin. Fumagillin significantly increased the expression on TXN in both Cos-1 and 293T cells. See FIG. 3. EC50 of fumagillin to increase TXN levels is < 10 nM. See FIG. 4.

Example 2: Oral administration of fumagillin reduces body fat in mammals

[0077] Oral administration of the MetAP2 inhibitor fumagillin causes an increase in circulating ketone bodies, as measured by concentration of beta hydroxybutyrate, which indicates an increase in the breakdown of stored fat. C57BL/6 mice conditioned on a high fat diet (also referred to as a diet-induced obese diet or DIO) were treated with fumagillin (ZGN-201) administered by oral gavage at doses of 0.3 or 3 mg/kg daily for 10 days resulting in an increase in beta hydroxybutyrate (FIG. 5A) concentrations, without an increase in free fatty acid concentration (FIG. 5B) as well as a reduction in body weight by 7 to 15 percent (data not shown). Increased hydroxybutyrate concentration persisted at longer time points, as was seen in C57BL/6 mice conditioned on a high fat diet and treated with fumagillin (ZGN-201) as a diet admixture at a dose of 1 mg/kg daily for 250 days (FIG. 5C).

[0078] In addition to showing an increase in beta hydroxybutyrate concentration, C57BL/6 mice conditioned on a high fat diet and treated with fumagillin (ZGN-201) as a diet admixture (1 mg/kg daily for 250 days) also experienced a reduction in body fat by approximately 40 percent (FIG. 6). Once attaining the maximum weight reduction, mice treated daily with 1 mg/kg fumagillin maintained a weight that was 25% less than that of age-matched mice maintained on normal mouse chow diet (lean mice, FIG. 6) for the duration of the experiment. The size and appearance of epididymal fat, peri-renal fat and liver in

fumagillin-treated mice was more similar to that of age-matched lean mice than that of untreated mice on a high fat diet (FIG 7). Retroperitoneal fat pad weight in fumagillin-treated mice was also more similar to that of age-matched lean mice than that of untreated mice on a high fat diet (FIG. 8).

[0079] Adipose tissue in mice fed a high-fat diet show distinctive crown-like structures not seen in lean mice. These crown-like structures were also absent from the adipose tissue of fumagillin-treated mice. Additionally, adipocyte size in fumagillin-treated mice was more similar to that of age-matched lean mice than that of untreated mice on a high fat diet.

[0080] Fasted glucose and insulin levels, as well as insulin-glucose product, in fumagillin-treated mice were more similar to that of age-matched lean mice than that of untreated mice on a high fat diet (FIG. 9), indicating that fumagillin normalizes sensitivity to insulin in mice on a high fat diet.

Example 3: Fumagillin reduces food intake during the peak of weight loss

[0081] High fat diet-fed C57BL/6 mice were treated with 1 mg/kg of the MetAP2 inhibitor fumagillin (ZGN-201, squares) or vehicle (diamonds) for 14 days (FIG. 10). Body weights (left panel) were determined daily, while food intake (right panel) was assessed every other day by measuring the food consumed by each cage of three mice over the two day period. Data shown in FIG. 10 are means ± SEM, n = 9 animals. As illustrated in FIG. 10, weight loss during treatment with MetAP2 inhibitors follows a sequence of three distinct phases. First, during the initial two to three days of treatment, no overt changes in food intake or body weight are observed. During this time period key changes in MetAP2 substrates occur, including glyceraldehyde-3-phosphate dehydrogenase and thioredoxin (data not shown). Second, the animals enter a period of rapid weight loss that proceeds until essentially all excess fat deposits are consumed. During this second period, fat oxidation is enhanced and food intake is inhibited.

[0082] Weight loss following treatment with therapeutically relevant doses of MetAP2 inhibitors stops once animals attain a normal body composition. Once weight loss reaches a nadir, fumagillin-treated animals enter a third phase in which their food intake returns to normal levels despite maintaining a normal body weight and improved metabolic control

evidenced by reduced insulin and glucose concentrations. This third phase can be maintained indefinitely provided the drug is continued (see FIG. 10), or presumably can be maintained in practice through other complementary pharmacologic, dietary, or lifestyle interventions.

Example 4: Oral administration of fumagillin results in fat loss with minimal change in lean tissue

[0083] C57BL/6 mice were conditioned on a low-fat diet (LF), a high fat diet (HF), or a high-fat diet and treated with fumagillin (ZGN-201) administered by oral gavage at doses of 0.1 or 0.3 mg/kg for 28 days. Either dose of fumagillin caused a reduction in body weight down to the level of low fat fed mice. To determine whether the weight loss resulted from loss of fat or loss of lean tissue, the percentage change in fat tissue and lean tissue was determined for LF mice, HF mice, HF mice treated with 0.1 mg/kg fumagillin, and HF mice treated with 0.3 mg/kg fumagillin. As shown in FIG. 11, HF mice treated with either dose of fumagillin experienced a 40-50% reduction in fat (compared to an increase in fat in both the LF and HF control mice), but only a 5-10% reduction in lean tissue, indicating that fumagillin-induced weight loss results primarily from loss of fat, rather than loss of lean tissue.

[0084] Upon histological examination, the studies of treated obese mice show no effect on growth of new blood vessels in growing fat depots and no effect on blood vessel size or density when compared to animals in which weight loss has been induced by simple energy (food) restriction.

Example 5: Orally administered compounds having MetAP-2 inhibitory cores cause weight loss in diet-induced obese mice

[0085] A weight loss study was conducted in obese mice. The mice in this study were not genetically obese, but prior to and during the study, obesity was induced by a high-fat diet. Twelve week-old C57BL/6NTac mice, maintained on a 60% fat diet prior to and during the study, were separated into seven groups, eight animals per group. Average body weight of the mice was approximately 47 g at the start of the study.

[0086] Each of six groups was orally administered 1.0 mg/kg of a compound (fumagillin and compounds B. C, D, E, F and G as disclosed herein), in 10% gelucire in deoinised water. Each of these six groups was administered a different compound. One group

was orally administered fumagillin at 1.0 mg/kg (group 201) in 10% gelucire in deoinised water, and one group was administered 10% gelucire in deoinised water (vehicle). Mice received administrations once a day for 7 days.

[0087] Data show that mice administered fumagillin lost the most weight over the course of the 8 days (Fig. 13). Mice in groups B, C, E, and E also lost weight over the course of the 8 days, with mice in group 233 losing the most weight of these four groups (Fig. 13). (Data analyzed by ANCOVA with body weight on Day 1 as covariate follwed by multiple tests against vehicle group: *p<0.05; **p<0.01, ***p<0.001.)

Example 6

[0088] A weight loss study was conducted in obese mice, similar to Example 5. Twelve week-old C57BL/6NTac mice, maintained on a 60% fat diet prior to and during the study, were separated into seven groups, eight animals per group. Average body weight of the mice was approximately 47 g at the start of the study.

[0089] The group was orally administered 100.0 mg/kg of a compound H, in 10% gelucire in deoinised water. Mice received administrations once a day for 7 days. After 7 days, the average weight loss of the mice was 13.2%.

Example 7 Normalization of Glucose Tolerance in Overweight Dogs

[0090] Feeding with a high-fat high-fructose (HFF) diet in dogs has been observed to induce a selective hepatic insulin resistance, reducing liver glucose extraction and causing glucose intolerance. The effects of an 8 week treatment with ZGN-201 (ZGN) on body weight and metabolic parameters in 6 overweight dogs (3 HFF and 3 chow-fed (OWC)) were evaluated. The study was comprised of three phases – pretreatment (PT: 4 weeks), treatment (ZGNTx: 8 weeks), and washout (WO: 4 weeks). FI, BW, blood chemistry and hematology, beta-hydroxybutyrate (BHB), and glycerol were assessed weekly. Oral glucose tolerance tests (OGTT, 0.9 g/kg initial BW) were performed at the end of PT, ZGNTx, and WO.

[0091] In HFF dogs, daily oral treatment with ZGN promoted loss of 81% of excess BW (resulting in a loss of approximately 8 percent of body weight, see Figure 14), whereas treatment in OWC dogs inhibited weight gain. ZGN reduced FI in HFF dogs by 29%, but had minimal effect (9% reduction) on FI in OWC dogs. After discontinuation of treatment, weight

loss in HFF dogs was sustained, where approximately one half of the initial weight loss was sustained after eight to ten weeks following the treatment discontinuation (See Figure 14). Glycerol levels increased, reflecting enhanced lipolysis, while BHB levels, reflecting fat oxidation, were increased in HFF only.

Endpoint	High-fat high fructose			Overweight chow		
	PT	ZGNTx	WO	PT	ZGNTx	WO
BW (kg)	33.5 ± 2.6	30.8 ± 3.4*	31.7 ± 2.9	17.7 ± 0.2	17.8 ± 0.5	18.6 ± 0.7
FI (kcal/d)	1677 ± 262	1187 ± 258*	1657 ± 147	1365 ± 60	1255 ± 64*	1116 ± 22*
Glycerol (μM)	72 ± 10	131 ± 54*	66 ± 25	94 ± 9	143 ± 26	86 ± 33
BHB (μM)	51 ± 13	76 ± 9*	39 ± 7	41 ± 9	48 ± 11	44 ± 5

*p<0.05
vs.PT

[0092] ZGN treatment reversed 75% of the abnormality in the plasma glucose excursion during the OGTT (see Figure 15) , despite a 32% reduction in insulin secretion (C-peptide AUC). After the drug was washed out, the improvement in plasma glucose was completely reversed. These results suggest that ZGN-201 treatment appears to correct the defect in hepatic glucose uptake seen in this model, and thereby decreases the demand for beta cell insulin secretion.

Incorporation by Reference

[0093] References and citations to other documents, such as patents, patent applications, patent publications, journals, books, papers, web contents, have been made throughout this disclosure. All such documents are hereby incorporated herein by reference in their entirety for all purposes, including PCT/US09/66816.

Equivalents

[0094] Various modifications of the invention and many further embodiments thereof, in addition to those shown and described herein, will become apparent to those skilled in the art

from the full contents of this document, including references to the scientific and patent literature cited herein. The subject matter herein contains important information, exemplification and guidance that can be adapted to the practice of this invention in its various embodiments and equivalents thereof.

What is claimed is:

1. A method of effecting weight loss in a patient in need thereof, comprising administering a pharmaceutically effective amount of a MetAP2 inhibitor to the patient, wherein upon discontinuation of administration of said MetAP2 inhibitor, the patient has durable weight loss maintained at least for about 1 month.

2. The method of claim 1, wherein the durable weight loss is maintained for at least about 3 months.

3. The method of claim 1 or 2, wherein the patient is overweight or obese.

4. A method of inhibiting further weight gain in an overweight or obese patient, comprising administering a pharmaceutically effective amount of a MetAP2 inhibitor to the patient.

5. The method of claim 4, wherein the patient is overweight.

6. The method of claim 5, wherein the patient is not obese.

7. A method of increasing and/or improving glucose tolerance and /or glycemic control in a patient in need thereof, comprising administering a pharmaceutically effective amount of a MetAP2 inhibitor to the patient.

8. The method of claim 7, wherein the patient is suffering from type 2 diabetes.

9. The method of claim 7, wherein the patient has impaired glucose tolerance and/or impaired fasting glucose.

10. The method of any one of claims 1-9, wherein said MetAP2 inhibitor is administered alone or in combination with another active agent.

11. The method of any one of claims 1-9, wherein said MetAP2 inhibitor is administered buccally, sublingually or transdermally.

12. The method of any one of claims 1-9, wherein said MetAP2 inhibitor is administered subcutaneously or intravenously.

13. The method of any one of claims 1-9, wherein said MetAP2 inhibitor is administered via inhalation.

14. The method of any one of claims 1-9, wherein said MetAP2 inhibitor is administered rectally.

15. The method of any one of claims 19, wherein said MetAP2 inhibitor is a substantially irreversible inhibitor.

16. The method of any one of claims 1-15, wherein said MetAP2 inhibitor is selected from the group consisting of a fumagillin, fumagillol or fumagillin ketone derivative, siRNA, shRNA, an antibody, or a antisense compound.

17. The method of any one of claims 1-16, wherein said MetAP2 inhibitor is selected from O-(4-dimethylaminoethoxycinnamoyl)fumagillol and pharmaceutically acceptable salts thereof.

18. The method of any one of claims 1-17, wherein said non-parenterally administering results in decreased body fat and a substantial maintenance of muscle mass in said patient.

19. The method of any one of claims 1-18, wherein upon administration, fat oxidation is enhanced as compared to a patient on a restricted food intake diet alone.

20. A method for reducing the amount or frequency of administering supplemental insulin in a patient suffering from type 2 diabetes, comprising a pharmaceutically effective amount of a MetAP2 inhibitor.

21. A method of maintaining a specified weight in a formerly obese patient, comprising administering a pharmaceutically effective amount of a MetAP2 inhibitor to said patient.

22. The method of claim 21, wherein the pharmaceutically effective amount is less as compared to the amount needed to reduce weight or body fat.

23. The method of any one of claims 20-22, wherein said pharmaceutically effective amount does not substantially modulate or suppress angiogenesis.

24. The method of any one of claims 20-23, wherein said MetAP2 inhibitor is a substantially irreversible inhibitor.

25. The method of any one of claims 20-24, wherein said MetAP2 inhibitor is selected from the group consisting of a fumagillin, fumagillol or fumagillin ketone derivative, siRNA, shRNA, an antibody, or an antisense compound.

26. The method of any one of claims 20-24, wherein said MetAP2 inhibitor is selected from O-(4-dimethylaminoethoxycinnamoyl)fumagillol and pharmaceutically acceptable salts thereof.

27. The method of any one of claims 1-26, further comprising co-administering an additional weight loss agent.

28. The method of any one of claims 1-27, further comprising administering a food restricted diet to said patient.

29. The method of any one of claims 1-28, wherein said administration occurs at least daily.

30. The method of any one of claims 1-28, wherein said administration occurs at least weekly.

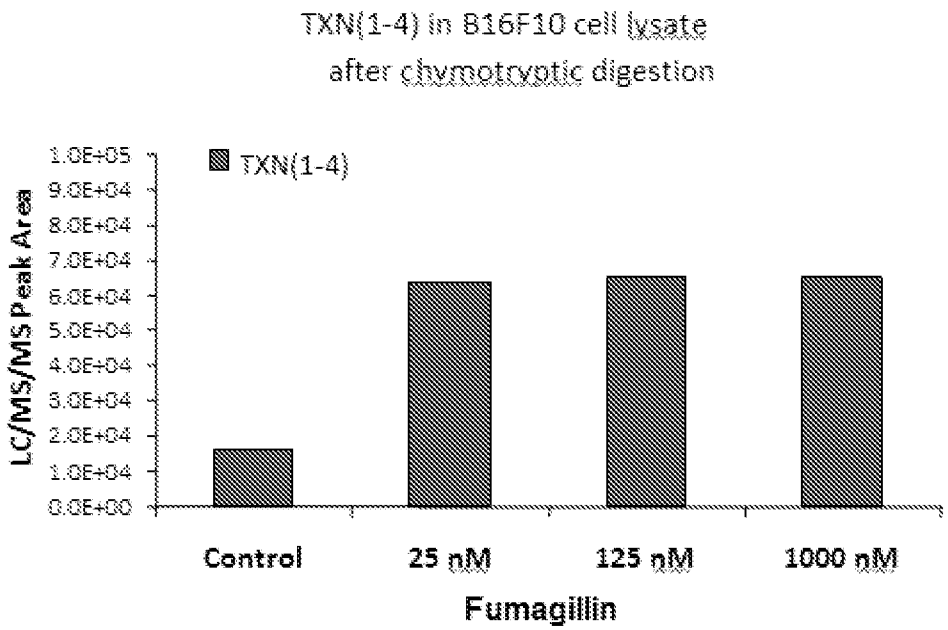

TXN(1-4) in B16F10 cell lysate
after chymotryptic digestion
TXN(1-4)
LC/MS/MS Peak Area
1.0E+05
9.0E+04
8.0E+04
7.0E+04
6.0E+04
5.0E+04
4.0E+04
3.0E+04
2.0E+04
1.0E+04
0.0E+00
Control
25 nM
125 nM
1000 nM
Fumagillin

FIG. 1

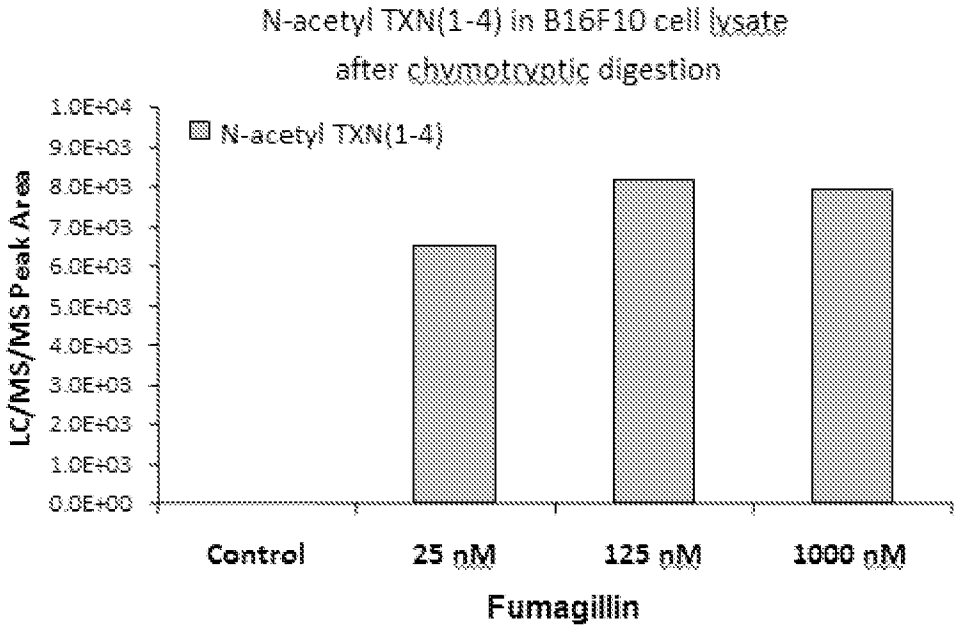

N-acetyl TXN(1-4) in B16F10 cell lysate
after chymotryptic digestion
N-acetyl TXN(1-4)
LC/MS/MS Peak Area
1.0E+04
9.0E+03
8.0E+03
7.0E+03
6.0E+03
5.0E+03
4.0E+03
3.0E+03
2.0E+03
1.0E+03
0.0E+00
Control
25 nM
125 nM
1000 nM
Fumagillin

FIG. 2

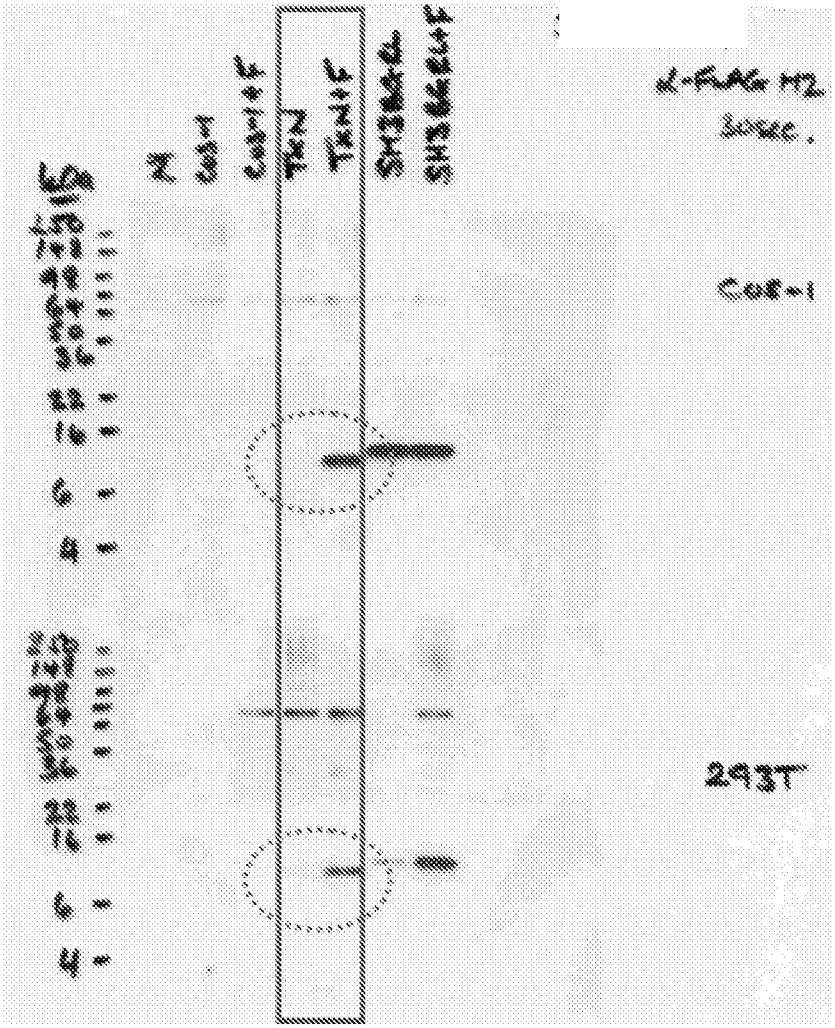


FIG. 3

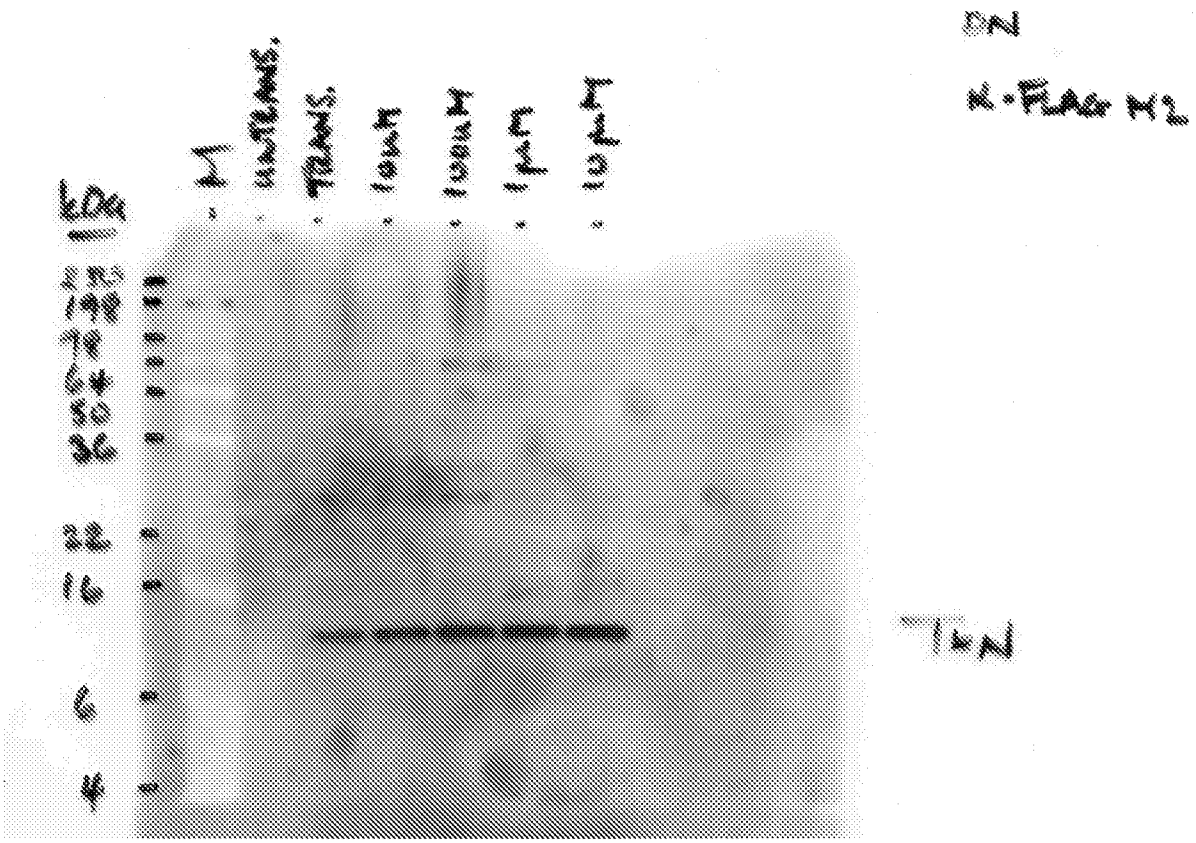
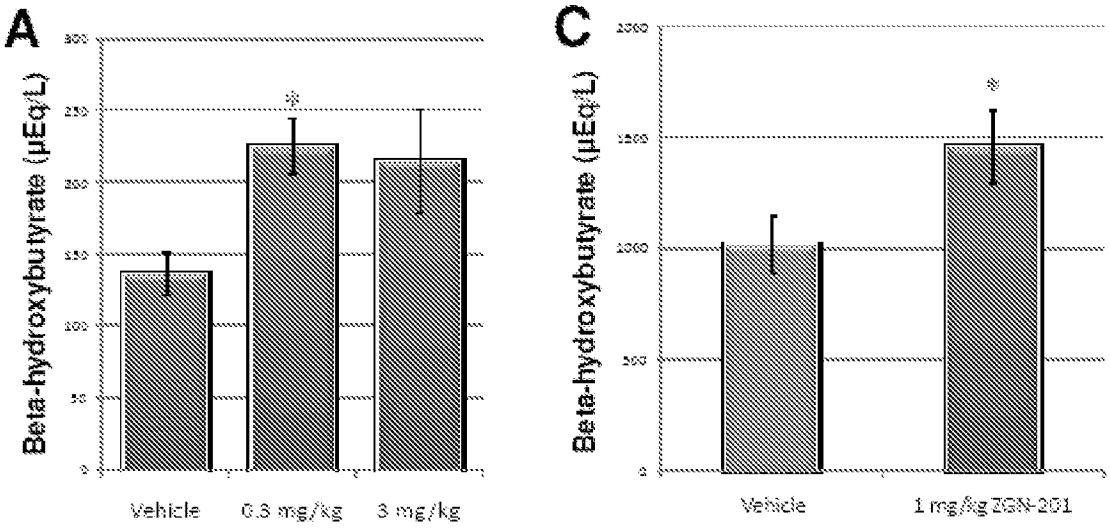

DN
K-FLAG H2
kDa
UNTRANS.
TRANS.
10μL
50μL
1μL
5μL
198
98
64
50
36
22
16
6
4
TN

FIG. 4


A
Beta-hydroxybutyrate (µEq/L)
Vehicle
0.3 mg/kg
3 mg/kg
C
Beta-hydroxybutyrate (µEq/L)
Vehicle
1 mg/kg ZGN-201

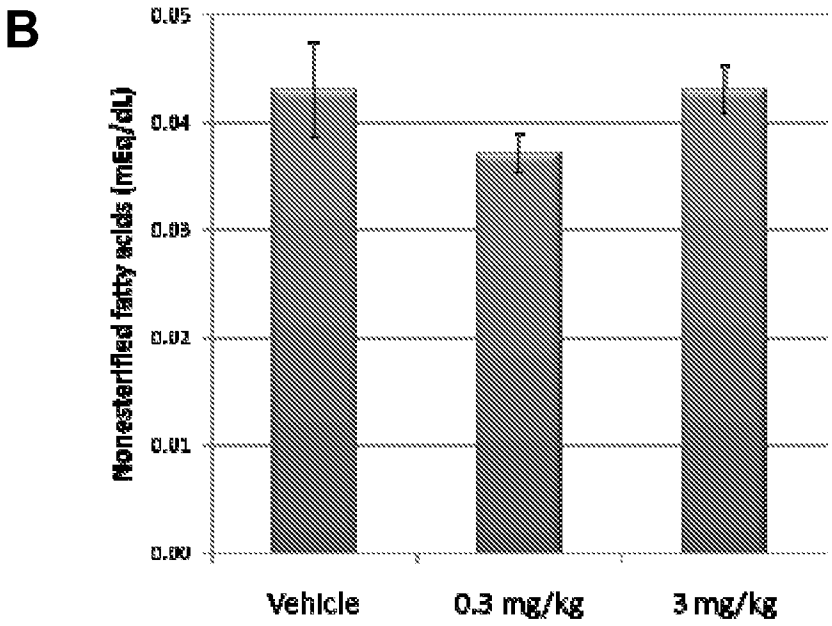

B
Nonesterified fatty acids (mEq/dL)
0.05
0.04
0.03
0.02
0.01
0.00
Vehicle
0.3 mg/kg
3 mg/kg

FIG. 5

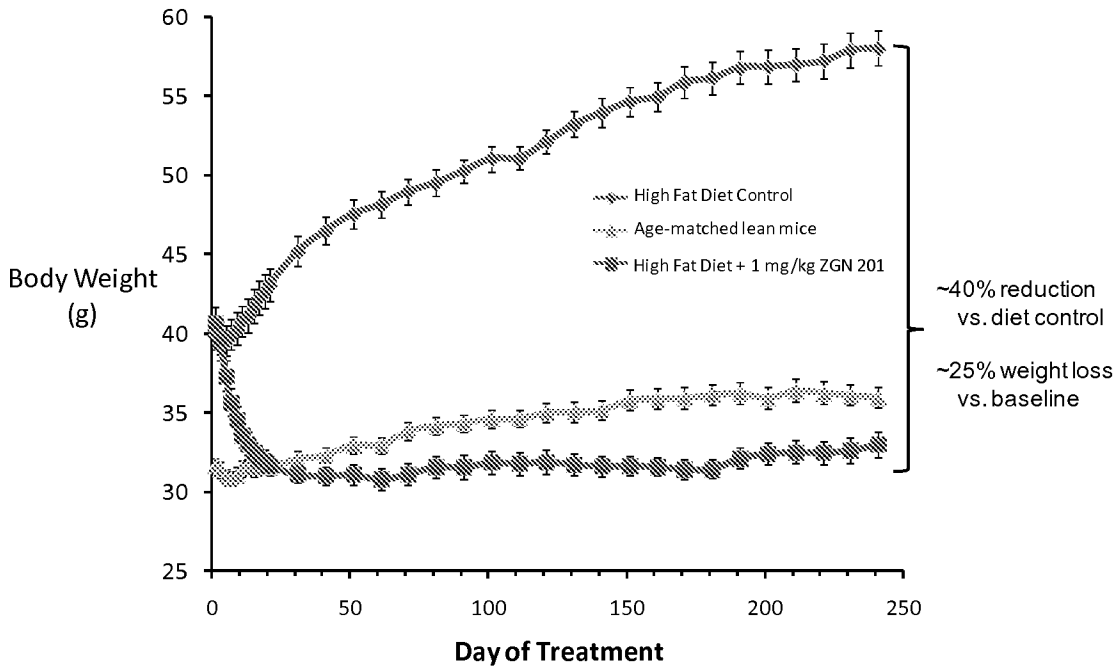

60
55
50
45
40
35
30
25
Body Weight (g)
0
50
100
150
200
250
Day of Treatment
High Fat Diet Control
Age-matched lean mice
High Fat Diet + 1 mg/kg ZGN 201
~40% reduction vs. diet control
~25% weight loss vs. baseline

FIG. 6

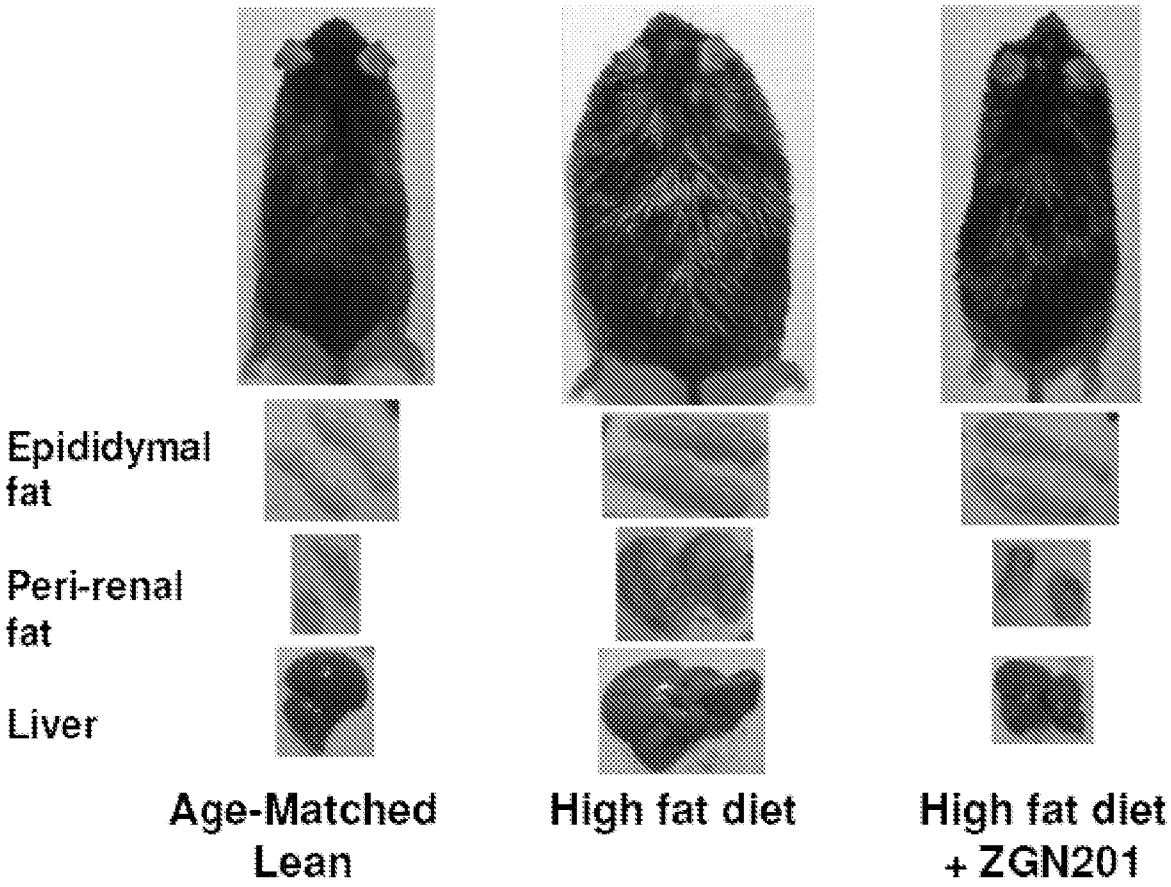

Epididymal fat
Peri-renal fat
Liver
Age-Matched Lean
High fat diet
High fat diet + ZGN201

FIG. 7

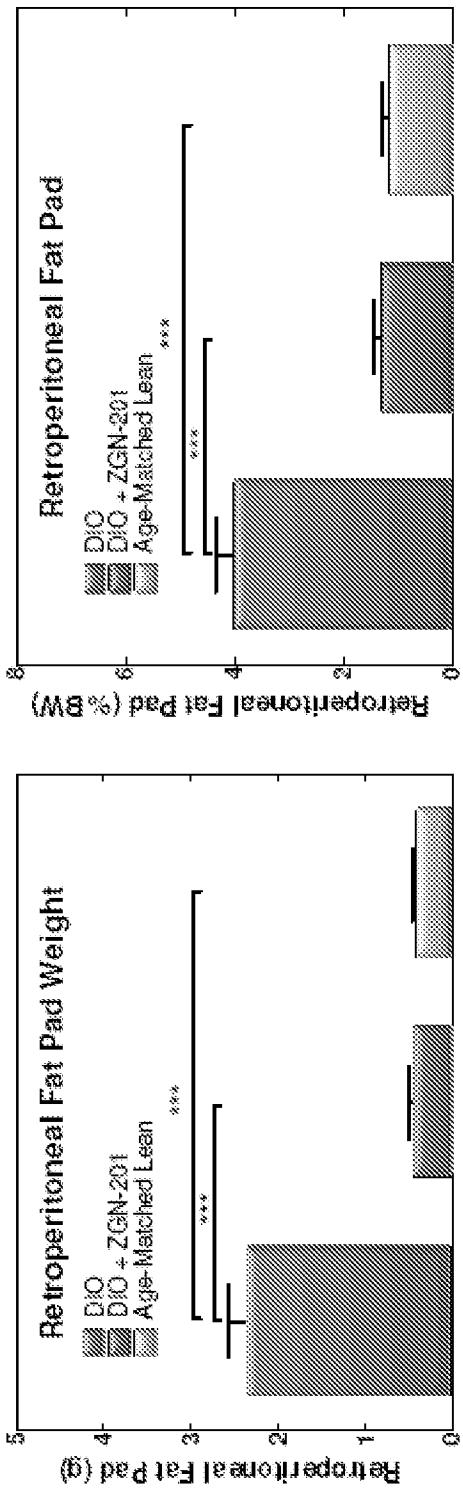

Retroperitoneal Fat Pad Weight
DIO
DIO + ZGN-201
Age-Matched Lean
***
***
Retroperitoneal Fat Pad (g)
0
1
2
3
4
5
Retroperitoneal Fat Pad
DIO
DIO + ZGN-201
Age-Matched Lean
***
***
Retroperitoneal Fat Pad (% BW)
0
2
4
6
8

FIG. 8

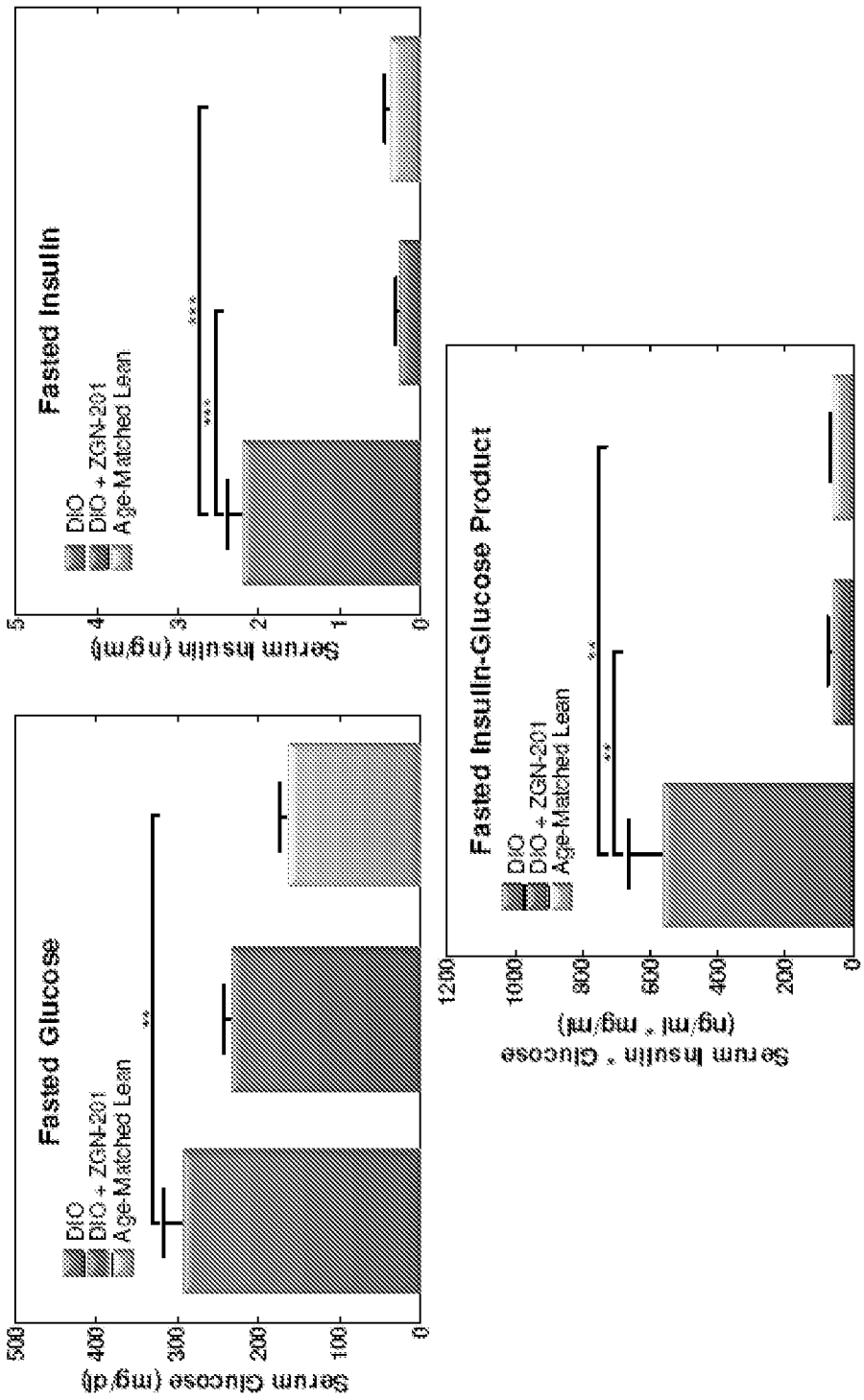

Fasted Glucose
DIO
DIO + ZGN-201
Age-Matched Lean
Serum Glucose (mg/dl)
Fasted Insulin
Serum Insulin (ng/ml)
Fasted Insulin-Glucose Product
Serum Insulin * Glucose
(ng/ml * mg/ml)

FIG. 9

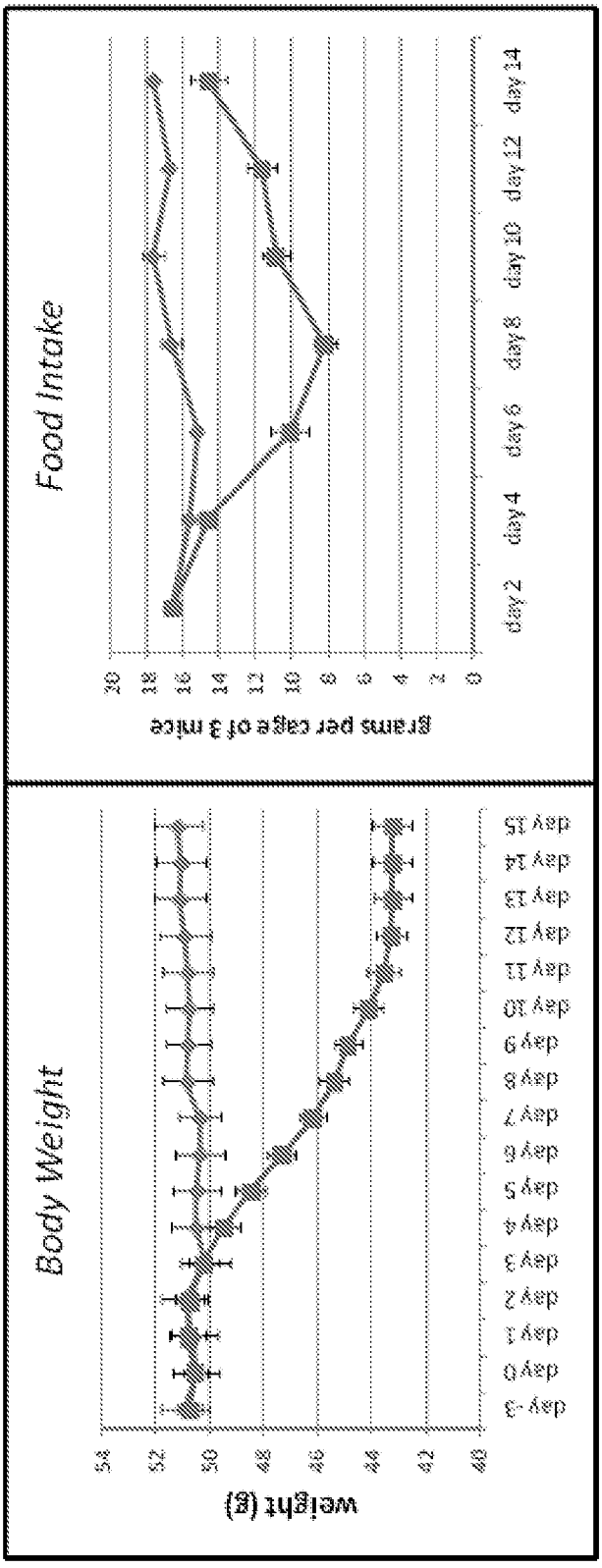

Body Weight
weight (g)
58
56
54
52
50
48
46
44
42
40
day -3
day 0
day 1
day 2
day 3
day 4
day 5
day 6
day 7
day 8
day 9
day 10
day 11
day 12
day 13
day 14
day 15
Food Intake
grams per cage of 3 mice
20
18
16
14
12
10
8
6
4
2
0
day 2
day 4
day 6
day 8
day 10
day 12
day 14

FIG. 10

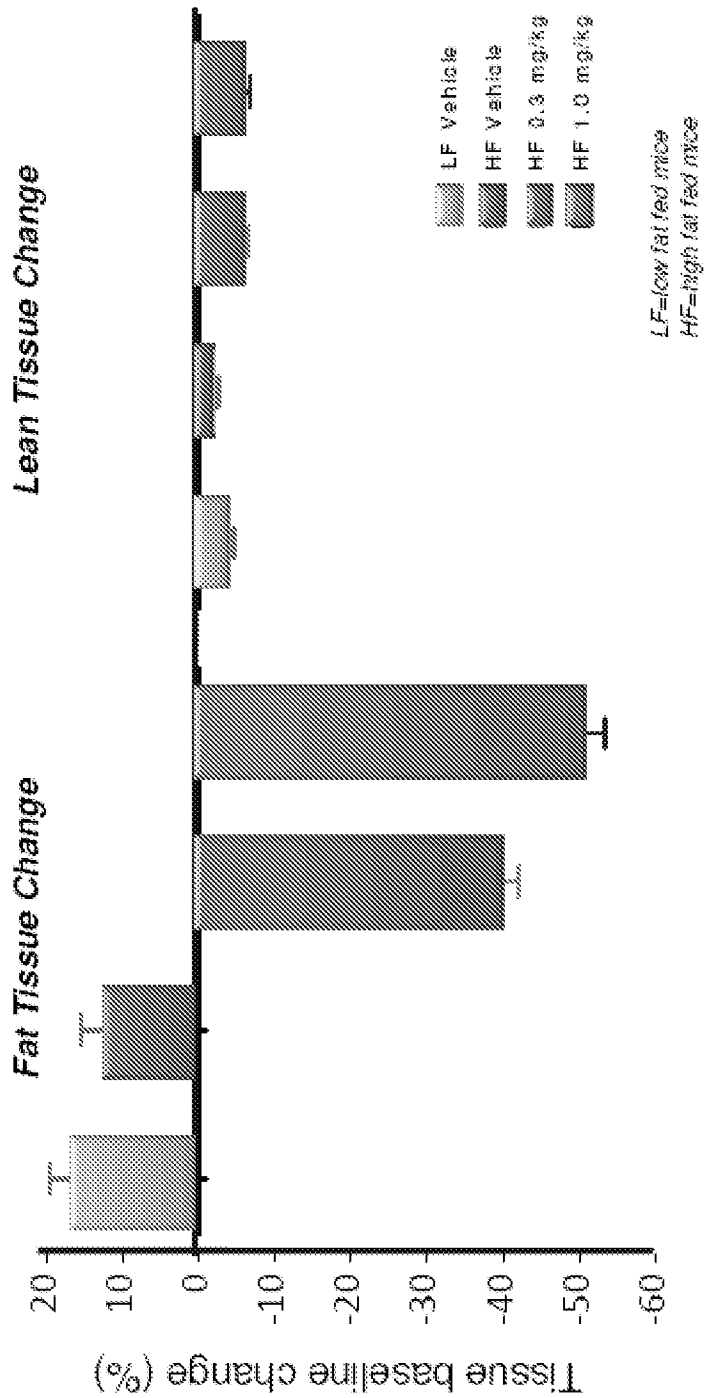

Fat Tissue Change
Lean Tissue Change
Tissue baseline change (%)
20
10
0
-10
-20
-30
-40
-50
-60
LF Vehicle
HF Vehicle
HF 0.3 mg/kg
HF 1.0 mg/kg
LF=low fat fed mice
HF=high fat fed mice

FIG. 11

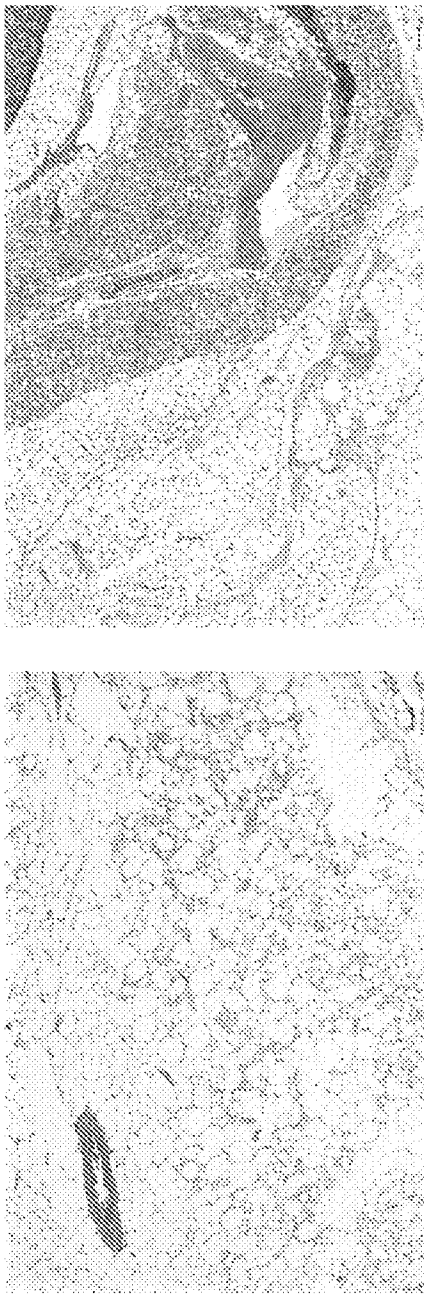


FIG. 12

FIGURE 13

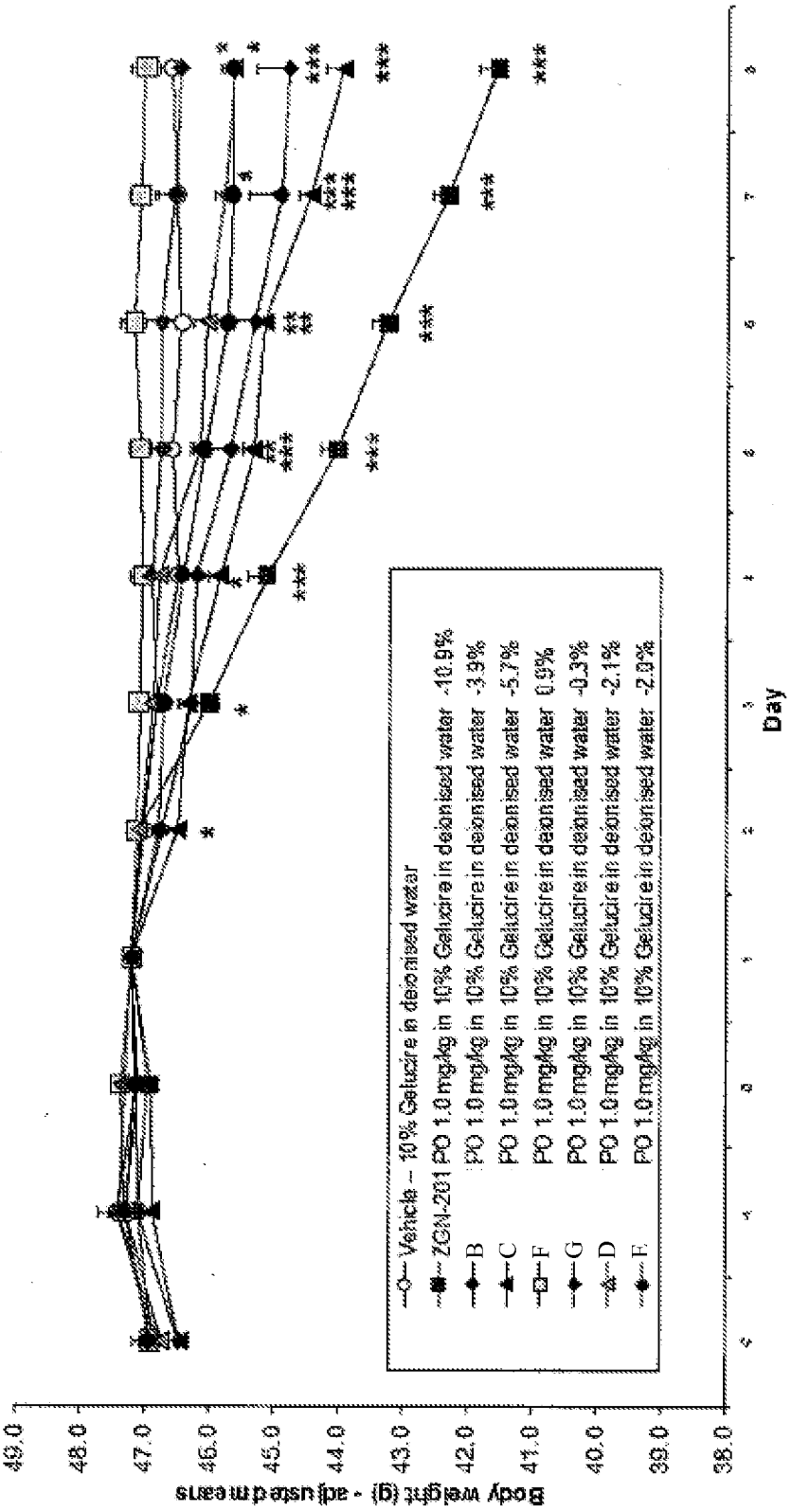

Body weight (g) - adjusted means
Day
Vehicle – 10% Gelucire in deionised water
ZGN-201 PO 1.0 mg/kg in 10% Gelucire in deionised water -10.9%
B PO 1.0 mg/kg in 10% Gelucire in deionised water -3.9%
C PO 1.0 mg/kg in 10% Gelucire in deionised water -5.7%
F PO 1.0 mg/kg in 10% Gelucire in deionised water 0.9%
G PO 1.0 mg/kg in 10% Gelucire in deionised water -0.3%
D PO 1.0 mg/kg in 10% Gelucire in deionised water -2.1%
E PO 1.0 mg/kg in 10% Gelucire in deionised water -2.8%

FIGURE 14

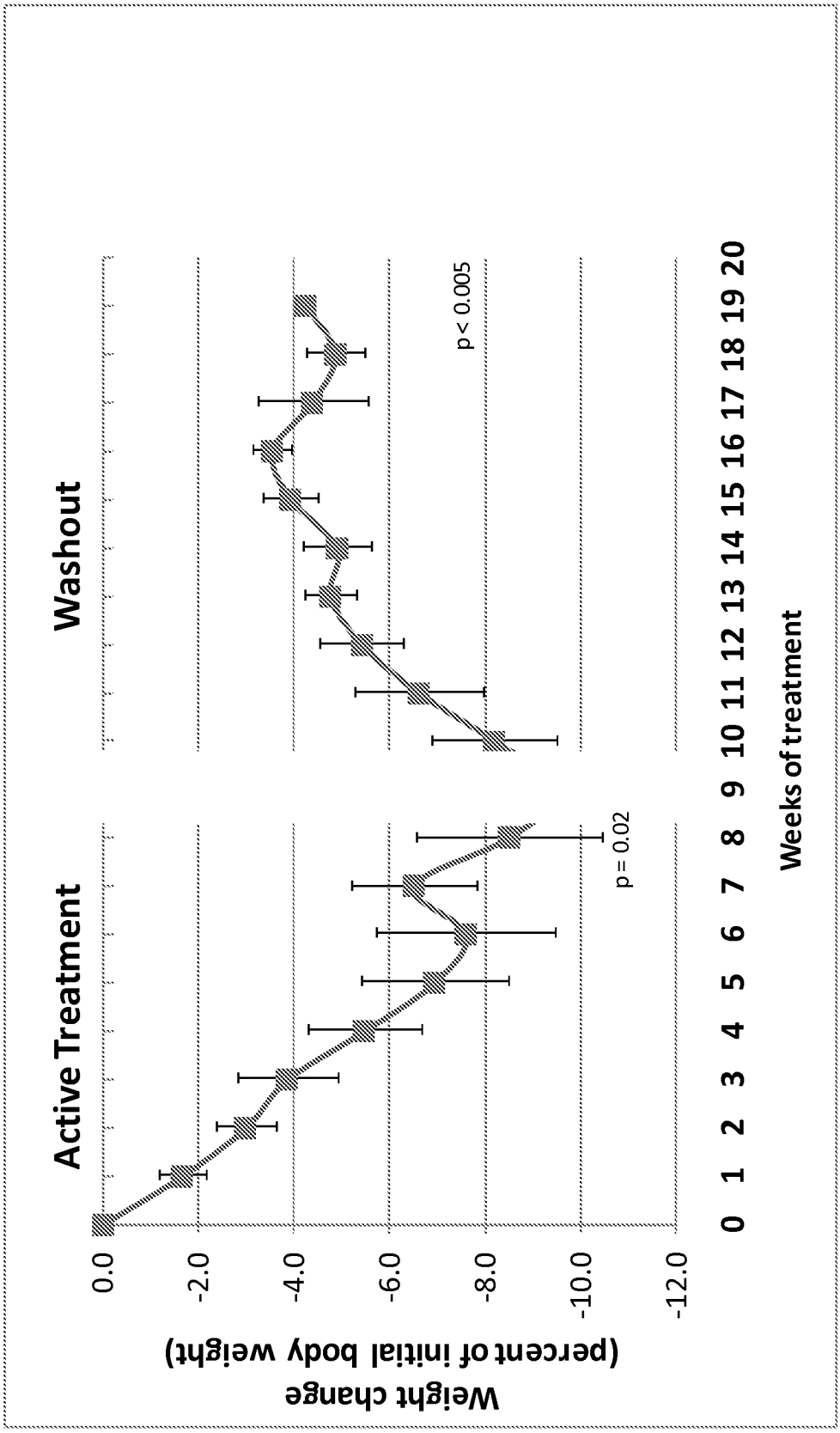
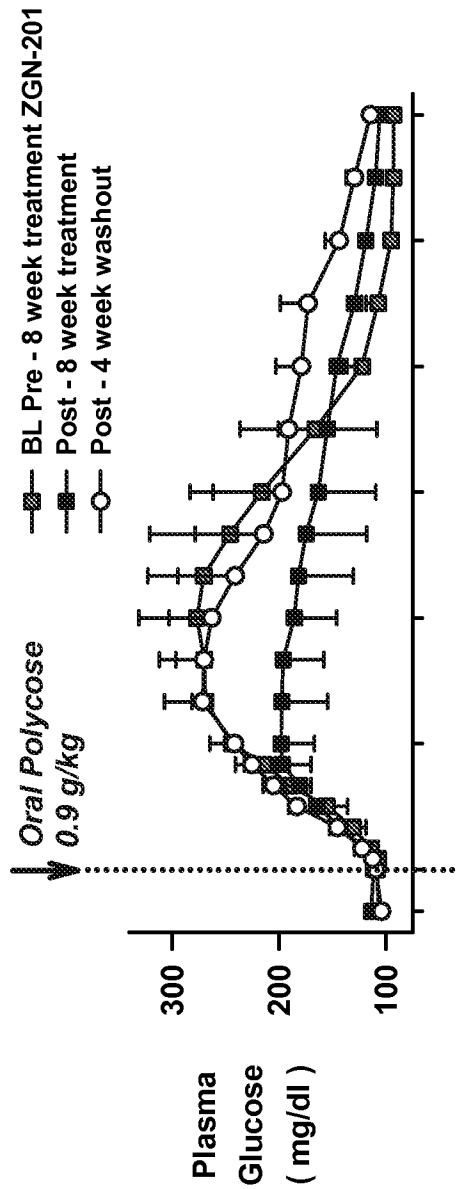

Active Treatment
Washout
Weight change
(percent of initial body weight)
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
-12.0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Weeks of treatment
p = 0.02
p < 0.005

FIGURE 15


Oral Polycose
0.9 g/kg
BL Pre - 8 week treatment ZGN-201
Post - 8 week treatment
Post - 4 week washout
Plasma Glucose (mg/dl)
300
200
100