



- (51) **International Patent Classification:**
A61K 31/417 (2006.01) A61P 3/00 (2006.01)
A61K 31/133 (2006.01)
- (21) **International Application Number:**
PCT/US2015/034833
- (22) **International Filing Date:**
9 June 2015 (09.06.2015)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
62/01 1,978 13 June 2014 (13.06.2014) US
62/141,491 1 April 2015 (01.04.2015) 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, BN, 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, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, 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, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, 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, KM, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

(54) **Title:** METHODS OF USING CARNOSINOL AND ANALOGS THEREOF

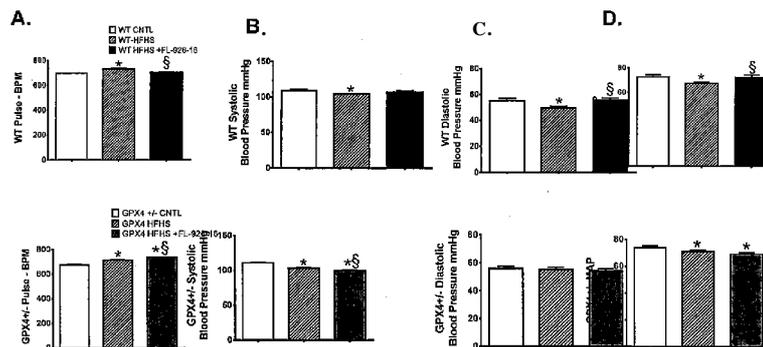


Fig. 1

(57) **Abstract:** Embodiments of the present invention relate to methods of preventing or treating diabetes, an obesity/diabetes-related condition, insulin sensitivity, heart disease, mitochondrial dysfunction or liver disease comprising administering carnosinol or an analog thereof to a subject in need thereof.

WO2015/191533 A1

Methods of Using Carnosinol and Analogs Thereof

Statement of Priority

[0001] This application claims priority to U.S. Provisional Application Serial Nos. 62/011,978, filed June 13, 2014 and 62/141,491, filed April 1, 2015. The disclosure of each of which is incorporated by reference in their entireties.

Reservation of Copyright

[0002] A portion of the disclosure of this patent document contains material which is subject to copyright protection. The copyright owner, East Carolina University, Greenville, North Carolina, a constituent institution of the University of North Carolina, has no objection to the reproduction by anyone of the patent document or the patent disclosure, as it appears in Patent Office patent files or records, but otherwise reserves all copyright rights whatsoever.

Field of the Invention

[0003] The present invention relates to methods of treatment and prevention of various disorders using carnosinol and/or analogs thereof.

Summary

[0004] Aspects of the present invention include the use of carnosinol and/or analogs thereof for the prevention or treatment of obesity, diabetes, a diabetes-related condition, insulin resistance, heart disease, mitochondrial dysfunction and liver disease.

[0005] Aspects of the present invention include the use of carnosinol and/or analogs thereof for the mitigation of cardiac remodeling.

[0006] Aspects of the present invention include the use of carnosinol and/or analogs thereof for the prevention or treatment of cardiomyopathy.

[0007] Aspects of the present invention include the use of carnosinol and/or analogs thereof for the restoration of insulin sensitivity.

[0008] Aspects of the present invention include the use of carnosinol and/or analogs thereof for the mitigation of liver steatosis.

[0009] Aspects of the present invention include the use of carnosinol and/or analogs thereof for the preparation of pharmaceutical and/or nutraceutical compositions and such

pharmaceutical and/or nutraceutical compositions for the prevention or treatment of the diseases and disorders described herein for human and veterinary purposes.

[0010] Aspects of the present invention further include a kit including carnosinol and/or analogs thereof in a physiologically acceptable carrier and a container suitable for delivery of the carnosinol and/or analogs thereof.

Brief Description of the Drawings

[0011] Fig. 1 shows blood pressure parameters after 16 weeks on a high fat/high sucrose (HFHS) diet, with and without carnosinol treatment.

[0012] Fig. 2 shows that carnosinol mitigates cardiac hypertrophy in HFHS diet-induced obese mice.

[0013] Fig. 3 shows heart histology (cardiomyocyte size) in mice with obesity and treated with carnosinol.

[0014] Fig. 4 shows that carnosinol mitigates cardiac fibrosis in HFHS diet-induced obese mice.

[0015] Fig. 5 shows that carnosinol improves mitochondrial function.

[0016] Fig. 6 shows that mitochondrial HNE-adducts are diminished with carnosinol treatment in HFHS diet-induced obese mice.

[0017] Fig. 7 shows the total body weight gain throughout the duration of the study (A) and percent body fat at termination of the study (B).

[0018] Fig. 8 shows that there was no difference in whole body energy expenditure occurring with carnosinol treatment in HFHS diet fed mice.

[0019] Fig. 9 shows that carnosinol improves whole body glycemic control in diet-induced obese mice.

[0020] Fig. 10 shows that carnosinol improves skeletal muscle insulin sensitivity in HFHS fed mice.

[0021] Fig. 11 shows that carnosinol mitigates liver steatosis in HFHS fed mice.

[0022] Fig. 12 shows that carnosinol mitigates liver fibrosis in HFHS diet-induced obese mice.

[0023] Fig. 13 shows that liver HNE-adducts are diminished with carnosinol treatment in HFHS diet-induced obese mice.

Detailed Description

[0024] This invention may be embodied in different forms and should not be construed as limited to the embodiments set forth herein. Rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the invention to those skilled in the art.

[0025] The terminology used in the description herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. As used in the description and the appended claims, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise.

[0026] Unless otherwise defined, all terms (including 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. It will be further understood that terms, such as those defined in commonly used dictionaries, should be interpreted as having a meaning that is consistent with their meaning in the context of the present application and relevant art and should not be interpreted in an idealized or overly formal sense unless expressly so defined herein. The terminology used in the description herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention.

[0027] All patent and patent application references referred to in this patent application are hereby incorporated by reference in their entirety as if set forth fully herein.

[0028] Also as used herein, "and/or" refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative ("or").

[0029] Unless the context indicates otherwise, it is specifically intended that the various features of the embodiments of the invention described herein may be used in any combination. For example, features described in relation to one embodiment may also be applicable to and combinable with other embodiments and aspects of the invention.

[0030] Moreover, the embodiments of the present invention also contemplate that in some embodiments, any feature or combination of features set forth herein may be excluded or omitted. To illustrate, if the specification states that a complex comprises components A, B and C, in some embodiments, any of A, B or C, or a combination thereof, may be omitted and disclaimed.

[0031] As used herein, the transitional phrase "consisting essentially of (and grammatical variants) is to be interpreted as encompassing the recited materials or steps "and those that do not materially affect the basic and novel characteristic(s)" of the claimed

invention. *See, In re Herz*, 537 F.2d 549, 551-52, 190 U.S.P.Q. 461, 463 (CCPA 1976) (emphasis in the original); *see also* MPEP § 2111.03. Thus, the term "consisting essentially of" as used herein should not be interpreted as equivalent to "comprising."

[0001] "Diabetes" is generally considered a chronic disease that occurs when the pancreas does not produce enough insulin, or the body cannot effectively use the insulin it produces. One of the main functions of insulin is to lower blood glucose levels by enabling glucose to enter the cells of the body, where it is used for energy or stored for future use. Diabetes can refer to a disease diagnosed as diabetes according to the diagnostic standard, for example, of WHO (World Health Organization), Japan Diabetes Society, American Diabetes Association or European Association for the Study of Diabetes and includes Type 1 diabetes, Type 2 diabetes, gestational or pregnancy diabetes, and the like. Type 2 diabetes can be characterized by its resistance to the action of insulin, i.e., "insulin resistance." Generally, a person who is insulin-sensitive requires a relatively small amount of insulin to maintain blood glucose levels in the normal range; however, a person who is insulin-resistant, may require significantly more insulin to achieve the same or similar blood-glucose-lowering effects.

[0002] "Insulin resistance" can mean a disease diagnosed as insulin resistance, based on the insulin resistance index (fasting blood sugar (mg/dL) x fasting insulin (microU/mL) ÷ 405) or on the results obtained by examination by glucose clamp method or the like and includes syndrome X additionally. In addition to Type 2 diabetes, diseases with "insulin resistance" include, for example, fatty liver, particularly non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), hyperglycemia, lipodosis, impaired glucose tolerance, hyperlipemia, pregnancy diabetes, polycystic ovary syndrome and the like.

[0003] Thus, a diabetes-related disorder can be hyperglycemia, hyperinsulinemia, impaired glucose tolerance, impaired fasting glucose, non-alcoholic fatty liver disease, dyslipidemia, hypertriglyceridemia, insulin resistance, and combinations thereof.

[0004] "Pre-diabetes" as used herein refers to elevated glucose levels, but not elevated to the level of a diagnosis of diabetes.

[0005] "Glycemic control" as used herein refers to maintaining blood glucose levels close to that of a person having normal blood glucose levels (e.g., a non-diabetic subject).

[0006] "Heart disease" or "cardiovascular disease" as used herein refers to a range of diseases or events that affect the heart and/or vasculature. Types of heart disease include, but are not limited to, coronary heart disease, cardiomyopathy, hypertrophy, ischemic heart disease, heart failure, inflammatory heart disease, fibrosis, valvular heart disease, aneurysm,

atherosclerotic disease, dyslipidemia, hypercholesterolemia, hypertension, coronary artery disease, and combinations thereof.

[0007] Heart disease can be assessed using clinical parameters and/or assessments known to those skilled in the art of diagnosing and/or treating the same, for example, physical examinations, detection of signs and symptoms of cardiovascular disease, electrocardiogram, echocardiogram, chest X-ray, magnetic resonance imaging, blood tests to detect cardiac biomarkers, etc. Biomarkers typically used in the clinical setting include, but are not limited to, cardiac troponins (C, T, and I), CK and CK-MB, and myoglobin.

[0008] "Cardiac remodeling" as used herein refers to the detrimental impact on the change in size, shape, structure and/or physiology of the heart after injury to the myocardium.

[0009] "Obesity" as used herein refers to a state of excess body fat mass. Obesity can include both excess body weight and excess adipose tissue mass in a subject. An obese subject may include a subject having a body mass index of about 30 kg/m². An obese subject may further include a subject whose body weight is twenty percent (20%) or more above the level considered by the U.S. Public Health Department, and/or similar organizations to be normal or healthy for the subject's age and gender. According to some standards, obesity is defined as a body mass index (BMI) equal to or more than about 30. *See* Center for Disease Control (CDC) standards for obesity. According to additional standards, generally, the normal amount of body fat (expressed as percentage of body fat) is between about 25-30% in women and about 18-23% in men. Women with over about 30% body fat and men with over about 25% body fat are considered obese. "Morbid obesity" also "clinically severe obesity," refers to a subject having a BMI of 35 or higher.

[0010] "Overweight" as used herein refers to a state wherein a subject has a body weight greater than the level considered by the U.S. Public Health Department, and/or similar organizations to be normal or healthy for the subject's age and gender. According to some standards, overweight is defined as a BMI equal to or more than about 25 where normal weight is defined as a BMI of about 18.5 to 24.0. According to some standards an adult who has a BMI between 25 and 29.9 is considered overweight. *See* Center for Disease Control (CDC) overweight definition.

[0011] Methods of estimating body fat and body fat distribution can include measurements of skinfold thickness and waist circumference, calculation of waist-to-hip circumference ratios, and techniques such as ultrasound, computed tomography, and magnetic resonance imaging (MRI). However, in any circumstance, a diagnosis of any disease or disorder described herein may be made by clinical observation and assessment

and/or through diagnostic testing recognized as acceptable by those skilled in the art for determining the amount and/or duration of therapy.

[0012] The term "metabolic syndrome" refers to a cluster of conditions including increased blood pressure, a high blood glucose level, excess body fat around the waist and abnormal cholesterol levels that occurring together can increasing the risk of heart disease, stroke and/or diabetes. "Metabolic syndrome" further includes a disease diagnosed as metabolic syndrome according to the diagnostic standard, for example, by the WHO, NCEP, IDF or the Committee for Diagnostic Standard of Metabolic Syndrome in the Japan Atherosclerosis Society.

[0013] "Mitochondrial dysfunction" or "mitochondrial disorders" are evidenced when the cellular supply of energy is unable to keep up with demand; symptoms predominate in tissues with the highest energy requirements (e.g., brain and muscle). Mitochondrial disorders are most commonly displayed as neuromuscular disorders, including developmental delay, seizure disorders, hypotonia, skeletal muscle weakness and cardiomyopathy. Other manifestations which have been reported include gastroesophageal reflux, apnea, optic atrophy, deafness, acute liver failure, diabetes mellitus, and other hormonal deficiencies.

[0014] "Liver disease" as used herein refers to inflammation, hepatitis, fibrosis, cirrhosis (scarring), non-alcoholic fatty liver disease (NAFLD), steatosis (accumulation of fats and/or lipids in the liver), steatohepatitis (inflammation and concurrent accumulation of fats and/or lipids in the liver) nonalcoholic steatohepatitis (NASH), and combinations thereof.

[0015] "Subjects" as used herein are generally human subjects and include, but are not limited to, "patients." The subjects may be male or female and may be of any race or ethnicity, including, but not limited to, Caucasian, African-American, African, Asian, Hispanic, Indian, etc. The subjects may be of any age, including newborn, neonate, infant, child, juvenile, adolescent, adult, and geriatric. Subjects may also include animal subjects, particularly mammalian subjects such as canines, felines, bovines, caprines, equines, ovines, porcines, rodents (*e.g.* rats and mice), lagomorphs, primates (including non-human primates), etc., for prevention and treatment purposes as well as veterinary medicine and/or pharmaceutical drug development purposes.

[0016] The term "treating" and grammatical variants thereof, as used herein, refer to any type of treatment that imparts a benefit to a subject, including delaying, and/or reducing the progression of one or more symptom(s) and/or condition(s), reducing the severity of one or more symptom(s) and/or condition(s), etc. Those skilled in the art will appreciate that the

benefit imparted by the treatment according to the methods of the present invention is not necessarily meant to imply cure or complete prevention.

[0017] "Prevent", "prevention", and grammatical variants thereof, as used herein, refer to avoiding the onset of a disease, disorder and/or a clinical symptom(s) in a subject relative to what would occur in the absence of the methods of the present invention.

[0018] The compounds and/or pharmaceutical or nutraceutical compositions of the present invention including carnosinol and/or analogs thereof may be administered to a subject by any suitable route, including, but not limited to, orally (inclusive of administration via the oral cavity), parenterally, by inhalation spray, topically, transdermally, rectally, nasally, sublingually, buccally, vaginally or via an implanted reservoir. The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques.

[0019] The compounds and/or pharmaceutical or nutraceutical compositions of the present invention including carnosinol or analogs thereof may be mixed with a vehicle and administered to the subject orally. The vehicle may be a liquid, semi-solid or solid substance for human or animal consumption.

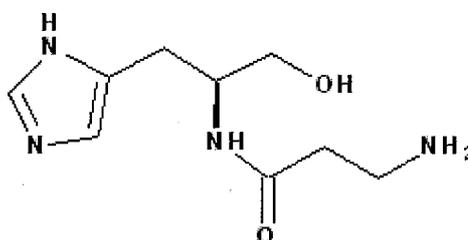
[0020] The compounds and/or pharmaceutical or nutraceutical compositions of the present invention including carnosinol and/or analogs thereof may be administered at the time of the onset of obesity, diabetes, the diabetes-related condition, insulin resistance, heart disease, mitochondrial dysfunction, metabolic syndrome or liver disease. In other embodiments, administration occurs after the onset of obesity, diabetes, the diabetes-related condition, insulin resistance, heart disease, mitochondrial dysfunction, metabolic syndrome or liver disease. In still other embodiments, administration occurs before the onset of the obesity, diabetes, the diabetes-related condition, insulin resistance, heart disease, mitochondrial dysfunction, metabolic syndrome or liver disease.

[0021] "Physiologically acceptable carrier" and "pharmaceutically acceptable carrier," which may be interchangeably used, refer to a carrier that does not cause significant irritation to an organism and does not abrogate the biological activity and/or properties of the administered compound.

[0022] "Kit" as used herein refers to an assembly of components. The assembly of components can be a partial or complete assembly. Instructions for use of the kit or use of various components of the kit are optionally included.

[0023] Embodiments of the present invention provide methods of preventing or treating obesity, diabetes, a diabetes-related condition, insulin sensitivity, heart disease, mitochondrial dysfunction, metabolic syndrome or liver disease comprising administering carnosinol or an analog thereof to a subject in need thereof.

[0024] Compounds suitable for use according to the present invention include compounds described in U.S. Patent No. 8,623,900 and WO2008001 175, WO2008001174 and WO2005120543, the disclosures of which are incorporated herein by reference. According to embodiments of the present invention, the compound used herein is carnosinol or analogs thereof. In some embodiments, the compound used herein is carnosinol and has the following structure:



[0025] In some embodiments, administration of carnosinol or an analog thereof enhances insulin-stimulated skeletal muscle glucose uptake. Administration of carnosinol or an analog thereof can improve glycemic control. Administration of carnosinol or an analog thereof can prevent or treat cardiac hypertrophy. Further, administration of carnosinol or an analog thereof can prevent or treat cardiac fibrosis. Administration of carnosinol or an analog thereof prevents or treats cardiac hypertrophy. Additionally, administration of carnosinol or an analog thereof can also improve cardiac mitochondrial function or cardiac remodeling. Such improvement can include an improvement or a decrease in infarct size, necrosis, apoptosis, autophagy, angiogenesis, chamber dilation, wall thinning, inflammation, reduction in markers of cardiomyocyte degradation or a combination thereof.

[0026] In particular embodiments, the heart disease is coronary heart disease, cardiomyopathy, hypertrophy, ischemic heart disease, heart failure, inflammatory heart disease, fibrosis, valvular heart disease, aneurysm, atherosclerotic disease, dyslipidemia, hypercholesterolemia, hypertension, coronary artery disease, and combinations thereof.

[0027] In some embodiments, the liver disease is inflammation, hepatitis, fibrosis, cirrhosis, non-alcoholic fatty liver disease (NAFLD), steatosis, steatohepatitis, nonalcoholic steatohepatitis (NASH), and combinations thereof.

[0028] Embodiments of the present invention are directed to use in subjects such as those described above. Additionally, subjects further include, but are not limited to, those who are overweight, obese or morbidly obese as well as those at risk for becoming overweight, obese or morbidly obese. Risk factors for obesity include, but are not limited to, poor diet, lack of physical activity, working varied shifts, certain medications, rare hereditary diseases, and hormonal imbalances (such as hypothyroid and Cushing's disease), cessation of smoking, increased age, genetic factors, race and cultural influences.

[0029] In some instances, the subject is pre-diabetic. In some instances the subject is diabetic. In some instances, the subject is not pre-diabetic or diabetic, but instead, the subject has a normal blood-glucose level.

[0001] Embodiments of the present invention relate to administering carnosinol and/or analogs thereof, which includes administration of carnosinol and/or analogs thereof in pharmaceutical or nutraceutical compositions.

[0030] In some embodiments, the carnosinol and/or an analog thereof is administered to the subject parenterally. In particular embodiments, parenteral delivery is via intravenous administration. In other embodiments, parenteral administration is through an intramyocardial administration device.

[0031] In some embodiments, the carnosinol and/or an analog thereof is administered to the subject orally. In other embodiments, the carnosinol and/or an analog thereof is mixed with a vehicle suitable for human or animal consumption. The vehicle may be a liquid, semi-solid or solid substance for human or animal consumption including, but not limited to, water, juice, milk, coffee, tea, carbonated beverages, non-carbonated beverages, sports drinks and the like as well as blended beverages such as smoothies, also yogurt, puddings, ice cream, sorbets and the like.

[0032] When administered in a pharmaceutical or nutraceutical composition, carnosinol and/or an analog thereof may be combined with a physiologically acceptable carrier. In some embodiments, the physiologically acceptable carrier can include, but is not limited to, sterile water, saline, glucose, dextrose, stabilizers (e.g., sugars and amino acids), preservatives, wetting agents, emulsifying agents, and pH buffering agents. Suitable carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin.

[0033] In some embodiments, the dosage of the compounds can be from about 0.1 to about 100 mg/kg, administered orally 1-4 times per day. In addition, compounds can be administered by injection at approximately 0.01 - 20 mg/kg per dose, with administration 1-4

times per day. Treatment could continue for weeks, months or longer. Determination of optimal dosages for a particular situation is within the capabilities of those skilled in the art.

Examples

[0034] Oxidation of polyunsaturated fatty acids (PUFAs) yields lipid peroxidation end products (LPPs), including α,β -unsaturated aldehydes, which are thought to contribute to a number of chronic diseases associated with obesity. However, the precise role of these LPPs and the mechanisms involved remain unknown. Glutathione Peroxidase 4 (GPx4) is an antioxidant enzyme with both cytosolic and mitochondrial isoforms, and is unique in that it specifically neutralizes lipid peroxides. In our investigations, GPx4 (GPx4^{+/-}) and their wild type (WT) littermates were fed a high fat high sucrose (HFHS) diet for 16 weeks in order to examine the role of LPPs in cardiac remodeling that occurs with obesity. To determine whether scavenging LPPs can mitigate this remodeling, GPx4^{+/-} and WT mice were treated with FL-926-16 (carnosinol), a novel carbonyl scavenging compound. FL-926-16 intervention (40mg/kg) began after 8 weeks on the HFHS diet.

Example 1

Cardiac and Mitochondrial Studies

[0035] Study design.

6 cohorts:

WT Control Diet

WT HFHS Diet

WT HFHS Diet + Carnosinol (45 mg/kg in drinking water beginning at 8 weeks into HFHS diet)

GPx4^{+/-} Control Diet

GPx4^{+/-} HFHS Diet

GPx4^{+/-} HFHS Diet + Carnosinol

[0036] Cardiac structure/function was assessed, along with cardiac mitochondrial function in permeabilized ventricular myofibers. Fig. 1 presents blood pressure parameters after 16 weeks on diet measured using tail cuff A. Pulse B. Systolic C. Diastolic. D. Mean Arterial Pressure (MAP). Data represented as mean \pm SEM, $p < 0.05$ using one way ANOVA followed by Newman-Keuls (n=6-8) * vs CNTL; § vs HFHS. Fig. 2 shows that carnosinol mitigates cardiac hypertrophy in HFHS diet-induced obese mice. (A) Heart: tibia ratio (B) Cardiomyocyte diameter (n = 2). Data shown as mean \pm SEM, $p < 0.05$ using one way

ANOVA followed by Newman-Keuls * vs CNTL; § vs HFHS. FL-926-16 mitigated HFHS-diet induced cardiac hypertrophy in WT only. Fig. 3 demonstrates cardiac histology (H & E staining) in mice with diet-induced obesity and treated with carnosinol. The results show the enlarged size of cells with a HFHS diet, and reduced size in carnosinol treated HFHS mice.

Fig. 4 shows that carnosinol mitigates cardiac fibrosis with HFHS diet-induced obesity.

[0037] FL-926-16 improved mitochondrial efficiency - the ratio of ATP production to O_2 consumption by two-fold, and restored mitochondrial fatty acid oxidation rates to levels comparable to control diet in both WT-HFHS and GPx4-HFHS groups. Fig. 5 shows that mitochondrial function in the heart improves with carnosinol treatment in HFHS diet-induced obese mice. The left panel presents the rates of O_2 consumption and ATP production in a preparation of permeabilized left ventricular myofibers prepared from mice in each of the 6 cohorts used in this study, under clamped submaximal phosphorylating state. The right panel presents the ratio of ATP produced to O_2 consumed under these conditions. Data are representative of N=6-8 mice. Fig. 6 shows that mitochondrial HNE-adducts are diminished with carnosinol treatment in HFHS diet-induced obese mice. Shown in the immunoblot are mitochondrial proteins that have been covalently modified with 4-hydroxynonenal, a lipid peroxide-derived aldehyde. The decrease in HNE adduct levels in both carnosinol-treated groups of HFHS-fed mice is comparable to the levels in lean control mice.

Example 2

Metabolic and Liver Studies

[0038] Study design.

6 cohorts:

WT Control Diet

WT (High fat, High sucrose) HFHS Diet

WT HFHS Diet + Carnosinol (45 mg/kg in drinking water beginning at 8 weeks into HFHS diet)

GPx4+/- Control Diet

GPx4+/- HFHS Diet

GPx4+/- HFHS Diet + Carnosinol

[0039] A number of whole body metabolic parameters were examined. GPx4 - HFHS mice were substantially more insulin resistant compared to WT- HFHS. FL-926-16 increased glucose tolerance by 60% and 22% in WT and GPx4+/- respectively. Fig. 7 shows the total

body weight gain throughout the duration of the study (A) and percent body fat at termination of the study (B) in all groups of mice (n=6-8). Data are mean \pm SEM, * p<0.05 vs CNTL within genotype using 2-way ANOVA followed by Dunnett's multiple comparisons test. Use of carnosinol does not impact body weight with diet-induced obesity. Fig. 8 shows whole body O_2 and CO_2 exchange demonstrating that there was no difference in whole body energy expenditure occurring with carnosinol treatment in HFHS diet fed mice. Indirect calorimetry in a metabolic cage system was used in week 14 of this study to measure whole body O_2 consumption (A), CO_2 production (B) and respiratory exchange ratio RER (C) in both WT (left panels) and GPx4^{+/-} mice, fed control (CNTL) diet, HFHS diet or HFHS + Carnosinol. N=4 mice per group. Fig. 9 shows that carnosinol improves whole body glycemic control in diet-induced obese mice. Blood glucose levels were measured following oral glucose challenge in WT (A) and GPx4^{+/-} (B) mice following HFHS diet with and without carnosinol. The area under the curve (AUC) of glucose tolerance test was calculated for each group of mice using the trapezoidal rule (C) (n = 6-8). Data shown as mean \pm SEM, * p<0.05 vs CNTL within genotype, † p<0.05 vs. WT-HFHS, using 2-way ANOVA followed by Dunnett's multiple comparisons test. Fig. 10 shows that carnosinol improves skeletal muscle insulin sensitivity in HFHS fed mice. The total uptake of 2-deoxyglucose (2-DOG) following an insulin exposure in whole, intact Soleus is shown in panel (A) and Extensor Digitorum Longus skeletal muscles prepared from the cohorts of mice in this study (B) (n = 5-6). Data shown as mean \pm SEM, * p<0.05 vs CNTL within genotype, † p<0.05 vs. HFHS only within genotype, using 2-way ANOVA followed by Dunnett's multiple comparisons test.

[0040] FL-926-16 also mitigated liver steatosis as evidenced by decreased lipid droplet accumulation in both WT and GPx4^{+/-} mice. In particular, Fig. 11 shows that carnosinol mitigates liver steatosis in HFHS fed mice, and in particular, the panels showing samples of liver that have been stained with Oil Red O, a red dye specific for triglycerides. There was an increase in number and size of red spheres in mice with a HFHS diet, and an improvement (i.e., diminished number and size) in these fat depositions with carnosinol treatment for 10 weeks. Fig. 12 shows that carnosinol mitigates cardiac fibrosis in HFHS diet-induced obese mice presenting samples of liver that have been stained with Picosirius Red, a dye that stains collagen. Fig. 13 shows that liver HNE-adducts are diminished with carnosinol treatment in HFHS diet-induced obese mice as shown in the immunoblots presenting liver GPx4 enzyme and HNE-adducts (proteins that have been covalently modified with 4-hydroxynonenal, a lipid peroxide-derived aldehyde). The decrease in HNE adduct levels in both carnosinol-treated groups of HFHS-fed mice is comparable the levels in lean control mice.

[0041] These findings provide evidence that carnosinol, a carbonyl scavenger, can be used as a therapy for cardiovascular diseases, mitochondrial dysfunction, metabolic syndrome, obesity, diabetes, diabetes-related disorders, insulin resistance, and liver disease.

[0042] The foregoing is illustrative of the present invention and is not to be construed as limited to the specific embodiments disclosed, and that modifications to the disclosed embodiments, as well as other embodiments, are intended to be included within the scope of the appended claims. The invention is defined by the following claims, with equivalents of the claims to be included therein.

That Which is Claimed is:

1. A method of preventing or treating diabetes, a diabetes-related condition, insulin sensitivity, heart disease, mitochondrial dysfunction, metabolic syndrome or liver disease comprising administering carnosinol or an analog thereof to a subject in need thereof.
2. The method of claim 1, wherein administration of carnosinol or an analog thereof enhances insulin-stimulated muscle glucose uptake.
3. The method of claim 1, wherein administration of carnosinol or an analog thereof improves glycemic control.
4. The method of claim 1, wherein the heart disease is cardiomyopathy.
5. The method of claim 1, wherein administration of carnosinol or an analog thereof prevents or treats cardiac hypertrophy.
6. The method of claim 1, wherein administration of carnosinol or an analog thereof prevents or treats cardiac fibrosis.
7. The method of claim 1, wherein administration of carnosinol or an analog thereof improves cardiac mitochondrial function or cardiac remodeling.
8. The method of claim 1, wherein the liver disease is nonalcoholic fatty liver disorder (NALD).
9. The method of claim 1, wherein the liver disease is cirrhosis.
10. The method of claim 1, wherein the liver disease is steatosis.
11. The method of claim 1, wherein the liver disease is nonalcoholic steatohepatitis (NASH).
12. The method of claim 1, wherein the carnosinol or an analog thereof is administered to the subject orally.
13. The method of claim 1, wherein the carnosinol or an analog thereof is mixed with a liquid and administered to the subject orally.

14. The method of claim 1, wherein the camosinol or an analog thereof is mixed with water, juice, milk, coffee, tea, a carbonated beverage, a non-carbonated beverage, a sports drink, a smoothie, yogurt, pudding, ice cream or sorbet.

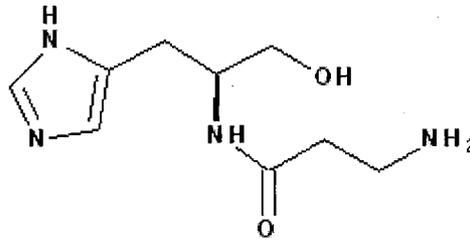
15. The method of claim 1, wherein the subject is overweight.

16. The method of claim 1, wherein the subject is obese.

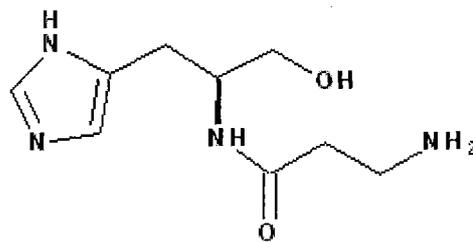
17. The method of claim 1, wherein the subject is morbidly obese.

18. The method of claim 1, wherein the subject is pre-diabetic.

19. The method of claim 1, wherein a compound having the following structure is administered to the subject:



20. The pharmaceutical or nutraceutical composition comprising a compound having the following structure:



and a physiologically acceptable carrier.

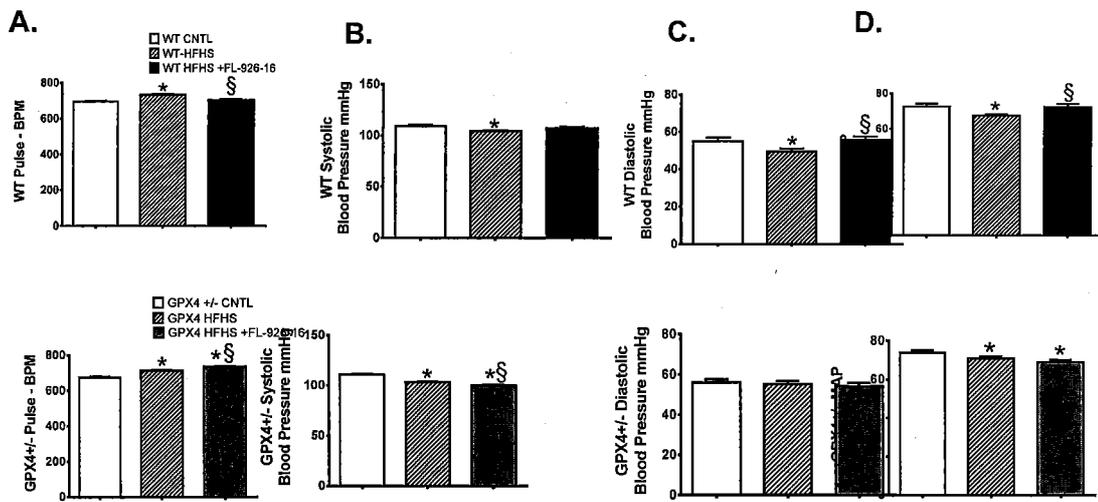
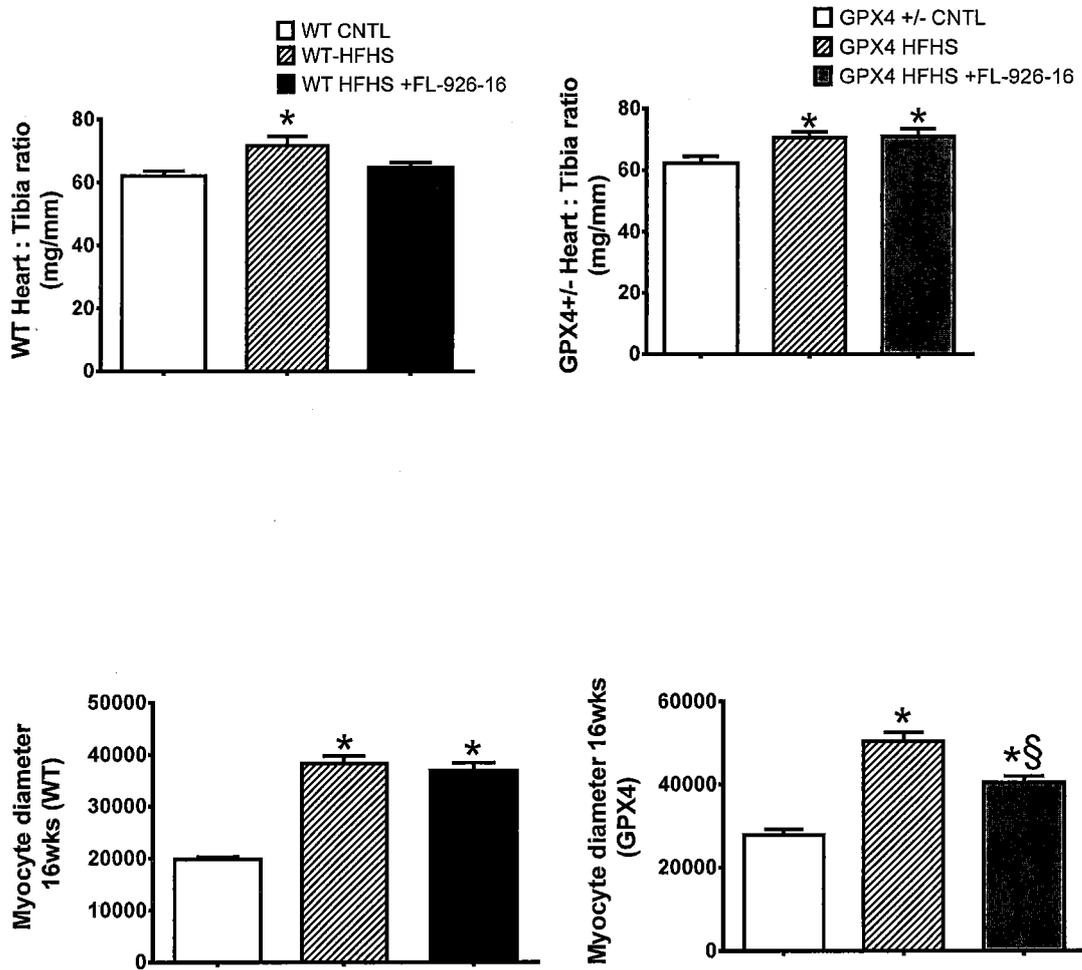


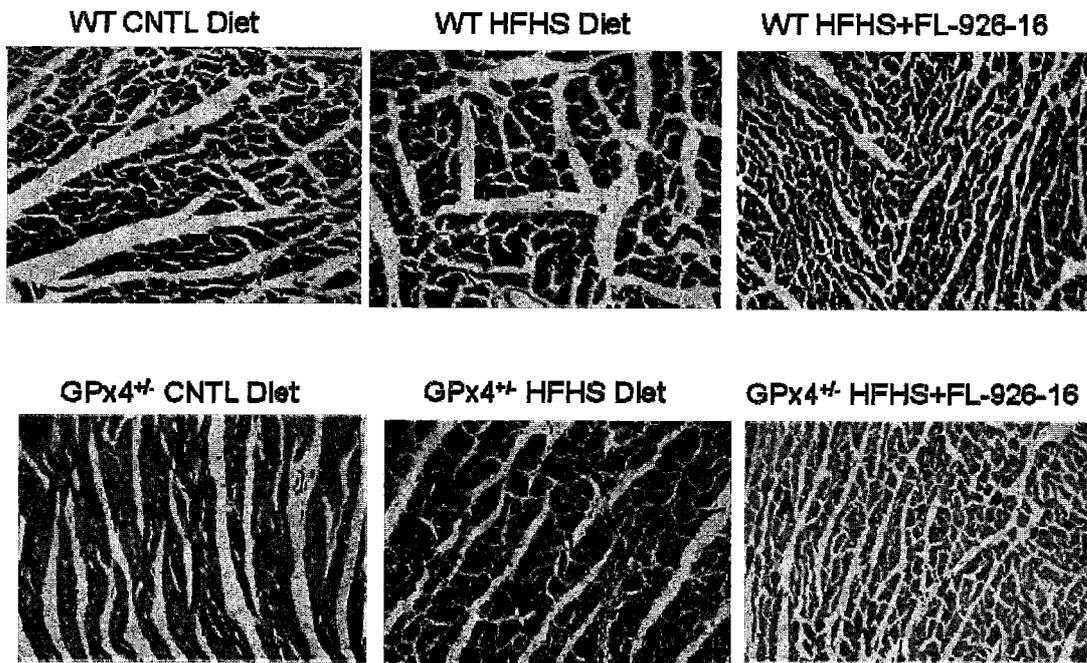
Fig. 1



Carnosinol mitigates cardiac hypertrophy in HFHS diet-induced obese mice. (A) Heart: tibia ratio (B) Cardiomyocyte diameter (n = 2). Data shown as means ± SEM, p<0.05 One way ANOVA followed by Newman-Keuls * vs CNTL ; § vs HFHS.

Fig. 2

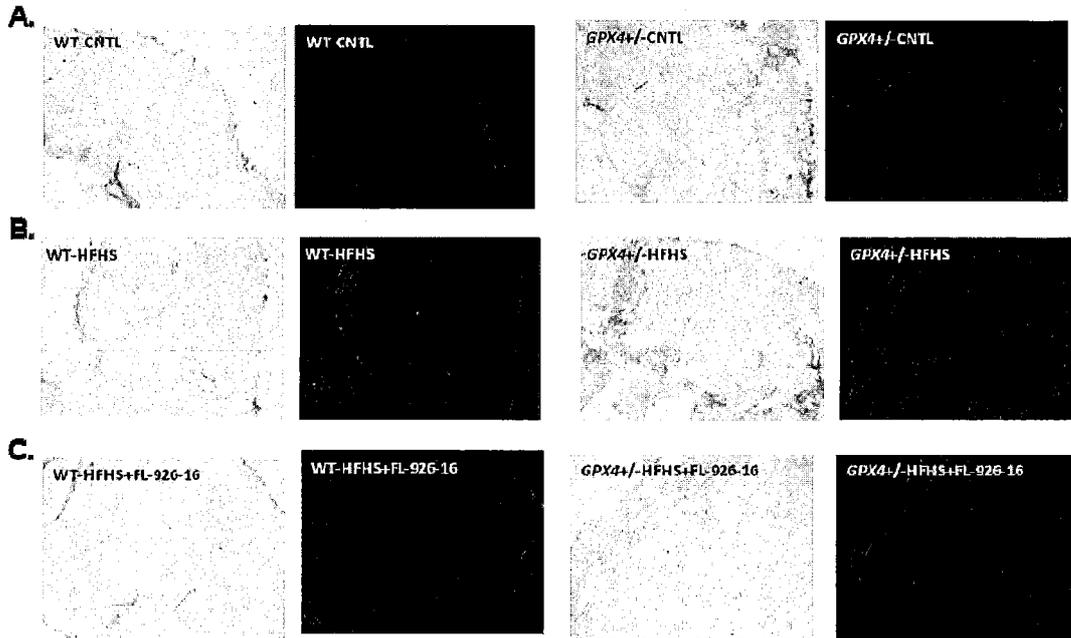
3/13



Cardiac histology (H & E staining) in mice with diet-induced obesity and treated with Carnosinol. Note the enlarged size of cells with HFHS diet, and reduced size in Carnosinol treated HFHS mice.

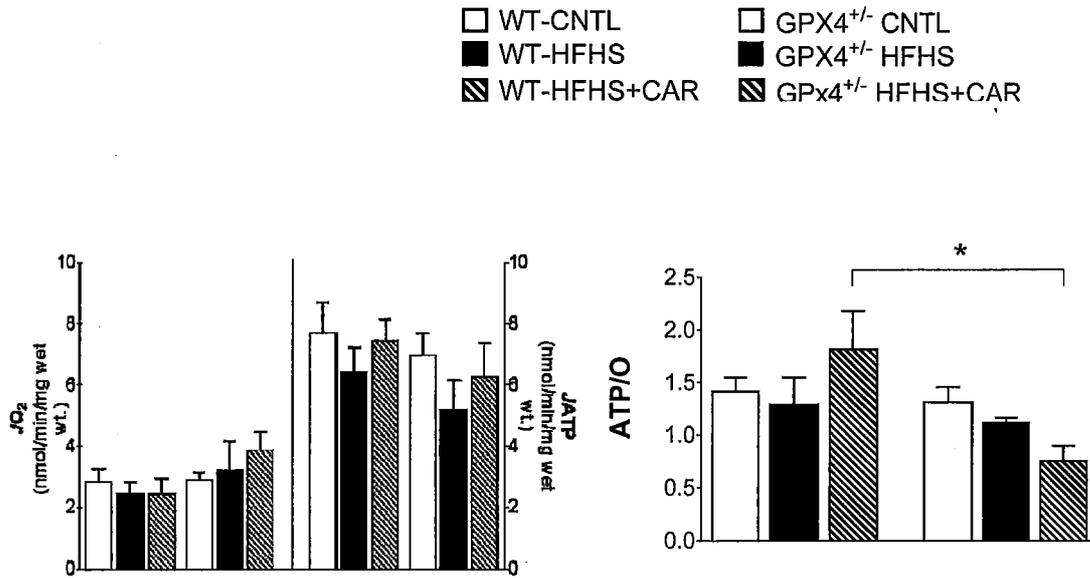
Fig. 3

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Carnosinol mitigates Cardiac Fibrosis with HFHS diet-induced obesity. in the WT and GPx4^{+/-} mice under control diet conditions (A), with HFHS diet-induced obesity (B), and with HFHS diet + Carnosinol treatment for 8 weeks (C), under bright field microscopy (left panels) and with Picosirius Red staining of collagen (fibrosis) under polarized light (right panels). Note the decreased collagen stain intensity with Carnosinol treatment in the obese groups.

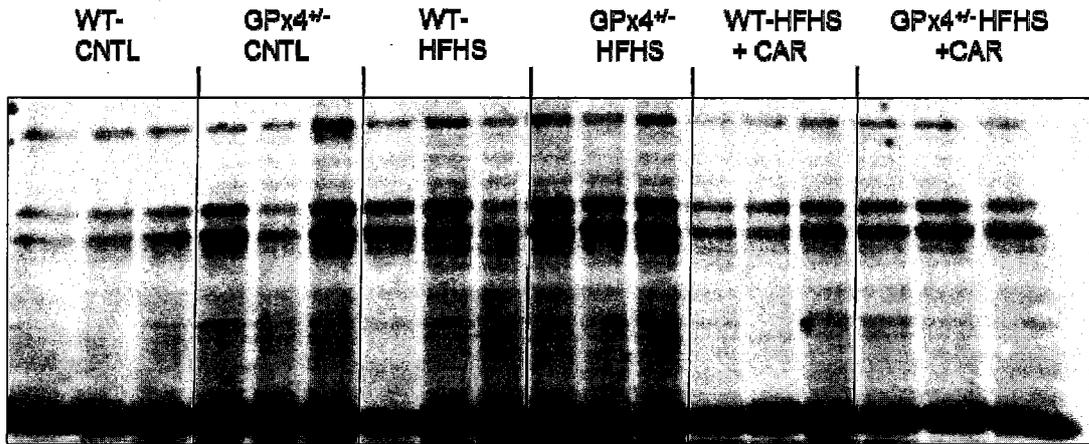
Fig. 4



Mitochondrial Function in Heart improves with Carnosinol treatment in HFHS diet-induced obese mice. Shown here in panel at left are the rates of O₂ consumption and ATP production in a preparation of permeabilized left ventricular myofibers prepared from mice in each of the 6 cohorts used in this study, under clamped submaximal phosphorylating state. In the panel at right is the ratio of ATP produced to O₂ consumed under these conditions. Data are representative of N=6-8 mice.

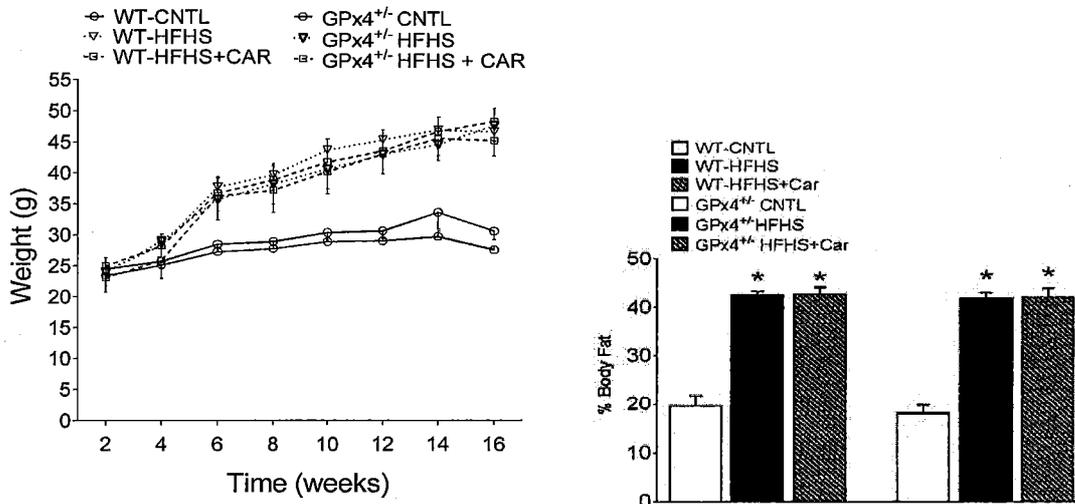
Fig. 5

6/13



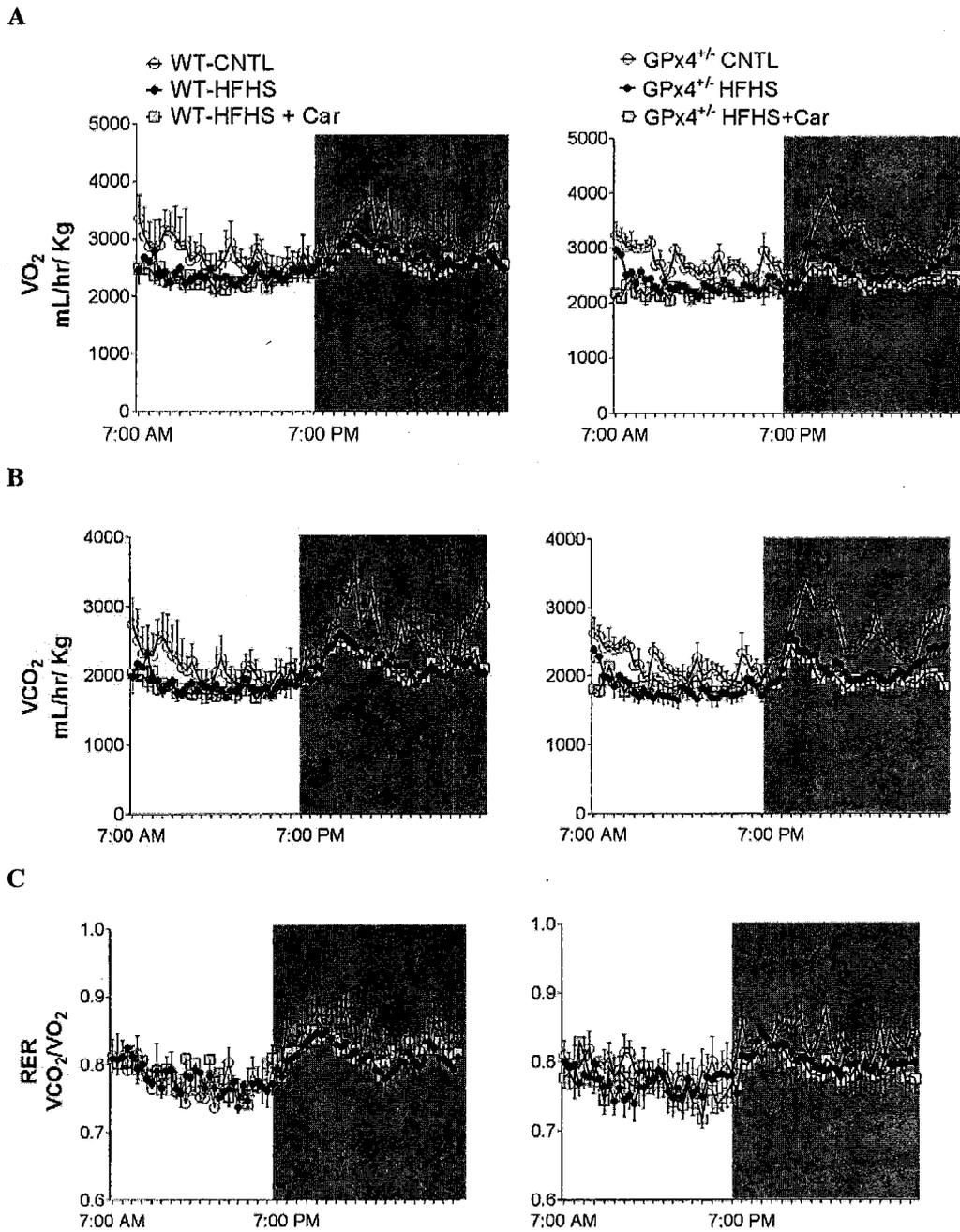
Cardiac histology (H & E staining) in mice with diet-induced obesity and treated with Carnosinol. Note the enlarged size of cells with HFHS diet, and reduced size in Carnosinol treated HFHS mice.

Fig. 6



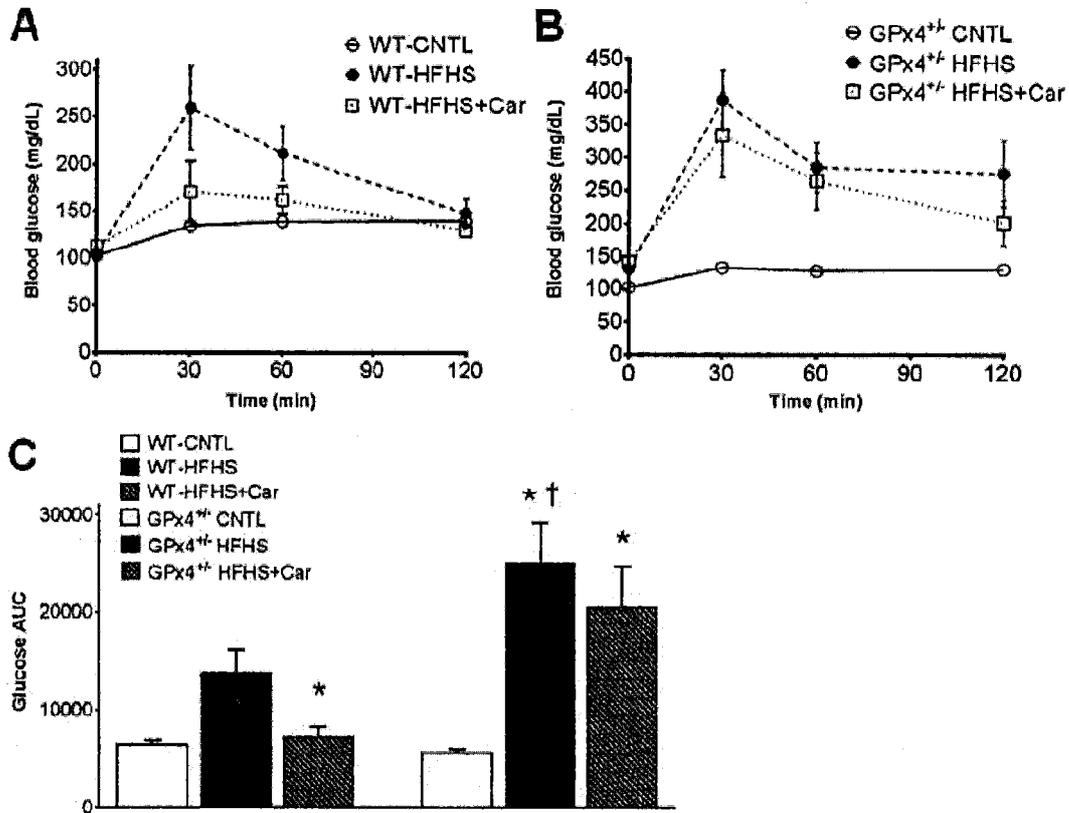
Body composition. Both total body weight gain throughout the duration of the study (A) and % body fat at termination of the study (B) in all groups of mice are shown. (n=6-8). Data are mean ± SEM, * p<0.05 vs CNTL within genotype using 2-way ANOVA followed by Dunnett's multiple comparisons test. Use of Carnosinol does not impact body weight with diet-induced obesity.

Fig. 7



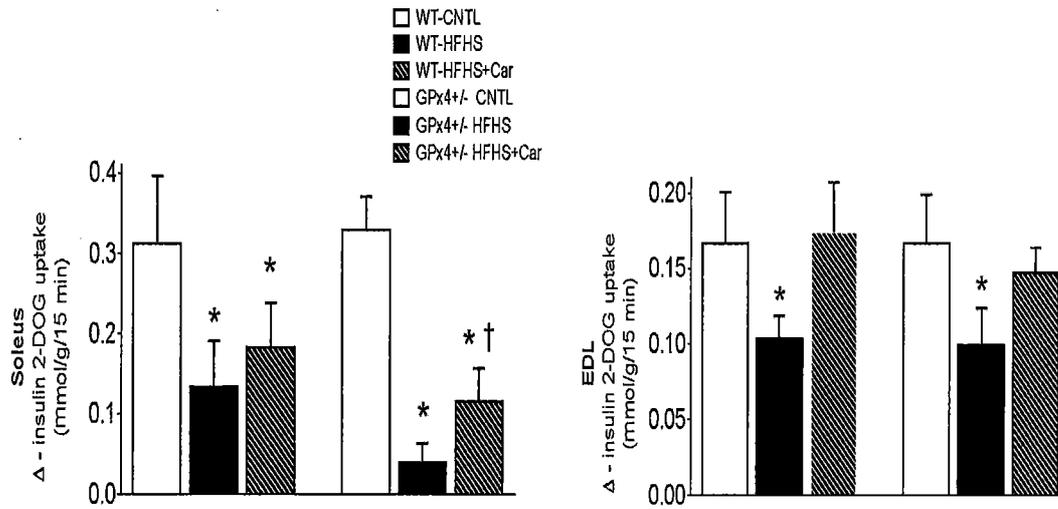
Whole body O₂ and CO₂ exchange Indirect calorimetry in metabolic cage system was used in week 14 of this study to measure whole body O₂ consumption (A), CO₂ production (B) and respiratory exchange ratio RER (C) in both WT (left panels) and GPx4^{+/-} mice, fed control (CNTL) diet, HFHS diet or HFHS + Carnosinol. N=4 mice per group. No differences in whole body energy expenditure occurs with Carnosinol treatment in HFHS diet fed mice.

Fig. 8



Carnosinol improves whole body glycemic control in diet-induced obese mice. Blood glucose levels were measured following oral glucose challenge in WT (A) and GPx4^{+/-} (B) mice following HFHS diet with and without Carnosinol. The area under the curve (AUC) of glucose tolerance test was calculated for each group of mice using the trapezoidal rule (C). (n = 6-8). Data shown as mean ± SEM, * p<0.05 vs CNTL within genotype, † p<0.05 vs. WT-HFHS, using 2-way ANOVA followed by Dunnett's multiple comparisons test.

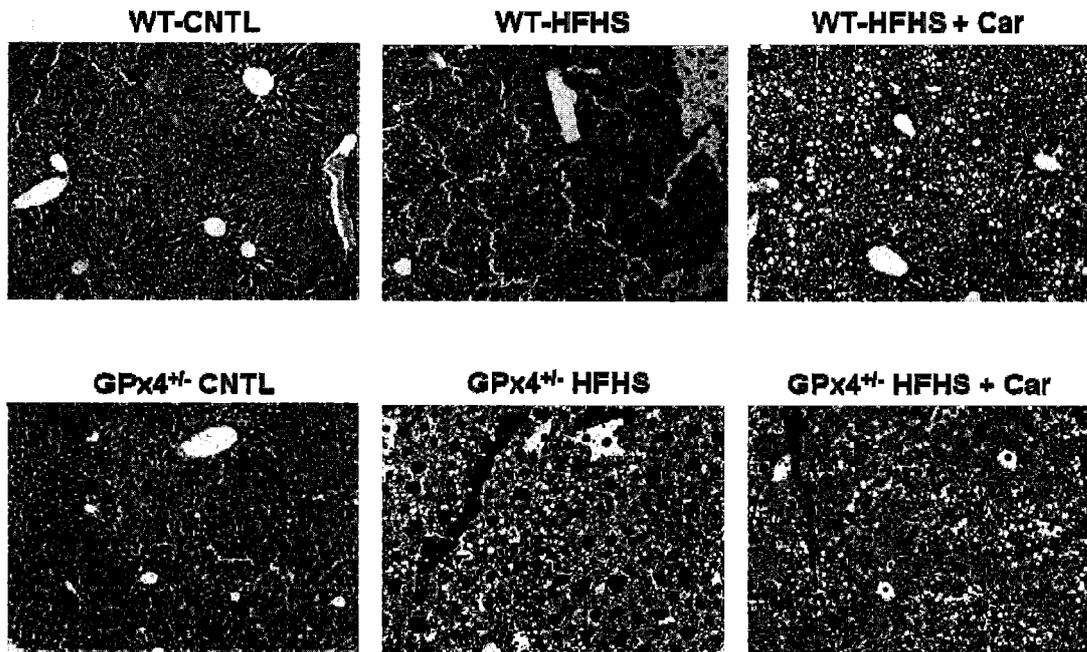
Fig. 9



Carnosinol improves skeletal muscle insulin sensitivity in HFHS fed mice. Shown above is the total uptake of 2-deoxyglucose (2-DOG) following an insulin exposure in whole, intact Soleus (A) and Extensor Digitorum Longus skeletal muscles prepared from the cohorts of mice in this study. (n = 5-6). Data shown as mean ± SEM, * p<0.05 vs CNTL within genotype, † p<0.05 vs. HFHS only within genotype, using 2-way ANOVA followed by Dunnett's multiple comparisons test.

Fig. 10

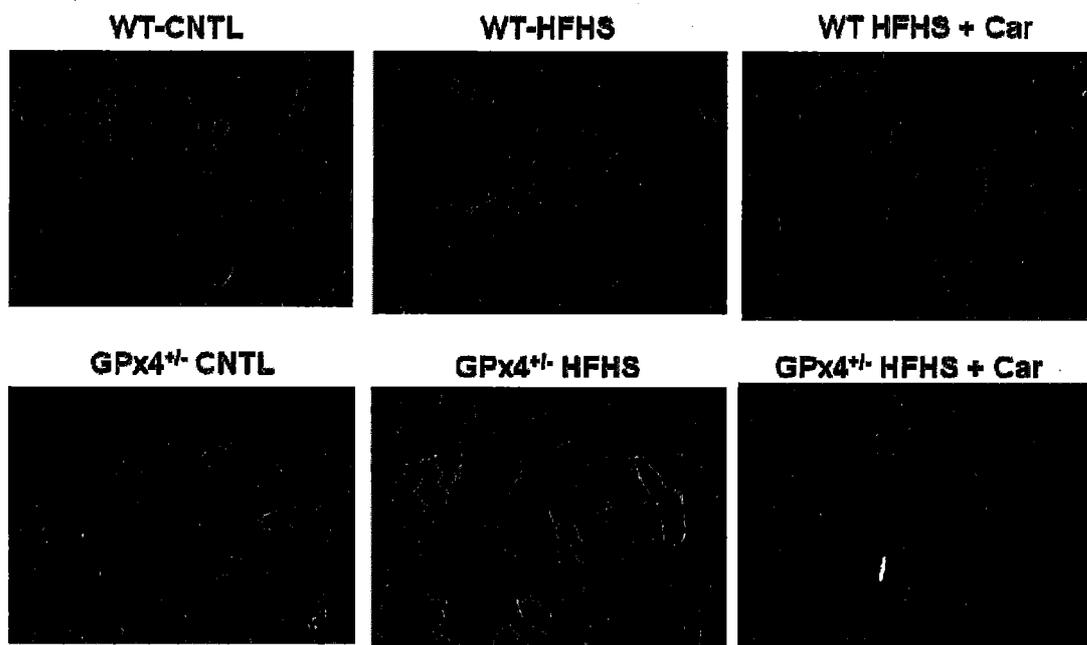
11/13



Carnosinol mitigates Liver Steatosis in HFHS fed mice. The panels above are showing samples of liver that have been stained with Oil Red O, a red dye specific for triglycerides. Note the large increase in number and size of red spheres in mice with HFHS diet, and the improvement (i.e., diminished number and size) in these fat depositions with Carnosinol treatment for 10 weeks.

Fig. 11

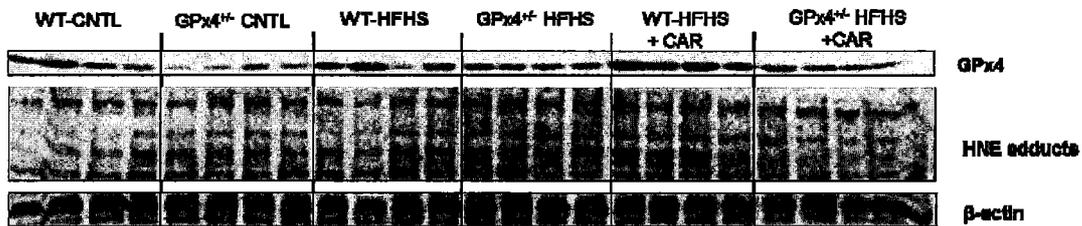
12/13



Liver Fibrosis. The panels above are showing samples of liver that have been stained with Picosirius Red, a dye that stains Collagen.

Fig. 12

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Liver HNE-adducts are diminished with Carnosinol treatment in HFHS diet-induced obese mice. Shown in the immunoblots above liver GPx4 enzyme, and HNE-adducts (proteins that have been covalently modified with 4-hydroxynonenal, a lipid peroxide-derived aldehyde). Note the decrease in HNE adduct levels in both carnosinol-treated groups of HFHS-fed mice to levels comparable to lean control mice.

Fig. 13

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 15/34833

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/417; A61K 31/133; A61P 3/00 (2015.01)
CPC - A61K 31/417; A61K 31/133; A61K 38/05
 According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
 Minimum documentation searched (classification system followed by classification symbols)
 IPC(8): A61K 31/417; A61K 31/133; A61P 3/00 (2015.01)
 CPC: A61K 31/417; A61K 31/133; A61K 38/05

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
 USPC: 514/21 .91 ; 514/399; 514/626 (key Word limited; see search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
 PatBase, Google Patents, Google Scholar
 Search terms used: carnosinol, carnosine, reduction/reduced, derivative, diabetes, metabolic disorder, obesity

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 201 1/080139 A 1 (Negrisoli et al.) 07 July 201 1 (07.07.201 1); pg 2 ln 27-pg 3 ln 15, pg 8 ln 8-14, pg 10 ln 9-10	1, 19-20 -----
Y		2-18
Y	WO 2012/047800 A 1 (Ivy et al.) 12 April 2012 (12.04.2012); para [0006], [0046]	2
Y	US 2009/00761 11 A 1 (Lee et al.) 19 March 2009 (19.03.2009); para [0027]	3, 18
Y	WO 2009/033755 A 2 (Bevec et al.) 19 March 2009 (19.03.2009); pg 2 ln 8-12, pg 10 ln 36-pg 11 ln 5, pg 30 ln 29-39	4-6
Y	US 201 1/0195977 A 1 (Fancelli et al.) 11 August 201 1 (11.08.201 1); (para [0063], para [0080], para [0312], para [0314]	7
Y	US 2006/0100189 A 1 (Gurtner et al.) 11 May 2006 (11.05.2006); para [0016], [0059]	8-1 1
Y	WO 2008/037740 A 2 (Bernasconi et al.) 03 April 2008 (03.04.2008); pg 2 ln 31-32, pg 3 ln 31-32	12-14
Y	US 2012/0177730 A 1 (Baron et al.) 12 July 2012 (12.07.2012); para [0391], [0462], [1169]	15-17

Further documents are listed in the continuation of Box C.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 08 August 2015 (08.08.2015)	Date of mailing of the international search report 0 2 SEP 2015
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Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774
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