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(71) Applicant(s)
University of Iowa Research Foundation;THE UNITED STATES OF AMERICA as represented by THE SECRETARY OF STATE OF THE DEPARTMENT OF VETERANS AFFAIRS

(72) Inventor(s)
Adams, Christopher M.;Kunkel, Steven D.;Welsh, Michael

(74) Agent / Attorney
Griffith Hack, GPO Box 1285, Melbourne, VIC, 3001, AU

(56) Related Art
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(71) Applicant *(for all designated States except US)*: **UNIVERSITY OF IOWA RESEARCH FOUNDATION** [US/US]; Iowa Centers For Enterprise, 2660 University Capitol Centre, Iowa City, IA 5242-5500 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ADAMS, Christopher, M.** [US/US]; 726 N. Van Buren, Iowa City, IA 52245 (US). **KUNKEL, Steven, D.** [US/US]; 1406 Oaklawn Avenue, Iowa City, IA 52245 (US). **WELSH, Michael** [US/US]; 3460 560th Street, Riverside, IA 52327 (US).

(74) Agent: **SHORTELLL, Brian D.**; BALLARD SPAHR LLP, 999 Peachtree Street, Suite 1000, Atlanta, GA 30309 (US).

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(54) **Title**: METHODS OF INHIBITING MUSCLE ATROPHY

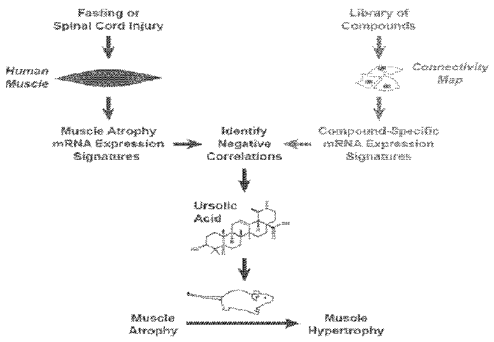

Figure 1

(57) **Abstract**: In one aspect, the invention relates to methods for treating muscle atrophy by providing to an animal in need thereof an effective amount of a compound. The compound can modulate the expression levels of multiple mRNA of a muscle atrophy signature. This abstract is intended as a scanning tool for purposes of searching in the particular art and is not intended to be limiting of the present invention. The disclosed compounds can treat muscle atrophy when administered in an effective amount to an animal, such as a mammal, fish or bird. For example, human. Also disclosed in a method of lowering blood glucose in an animal by administering ursolic acid or ursolic acid analogs, such as betulininc acid analogs, and narigenin analogs, such as naringenin, in an effective amount to an animal. Also disclosed in a method of lowering blood glucose in an animal by administering ungerine/hippeastrine analogs, such as hippeastrine, in an effective amount to an animal. The disclosed compounds can also promote muscle health, promote normal muscle function, and/or promote healthy aging muscles by providing to a subject in need thereof an effective amount of a disclosed compound.

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METHODS FOR INHIBITING MUSCLE ATROPHY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This Application claims the benefit of U.S. Provisional Application No. 61/493,969, filed on June 6, 2011; which is incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with government support under grant VA Career Development Award-2 to Christopher M. Adams; support from a VA Research Enhancement Award Program to Steven D. Kunkel; and grant IBX000976A awarded by the Department of Veterans Affairs Biomedical Laboratory Research & Development Service and grant 1R01AR059115-01 awarded by the National Institutes of Health and National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIH/NIAMS). The United States government has certain rights in the invention.

BACKGROUND

[0003] Skeletal muscle atrophy is characteristic of starvation and a common effect of aging. It is also a nearly universal consequence of severe human illnesses, including cancer, chronic renal failure, congestive heart failure, chronic respiratory disease, insulin deficiency, acute critical illness, chronic infections such as HIV/AIDS, muscle denervation, and many other medical and surgical conditions that limit muscle use. However, medical therapies to prevent or reverse skeletal muscle atrophy in human patients do not exist. As a result, millions of individuals suffer sequelae of muscle atrophy, including weakness, falls, fractures, opportunistic respiratory infections, and loss of independence. The burden that skeletal muscle atrophy places on individuals, their families, and society in general, is tremendous.

[0004] The pathogenesis of skeletal muscle atrophy is not well understood. Nevertheless, important advances have been made. For example, it has been described previously that insulin/IGF1 signaling promotes muscle hypertrophy and inhibits muscle atrophy, but is reduced by atrophy-inducing stresses such as fasting or muscle denervation (Bodine SC, *et al.* (2001) *Nat Cell Biol* 3(11):1014-1019; Sandri M, *et al.* (2004) *Cell* 117(3):399-412l; Stitt TN, *et al.* (2004) *Mol Cell* 14(3):395-403; Hu Z, *et al.* (2009) *The Journal of clinical investigation* 119(10):3059-3069; Dobrowolny G, *et al.* (2005) *The*

Journal of cell biology 168(2):193-199; Kandarian SC & Jackman RW (2006) *Muscle & nerve* 33(2):155-165; Hirose M, *et al.* (2001) *Metabolism: clinical and experimental* 50(2):216-222; Pallafacchina G, *et al.* (2002) *Proceedings of the National Academy of Sciences of the United States of America* 99(14):9213-9218). The hypertrophic and anti-atrophic effects of insulin/IGF1 signaling are mediated at least in part through increased activity of phosphoinositide 3-kinase (PI3K) and its downstream effectors, including Akt and mammalian target of rapamycin complex 1 (mTORCl) Sandri M (2008) *Physiology (Bethesda)* 23:160-170; Glass DJ (2005) *The international journal of biochemistry & cell biology* 37(10):1974-1984).

[0005] Another important advance came from microarray studies of atrophying rodent muscle (Lecker SH, *et al.* (2004) *Faseb J* 18(1):39-51; Sacheck JM, *et al.* (2007) *Faseb J* 21(1):140-155; Jagoe RT, *et al. Faseb J* 16(13):1697-1712). Those studies showed that several seemingly disparate atrophy-inducing stresses (including fasting, muscle denervation and severe systemic illness) generated many common changes in skeletal muscle mRNA expression. Some of those atrophy-associated changes promote muscle atrophy in mice; these include induction of the mRNAs encoding atroginI/MAFbx and MuRF1 (two E3 ubiquitin ligases that catalyze proteolytic events), and repression of the mRNA encoding PGC-l α (a transcriptional co-activator that inhibits muscle atrophy) (Sandri M, *et al.* (2006) *Proceedings of the National Academy of Sciences of the United States of America* 103(44):16260-16265; Wenz T, *et al. Proceedings of the National Academy of Sciences of the United States of America* 106(48):20405-20410; Bodine SC, *et al.* (2001) *Science (New York, N.Y* 294(5547):1704-1708; Lagirand-Cantaloube J, *et al.* (2008) *The EMBO journal* 27(8):1266-1276; Cohen S, *et al.* (2009) *The Journal of cell biology* 185(6):1083-1095; Adams V, *et al.* (2008) *Journal of molecular biology* 384(1):48-59). However, the roles of many other mRNAs that are increased or decreased in atrophying rodent muscle are not yet defined. Data on the mechanisms of human muscle atrophy are even more limited, although atrogin-l and MuRF1 are likely to be involved (Leger B, *et al.* (2006) *Faseb J* 20(3):583-585; Doucet M, *et al.* (2007) *American journal of respiratory and critical care medicine* 176(3):261-269; Levine S, *et al.* (2008) *The New England journal of medicine* 358(13):1327-1335).

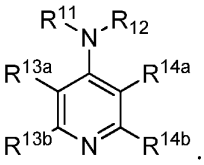
[0006] Despite advances in understanding the physiology and pathophysiology of muscle atrophy, there is still a scarcity of compounds that are both potent, efficacious, and selective modulators of muscle growth and also effective in the treatment of muscle atrophy associated and diseases in which the muscle atrophy or the need to increase muscle mass is involved.

These needs and other needs are satisfied by the present invention.

SUMMARY

[0007] In accordance with the purpose(s) of the invention, as embodied and broadly described herein, the invention, in one aspect, relates to compounds useful in methods to treat muscle atrophy. The compounds can be selected from a tacrine and analogs, naringenin and analogs, allantoin and analogs, conessine and analogs, tomatidine and analogs, ungerine/hippeastrine and analogs, and betulinic acid and analogs, or a mixture thereof.

[0008] Tacrine and analogs can have the structure:



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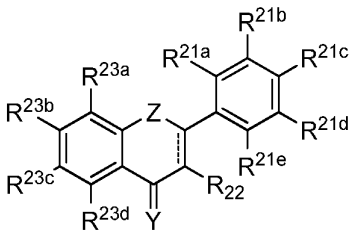
[0009] Naringenin and analogs can have the structure:



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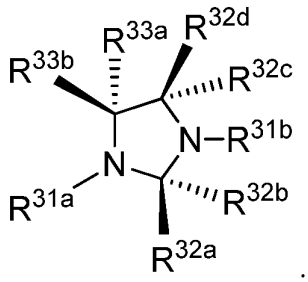
[0010] Allantoin and analogs can have the structure:



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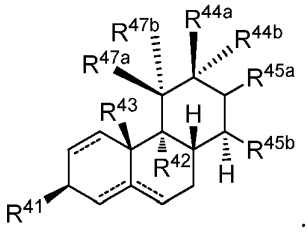
[0011] Conessine and analogs can have the structure:



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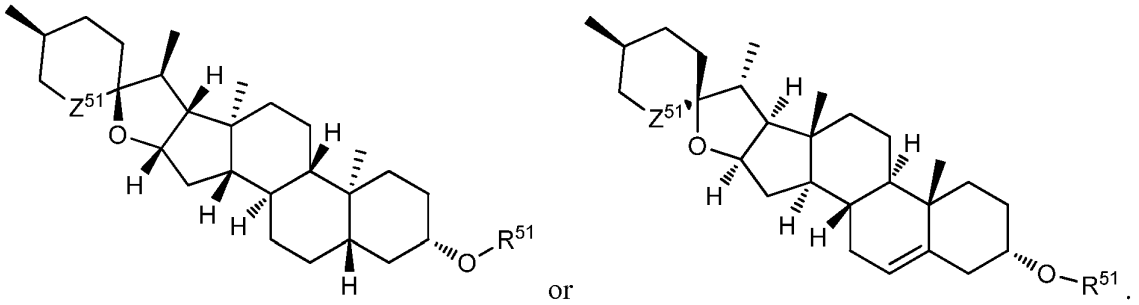
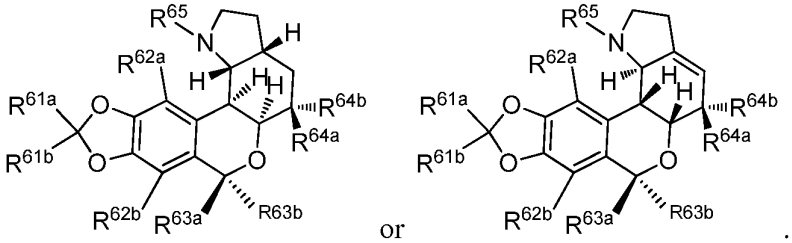
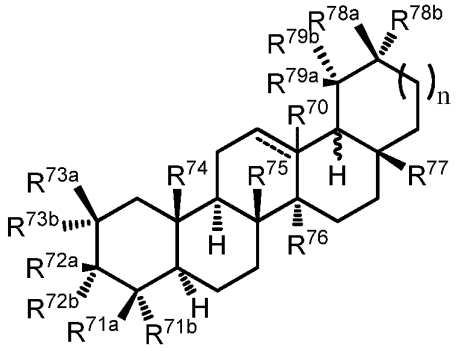
[0012] Tomatidine and analogs can have the structure:



[0013] Ungerine/hippeastrine and analogs can have the structure:



[0014] Betulinic acid and analogs can have the structure:



[0015] The disclosed compounds can treat muscle atrophy when administered in an effective amount to an animal, such as a mammal, fish or bird. For example, human.

[0016] Also disclosed in a method of lowering blood glucose in an animal by administering ursolic acid or ursolic acid analogs, such as betulininc acid analogs, and narigenin analogs, such as naringenin, in an effective amount to an animal.

[0017] Also disclosed in a method of lowering blood glucose in an animal by administering ungerine/hippeastrine analogs, such as hippeastrine, in an effective amount to an animal.

[0018] The disclosed compounds can also promote muscle health, promote normal muscle function, and/or promote healthy aging muscles by providing to a subject in need thereof an effective amount of a disclosed compound.

[0019] Also disclosed herein are pharmaceutical compositions comprising compounds used in the methods. Also disclosed herein are kits comprising compounds used in the

methods.

[0020] In further aspects, In a further aspect, the invention relates to compounds identified using muscle atrophy signature-1, muscle atrophy signature-2 or both muscle atrophy signatures. In still further aspects, the purpose(s) of the invention, as embodied and broadly described herein, the invention, in one aspect, relates to compounds useful in methods to modulate muscle health promote normal muscle function, and/or promote healthy aging muscles, methods to inhibit muscle atrophy, methods to increase insulin/IGF-I signaling, methods to reduce body fat; methods to reduce blood glucose, methods to reduce blood triglycerides, methods to reduce blood cholesterol, methods to reduce obesity, methods to reduce fatty liver disease, and methods to reduce diabetes, and pharmaceutical compositions comprising compounds used in the methods.

[0021] Disclosed are methods for treating muscle atrophy in a mammal, the method comprising administering to the mammal an effective amount of a compound, wherein the compound: (a) down regulates multiple induced mRNAs of a Muscle Atrophy Signature, compared to expression levels of the induced mRNAs of the Muscle Atrophy Signature in the same type of the muscle cell in the absence of the compound, and/or (b) up regulates multiple repressed mRNAs of the Muscle Atrophy Signature, compared to expression levels of the repressed mRNAs of the Muscle Atrophy Signature in the same type of the muscle cell in the absence of the compound, thereby inhibiting muscle atrophy in the mammal.

[0022] Also disclosed are methods for identifying a compound that inhibits muscle atrophy when administered in a effective amount to a animal in need of treatment thereof, the method comprising the steps of:(i) selecting a candidate compound; (ii) determining the effect of the candidate compound on a cell's expression levels of a plurality of induced mRNAs and/or repressed mRNAs of a Muscle Atrophy Signature, wherein the candidate compound is identified as suitable for muscle atrophy inhibition if: (a) more than one of the induced mRNAs of the Muscle Atrophy Signature are down regulated, compared to expression levels of the induced mRNAs of the Muscle Atrophy Signature in the same type of cell in the absence of the candidate compound; and/or (b) more than one of the repressed mRNAs of the Muscle Atrophy Signature are up regulated, compared to expression levels of the repressed mRNAs of the Muscle Atrophy Signature in the same type of cell in the absence of the candidate compound. In one aspect, the method further comprises administering the candidate compound to an animal. The candidate compound can be tacrine and analogs, naringenin and analogs, allantoin and analogs, conessine and analogs, tomatidine and analogs, ungerine/hippeastrine and analogs, and betulinic acid and analogs, or

a mixture thereof.

[0023] Also disclosed are methods for manufacturing a medicament associated with muscle atrophy or the need to promote muscle health, promote normal muscle function, and/or promote healthy aging muscles comprising combining at least one disclosed compound or at least one disclosed product with a pharmaceutically acceptable carrier or diluent.

[0024] Also disclosed are uses of a disclosed compound or a disclosed product in the manufacture of a medicament for the treatment of a disorder associated with muscle atrophy or the need to promote muscle health, promote normal muscle function, and/or promote healthy aging muscles.

[0025] While aspects of the present invention can be described and claimed in a particular statutory class, such as the system statutory class, this is for convenience only and one of skill in the art will understand that each aspect of the present invention can be described and claimed in any statutory class. Unless otherwise expressly stated, it is in no way intended that any method or aspect set forth herein be construed as requiring that its steps be performed in a specific order. Accordingly, where a method claim does not specifically state in the claims or descriptions that the steps are to be limited to a specific order, it is no way intended that an order be inferred, in any respect. This holds for any possible non-express basis for interpretation, including matters of logic with respect to arrangement of steps or operational flow, plain meaning derived from grammatical organization or punctuation, or the number or type of aspects described in the specification.

BRIEF DESCRIPTION OF THE FIGURES

[0026] The accompanying figures, which are incorporated in and constitute a part of this specification, illustrate several aspects and together with the description serve to explain the principles of the invention.

[0027] **Figure 1** shows a schematic overview of the discovery process leading to a pharmacological compound that promotes skeletal muscle growth and inhibits skeletal muscle atrophy.

[0028] **Figure 2** shows human muscle atrophy signature-1.

[0029] **Figure 3** shows human muscle atrophy signature-2.

[0030] **Figure 4** shows representative data on the effect of fasting on skeletal muscle mRNA expression in healthy human adults.

[0031] **Figure 5** shows qPCR analysis of representative fasting-responsive mRNAs from human skeletal muscle.

[0032] **Figure 6** shows representative data on the identification of ursolic acid as an inhibitor of fasting-induced skeletal muscle atrophy.

[0033] **Figure 7** shows representative data on the identification of ursolic acid as an inhibitor of denervation-induced muscle atrophy.

[0034] **Figure 8** shows representative data on ursolic acid-mediated induction of muscle hypertrophy.

[0035] **Figure 9** shows representative data on the effect of ursolic acid on mouse skeletal muscle specific tetanic force.

[0036] **Figure 10** shows representative data on the effect of ursolic acid on muscle growth, atrophic gene expression, trophic gene expression, and skeletal muscle IGF-I signaling.

[0037] **Figure 11** shows representative data on the effect of ursolic acid on skeletal muscle expression of IGF1 gene exons, adipose IGF1 mRNA expression, and skeletal muscle insulin signaling.

[0038] **Figure 12** shows representative data on the effect of ursolic acid on adiposity and plasma lipids.

[0039] **Figure 13** shows representative data on the effect of ursolic acid on food consumption, liver weight, kidney weight, and plasma ALT, bilirubin, and creatinine concentrations.

[0040] **Figure 14** shows representative data on the effect of ursolic acid on weight gain, white adipose tissue weight, skeletal muscle weight, brown adipose tissue weight and energy expenditure in a mouse model of obesity and metabolic syndrome.

[0041] **Figure 15** shows representative data on the effect of ursolic acid on obesity-related pre-diabetes, diabetes, fatty liver disease and hyperlipidemia in a mouse model of obesity and metabolic syndrome.

[0042] **Figure 16** shows representative data that oleanolic acid and metformin do not reduce skeletal muscle atrophy.

[0043] **Figure 17** shows representative data that targeted inhibition of PTP1B does not inhibit skeletal muscle atrophy.

[0044] **Figure 18** shows representative data on the effect of ursolic acid serum concentration on muscle mass and adiposity.

[0045] **Figure 19** shows that betulinic acid, like ursolic acid, reduces immobilization-induced skeletal muscle atrophy. Mice were administered vehicle (corn oil) or the indicated concentration of ursolic acid (A) or betulinic acid (B) via intraperitoneal injection twice a day

for two days. One tibialis anterior (TA) muscle was immobilized with a surgical staple, leaving the contralateral mobile TA as an intrasubject control. Vehicle, or the same dose of ursolic acid or betulinic acid was administered via i.p. injection twice daily for six days before comparing weights of the immobile and mobile TAs. Data are means ± SEM from 9-10 mice per condition. A, ursolic acid dose-response relationship. B, betulinic acid dose-response relationship.

[0046] **Figure 20** shows that naringenin reduces immobilization-induced skeletal muscle atrophy. Mice were administered vehicle (corn oil), ursolic acid (200 mg/kg), naringenin (200 mg/kg) or ursolic acid plus naringenin (both at 200 mg/kg) via intraperitoneal injection twice a day for two days. One tibialis anterior (TA) muscle was immobilized with a surgical staple, leaving the contralateral mobile TA as an intrasubject control. Vehicle, or the same dose of ursolic acid and/or naringenin was administered via i.p. injection twice daily for six days before comparing weights of the immobile and mobile TAs. Data are means ± SEM from 9-10 mice per condition.

[0047] **Figure 21** shows that the combination of ursolic acid and naringenin normalizes fasting blood glucose levels in a mouse model of glucose intolerance, obesity and fatty liver disease. Mice were fed standard chow, high fat diet (HFD) plus the indicated concentrations of naringenin, or HFD containing 0.15% ursolic acid (UA) plus the indicated concentrations of naringenin for 5 weeks before measurement of fasting blood glucose (A), total body weight (B), fat mass by NMR (C), liver weight (D), grip strength (E) and skeletal muscle weight (bilateral tibialis anterior, gastrcocnemius, soleus, quadriceps and triceps muscle; F). Dashed line indicates levels in control mice that were fed standard chow. Open symbols indicate levels in mice fed HFD containing the indicated concentrations of naringenin. Closed symbols indicate levels in mice fed HFD containing 0.15% UA plus the indicated concentrations of naringenin. Data are means ± SEM from ≥ 12 mice per condition.

[0048] **Figure 22** shows that tomatidine reduces immobilization-induced muscle atrophy. Mice were administered vehicle (corn oil) or the indicated concentration of tomatidine via intraperitoneal injection twice a day for two days. One tibialis anterior (TA) muscle was immobilized with a surgical staple, leaving the contralateral mobile TA as an intrasubject control. Vehicle, or the same dose of tomatidine was administered via i.p. injection twice daily for six days before comparing weights of the immobile and mobile TAs. Data are means ± SEM from 9-10 mice per condition. A, effects of 50, 100 and 200 mg/kg tomatidine. B, effects of 5, 15 and 50 mg/kg tomatidine.

[0049] **Figure 23** shows that tomatidine reduces fasting-induced muscle atrophy. Data

are means ± SEM from 9-12 mice per condition. Food was withdrawn from mice, and then vehicle (corn oil), or the indicated concentrations of ursolic acid or tomatidine, were administered by i.p. injection. Twelve hours later, mice received another i.p. injection of vehicle or the same dose of ursolic acid or tomatidine. Twelve hours later, skeletal muscles (bilateral tibialis anterior, gastrcocnemius, soleus, quadriceps muscles) were harvested and weighed. A, comparison of 200 mg/kg ursolic acid and 50 mg/kg tomatidine. B, effects of 5, 15 and 50 mg/kg tomatidine.

[0050] **Figure 24** shows that allantoin, tacrine, ungerine, hippeastrine and conessine reduce fasting-induced muscle atrophy. Food was withdrawn from mice, and then vehicle or the indicated dose of ursolic acid, tomatidine, allantoin, tacrine, ungerine, hippeastrine or conessine was administered by i.p. injection. Twelve hours later, mice received another i.p. injection of vehicle or the same dose of ursolic acid, tomatidine, allantoin, tacrine, ungerine, hippeastrine or conessine. Twelve hours later, skeletal muscles (bilateral tibialis anterior, gastrcocnemius and soleus muscles) were harvested and weighed. Data are means ± SEM from ≥ 9 mice per condition and show the percent change in skeletal muscle weight relative to vehicle-treated animals in the same experiment. The vehicle for ursolic acid, tomatidine, ungerine, hippeastrine and conessine was corn oil. The vehicle for tacrine and allantoin was saline.

[0051] **Figure 25** shows that hippeastrine and conessine reduce fasting blood glucose. Food was withdrawn from mice, and then vehicle or the indicated dose of hippeastrine or conessine was administered by i.p. injection. Twelve hours later, mice received another i.p. injection of vehicle or the same dose of hippeastrine or conessine. Twelve hours later, blood glucose was measured via tail vein. Data are means ± SEM from ≥ 9 mice per condition.

[0052] Additional advantages of the invention will be set forth in part in the description which follows, and in part will be obvious from the description, or can be learned by practice of the invention. The advantages of the invention will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

DESCRIPTION

[0053] The present invention can be understood more readily by reference to the following detailed description of the invention and the Examples included therein.

[0054] Before the present compounds, compositions, articles, systems, devices, and/or methods are disclosed and described, it is to be understood that they are not limited to

specific synthetic methods unless otherwise specified, or to particular reagents unless otherwise specified, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only and is not intended to be limiting. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, example methods and materials are now described.

[0055] All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided herein can be different from the actual publication dates, which can require independent confirmation.

A. DEFINITIONS

[0056] As used herein, nomenclature for compounds, including organic compounds, can be given using common names, IUPAC, IUBMB, or CAS recommendations for nomenclature. When one or more stereochemical features are present, Cahn-Ingold-Prelog rules for stereochemistry can be employed to designate stereochemical priority, *E/Z* specification, and the like. One of skill in the art can readily ascertain the structure of a compound if given a name, either by systemic reduction of the compound structure using naming conventions, or by commercially available software, such as CHEMDRAW™ (Cambridgesoft Corporation, U.S.A.).

[0057] As used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a functional group," "an alkyl," or "a residue" includes mixtures of two or more such functional groups, alkyls, or residues, and the like.

[0058] Ranges can be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, a further aspect includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms a further aspect. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint. It is also understood that there are a number of values disclosed herein, and that each value is also herein disclosed as "about" that particular value in addition to the

value itself. For example, if the value "10" is disclosed, then "about 10" is also disclosed. It is also understood that each unit between two particular units are also disclosed. For example, if 10 and 15 are disclosed, then 11, 12, 13, and 14 are also disclosed.

[0059] References in the specification and concluding claims to parts by weight of a particular element or component in a composition denotes the weight relationship between the element or component and any other elements or components in the composition or article for which a part by weight is expressed. Thus, in a compound containing 2 parts by weight of component X and 5 parts by weight component Y, X and Y are present at a weight ratio of 2:5, and are present in such ratio regardless of whether additional components are contained in the compound.

[0060] A weight percent (wt. %) of a component, unless specifically stated to the contrary, is based on the total weight of the formulation or composition in which the component is included.

[0061] As used herein, the terms "optional" or "optionally" means that the subsequently described event or circumstance can or can not occur, and that the description includes instances where said event or circumstance occurs and instances where it does not.

[0062] As used herein, the term "muscle atrophy signature-1" refers to the set of mRNAs with an altered expression pattern associated with muscle atrophy. The mRNAs comprise mRNAs that are either induced or repressed during the pathophysiology of muscle atrophy and which were identified using the methods described herein. For clarity, muscle atrophy signature-1 comprise the induced and repressed mRNAs described in Figure 2.

[0063] As used herein, the term "muscle atrophy signature-2" refers to the set of mRNAs with an altered expression pattern associated with muscle atrophy. The mRNAs comprise mRNAs that are either induced or repressed during the pathophysiology of muscle atrophy and which were identified using the methods described herein. For clarity, muscle atrophy signature-2 comprise the induced and repressed mRNAs described in Figure 3.

[0064] As used herein, the term "muscle atrophy signature-3" refers to the set of mRNAs with an altered expression pattern associated with muscle atrophy. The mRNAs comprise mRNAs that are either induced or repressed during the pathophysiology of muscle atrophy and which were identified using the methods described herein. For clarity, muscle atrophy signature-3 comprise the induced and repressed mRNAs described in Example 23.

[0065] As used herein, the term "muscle atrophy signature-4" refers to the set of mRNAs with an altered expression pattern associated with muscle atrophy. The mRNAs comprise mRNAs that are either induced or repressed during the pathophysiology of muscle atrophy

and which were identified using the methods described herein. For clarity, muscle atrophy signature-4 comprise the induced and repressed mRNAs described in Example 24.

[0066] As used herein, the term "subject" refers to the target of administration, e.g. an animal. Thus the subject of the herein disclosed methods can be a vertebrate, such as a mammal, a fish, a bird, a reptile, or an amphibian. Alternatively, the subject of the herein disclosed methods can be a human, non-human primate, horse, pig, rabbit, dog, sheep, goat, cow, cat, guinea pig, fish, bird, or rodent. The term does not denote a particular age or sex. Thus, adult and newborn subjects, as well as fetuses, whether male or female, are intended to be covered. In one aspect, the subject is a mammal. A patient refers to a subject afflicted with a disease or disorder. The term "patient" includes human and veterinary subjects. In some aspects of the disclosed methods, the subject has been diagnosed with a need for treatment of one or more muscle disorders prior to the administering step. In some aspects of the disclosed method, the subject has been diagnosed with a need for promoting muscle health prior to the administering step. In some aspects of the disclosed method, the subject has been diagnosed with a need for promoting muscle health prior, promote normal muscle function, and/or promote healthy aging muscles to the administering step.

[0067] As used herein, the term "treatment" refers to the medical management of a patient with the intent to cure, ameliorate, stabilize, or prevent a disease, pathological condition, or disorder. This term includes the use for astetic and self improvement purposes, for example, such uses include, but are not limited to, the administration of the disclosed compound in nutraceuticals, medicinal food, energy bar, energy drink, supplements (such as multivitamins). This term includes active treatment, that is, treatment directed specifically toward the improvement of a disease, pathological condition, or disorder, and also includes causal treatment, that is, treatment directed toward removal of the cause of the associated disease, pathological condition, or disorder. In addition, this term includes palliative treatment, that is, treatment designed for the relief of symptoms rather than the curing of the disease, pathological condition, or disorder; preventative treatment, that is, treatment directed to minimizing or partially or completely inhibiting the development of the associated disease, pathological condition, or disorder; and supportive treatment, that is, treatment employed to supplement another specific therapy directed toward the improvement of the associated disease, pathological condition, or disorder. In various aspects, the term covers any treatment of a subject, including a mammal (e.g., a human), and includes: (i) preventing the disease from occurring in a subject that can be predisposed to the disease but has not yet been diagnosed as having it; (ii) inhibiting the disease, i.e., arresting its development; or (iii)

relieving the disease, i.e., causing regression of the disease. In one aspect, the subject is a mammal such as a primate, and, in a further aspect, the subject is a human. The term "subject" also includes domesticated animals (e.g., cats, dogs, etc.), livestock (e.g., cattle, horses, pigs, sheep, goats, fish, bird, etc.), and laboratory animals (e.g., mouse, rabbit, rat, guinea pig, fruit fly, etc.).

[0068] As used herein, the term "prevent" or "preventing" refers to precluding, averting, obviating, forestalling, stopping, or hindering something from happening, especially by advance action. It is understood that where reduce, inhibit or prevent are used herein, unless specifically indicated otherwise, the use of the other two words is also expressly disclosed.

[0069] As used herein, the term "diagnosed" means having been subjected to a physical examination by a person of skill, for example, a physician, and found to have a condition that can be diagnosed or treated by the compounds, compositions, or methods disclosed herein. For example, "diagnosed with a muscle atrophy disorder" means having been subjected to a physical examination by a person of skill, for example, a physician, and found to have a condition that can be diagnosed or treated by a compound or composition that can promote muscle health, promote normal muscle function, and/or promote healthy aging muscles. As a further example, "diagnosed with a need for promoting muscle health" refers to having been subjected to a physical examination by a person of skill, for example, a physician, and found to have a condition characterized by muscle atrophy or other disease wherein promoting muscle health, promoting normal muscle function, and/or promoting healthy aging muscles would be beneficial to the subject. Such a diagnosis can be in reference to a disorder, such as muscle atrophy, and the like, as discussed herein.

[0070] As used herein, the phrase "identified to be in need of treatment for a disorder," or the like, refers to selection of a subject based upon need for treatment of the disorder. For example, a subject can be identified as having a need for treatment of a disorder (e.g., a disorder related to muscle atrophy) based upon an earlier diagnosis by a person of skill and thereafter subjected to treatment for the disorder. It is contemplated that the identification can, in one aspect, be performed by a person different from the person making the diagnosis. It is also contemplated, in a further aspect, that the administration can be performed by one who subsequently performed the administration.

[0071] As used herein, the terms "administering" and "administration" refer to any method of providing a pharmaceutical preparation to a subject. Such methods are well known to those skilled in the art and include, but are not limited to, oral administration, transdermal administration, administration by inhalation, nasal administration, topical

administration, intravaginal administration, ophthalmic administration, intraaural administration, intracerebral administration, rectal administration, sublingual administration, buccal administration, and parenteral administration, including injectable such as intravenous administration, intra-arterial administration, intramuscular administration, and subcutaneous administration. Administration can be continuous or intermittent. In various aspects, a preparation can be administered therapeutically; that is, administered to treat an existing disease or condition. In further various aspects, a preparation can be administered prophylactically; that is, administered for prevention of a disease or condition.

[0072] The term "contacting" as used herein refers to bringing a disclosed compound and a cell, target receptor, or other biological entity together in such a manner that the compound can affect the activity of the target (e.g., receptor, transcription factor, cell, etc.), either directly; i.e., by interacting with the target itself, or indirectly; i.e., by interacting with another molecule, co-factor, factor, or protein on which the activity of the target is dependent.

[0073] As used herein, the terms "effective amount" and "amount effective" refer to an amount that is sufficient to achieve the desired result or to have an effect on an undesired condition. For example, a "therapeutically effective amount" refers to an amount that is sufficient to achieve the desired therapeutic result or to have an effect on undesired symptoms, but is generally insufficient to cause adverse side affects. The specific therapeutically effective dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration; the route of administration; the rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed and like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of a compound at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. If desired, the effective daily dose can be divided into multiple doses for purposes of administration. Consequently, single dose compositions can contain such amounts or submultiples thereof to make up the daily dose. The dosage can be adjusted by the individual physician in the event of any contraindications. Dosage can vary, and can be administered in one or more dose administrations daily, for one or several days. Guidance can be found in the literature for appropriate dosages for given classes of pharmaceutical products. In further various aspects, a preparation can be administered in a "prophylactically effective amount"; that is, an amount effective for

prevention of a disease or condition.

[0074] As used herein, "EC_{50}," is intended to refer to the concentration or dose of a substance (e.g., a compound or a drug) that is required for 50% enhancement or activation of a biological process, or component of a process, including a protein, subunit, organelle, ribonucleoprotein, etc. EC_{50} also refers to the concentration or dose of a substance that is required for 50% enhancement or activation *in vivo*, as further defined elsewhere herein. Alternatively, EC_{50} can refer to the concentration or dose of compound that provokes a response halfway between the baseline and maximum response. The response can be measured in a *in vitro* or *in vivo* system as is convenient and appropriate for the biological response of interest. For example, the response can be measured *in vitro* using cultured muscle cells or in an *ex vivo* organ culture system with isolated muscle fibers. Alternatively, the response can be measured *in vivo* using an appropriate research model such as rodent, including mice and rats. The mouse or rat can be an inbred strain with phenotypic characteristics of interest such as obesity or diabetes. As appropriate, the response can be measured in a transgenic or knockout mouse or rat wherein the a gene or genes has been introduced or knocked-out, as appropriate, to replicate a disease process.

[0075] As used herein, "IC_{50}," is intended to refer to the concentration or dose of a substance (e.g., a compound or a drug) that is required for 50% inhibition or diminuation of a biological process, or component of a process, including a protein, subunit, organelle, ribonucleoprotein, etc. IC_{50} also refers to the concentration or dose of a substance that is required for 50% inhibition or diminuation *in vivo*, as further defined elsewhere herein. Alternatively, IC_{50} also refers to the half maximal (50%) inhibitory concentration (IC) or inhibitory dose of a substance. The response can be measured in a *in vitro* or *in vivo* system as is convenient and appropriate for the biological response of interest. For example, the response can be measured *in vitro* using cultured muscle cells or in an *ex vivo* organ culture system with isolated muscle fibers. Alternatively, the response can be measured *in vivo* using an appropriate research model such as rodent, including mice and rats. The mouse or rat can be an inbred strain with phenotypic characteristics of interest such as obesity or diabetes. As appropriate, the response can be measured in a transgenic or knockout mouse or rat wherein the a gene or genes has been introduced or knocked-out, as appropriate, to replicate a disease process.

[0076] The term "pharmaceutically acceptable" describes a material that is not biologically or otherwise undesirable, i.e., without causing an unacceptable level of undesirable biological effects or interacting in a deleterious manner.

[0077] As used herein, the term "derivative" refers to a compound having a structure derived from the structure of a parent compound (e.g., a compound disclosed herein) and whose structure is sufficiently similar to those disclosed herein and based upon that similarity, would be expected by one skilled in the art to exhibit the same or similar activities and utilities as the claimed compounds, or to induce, as a precursor, the same or similar activities and utilities as the claimed compounds. Exemplary derivatives include salts, esters, amides, salts of esters or amides, and N-oxides of a parent compound.

[0078] As used herein, the term "pharmaceutically acceptable carrier" refers to sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, as well as sterile powders for reconstitution into sterile injectable solutions or dispersions just prior to use. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents or vehicles include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol and the like), carboxymethylcellulose and suitable mixtures thereof, vegetable oils (such as olive oil) and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials such as lecithin, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants. These compositions can also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms can be ensured by the inclusion of various antibacterial and antifungal agents such as paraben, chlorobutanol, phenol, sorbic acid and the like. It can also be desirable to include isotonic agents such as sugars, sodium chloride and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the inclusion of agents, such as aluminum monostearate and gelatin, which delay absorption. Injectable depot forms are made by forming microencapsule matrices of the drug in biodegradable polymers such as polylactide-polyglycolide, poly(orthoesters) and poly(anhydrides). Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be controlled. Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissues. The injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable media just prior to use. Suitable inert carriers can include sugars such as lactose. Desirably, at least 95% by weight of the particles of the active ingredient have an effective particle size in the range of 0.01 to 10 micrometers.

[0079] A residue of a chemical species, as used in the specification and concluding

claims, refers to the moiety that is the resulting product of the chemical species in a particular reaction scheme or subsequent formulation or chemical product, regardless of whether the moiety is actually obtained from the chemical species. Thus, an ethylene glycol residue in a polyester refers to one or more -OCH_2CH_2O- units in the polyester, regardless of whether ethylene glycol was used to prepare the polyester. Similarly, a sebacic acid residue in a polyester refers to one or more -$CO(CH_2)_8CO$- moieties in the polyester, regardless of whether the residue is obtained by reacting sebacic acid or an ester thereof to obtain the polyester.

[0080] As used herein, the term "substituted" is contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, and aromatic and nonaromatic substituents of organic compounds. Illustrative substituents include, for example, those described below. The permissible substituents can be one or more and the same or different for appropriate organic compounds. For purposes of this disclosure, the heteroatoms, such as nitrogen, can have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valences of the heteroatoms. This disclosure is not intended to be limited in any manner by the permissible substituents of organic compounds. Also, the terms "substitution" or "substituted with" include the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, *e.g.*, a compound that does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc. It is also contemplated that, in certain aspects, unless expressly indicated to the contrary, individual substituents can be further optionally substituted (i.e., further substituted or unsubstituted).

[0081] In defining various terms, "A^1," "A^2," "A^3," and "A^4" are used herein as generic symbols to represent various specific substituents. These symbols can be any substituent, not limited to those disclosed herein, and when they are defined to be certain substituents in one instance, they can, in another instance, be defined as some other substituents.

[0082] The term "alkyl" as used herein is a branched or unbranched saturated hydrocarbon group of 1 to 24 carbon atoms, such as methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, isobutyl, *s*-butyl, *t*-butyl, *n*-pentyl, isopentyl, *s*-pentyl, neopentyl, hexyl, heptyl, octyl, nonyl, decyl, dode cyl, tetradecyl, hexadecyl, eicosyl, tetracosyl, and the like. The alkyl group can be cyclic or acyclic. The alkyl group can be branched or unbranched. The alkyl group can also be substituted or unsubstituted. For example, the alkyl group can be

substituted with one or more groups including, but not limited to, alkyl, cycloalkyl, alkoxy, amino, ether, halide, hydroxy, nitro, silyl, sulfo-oxo, or thiol, as described herein. A "lower alkyl" group is an alkyl group containing from one to six (e.g., from one to four) carbon atoms.

[0083] Throughout the specification "alkyl" is generally used to refer to both unsubstituted alkyl groups and substituted alkyl groups; however, substituted alkyl groups are also specifically referred to herein by identifying the specific substituent(s) on the alkyl group. For example, the term "halogenated alkyl" or "haloalkyl" specifically refers to an alkyl group that is substituted with one or more halide, *e.g.*, fluorine, chlorine, bromine, or iodine. The term "alkoxyalkyl" specifically refers to an alkyl group that is substituted with one or more alkoxy groups, as described below. The term "alkylamino" specifically refers to an alkyl group that is substituted with one or more amino groups, as described below, and the like. When "alkyl" is used in one instance and a specific term such as "alkylalcohol" is used in another, it is not meant to imply that the term "alkyl" does not also refer to specific terms such as "alkylalcohol" and the like.

[0084] This practice is also used for other groups described herein. That is, while a term such as "cycloalkyl" refers to both unsubstituted and substituted cycloalkyl moieties, the substituted moieties can, in addition, be specifically identified herein; for example, a particular substituted cycloalkyl can be referred to as, *e.g.*, an "alkylcycloalkyl." Similarly, a substituted alkoxy can be specifically referred to as, *e.g.*, a "halogenated alkoxy," a particular substituted alkenyl can be, *e.g.*, an "alkenylalcohol," and the like. Again, the practice of using a general term, such as "cycloalkyl," and a specific term, such as "alkylcycloalkyl," is not meant to imply that the general term does not also include the specific term.

[0085] The term "cycloalkyl" as used herein is a non-aromatic carbon-based ring composed of at least three carbon atoms. Examples of cycloalkyl groups include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, norbornyl, and the like. The term "heterocycloalkyl" is a type of cycloalkyl group as defined above, and is included within the meaning of the term "cycloalkyl," where at least one of the carbon atoms of the ring is replaced with a heteroatom such as, but not limited to, nitrogen, oxygen, sulfur, or phosphorus. The cycloalkyl group and heterocycloalkyl group can be substituted or unsubstituted. The cycloalkyl group and heterocycloalkyl group can be substituted with one or more groups including, but not limited to, alkyl, cycloalkyl, alkoxy, amino, ether, halide, hydroxy, nitro, silyl, sulfo-oxo, or thiol as described herein.

[0086] The term "polyalkylene group" as used herein is a group having two or more CH_2

groups linked to one another. The polyalkylene group can be represented by the formula —$(CH_2)_a$—, where "a" is an integer of from 2 to 500.

[0087] The terms "alkoxy" and "alkoxyl" as used herein to refer to an alkyl or cycloalkyl group bonded through an ether linkage; that is, an "alkoxy" group can be defined as —OA^1 where A^1 is alkyl or cycloalkyl as defined above. "Alkoxy" also includes polymers of alkoxy groups as just described; that is, an alkoxy can be a polyether such as —OA^1—OA^2 or —OA^1—$(OA^2)_a$—OA^3, where "a" is an integer of from 1 to 200 and A^1, A^2, and A^3 are alkyl and/or cycloalkyl groups.

[0088] The term "alkenyl" as used herein is a hydrocarbon group of from 2 to 24 carbon atoms with a structural formula containing at least one carbon-carbon double bond. Asymmetric structures such as $(A^1A^2)C=C(A^3A^4)$ are intended to include both the *E* and *Z* isomers. This can be presumed in structural formulae herein wherein an asymmetric alkene is present, or it can be explicitly indicated by the bond symbol C=C. The alkenyl group can be substituted with one or more groups including, but not limited to, alkyl, cycloalkyl, alkoxy, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, heteroaryl, aldehyde, amino, carboxylic acid, ester, ether, halide, hydroxy, ketone, azide, nitro, silyl, sulfo-oxo, or thiol, as described herein.

[0089] The term "cycloalkenyl" as used herein is a non-aromatic carbon-based ring composed of at least three carbon atoms and containing at least one carbon-carbon double bound, *i.e.*, C=C. Examples of cycloalkenyl groups include, but are not limited to, cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclopentadienyl, cyclohexenyl, cyclohexadienyl, norbornenyl, and the like. The term "heterocycloalkenyl" is a type of cycloalkenyl group as defined above, and is included within the meaning of the term "cycloalkenyl," where at least one of the carbon atoms of the ring is replaced with a heteroatom such as, but not limited to, nitrogen, oxygen, sulfur, or phosphorus. The cycloalkenyl group and heterocycloalkenyl group can be substituted or unsubstituted. The cycloalkenyl group and heterocycloalkenyl group can be substituted with one or more groups including, but not limited to, alkyl, cycloalkyl, alkoxy, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, heteroaryl, aldehyde, amino, carboxylic acid, ester, ether, halide, hydroxy, ketone, azide, nitro, silyl, sulfo-oxo, or thiol as described herein.

[0090] The term "alkynyl" as used herein is a hydrocarbon group of 2 to 24 carbon atoms with a structural formula containing at least one carbon-carbon triple bond. The alkynyl group can be unsubstituted or substituted with one or more groups including, but not limited to, alkyl, cycloalkyl, alkoxy, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, heteroaryl,

aldehyde, amino, carboxylic acid, ester, ether, halide, hydroxy, ketone, azide, nitro, silyl, sulfo-oxo, or thiol, as described herein.

[0091] The term "cycloalkynyl" as used herein is a non-aromatic carbon-based ring composed of at least seven carbon atoms and containing at least one carbon-carbon triple bound. Examples of cycloalkynyl groups include, but are not limited to, cycloheptynyl, cyclooctynyl, cyclononynyl, and the like. The term "heterocycloalkynyl" is a type of cycloalkenyl group as defined above, and is included within the meaning of the term "cycloalkynyl," where at least one of the carbon atoms of the ring is replaced with a heteroatom such as, but not limited to, nitrogen, oxygen, sulfur, or phosphorus. The cycloalkynyl group and heterocycloalkynyl group can be substituted or unsubstituted. The cycloalkynyl group and heterocycloalkynyl group can be substituted with one or more groups including, but not limited to, alkyl, cycloalkyl, alkoxy, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, heteroaryl, aldehyde, amino, carboxylic acid, ester, ether, halide, hydroxy, ketone, azide, nitro, silyl, sulfo-oxo, or thiol as described herein.

[0092] The term "aryl" as used herein is a group that contains any carbon-based aromatic group including, but not limited to, benzene, naphthalene, phenyl, biphenyl, phenoxybenzene, and the like. The term "aryl" also includes "heteroaryl," which is defined as a group that contains an aromatic group that has at least one heteroatom incorporated within the ring of the aromatic group. Examples of heteroatoms include, but are not limited to, nitrogen, oxygen, sulfur, and phosphorus. Likewise, the term "non-heteroaryl," which is also included in the term "aryl," defines a group that contains an aromatic group that does not contain a heteroatom. The aryl group can be substituted or unsubstituted. The aryl group can be substituted with one or more groups including, but not limited to, alkyl, cycloalkyl, alkoxy, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, heteroaryl, aldehyde, amino, carboxylic acid, ester, ether, halide, hydroxy, ketone, azide, nitro, silyl, sulfo-oxo, or thiol as described herein. The term "biaryl" is a specific type of aryl group and is included in the definition of "aryl." Biaryl refers to two aryl groups that are bound together *via* a fused ring structure, as in naphthalene, or are attached *via* one or more carbon-carbon bonds, as in biphenyl.

[0093] The term "aldehyde" as used herein is represented by the formula —C(O)H. Throughout this specification "C(O)" is a short hand notation for a carbonyl group, *i.e.*, C=O.

[0094] The terms "amine" or "amino" as used herein are represented by the formula —NA^1A^2, where A^1 and A^2 can be, independently, hydrogen or alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, or heteroaryl group as described herein.

[0095] The term "alkylamino" as used herein is represented by the formula —NH(-alkyl)

where alkyl is a described herein. Representative examples include, but are not limited to, methylamino group, ethylamino group, propylamino group, isopropylamino group, butylamino group, isobutylamino group, (sec-butyl)amino group, (tert-butyl)amino group, pentylamino group, isopentylamino group, (tert-pentyl)amino group, hexylamino group, and the like.

[0096] The term "dialkylamino" as used herein is represented by the formula —N(-alkyl)$_2$ where alkyl is a described herein. Representative examples include, but are not limited to, dimethylamino group, diethylamino group, dipropylamino group, diisopropylamino group, dibutylamino group, diisobutylamino group, di(sec-butyl)amino group, di(tert-butyl)amino group, dipentylamino group, diisopentylamino group, di(tert-pentyl)amino group, dihexylamino group, N-ethyl-N-methylamino group, N-methyl-N-propylamino group, N-ethyl-N-propylamino group and the like.

[0097] The term "carboxylic acid" as used herein is represented by the formula —C(O)OH.

[0098] The term "ester" as used herein is represented by the formula —OC(O)A^1 or —C(O)OA^1, where A^1 can be alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, or heteroaryl group as described herein. The term "polyester" as used herein is represented by the formula —(A^1O(O)C-A^2-C(O)O)$_a$— or —(A^1O(O)C-A^2-OC(O))$_a$—, where A^1 and A^2 can be, independently, an alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, or heteroaryl group described herein and "a" is an interger from 1 to 500. "Polyester" is as the term used to describe a group that is produced by the reaction between a compound having at least two carboxylic acid groups with a compound having at least two hydroxyl groups.

[0099] The term "ether" as used herein is represented by the formula A^1OA^2, where A^1 and A^2 can be, independently, an alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, or heteroaryl group described herein. The term "polyether" as used herein is represented by the formula —(A^1O-A^2O)$_a$—, where A^1 and A^2 can be, independently, an alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, or heteroaryl group described herein and "a" is an integer of from 1 to 500. Examples of polyether groups include polyethylene oxide, polypropylene oxide, and polybutylene oxide.

[00100] The term "halide" as used herein refers to the halogens fluorine, chlorine, bromine, and iodine.

[00101] The term "heterocycle," as used herein refers to single and multi-cyclic aromatic or non-aromatic ring systems in which at least one of the ring members is other than carbon.

Heterocycle includes azetidine, dioxane, furan, imidazole, isothiazole, isoxazole, morpholine, oxazole, oxazole, including, 1,2,3-oxadiazole, 1,2,5-oxadiazole and 1,3,4-oxadiazole, piperazine, piperidine, pyrazine, pyrazole, pyridazine, pyridine, pyrimidine, pyrrole, pyrrolidine, tetrahydrofuran, tetrahydropyran, tetrazine, including 1,2,4,5-tetrazine, tetrazole, including 1,2,3,4-tetrazole and 1,2,4,5-tetrazole, thiadiazole, including, 1,2,3-thiadiazole, 1,2,5-thiadiazole, and 1,3,4-thiadiazole, thiazole, thiophene, triazine, including 1,3,5-triazine and 1,2,4-triazine, triazole, including, 1,2,3-triazole, 1,3,4-triazole, and the like.

[00102] The term "hydroxyl" as used herein is represented by the formula —OH.

[00103] The term "ketone" as used herein is represented by the formula $A^1C(O)A^2$, where A^1 and A^2 can be, independently, an alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, or heteroaryl group as described herein.

[00104] The term "azide" as used herein is represented by the formula $—N_3$.

[00105] The term "nitro" as used herein is represented by the formula $—NO_2$.

[00106] The term "nitrile" as used herein is represented by the formula —CN.

[00107] The term "silyl" as used herein is represented by the formula $—SiA^1A^2A^3$, where A^1, A^2, and A^3 can be, independently, hydrogen or an alkyl, cycloalkyl, alkoxy, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, or heteroaryl group as described herein.

[00108] The term "sulfo-oxo" as used herein is represented by the formulas $—S(O)A^1$, $—S(O)_2A^1$, $—OS(O)_2A^1$, or $—OS(O)_2OA^1$, where A^1 can be hydrogen or an alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, or heteroaryl group as described herein. Throughout this specification "S(O)" is a short hand notation for S=O. The term "sulfonyl" is used herein to refer to the sulfo-oxo group represented by the formula $—S(O)_2A^1$, where A^1 can be hydrogen or an alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, or heteroaryl group as described herein. The term "sulfone" as used herein is represented by the formula $A^1S(O)_2A^2$, where A^1 and A^2 can be, independently, an alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, or heteroaryl group as described herein. The term "sulfoxide" as used herein is represented by the formula $A^1S(O)A^2$, where A^1 and A^2 can be, independently, an alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, or heteroaryl group as described herein.

[00109] The term "thiol" as used herein is represented by the formula —SH.

[00110] "R^1," "R^2," "R^3," "R^n," where n is an integer, as used herein can, independently, possess one or more of the groups listed above. For example, if R^1 is a straight chain alkyl group, one of the hydrogen atoms of the alkyl group can optionally be substituted with a hydroxyl group, an alkoxy group, an alkyl group, a halide, and the like. Depending upon the

groups that are selected, a first group can be incorporated within second group or, alternatively, the first group can be pendant (*i.e.*, attached) to the second group. For example, with the phrase "an alkyl group comprising an amino group," the amino group can be incorporated within the backbone of the alkyl group. Alternatively, the amino group can be attached to the backbone of the alkyl group. The nature of the group(s) that is (are) selected will determine if the first group is embedded or attached to the second group.

[00111] As described herein, compounds of the invention may contain "optionally substituted" moieties. In general, the term "substituted," whether preceded by the term "optionally" or not, means that one or more hydrogens of the designated moiety are replaced with a suitable substituent. Unless otherwise indicated, an "optionally substituted" group may have a suitable substituent at each substitutable position of the group, and when more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at every position. Combinations of substituents envisioned by this invention are preferably those that result in the formation of stable or chemically feasible compounds. In is also contemplated that, in certain aspects, unless expressly indicated to the contrary, individual substituents can be further optionally substituted (i.e., further substituted or unsubstituted).

[00112] The term "stable," as used herein, refers to compounds that are not substantially altered when subjected to conditions to allow for their production, detection, and, in certain aspects, their recovery, purification, and use for one or more of the purposes disclosed herein.

[00113] Suitable monovalent substituents on a substitutable carbon atom of an "optionally substituted" group are independently halogen; $-(CH_2)_{0-4}R^\circ$; $-(CH_2)_{0-4}OR^\circ$; $-O(CH_2)_{0-4}R^\circ$, $-O-(CH_2)_{0-4}C(O)OR^\circ$; $-(CH_2)_{0-4}CH(OR^\circ)_2$; $-(CH_2)_{0-4}SR^\circ$; $-(CH_2)_{0-4}Ph$, which may be substituted with R°; $-(CH_2)_{0-4}O(CH_2)_{0-1}Ph$ which may be substituted with R°; $-CH=CHPh$, which may be substituted with R°; $-(CH_2)_{0-4}O(CH_2)_{0-1}$-pyridyl which may be substituted with R°; $-NO_2$; $-CN$; $-N_3$; $-(CH_2)_{0-4}N(R^\circ)_2$; $-(CH_2)_{0-4}N(R^\circ)C(O)R^\circ$; $-N(R^\circ)C(S)R^\circ$; $-(CH_2)_{0-4}N(R^\circ)C(O)NR^\circ_2$; $-N(R^\circ)C(S)NR^\circ_2$; $-(CH_2)_{0-4}N(R^\circ)C(O)OR^\circ$; $-N(R^\circ)N(R^\circ)C(O)R^\circ$; $-N(R^\circ)N(R^\circ)C(O)NR^\circ_2$; $-N(R^\circ)N(R^\circ)C(O)OR^\circ$; $-(CH_2)_{0-4}C(O)R^\circ$; $-C(S)R^\circ$; $-(CH_2)_{0-4}C(O)OR^\circ$; $-(CH_2)_{0-4}C(O)SR^\circ$; $-(CH_2)_{0-4}C(O)OSiR^\circ_3$; $-(CH_2)_{0-4}OC(O)R^\circ$; $-OC(O)(CH_2)_{0-4}SR-$, $SC(S)SR^\circ$; $-(CH_2)_{0-4}SC(O)R^\circ$; $-(CH_2)_{0-4}C(O)NR^\circ_2$; $-C(S)NR^\circ_2$; $-C(S)SR^\circ$; $-SC(S)SR^\circ$, $-(CH_2)_{0-4}OC(O)NR^\circ_2$; $-C(O)N(OR^\circ)R^\circ$; $-C(O)C(O)R^\circ$; $-C(O)CH_2C(O)R^\circ$; $-C(NOR^\circ)R^\circ$; $-(CH_2)_{0-4}SSR^\circ$; $-(CH_2)_{0-4}S(O)_2R^\circ$; $-(CH_2)_{0-4}S(O)_2OR^\circ$; $-(CH_2)_{0-4}OS(O)_2R^\circ$; $-S(O)_2NR^\circ_2$; $-(CH_2)_{0-4}S(O)R^\circ$; $-N(R^\circ)S(O)_2NR^\circ_2$; $-N(R^\circ)S(O)_2R^\circ$; $-N(OR^\circ)R^\circ$; –

$C(NH)NR^{\circ}_2$; $-P(O)_2R^{\circ}$; $-P(O)R^{\circ}_2$; $-OP(O)R^{\circ}_2$; $-OP(O)(OR^{\circ})_2$; SiR°_3; $-(C_{1-4}$ straight or branched alkylene$)O-N(R^{\circ})_2$; or $-(C_{1-4}$ straight or branched alkylene$)C(O)O-N(R^{\circ})_2$, wherein each R° may be substituted as defined below and is independently hydrogen, C_{1-6} aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, $-CH_2$-(5-6 membered heteroaryl ring), or a 5–6–membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or, notwithstanding the definition above, two independent occurrences of R°, taken together with their intervening atom(s), form a 3–12–membered saturated, partially unsaturated, or aryl mono– or bicyclic ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, which may be substituted as defined below.

[00114] Suitable monovalent substituents on R° (or the ring formed by taking two independent occurrences of R° together with their intervening atoms), are independently halogen, $-(CH_2)_{0-2}R^{\bullet}$, $-(haloR^{\bullet})$, $-(CH_2)_{0-2}OH$, $-(CH_2)_{0-2}OR^{\bullet}$, $-(CH_2)_{0-2}CH(OR^{\bullet})_2$; $-O(haloR^{\bullet})$, $-CN$, $-N_3$, $-(CH_2)_{0-2}C(O)R^{\bullet}$, $-(CH_2)_{0-2}C(O)OH$, $-(CH_2)_{0-2}C(O)OR^{\bullet}$, $-(CH_2)_{0-2}SR^{\bullet}$, $-(CH_2)_{0-2}SH$, $-(CH_2)_{0-2}NH_2$, $-(CH_2)_{0-2}NHR^{\bullet}$, $-(CH_2)_{0-2}NR^{\bullet}_2$, $-NO_2$, $-SiR^{\bullet}_3$, $-OSiR^{\bullet}_3$, $-C(O)SR^{\bullet}$, $-(C_{1-4}$ straight or branched alkylene$)C(O)OR^{\bullet}$, or $-SSR^{\bullet}$ wherein each $R^{\bullet}$ is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently selected from C_{1-4} aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, or a 5–6–membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Suitable divalent substituents on a saturated carbon atom of R° include =O and =S.

[00115] Suitable divalent substituents on a saturated carbon atom of an "optionally substituted" group include the following: =O, =S, $=NNR^*_2$, $=NNHC(O)R^*$, $=NNHC(O)OR^*$, $=NNHS(O)_2R^*$, $=NR^*$, $=NOR^*$, $-O(C(R^*_2))_{2-3}O-$, or $-S(C(R^*_2))_{2-3}S-$, wherein each independent occurrence of R^* is selected from hydrogen, C_{1-6} aliphatic which may be substituted as defined below, or an unsubstituted 5–6–membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Suitable divalent substituents that are bound to vicinal substitutable carbons of an "optionally substituted" group include: $-O(CR^*_2)_{2-3}O-$, wherein each independent occurrence of R^* is selected from hydrogen, C_{1-6} aliphatic which may be substituted as defined below, or an unsubstituted 5–6–membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00116] Suitable substituents on the aliphatic group of R^* include halogen, $–R^•$, -(halo$R^•$), -OH, $–OR^•$, –O(halo$R^•$), –CN, –C(O)OH, $–C(O)OR^•$, $–NH_2$, $–NHR^•$, $–NR^•_2$, or $–NO_2$, wherein each $R^•$ is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently $C_{1–4}$ aliphatic, $–CH_2Ph$, $–O(CH_2)_{0–1}Ph$, or a 5–6–membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00117] Suitable substituents on a substitutable nitrogen of an "optionally substituted" group include $–R^†$, $–NR^†_2$, $–C(O)R^†$, $–C(O)OR^†$, $–C(O)C(O)R^†$, $–C(O)CH_2C(O)R^†$, $–S(O)_2R^†$, $-S(O)_2NR^†_2$, $–C(S)NR^†_2$, $–C(NH)NR^†_2$, or $–N(R^†)S(O)_2R^†$; wherein each $R^†$ is independently hydrogen, $C_{1–6}$ aliphatic which may be substituted as defined below, unsubstituted –OPh, or an unsubstituted 5–6–membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or, notwithstanding the definition above, two independent occurrences of $R^†$, taken together with their intervening atom(s) form an unsubstituted 3–12–membered saturated, partially unsaturated, or aryl mono– or bicyclic ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00118] Suitable substituents on the aliphatic group of $R^†$ are independently halogen, $–R^•$, -(halo$R^•$), –OH, $–OR^•$, –O(halo$R^•$), –CN, –C(O)OH, $–C(O)OR^•$, $–NH_2$, $–NHR^•$, $–NR^•_2$, or $-NO_2$, wherein each $R^•$ is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently $C_{1–4}$ aliphatic, $–CH_2Ph$, $–O(CH_2)_{0–1}Ph$, or a 5–6–membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

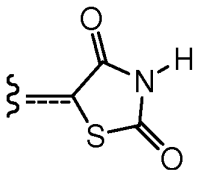
[00119] The term "leaving group" refers to an atom (or a group of atoms) with electron withdrawing ability that can be displaced as a stable species, taking with it the bonding electrons. Examples of suitable leaving groups include halides and sulfonate esters, including, but not limited to, triflate, mesylate, tosylate, and brosylate.

[00120] The terms "hydrolysable group" and "hydrolysable moiety" refer to a functional group capable of undergoing hydrolysis, e.g., under basic or acidic conditions. Examples of hydrolysable residues include, without limitatation, acid halides, activated carboxylic acids, and various protecting groups known in the art (see, for example, "Protective Groups in Organic Synthesis," T. W. Greene, P. G. M. Wuts, Wiley-Interscience, 1999).

[00121] The term "organic residue" defines a carbon containing residue, i.e., a residue comprising at least one carbon atom, and includes but is not limited to the carbon-containing groups, residues, or radicals defined hereinabove. Organic residues can contain various

heteroatoms, or be bonded to another molecule through a heteroatom, including oxygen, nitrogen, sulfur, phosphorus, or the like. Examples of organic residues include but are not limited alkyl or substituted alkyls, alkoxy or substituted alkoxy, mono or di-substituted amino, amide groups, etc. Organic residues can preferably comprise 1 to 18 carbon atoms, 1 to 15, carbon atoms, 1 to 12 carbon atoms, 1 to 8 carbon atoms, 1 to 6 carbon atoms, or 1 to 4 carbon atoms. In a further aspect, an organic residue can comprise 2 to 18 carbon atoms, 2 to 15, carbon atoms, 2 to 12 carbon atoms, 2 to 8 carbon atoms, 2 to 4 carbon atoms, or 2 to 4 carbon atoms.

[00122] A very close synonym of the term "residue" is the term "radical," which as used in the specification and concluding claims, refers to a fragment, group, or substructure of a molecule described herein, regardless of how the molecule is prepared. For example, a 2,4-thiazolidinedione radical in a particular compound has the structure



regardless of whether thiazolidinedione is used to prepare the compound. In some embodiments the radical (for example an alkyl) can be further modified (i.e., substituted alkyl) by having bonded thereto one or more "substituent radicals." The number of atoms in a given radical is not critical to the present invention unless it is indicated to the contrary elsewhere herein.

[00123] "Organic radicals," as the term is defined and used herein, contain one or more carbon atoms. An organic radical can have, for example, 1-26 carbon atoms, 1-18 carbon atoms, 1-12 carbon atoms, 1-8 carbon atoms, 1-6 carbon atoms, or 1-4 carbon atoms. In a further aspect, an organic radical can have 2-26 carbon atoms, 2-18 carbon atoms, 2-12 carbon atoms, 2-8 carbon atoms, 2-6 carbon atoms, or 2-4 carbon atoms. Organic radicals often have hydrogen bound to at least some of the carbon atoms of the organic radical. One example, of an organic radical that comprises no inorganic atoms is a 5, 6, 7, 8-tetrahydro-2-naphthyl radical. In some embodiments, an organic radical can contain 1-10 inorganic heteroatoms bound thereto or therein, including halogens, oxygen, sulfur, nitrogen, phosphorus, and the like. Examples of organic radicals include but are not limited to an alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, mono-substituted amino, di-substituted amino, acyloxy, cyano, carboxy, carboalkoxy, alkylcarboxamide, substituted alkylcarboxamide, dialkylcarboxamide, substituted dialkylcarboxamide, alkylsulfonyl,

alkylsulfinyl, thioalkyl, thiohaloalkyl, alkoxy, substituted alkoxy, haloalkyl, haloalkoxy, aryl, substituted aryl, heteroaryl, heterocyclic, or substituted heterocyclic radicals, wherein the terms are defined elsewhere herein. A few non-limiting examples of organic radicals that include heteroatoms include alkoxy radicals, trifluoromethoxy radicals, acetoxy radicals, dimethylamino radicals and the like.

[00124] "Inorganic radicals," as the term is defined and used herein, contain no carbon atoms and therefore comprise only atoms other than carbon. Inorganic radicals comprise bonded combinations of atoms selected from hydrogen, nitrogen, oxygen, silicon, phosphorus, sulfur, selenium, and halogens such as fluorine, chlorine, bromine, and iodine, which can be present individually or bonded together in their chemically stable combinations. Inorganic radicals have 10 or fewer, or preferably one to six or one to four inorganic atoms as listed above bonded together. Examples of inorganic radicals include, but not limited to, amino, hydroxy, halogens, nitro, thiol, sulfate, phosphate, and like commonly known inorganic radicals. The inorganic radicals do not have bonded therein the metallic elements of the periodic table (such as the alkali metals, alkaline earth metals, transition metals, lanthanide metals, or actinide metals), although such metal ions can sometimes serve as a pharmaceutically acceptable cation for anionic inorganic radicals such as a sulfate, phosphate, or like anionic inorganic radical. Inorganic radicals do not comprise metalloids elements such as boron, aluminum, gallium, germanium, arsenic, tin, lead, or tellurium, or the noble gas elements, unless otherwise specifically indicated elsewhere herein.

[00125] Compounds described herein can contain one or more double bonds and, thus, potentially give rise to cis/trans (E/Z) isomers, as well as other conformational isomers. Unless stated to the contrary, the invention includes all such possible isomers, as well as mixtures of such isomers.

[00126] Unless stated to the contrary, a formula with chemical bonds shown only as solid lines and not as wedges or dashed lines contemplates each possible isomer, *e.g.*, each enantiomer and diastereomer, and a mixture of isomers, such as a racemic or scalemic mixture. Compounds described herein can contain one or more asymmetric centers and, thus, potentially give rise to diastereomers and optical isomers. Unless stated to the contrary, the present invention includes all such possible diastereomers as well as their racemic mixtures, their substantially pure resolved enantiomers, all possible geometric isomers, and pharmaceutically acceptable salts thereof. Mixtures of stereoisomers, as well as isolated specific stereoisomers, are also included. During the course of the synthetic procedures used to prepare such compounds, or in using racemization or epimerization procedures known to

those skilled in the art, the products of such procedures can be a mixture of stereoisomers.

[00127] Many organic compounds exist in optically active forms having the ability to rotate the plane of plane-polarized light. In describing an optically active compound, the prefixes D and L or R and S are used to denote the absolute configuration of the molecule about its chiral center(s). The prefixes d and l or (+) and (-) are employed to designate the sign of rotation of plane-polarized light by the compound, with (-) or meaning that the compound is levorotatory. A compound prefixed with (+) or d is dextrorotatory. For a given chemical structure, these compounds, called stereoisomers, are identical except that they are non-superimposable mirror images of one another. A specific stereoisomer can also be referred to as an enantiomer, and a mixture of such isomers is often called an enantiomeric mixture. A 50:50 mixture of enantiomers is referred to as a racemic mixture. Many of the compounds described herein can have one or more chiral centers and therefore can exist in different enantiomeric forms. If desired, a chiral carbon can be designated with an asterisk (*). When bonds to the chiral carbon are depicted as straight lines in the disclosed formulas, it is understood that both the (R) and (S) configurations of the chiral carbon, and hence both enantiomers and mixtures thereof, are embraced within the formula. As is used in the art, when it is desired to specify the absolute configuration about a chiral carbon, one of the bonds to the chiral carbon can be depicted as a wedge (bonds to atoms above the plane) and the other can be depicted as a series or wedge of short parallel lines is (bonds to atoms below the plane). The Cahn-Inglod-Prelog system can be used to assign the (R) or (S) configuration to a chiral carbon.

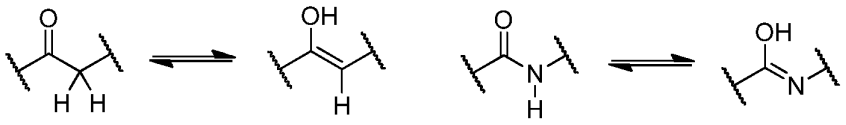
[00128] Compounds described herein comprise atoms in both their natural isotopic abundance and in non-natural abundance. The disclosed compounds can be isotopically-labelled or isotopically-substituted compounds identical to those described, but for the fact that one or more atoms are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number typically found in nature. Examples of isotopes that can be incorporated into compounds of the invention include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorous, fluorine and chlorine, such as ^{2}H, ^{3}H, ^{13}C, ^{14}C, ^{15}N, ^{18}O, ^{17}O, ^{35}S, ^{18}F and ^{36}Cl, respectively. Compounds further comprise prodrugs thereof, and pharmaceutically acceptable salts of said compounds or of said prodrugs which contain the aforementioned isotopes and/or other isotopes of other atoms are within the scope of this invention. Certain isotopically-labelled compounds of the present invention, for example those into which radioactive isotopes such as ^{3}H and ^{14}C are incorporated, are useful in drug and/or substrate tissue distribution assays. Tritiated, i.e., ^{3}H,

and carbon-14, i.e., ^{14}C, isotopes are particularly preferred for their ease of preparation and detectability. Further, substitution with heavier isotopes such as deuterium, i.e., ^{2}H, can afford certain therapeutic advantages resulting from greater metabolic stability, for example increased in vivo half-life or reduced dosage requirements and, hence, may be preferred in some circumstances. Isotopically labelled compounds of the present invention and prodrugs thereof can generally be prepared by carrying out the procedures below, by substituting a readily available isotopically labelled reagent for a non- isotopically labelled reagent.

[00129] The compounds described in the invention can be present as a solvate. In some cases, the solvent used to prepare the solvate is an aqueous solution, and the solvate is then often referred to as a hydrate. The compounds can be present as a hydrate, which can be obtained, for example, by crystallization from a solvent or from aqueous solution. In this connection, one, two, three or any arbitrary number of solvate or water molecules can combine with the compounds according to the invention to form solvates and hydrates. Unless stated to the contrary, the invention includes all such possible solvates.

[00130] The term "co-crystal" means a physical association of two or more molecules which owe their stability through non-covalent interaction. One or more components of this molecular complex provide a stable framework in the crystalline lattice. In certain instances, the guest molecules are incorporated in the crystalline lattice as anhydrates or solvates, see e.g. "Crystal Engineering of the Composition of Pharmaceutical Phases. Do Pharmaceutical Co-crystals Represent a New Path to Improved Medicines?" Almarasson, O., et. al., The Royal Society of Chemistry, 1889-1896, 2004. Examples of co-crystals include p-toluenesulfonic acid and benzenesulfonic acid.

[00131] It is also appreciated that certain compounds described herein can be present as an equilibrium of tautomers. For example, ketones with an α-hydrogen can exist in an equilibrium of the keto form and the enol form.



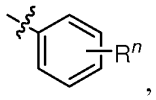
keto form enol form amide form imidic acid form

[00132] Likewise, amides with an N-hydrogen can exist in an equilibrium of the amide form and the imidic acid form. Unless stated to the contrary, the invention includes all such possible tautomers.

[00133] It is known that chemical substances form solids which are present in different states of order which are termed polymorphic forms or modifications. The different

modifications of a polymorphic substance can differ greatly in their physical properties. The compounds according to the invention can be present in different polymorphic forms, with it being possible for particular modifications to be metastable. Unless stated to the contrary, the invention includes all such possible polymorphic forms.

[00134] In some aspects, a structure of a compound can be represented by a formula:



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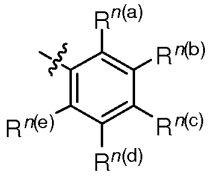
which is understood to be equivalent to a formula:



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wherein n is typically an integer. That is, R^n is understood to represent five independent substituents, $R^{n(a)}$, $R^{n(b)}$, $R^{n(c)}$, $R^{n(d)}$, $R^{n(e)}$. By "independent substituents," it is meant that each R substituent can be independently defined. For example, if in one instance $R^{n(a)}$ is halogen, then $R^{n(b)}$ is not necessarily halogen in that instance.

[00135] Certain materials, compounds, compositions, and components disclosed herein can be obtained commercially or readily synthesized using techniques generally known to those of skill in the art. For example, the starting materials and reagents used in preparing the disclosed compounds and compositions are either available from commercial suppliers such as Aldrich Chemical Co., (Milwaukee, Wis.), Acros Organics (Morris Plains, N.J.), Fisher Scientific (Pittsburgh, Pa.), or Sigma (St. Louis, Mo.) or are prepared by methods known to those skilled in the art following procedures set forth in references such as Fieser and Fieser's Reagents for Organic Synthesis, Volumes 1-17 (John Wiley and Sons, 1991); Rodd's Chemistry of Carbon Compounds, Volumes 1-5 and Supplementals (Elsevier Science Publishers, 1989); Organic Reactions, Volumes 1-40 (John Wiley and Sons, 1991); March's Advanced Organic Chemistry, (John Wiley and Sons, 4th Edition); and Larock's Comprehensive Organic Transformations (VCH Publishers Inc., 1989).

[00136] Unless otherwise expressly stated, it is in no way intended that any method set forth herein be construed as requiring that its steps be performed in a specific order. Accordingly, where a method claim does not actually recite an order to be followed by its steps or it is not otherwise specifically stated in the claims or descriptions that the steps are to be limited to a specific order, it is no way intended that an order be inferred, in any respect. This holds for any possible non-express basis for interpretation, including: matters of logic

with respect to arrangement of steps or operational flow; plain meaning derived from grammatical organization or punctuation; and the number or type of embodiments described in the specification.

[00137] Disclosed are the components to be used to prepare the compositions of the invention as well as the compositions themselves to be used within the methods disclosed herein. These and other materials are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, etc. of these materials are disclosed that while specific reference of each various individual and collective combinations and permutation of these compounds can not be explicitly disclosed, each is specifically contemplated and described herein. For example, if a particular compound is disclosed and discussed and a number of modifications that can be made to a number of molecules including the compounds are discussed, specifically contemplated is each and every combination and permutation of the compound and the modifications that are possible unless specifically indicated to the contrary. Thus, if a class of molecules A, B, and C are disclosed as well as a class of molecules D, E, and F and an example of a combination molecule, A-D is disclosed, then even if each is not individually recited each is individually and collectively contemplated meaning combinations, A-E, A-F, B-D, B-E, B-F, C-D, C-E, and C-F are considered disclosed. Likewise, any subset or combination of these is also disclosed. Thus, for example, the sub-group of A-E, B-F, and C-E would be considered disclosed. This concept applies to all aspects of this application including, but not limited to, steps in methods of making and using the compositions of the invention. Thus, if there are a variety of additional steps that can be performed it is understood that each of these additional steps can be performed with any specific embodiment or combination of embodiments of the methods of the invention.

[00138] It is understood that the compositions disclosed herein have certain functions. Disclosed herein are certain structural requirements for performing the disclosed functions, and it is understood that there are a variety of structures that can perform the same function that are related to the disclosed structures, and that these structures will typically achieve the same result.

B. COMPOUNDS

[00139] In one aspect, the invention relates to compounds useful in methods to inhibit muscle atrophy by providing to a subject in need thereof an effective amount of a compound or an analog thereof selected from among the compounds described herein, and pharmaceutical compositions comprising compounds used in the methods. In a further aspect,

the invention relates to compounds identified using muscle atrophy signature-1, muscle atrophy signature-2, or both muscle atrophy signatures. In a further aspect, the invention relates to compounds useful in methods to modulate muscle health, methods to inhibit muscle atrophy, methods to increase insulin/IGF-I signaling, methods to reduce body fat; methods to reduce blood glucose, methods to reduce blood triglycerides, methods to reduce blood cholesterol, methods to reduce obesity, methods to reduce fatty liver disease, and methods to reduce diabetes, and pharmaceutical compositions comprising compounds used in the methods.

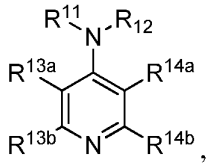
[00140] In one aspect, the compounds of the invention are useful in the treatment of muscle disorders. In a further aspect, the muscle disorder can be skeletal muscle atrophy secondary to malnutrition, muscle disuse (secondary to voluntary or involuntary bedrest), neurologic disease (including multiple sclerosis, amyotrophic lateral sclerosis, spinal muscular atrophy, critical illness neuropathy, spinal cord injury or peripheral nerve injury), orthopedic injury, casting, and other post-surgical forms of limb immobilization, chronic disease (including cancer, congestive heart failure, chronic pulmonary disease, chronic renal failure, chronic liver disease, diabetes mellitus, Cushing syndrome, growth hormone deficiency, IGF-I deficiency, androgen deficiency, estrogen deficiency, and chronic infections such as HIV/AIDS or tuberculosis), burns, sepsis, other illnesses requiring mechanical ventiliation, drug-induced muscle disease (such as glucorticoid-induced myopathy and statin-induced myopathy), genetic diseases that primarily affect skeletal muscle (such as muscular dystrophy and myotonic dystrophy), autoimmune diseases that affect skeletal muscle (such as polymyositis and dermatomyositis), spaceflight, or age-related sarcopenia.

[00141] It is contemplated that each disclosed derivative can be optionally further substituted. It is also contemplated that any one or more derivative can be optionally omitted from the invention. It is understood that a disclosed compound can be provided by the disclosed methods. It is also understood that the disclosed compounds can be employed in the disclosed methods of using.

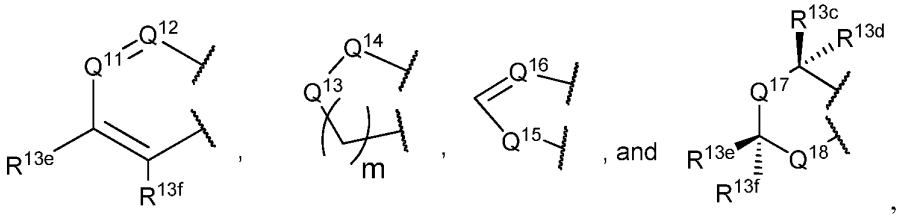
1. TACRINE AND ANALOGS

[00142] In one aspect, the compound can be a tacrine analogs.

[00143] In one aspect, the tacrine analogs has a structure represented by a formula:



wherein R^{13a} and R^{13b} together comprise a cycle selected from:



wherein Q^{11} is selected from N and CR^{13c};

wherein Q^{12} is selected from N and CR^{13d};

wherein Q^{13} and Q^{14} are independently selected from $CR^{13c}R^{13d}$, O, S, and NR^{14c};

wherein Q^{15} is selected from $CR^{13c}R^{13d}$, O, S, and NR^{14c};

wherein Q^{16} is selected from N and CR^{13c};

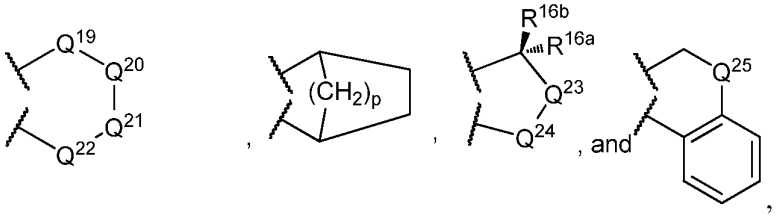
wherein Q^{17} and Q^{18} are independently selected from $CR^{13c}R^{13d}$, O, S, and NR^{14c};

wherein R^{11} and R^{12} are independently selected from H and C1-C6 alkyl;

wherein R^{13c}, R^{13d}, R^{13e}, and R^{13f} are independently selected from H, C1-C6 alkyl, C1-C6 alkoxy, halo, hydroxyl, nitro, amino, cyano, $NHCOR^{15}$, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C1-C6 alkylamino, C1-C6 dialkylamino, C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl, wherein C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl are independently are substituted with 0, 1, 2, or 3 substituents selected from halogen, hydroxyl, cyano, amino, C1-C6 alkyl, C1-C6 alkoxy, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C1-C6 alkylamino, and C1-C6 dialkylamino;

wherein each R^{14c} is independently selected from H and C1-C6 alkyl;

wherein R^{14a} and R^{14b} together comprise a cycle selected from:



wherein each of Q^{19}, Q^{20}, Q^{21}, Q^{22}, Q^{23}, Q^{24}, and Q^{25} are independently selected from $CR^{17a}R^{17b}$, O, S, and NR^{18};

wherein R^{16a} and R^{16a} are independently selected from H, C1-C6 alkyl, C1-C6 alkoxy, halo, hydroxyl, nitro, amino, cyano, $NHCOR^{15}$, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl,

C1-C6 alkylamino, C1-C6 dialkylamino, C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl, wherein C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl are independently are substituted with 0, 1, 2, or 3 substituents selected from halogen, hydroxyl, cyano, amino, C1-C6 alkyl, C1-C6 alkoxy, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C1-C6 alkylamino, and C1-C6 dialkylamino;

wherein R^{17a} and R^{17b} are independently selected from H, C1-C6 alkyl, C1-C6 alkoxy, halo, hydroxyl, nitro, amino, cyano, $NHCOR^{15}$, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C1-C6 alkylamino, C1-C6 dialkylamino, C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl, wherein C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl are independently are substituted with 0, 1, 2, or 3 substituents selected from halogen, hydroxyl, cyano, amino, C1-C6 alkyl, C1-C6 alkoxy, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C1-C6 alkylamino, and C1-C6 dialkylamino;

wherein each R^{18} is independently selected from H and C1-C6 alkyl;

wherein each R^{15} is independently selected from H and C1-C6 alkyl;

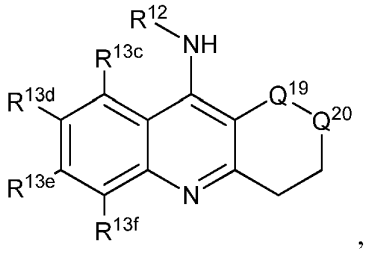
wherein each n is independently selected from 0, 1, and 2;

wherein m is selected from 1 and 2; and

wherein p is selected from 1, 2 and 3; or

a stereoisomer, tautomer, solvate, or pharmaceutically acceptable salt thereof.

[00144] In one aspect, compound (A) has the structure represented by the formula:



,

wherein R^{12} is selected from H and C1-C6 alkyl;

wherein R^{13c}, R^{13d}, R^{13e}, and R^{13f} are independently selected from H, C1-C6 alkyl, C1-C6 alkoxy, halo, hydroxyl, nitro, amino, cyano, $NHCOR^{15}$, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C1-C6 alkylamino, C1-C6 dialkylamino, C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl, wherein C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl are independently are substituted with 0, 1, 2, or 3 substituents selected from halogen, hydroxyl, cyano, amino, C1-C6 alkyl, C1-C6 alkoxy, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C1-C6 alkylamino, and C1-C6 dialkylamino;

wherein Q^{19} and Q^{20} are independently selected from $CR^{17a}R^{17b}$, O, S, and NR^{18};

wherein R^{17a} and R^{17b} are independently selected from H, C1-C6 alkyl, C1-C6 alkoxy, halo, hydroxyl, nitro, amino, cyano, $NHCOR^{15}$, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C1-C6 alkylamino, C1-C6 dialkylamino, C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, C2-C9 heterocyclyl, wherein C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl are independently are substituted with 0, 1, 2, or 3 substituents selected from halogen, hydroxyl, cyano, amino, C1-C6 alkyl, C1-C6 alkoxy, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C1-C6 alkylamino, and C1-C6 dialkylamino;

wherein each R^{18} is independently selected from H and C1-C6 alkyl; and

wherein n is selected from 0, 1, and 2.

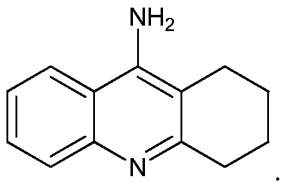
[00145] In another aspect, R^{12} is H; R^{13c}, R^{13d}, R^{13e}, and R^{13f} are independently selected from H, C1-C6 alkyl, C1-C6 alkoxy, halo, hydroxyl, nitro, and amino; Q^{19} and Q^{20} are independently selected from C $R^{17a}R^{17b}$, O, S, and NR^{18}; wherein R^{17a} and R^{17b} are independently selected from H, C1-C6 alkyl, C1-C6 alkoxy, halo, hydroxyl, nitro, and amino; wherein each R^{18} is independently H; and n is selected from 0, 1, and 2.

[00146] In another aspect, R^{12} is H; R^{13c}, R^{13d}, R^{13e}, and R^{13f} are independently selected from H, C1-C6 alkyl, C1-C6 alkoxy, halo, hydroxyl, nitro, and amino; Q^{19} and Q^{20} are independently selected from C $R^{17a}R^{17b}$; wherein R^{17a} and R^{17b} are independently selected from H, C1-C6 alkyl, C1-C6 alkoxy, halo, hydroxyl, nitro, and amino; and n is 1.

[00147] In another aspect, R^{12} is H; R^{13c}, R^{13d}, R^{13e}, and R^{13f} are independently selected from H, C1-C6 alkyl, C1-C6 alkoxy, halo, and hydroxyl; Q^{19} and Q^{20} are independently selected from $CR^{17a}R^{17b}$; wherein R^{17a} and R^{17b} are independently H; and n is 1.

[00148] In another aspect, R^{12} is H; R^{12} is H; R^{13c}, R^{13d}, R^{13e}, and R^{13f} are independently selected from H, C1-C6 alkyl, and halo; Q^{19} and Q^{20} are independently $CR^{17a}R^{17b}$; wherein R^{17a} and R^{17b} are independently H; and wherein n is 1.

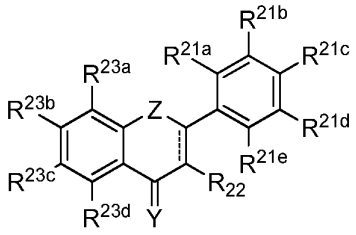
[00149] In another aspect, the formula is:]



2. NARINGENIN AND ANALOG

[00150] In one aspect, the compound can be a naringenin analog.

[00151] In one aspect, the naringenin analog has a structure represented by a formula:



,

wherein each ----- represents a covalent bond selected from a single or double bond;

wherein R^{21a}, R^{21b}, R^{21c}, R^{21d}, and R^{21e} are independently selected from H, OH, O-Glucosyl, halo, cyano, amino, nitro, nitroso, $NHCOR^{15}$, C1-C6 alkyl, C1-C6 alkoxy, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C1-C6 alkylamino, acyl, phenyl- C1-C6 alkoxy, benzyl- C1-C6 alkoxy, and C1-C6 dialkylamino;

wherein R^{22} is selected from H, OH, O- Glucosyl, halo, cyano, amino, nitro, nitroso, $NHCOR^{15}$, C1-C6 alkyl, C1-C6 alkoxy, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C1-C6 alkylamino, acyl phenyl- C1-C6 alkoxy, benzyl- C1-C6 alkoxy, and C1-C6 dialkylamino;

wherein R^{23a}, R^{23b}, R^{23c}, and R^{23d} are independently selected from H, OH, O-Glucosyl halo, cyano, amino, nitro, nitroso, $NHCOR^{15}$, C1-C20 alkyl, C1-C20 alkenyl, C1-C20 alkynyl, C1-C20 alkenynyl, C1-C20 alkoxy, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C1-C6 alkylamino, acyl, phenyl- C1-C6 alkoxy, benzyl- C1-C6 alkoxy, and C1-C6 dialkylamino;

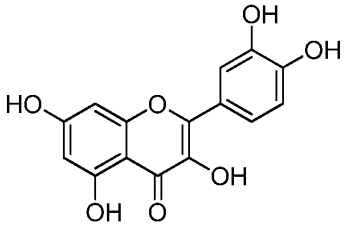
wherein R^{15} is selected from H and C1-C6 alkyl;

wherein Z is selected from O and S; and

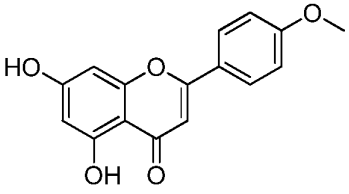
wherein Y is selected from O and S; or

a stereoisomer, tautomer, solvate, or pharmaceutically acceptable salt thereof;

wherein the compound does not have the structure:



or



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[00152] In one aspect, ----- indicates a covalent single bond. In another aspect, ----- indicates a covalent double bond.

[00153] In another aspect, Z is O, and Y is O.

[00154] In another aspect, R^{21a}, R^{21b}, R^{21c}, R^{21d}, and R^{21e} are independently selected from H and OH; wherein R^{22} is selected from H and OH; and wherein R^{23a}, R^{23b}, R^{23c}, R^{23d}, and R^{23e} are independently selected from H and OH.

[00155] In another aspect, R^{21a}, R^{21b}, R^{21c}, R^{21d}, and R^{21e} are independently selected from H, OH, O-Glucosyl, halo, cyano, amino, nitro, and nitroso.

[00156] In another aspect, R^{22} is H.

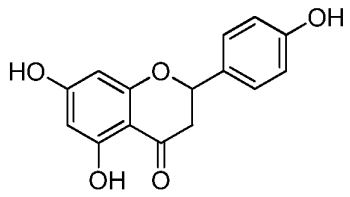
[00157] In another aspect, R^{21a}, R^{21b}, R^{21d}, and R^{21e} are H, and R^{21c} is OH.

[00158] In another aspect, R^{23a} and R^{23c} are H, and R^{23b} and R^{23d} are OH.

[00159] In another aspect, R^{21a}, R^{21b}, R^{21d}, and R^{21e} are H, R^{21c} is OH, R^{23a} and R^{23c} are H, and R^{23b} and R^{23d} are OH.

[00160] In another aspect, R^{21a}, R^{21d}, and R^{21e} are H, R^{21b} and R^{21c} are OH, R^{23a} and R^{23c} are H, and R^{23b} and R^{23d} are OH.

[00161] In another aspect, the compound has the structure:



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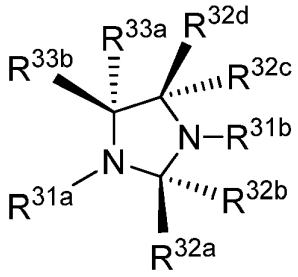
3. ALLANTOIN AND ANALOGS

[00162] In one aspect, the compound can be a allantoin analog.

[00163] In one aspect, the allantoin analog has a structure represented by a formula:



,

wherein R^{31a} and R^{31b} are independently selected from H, C1-C6 alkyl, C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl, wherein C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl are independently are substituted with 0, 1, 2, or 3 substituents selected from halogen, hydroxyl, cyano, amino, C1-C6 alkyl, C1-C6 alkoxy, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C1-C6 alkylamino, and C1-C6 dialkylamino;

wherein R^{32a} and R^{32b} are independently selected from H, C1-C6 alkyl, $OCl(OH)_4Al_2$, $OAl(OH)_2$, C1-C6 alkoxy, halo, hydroxyl, nitro, amino, cyano, $NHCOR^{15}$, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C1-C6 alkylamino, C1-C6 dialkylamino, C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl or taken together to form a double bond selected from =O and =S, wherein C6-C10 aryl, C3-C10 cycloalkyl, C5-C9

heteroaryl, and C2-C9 heterocyclyl are independently are substituted with 0, 1, 2, or 3 substituents selected from halogen, hydroxyl, cyano, amino, C1-C6 alkyl, C1-C6 alkoxy, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C1-C6 alkylamino, and C1-C6 dialkylamino;

wherein R^{32c} and R^{32d} are independently selected from H, C1-C6 alkyl, $OCl(OH)_4Al_2$, $OAl(OH)_2$, C1-C6 alkoxy, halo, hydroxyl, nitro, amino, cyano, $NHCOR^{15}$, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C1-C6 alkylamino, C1-C6 dialkylamino, C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl or taken together to form a double bond selected from =O and =S, wherein C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl are independently are substituted with 0, 1, 2, or 3 substituents selected from halogen, hydroxyl, cyano, amino, C1-C6 alkyl, C1-C6 alkoxy, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C1-C6 alkylamino, and C1-C6 dialkylamino;

wherein R^{33a} and R^{33b} are independently selected from H, $NR^{34a}CONR^{34b}R^{34c}$, C1-C6 alkyl, C1-C6 alkoxy, halo, hydroxyl, nitro, amino, cyano, $NHCOR^{15}$, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C1-C6 alkylamino, C1-C6 dialkylamino, C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl, wherein C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl are independently are substituted with 0, 1, 2, or 3 substituents selected from halogen, hydroxyl, cyano, amino, C1-C6 alkyl, C1-C6 alkoxy, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C1-C6 alkylamino, and C1-C6 dialkylamino; and

wherein R^{34a} R^{34b} and R^{34c} are independently selected from H, C1-C6 alkyl, C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl, wherein C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl are independently are substituted with 0, 1, 2, or 3 substituents selected from halogen, hydroxyl, cyano, amino, C1-C6 alkyl, C1-C6 alkoxy, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C1-C6 alkylamino, and C1-C6 dialkylamino; or

a stereoisomer, tautomer, solvate, or pharmaceutically acceptable salt thereof.

[00164] In one aspect, R^{31a} and R^{31b} are H.

[00165] In another aspect, R^{32a} and R^{32b} are taken together to form =O.

[00166] In another aspect, R^{32c} and R^{32d} are taken together to form =O.

[00167] In another aspect, R^{32a} and R^{32b} are taken together to form =O, and R^{32c} and R^{32d} are taken together to form =O.

[00168] In another aspect, R^{31a} is H, R^{31b} is H, R^{32a}, and R^{32b} are taken together to form =O, and R^{32c} and R^{32d} are taken together to form =O.

[00169] In another aspect, R^{31a} is H, R^{31b} is H, R^{32a} and R^{32b} are taken together to form =O,

R^{32c} and R^{32d} are taken together to form =O, and one of R^{33a} and R^{33b} is $NR^{34a}CONR^{34b}R^{34c}$ and the other one of R^{33a} and R^{33b} is H.

[00170] In another aspect, one of R^{33a} and R^{33b} is $NR^{34a}CONR^{34b}R^{34c}$ and the other one of R^{33a} and R^{33b} is H.

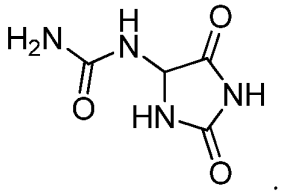
[00171] In another aspect, one of R^{32a} and R^{32b} is $OCl(OH)_4Al_2$ and the other one of R^{32a} and R^{32b} is H.

[00172] In another aspect, one of R^{32c} and R^{32d} is $OCl(OH)_4Al_2$ and the other one of R^{32c} and R^{32d} is H.

[00173] In another aspect, one of R^{32a} and R^{32b} is $OAl(OH)_2$ and the other one of R^{32a} and R^{32b} is H.

[00174] In another aspect, one of R^{32c} and R^{32d} is $OAl(OH)_2$ and the other one of R^{32c} and R^{32d} is H.

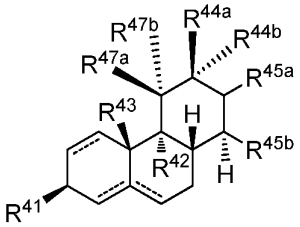
[00175] In another aspect, the compound has the structure:



4. CONESSINE AND ANALOGS

[00176] In one aspect, the compound can be a conessine analog.

[00177] In one aspect, the conessine analog has a structure represented by a formula:



wherein each ----- represents a covalent bond independently selected from a single or double bond, wherein valency is satisfied;

wherein R^{41} is selected from $NR^{48a}R^{48b}$, =O, =S, C1-C6 alkoxy and hydroxyl;

wherein R^{48a} and R^{48b} are independently selected from H, C1-C6 alkyl, C1-C6 heteroalkyl, C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl, wherein C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl are independently substituted with 0, 1, 2, or 3 substituents selected from halogen, hydroxyl, cyano, amino, C1-C6 alkyl, C1-C6 alkoxy, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C1-C6 alkylamino, and C1-C6 dialkylamino;

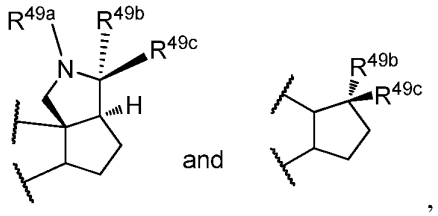
wherein R^{42} is selected from H, C1-C6 alkoxy and hydroxyl;

wherein R^{43} is selected from H and C1-C6 alkyl;

wherein R^{44a} and R^{44b} are independently selected from are independently selected from H, hydroxyl, and C1-C6 alkoxy;

wherein R^{47a} and R^{47b} are independently selected from are independently selected from H, hydroxyl, and C1-C6 alkoxy;

wherein R^{45a} and R^{45b} together comprise a cycle selected from:



,

wherein R^{49a} is selected from H and C1-C6 alkyl; and

wherein R^{49b} and R^{49c} are independently selected from H and C1-C6 alkyl, or taken together to form =O; or

a stereoisomer, tautomer, solvate, or pharmaceutically acceptable salt thereof.

[00178] In one aspect, R^{47a} and R^{47b} are independently selected from H, hydroxyl, and C1-C6 alkoxy.

[00179] In another aspect, R^{44a} and R^{44b} are independently selected from H, hydroxyl, and C1-C6 alkoxy.

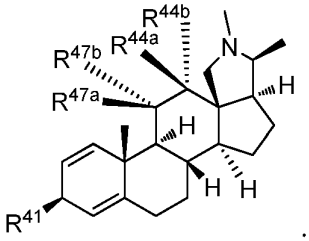
[00180] In another aspect, R^{42} is H.

[00181] In another aspect, R^{47a} and R^{47b} are selected from H, hydroxyl, and C1-C6 alkoxy; R^{44a} and R^{44b} are H.

[00182] In another aspect, R^{41} is selected from $NR^{48a}R^{48b}$ and =O, wherein R^{48a} and R^{48b} are independently selected from H and C1-C6 alkyl.

[00183] In another aspect, R^{43} is C1 alkyl.

[00184] In another aspect, the formula has the structure:



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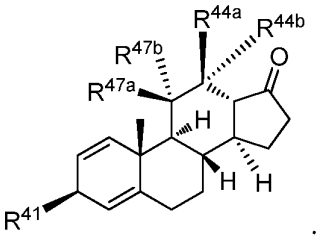
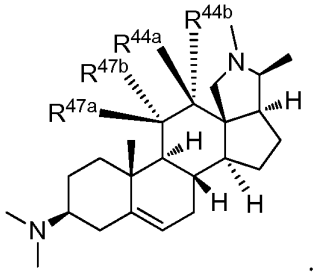
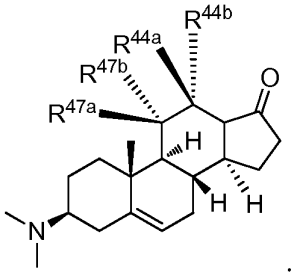
[00185] In another aspect, the formula has the structure:



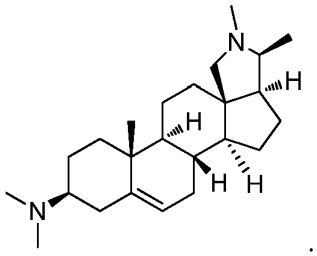
[00186] In another aspect, the formula has the structure:



[00187] In another aspect, the formula has the structure:



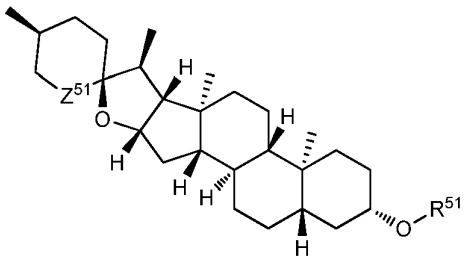
[00188] In another aspect, the formula has the structure:



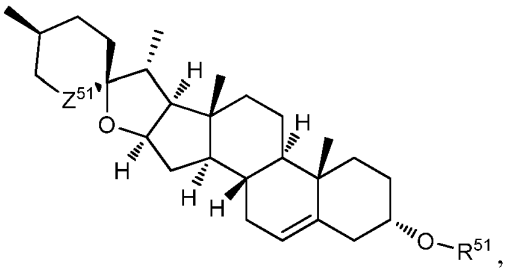
5. TOMATIDINE AND ANALOGS

[00189] In one aspect, the compound can be a tomatidine analog.

[00190] In one aspect, the tomatidine analog has a structure represented by a formula:



or



wherein R^{51} is selected from H, C1-C6 alkyl, COR^{53}, C1-C6 alkylamino, C1-C6 dialkylamino, C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl, wherein C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl are independently substituted with 0, 1, 2, or 3 substituents selected from halogen, hydroxyl, cyano, amino, C1-C6 alkyl, C1-C6 alkoxy, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C1-C6 alkylamino, and C1-C6 dialkylamino;

wherein R^{53} is selected from C1-C6 alkyl, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl, wherein C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl are independently substituted with 0, 1, 2, or 3 substituents selected from halogen, hydroxyl, cyano, amino, C1-C6 alkyl, C1-C6 alkoxy, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C1-C6 alkylamino, and C1-C6 dialkylamino;

wherein Z^{51} is selected from O, S, and NR^{54};

wherein R^{54} is selected from H, C1-C6 alkyl, COR^{55}, C1-C6 alkylamino, C1-C6 dialkylamino, C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl, wherein C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl are independently substituted with 0, 1, 2, or 3 substituents selected from halogen, hydroxyl, cyano, amino, C1-C6 alkyl, C1-C6 alkoxy, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C1-C6 alkylamino, and C1-C6 dialkylamino;

wherein R^{55} is selected from C1-C6 alkyl, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl, wherein C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl are independently substituted with 0, 1, 2, or 3 substituents selected from halogen, hydroxyl, cyano, amino, C1-C6 alkyl, C1-C6 alkoxy, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C1-C6 alkylamino, and C1-C6 dialkylamino; or

a stereoisomer, tautomer, solvate, or pharmaceutically acceptable salt thereof.

[00191] In one aspect, R^{51} is selected from H, C1-C6 alkyl and COR^{53}, wherein R^{53} is C1-C6 alkyl.

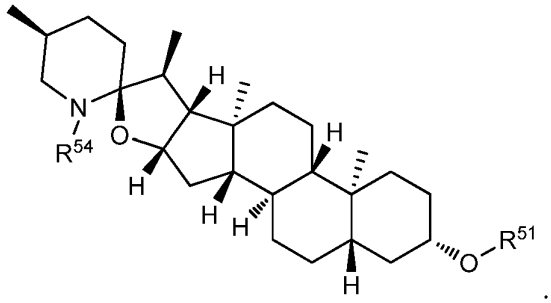
[00192] In another aspect, R^{51} is H.

[00193] In another aspect, Z^{51} is NR^{54}. In another aspect, Z^{51} is NR^{54}, wherein R^{54} is selected from H, C1-C6 alkyl, and COR^{55}, wherein R^{55} is C1-C6 alkyl.

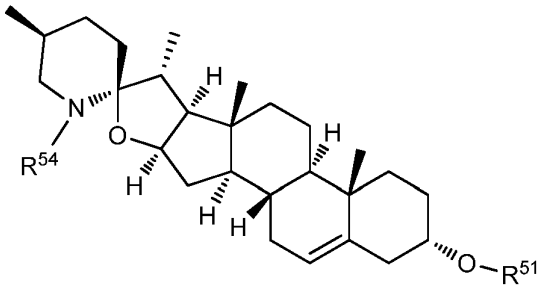
[00194] In another aspect, R^{51} is selected from H, C1-C6 alkyl and COR^{53}, wherein R^{53} is C1-C6 alkyl; and Z^{51} is NR^{54}, wherein R^{54} is selected from H, C1-C6 alkyl, and COR^{55}, wherein R^{55} is C1-C6 alkyl.

[00195] In another aspect, R^{51} and R^{54} are identical.

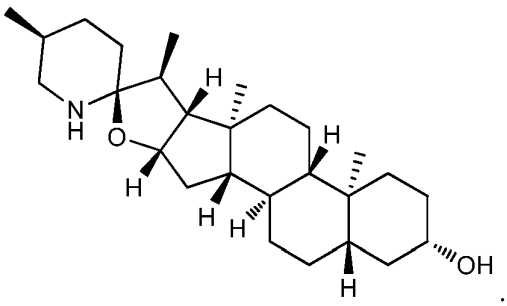
[00196] In another aspect, the structure is represented by the formula:



[00197] In another aspect, the structure is represented by the formula:



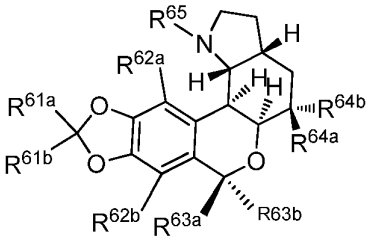
[00198] In another aspect, the formula has the structure:



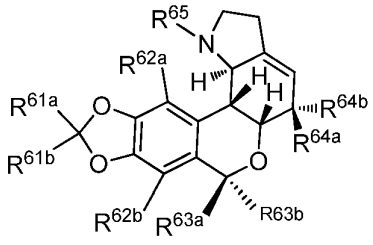
6. UNGERINE/HIPPEASTRINE AND ANALOGS

[00199] In one aspect, the compound can be a ungerine/hippeastrine analog.

[00200] In one aspect, the ungerine/hippeastrine has a structure represented by a formula:



or



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wherein R^{61a} and R^{61b} are independently selected from H, C1-C6 alkyl, C1-C6 alkoxy, halo, hydroxyl, nitro, amino, cyano, $NHCOR^{15}$, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C1-C6 alkylamino, C1-C6 dialkylamino, C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl,

and C2-C9 heterocyclyl, wherein C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl are independently substituted with 0, 1, 2, or 3 substituents selected from halogen, hydroxyl, cyano, amino, C1-C6 alkyl, C1-C6 alkoxy, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C1-C6 alkylamino, and C1-C6 dialkylamino;

wherein R^{62a} and R^{62b} are independently selected from H, C1-C6 alkyl, C1-C6 alkoxy, halo, hydroxyl, nitro, amino, cyano, $NHCOR^{15}$, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C1-C6 alkylamino, C1-C6 dialkylamino, C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl, wherein C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl are independently substituted with 0, 1, 2, or 3 substituents selected from halogen, hydroxyl, cyano, amino, C1-C6 alkyl, C1-C6 alkoxy, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C1-C6 alkylamino, and C1-C6 dialkylamino;

wherein R^{63a} and R^{63b} are independently selected from H, C1-C6 alkyl, C1-C6 alkoxy, halo, hydroxyl, nitro, amino, cyano, $NHCOR^{15}$, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C1-C6 alkylamino, C1-C6 dialkylamino, C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl, or taken together to form a group selected from =O and =S, wherein C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl are independently substituted with 0, 1, 2, or 3 substituents selected from halogen, hydroxyl, cyano, amino, C1-C6 alkyl, C1-C6 alkoxy, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C1-C6 alkylamino, and C1-C6 dialkylamino;

wherein R^{64a} and R^{64b} are independently selected from H, OR^{67}, C1-C6 alkyl, C1-C6 alkoxy, halo, hydroxyl, nitro, amino, cyano, $NHCOR^{15}$, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C1-C6 alkylamino, C1-C6 dialkylamino, C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl, wherein C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl are independently ubstituted with 0, 1, 2, or 3 substituents selected from halogen, hydroxyl, cyano, amino, C1-C6 alkyl, C1-C6 alkoxy, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C1-C6 alkylamino, and C1-C6 dialkylamino;

wherein R^{65} is selected from H, C1-C6 alkyl, C1-C6 alkoxy, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C1-C6 alkylamino, C1-C6 dialkylamino, COR^{66}, C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl, wherein C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl are independently substituted with 0, 1, 2, or 3 substituents selected from halogen, hydroxyl, cyano, amino, C1-C6 alkyl, C1-C6 alkoxy, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C1-C6 alkylamino, and C1-C6 dialkylamino;

wherein R^{66} is selected from C1-C6 alkyl, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl, wherein C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl are independently substituted with 0, 1, 2, or 3 substituents selected from halogen, hydroxyl, cyano, amino, C1-C6 alkyl, C1-C6 alkoxy, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C1-C6 alkylamino, and C1-C6 dialkylamino;

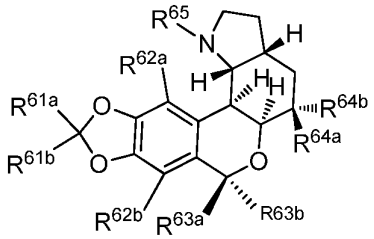
wherein R^{67} is selected from C1-C6 alkyl, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl, wherein C6-C10 aryl, C3-C10 cycloalkyl, C5-C9 heteroaryl, and C2-C9 heterocyclyl are independently substituted with 0, 1, 2, or 3 substituents selected from halogen, hydroxyl, cyano, amino, C1-C6 alkyl, C1-C6 alkoxy, C1-C6 monohaloalkyl, C1-C6 polyhaloalkyl, C1-C6 alkylamino, and C1-C6 dialkylamino;

wherein each R^{15} is independently selected from H and C1-C6 alkyl; or

a stereoisomer, tautomer, solvate, or pharmaceutically acceptable salt thereof,

wherein the compound is present in an effective amount.

[00201] In one aspect, the structure is represented by a formula:



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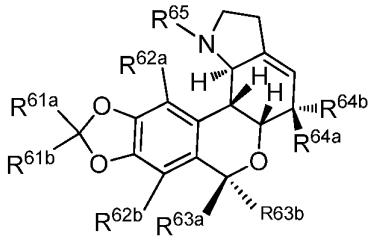
[00202] In another aspect, the structure is represented by a formula:



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[00203] In another aspect, R^{61a}, R^{61b}, R^{62a}, and R^{62b} are H.

[00204] In another aspect, one of R^{63a} and R^{63b} is hydroxyl and the other one of R^{63a} and R^{63b} is H.

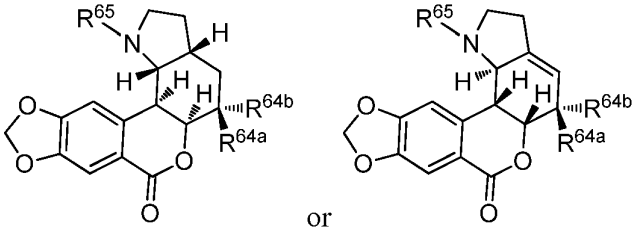
[00205] In another aspect, R^{63a} and R^{63b} are taken together and form =O.

[00206] In another aspect, one of R^{64a} and R^{64b} is hydroxyl or OR^{67} and the other one of R^{64a} and R^{64b} is H.

[00207] In another aspect, one of R^{64a} and R^{64b} is hydroxyl or OR^{67} and the other one of R^{64a} and R^{64b} is H, wherein R^{67} is C1-C6 alkyl.

[00208] In another aspect, R^{65} is C1-C6 alkyl.

[00209] In another aspect, the structure is represented by a formula:



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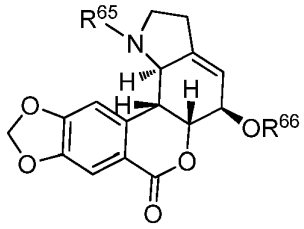
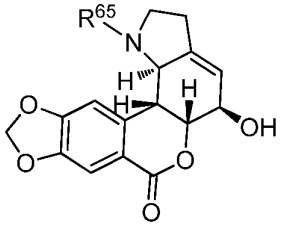
[00210] In another aspect, the structure is represented by a formula:



.

[00211] In another aspect, the structure is represented by a formula:



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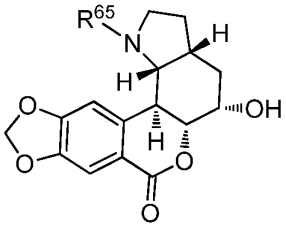
[00212] In another aspect, the structure is represented by a formula:



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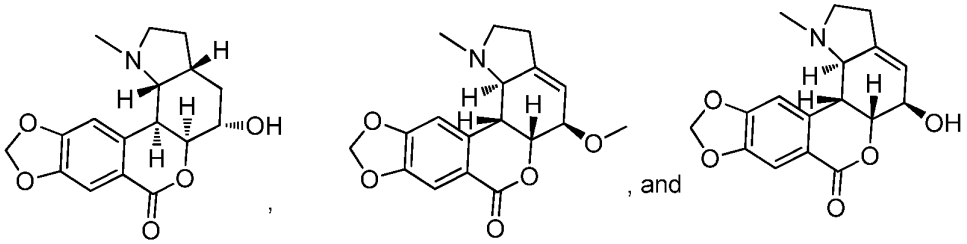
[00213] In another aspect, the structure is represented by a formula:



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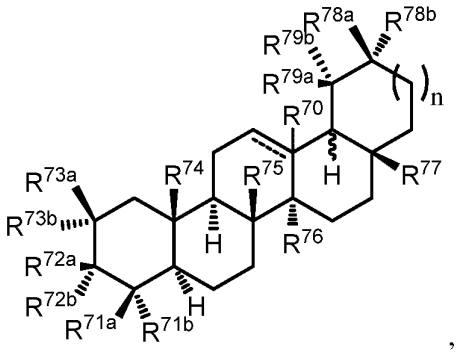
7. BETULINIC ACID AND ANALOGS

[00214] In one aspect, the compound can be a betulinic acid derivative.

[00215] In one aspect, has a structure represented by a formula:



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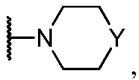
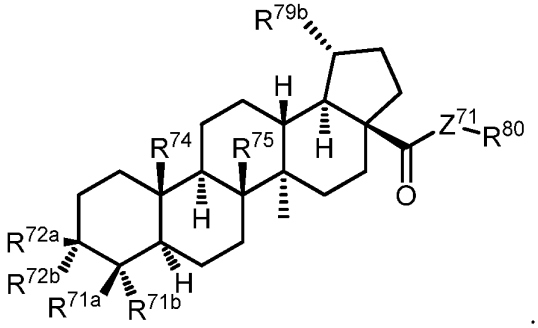
wherein ----- is an covalent bond selected from a single bond and a double bond, wherein valency is satisfied, and R^{70} is optionally present; wherein n is 0 or 1; wherein R^{70}, when present, is hydrogen; wherein R^{71a} is selected from C1-C6 alkyl and $-C(O)ZR^{82}$; wherein R^{71b} is selected from C1-C6 alkyl, or wherein R^{71a} and R^{71b} are optionally covalently bonded and, together with the intermediate carbon, comprise an optionally substituted C3-C5 cycloalkyl or C2-C5 heterocycloalkyl; wherein one of R^{72a} and R^{72b} is $-Z^{72}$, and the other is hydrogen, or R^{72a} and R^{72b} together comprise =O; wherein each of R^{73a} and R^{73b} is independently selected from hydrogen, hydroxyl, C1-C6 alkyl, and C1-C6 alkoxyl, provided that R^{73a} and R^{73b} are not simultaneously hydroxyl, wherein R^{73a} and R^{73b} are optionally covalently bonded and, together with the intermediate carbon, comprise an optionally substituted C3-C5 cycloalkyl or C2-C5 heterocycloalkyl; wherein each of R^{74}, R^{75}, and R^{76} is independently selected from C1-C6 alkyl; wherein R^{77} is selected from C1-C6 alkyl, and $-C(O)Z^{71}R^{80}$; wherein R^{80} is selected from hydrogen and C1-C6 alkyl; wherein R^{78a} and R^{78b} are independently selected from hydrogen and C1-C6 alkyl; wherein each of R^{79a} and R^{79b} is independently selected from hydrogen and C1-C6 alkyl, C2-C6 alkenyl, and C2-C6 alkynyl, provided that R^{79a} and R^{79b} are not simultaneously hydrogen; or wherein R^{79a} and R^{79b} are covalently bonded and, along with the intermediate carbon, together comprise C3-C5 cycloalkyl or C2-C5 heterocycloalkyl; wherein R^{82} is selected from hydrogen and C1-C6 alkyl; wherein Z^{71} and Z^{72} are independently selected from $-OR^{81}-$ and $-NR^{83}-$; wherein R^{83} and R^{83} are independently selected from hydrogen and C1-C4 alkyl; or, wherein Z^{71} and Z^{72} are independently N, R^{84} and R^{85} are covalently bonded and $-NR^{84}R^{85}$ comprises a moiety of the formula:



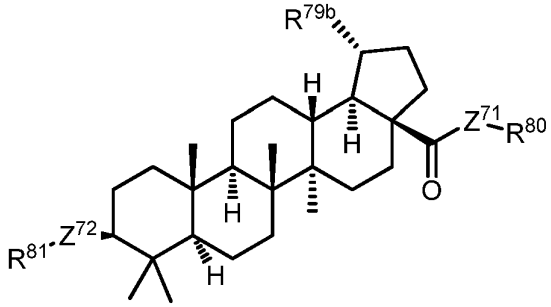
,

wherein Y is selected from –O–, –S–, –SO–, $–SO_2–$, –NH–, $–NCH_3–$, or a stereoisomer, tautomer, solvate, or pharmaceutically acceptable salt thereof.

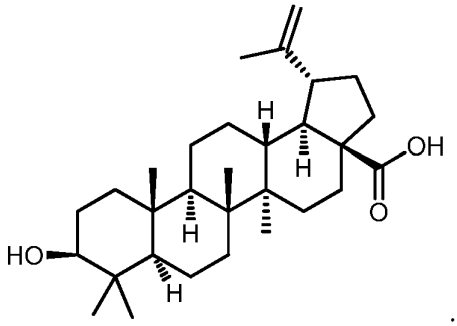
[00216] In another aspect, the formula has the structure:



[00217] In another aspect, the formula has the structure:



[00218] In another aspect, the formula has the structure:



[00219] In one aspect, ----- is a single bond. In another aspect, ----- is a double bond.

[00220] In one aspect, n is 0. In another aspect, n is 1.

[00221] In another aspect, R^{71a} is C1-C6 alkyl; R^{71b} is selected from C1-C6 alkyl; one of R^{72a} is $–Z^{72}$, and R^{72b} is hydrogen; R^{74}, R^{75} are independently selected from C1-C6 alkyl; wherein R^{79b} is selected from C1-C6 alkyl, C2-C6 alkenyl, and C2-C6 alkynyl; Z^{71} is –O-; and Z^{72} is selected from $–OR^8$ and $–NR^{83}–$; $R^{81}$and R^{83} are independently selected from hydrogen and C1-C4 alkyl.

[00222] In another aspect, R^{71a} is C1 alkyl; R^{71b} is C1 alkyl; R^{72a} is $–Z^{72}$, and R^{72b} is hydrogen; R^{74}, R^{75} are independently selected from C1 alkyl; wherein R^{79b} is selected from

C1-C6 alkyl, C2-C6 alkenyl, and C2-C6 alkynyl; Z^{71} is –O-; and Z^{72} is selected from $–OR^{81}$ and $–NR^{83}–$; wherein $R^{81}$and R^{83} are hydrogen.

[00223] In another aspect, R^{71a} is C1 alkyl; R^{71b} is C1 alkyl; R^{72a} is $–Z^{72}$, and R^{72b} is hydrogen; R^{74}, R^{75} are independently selected from C1 alkyl; R^{79b} is C2-C6 alkenyl; Z^{71} is –O-; and Z^{72} is selected from $–OR^{81}$ and $–NR^{83}–$; wherein $R^{81}$and R^{83} are hydrogen.

8. Compounds Identified by Muscle Atrophy Signature-1 and Muscle Atrophy Signature-2.

[00224] In various aspects, the invention relates to uses of one or more compounds selected from tacrine analogs, naringenin analogs, allantoin analogs, conessine analogs, tomatidine analogs, hippeastrine/ungerine analogs and betulinic acid analogs..

a. Muscle Atrophy Signature-1

[00225] In one aspect, the disclosed compounds comprise compounds identified using muscle atrophy signature-1. Such compounds include, but are not limited to, allantoin; conessine; naringenin; tacrine; tomatidine or a pharmaceutically acceptable salt, tautomer, stereoisomer, hydrate, solvate, or polymorph thereof. In a yet further aspect, the compound is an analog of one the preceding compounds as defined above.

b. Muscle Atrophy Signature-2

[00226] In a further aspect, the disclosed compounds comprise compounds identified using muscle atrophy signature-2. Such compounds include, but are not limited to, allantoin; betulinic acid; conessine; naringenin; tacrine; tomatidine or a pharmaceutically acceptable salt, tautomer, stereoisomer, hydrate, solvate, or polymorph thereof. In a yet further aspect, the compound is an analog of one the preceding compounds as defined above.

c. Muscle Atrophy Signature-1 or Muscle Atrophy Signature-2

[00227] In a further aspect, the disclosed compounds comprise compounds identified using either muscle atrophy signature-1 or muscle atrophy signature-2. Such compounds include, but are not limited to, allantoin; betulinic acid; conessine; naringenin; tacrine; tomatidine or a pharmaceutically acceptable salt, tautomer, stereoisomer, hydrate, solvate, or polymorph thereof. In a yet further aspect, the compound is an analog of one the preceding compounds as defined above.

d. Muscle Atrophy Signature-1 and Muscle Atrophy Signature-2

[00228] In a further aspect, the disclosed compounds comprise compounds identified using both muscle atrophy signature-1 and muscle atrophy signature-2, and is a compound associated with both muscle atrophy signatures. Such compounds include, but are not limited to, allantoin; conessine; naringenin; tacrine; tomatidine or a pharmaceutically acceptable salt,

tautomer, stereoisomer, hydrate, solvate, or polymorph thereof. In a yet further aspect, the compound is an analog of one the preceding compounds as defined above.

9. INHIBITION OF MUSCLE ATROPHY

[00229] In one aspect, the disclosed compounds inhibit muscle atrophy. In a further aspect, the disclosed compounds promoting muscle health, promoting normal muscle function, and/or promoting healthy aging muscles. In a yet further aspect, the disclosed compounds inhibit of muscle atrophy and promote muscle health, promote normal muscle function, and/or promote healthy aging muscles. In a further aspect, the inhibition of muscle atrophy is in an animal. In an even further aspect, the promoting muscle health, promoting normal muscle function, and/or promoting healthy aging muscles is in an animal. In a still further aspect, the animal is a mammal, In a yet further aspect, the mammal is a human. In a further aspect, the mammal is a mouse. In a yet further aspect, the mammal is a rodent.

[00230] In a further aspect, the disclosed compounds inhibit muscle atrophy when administered at an oral dose of greater than about 5 mg per day in a human. In a further aspect, the disclosed compounds inhibit muscle atrophy when administered at an oral dose of greater than about 10 mg per day in a human. In a further aspect, the disclosed compounds inhibit muscle atrophy when administered at an oral dose of greater than about 25 mg per day in a human. In a further aspect, the disclosed compounds inhibit muscle atrophy when administered at an oral dose of greater than about 50 mg per day in a human. In a further aspect, the disclosed compounds inhibit muscle atrophy when administered at an oral dose of greater than about 75 mg per day in a human. In a further aspect, the disclosed compounds inhibit muscle atrophy when administered at an oral dose of greater than about 100 mg per day in a human. In a further aspect, the disclosed compounds inhibit muscle atrophy when administered at an oral dose of greater than about 150 mg per day in a human. In a further aspect, the disclosed compounds inhibit muscle atrophy when administered at an oral dose of greater than about 200 mg per day in a human. In a further aspect, the disclosed compounds inhibit muscle atrophy when administered at an oral dose of greater than about 250 mg per day in a human. In a yet further aspect, the disclosed compounds inhibit muscle atrophy when administered at an oral dose of greater than about 300 mg per day in a human. In a still further aspect, the disclosed compounds inhibit muscle atrophy when administered at an oral dose of greater than about 400 mg per day in a human. In an even further aspect, the disclosed compounds inhibit muscle atrophy when administered at an oral dose of greater than about 500 mg per day in a human. In a further aspect, the disclosed compounds inhibit muscle atrophy when administered at an oral dose of greater than about 750 mg per day in a

human. In a yet further aspect, the disclosed compounds inhibit muscle atrophy when administered at an oral dose of greater than about 1000 mg per day in a human. In a still further aspect, the disclosed compounds inhibit muscle atrophy when administered at an oral dose of greater than about 1500 mg per day in a human. In an even further aspect, the disclosed compounds inhibit muscle atrophy when administered at an oral dose of greater than about 2000 mg per day in a human.

[00231] It is contemplated that one or more compounds can optionally be omitted from the disclosed invention.

C. PHARMACEUTICAL COMPOSITIONS

[00232] In one aspect, the invention relates to pharmaceutical compositions comprising the disclosed compounds. That is, a pharmaceutical composition can be provided comprising a therapeutically effective amount of at least one disclosed compound. In another example, a pharmaceutical composition can be provided comprising a prophylactically effective amount of at least one disclosed compound

[00233] In one aspect, the invention relates to pharmaceutical compositions comprising a pharmaceutically acceptable carrier and a compound, wherein the compound is present in an effective amount. The compound can be selected from a tacrine analog, allantoin analog, naringenin analog, conessine analog, tomatidine analog, ungerine/hippeastrine analog and betulinic acid analog. For example, the compound can be a tacrine analog. In another example, the compound can be a naringenin analog. In another example, the compound can be a conessine analog. In another example, the compound can be a tomatidine analog. In another example, the compound can be an ungerine/hippeastrine analog. In another example, the compound can be a betulinic acid analog.

[00234] In one aspect, the compound is present in an amount greater than about an amount selected from 5 mg, 10 mg, 25 mg, 50 mg, 100 mg, 150 mg, 200 mg, 250 mg, 300 mg, 400, mg, 500 mg, 750 mg, 1000 mg, 1,500 mg, or 2,000 mg.

[00235] A pharmaceutical composition comprising a pharmaceutically acceptable carrier and an effective amount of one or more of: (a) a compound selected from a tacrine analog, allantoin analog, naringenin analog, conessine analog, tomatidine analog, ungerine/hippeastrine analog and betulinic acid analog; (b) a compound that down regulates multiple induced mRNAs of Muscle Atrophy Signature 1, compared to expression levels in the same type of the muscle cell in the absence of the compound; (c) a compound that up regulates multiple repressed mRNAs of Muscle Atrophy Signature 1, compared to expression levels in the same type of the muscle cell in the absence of the compound; (d) a compound

that down regulates multiple induced mRNAs of Muscle Atrophy Signature 2, compared to expression levels in the same type of the muscle cell in the absence of the compound; and/or (e) a compound that up regulates multiple mRNAs of Muscle Atrophy Signature 2, compared to expression levels in the same type of the muscle cell in the absence of the compound.

[00236] In a further aspect, the amount is a therapeutically effective amount. In a still further aspect, the amount is a prophylactically effective amount.

[00237] In a further aspect, pharmaceutical composition is administered to an animal. In a still further aspect, the animal is a mammal, fish or bird. In a yet further aspect, the mammal is a primate. In a still further aspect, the mammal is a human. In an even further aspect, the human is a patient.

[00238] In a further aspect, the pharmaceutical composition comprises a compound identified using muscle atrophy signature-1. In a yet further aspect, the pharmaceutical composition comprises a compound identified using muscle atrophy signature-2. In a yet further aspect, the pharmaceutical composition comprises a compound identified using both muscle atrophy signature-1 and muscle atrophy signature-2.

[00239] In a further aspect, the animal is a domesticated animal. In a still further aspect, the domesticated animal is a domesticated fish, domesticated crustacean, or domesticated mollusk. In a yet further aspect, the domesticated animal is poultry. In an even further aspect, the poultry is selected from chicken, turkey, duck, and goose. In a still further aspect, the domesticated animal is livestock. In a yet further aspect, the livestock animal is selected from pig, cow, horse, goat, bison, and sheep.

[00240] In a further aspect, the effective amount is a therapeutically effective amount. In a still further aspect, the effective amount is a prophylactically effective amount. In a yet further aspect, the muscle disorder is muscle atrophy . In an even further aspect, the muscle disorder is a condition in need of promoting muscle health, promoting normal muscle function, and/or promoting healthy aging muscles.

[00241] In a further aspect, the pharmaceutical composition is administered following identification of the mammal in need of treatment of muscle atrophy. In a still further aspect, the pharmaceutical composition is administered following identification of the mammal in need of prevention of muscle atrophy. In an even further aspect, the mammal has been diagnosed with a need for treatment of muscle atrophy prior to the administering step.

[00242] In certain aspects, the disclosed pharmaceutical compositions comprise the disclosed compounds (including pharmaceutically acceptable salt(s) thereof) as an active ingredient, a pharmaceutically acceptable carrier, and, optionally, other therapeutic

ingredients or adjuvants. The instant compositions include those suitable for oral, rectal, topical, and parenteral (including subcutaneous, intramuscular, and intravenous) administration, although the most suitable route in any given case will depend on the particular host, and nature and severity of the conditions for which the active ingredient is being administered. The pharmaceutical compositions can be conveniently presented in unit dosage form and prepared by any of the methods well known in the art of pharmacy.

[00243] As used herein, the term "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable non-toxic bases or acids. When the compound of the present invention is acidic, its corresponding salt can be conveniently prepared from pharmaceutically acceptable non-toxic bases, including inorganic bases and organic bases. Salts derived from such inorganic bases include aluminum, ammonium, calcium, copper (-ic and -ous), ferric, ferrous, lithium, magnesium, manganese (-ic and -ous), potassium, sodium, zinc and the like salts. Particularly preferred are the ammonium, calcium, magnesium, potassium and sodium salts. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary, and tertiary amines, as well as cyclic amines and substituted amines such as naturally occurring and synthesized substituted amines. Other pharmaceutically acceptable organic non-toxic bases from which salts can be formed include ion exchange resins such as, for example, arginine, betaine, caffeine, choline, N,N'-dibenzylethylenediamine, diethylamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethylmorpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine, tripropylamine, tromethamine and the like.

[00244] As used herein, the term "pharmaceutically acceptable non-toxic acids", includes inorganic acids, organic acids, and salts prepared thereof, for example, acetic, benzenesulfonic, benzoic, camphorsulfonic, citric, ethanesulfonic, fumaric, gluconic, glutamic, hydrobromic, hydrochloric, isethionic, lactic, maleic, malic, mandelic, methanesulfonic, mucic, nitric, pamoic, pantothenic, phosphoric, succinic, sulfuric, tartaric, p-toluenesulfonic acid and the like. Preferred are citric, hydrobromic, hydrochloric, maleic, phosphoric, sulfuric, and tartaric acids.

[00245] In practice, the compounds of the invention, or pharmaceutically acceptable salts thereof, of this invention can be combined as the active ingredient in intimate admixture with a pharmaceutical carrier according to conventional pharmaceutical compounding techniques.

The carrier can take a wide variety of forms depending on the form of preparation desired for administration, e.g., oral or parenteral (including intravenous). Thus, the pharmaceutical compositions of the present invention can be presented as discrete units suitable for oral administration such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient. Further, the compositions can be presented as a powder, as granules, as a solution, as a suspension in an aqueous liquid, as a non-aqueous liquid, as an oil-in-water emulsion or as a water-in-oil liquid emulsion. In addition to the common dosage forms set out above, the compounds of the invention, and/or pharmaceutically acceptable salt(s) thereof, can also be administered by controlled release means and/or delivery devices. The compositions can be prepared by any of the methods of pharmacy. In general, such methods include a step of bringing into association the active ingredient with the carrier that constitutes one or more necessary ingredients. In general, the compositions are prepared by uniformly and intimately admixing the active ingredient with liquid carriers or finely divided solid carriers or both. The product can then be conveniently shaped into the desired presentation.

[00246] Thus, the pharmaceutical compositions of this invention can include a pharmaceutically acceptable carrier and a compound or a pharmaceutically acceptable salt of the compounds of the invention. The compounds of the invention, or pharmaceutically acceptable salts thereof, can also be included in pharmaceutical compositions in combination with one or more other therapeutically active compounds.

[00247] The pharmaceutical carrier employed can be, for example, a solid, liquid, or gas. Examples of solid carriers include lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, and stearic acid. Examples of liquid carriers are sugar syrup, peanut oil, olive oil, and water. Examples of gaseous carriers include carbon dioxide and nitrogen.

[00248] In preparing the compositions for oral dosage form, any convenient pharmaceutical media can be employed. For example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents and the like can be used to form oral liquid preparations such as suspensions, elixirs and solutions; while carriers such as starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disintegrating agents, and the like can be used to form oral solid preparations such as powders, capsules and tablets. Because of their ease of administration, tablets and capsules are the preferred oral dosage units whereby solid pharmaceutical carriers are employed. Optionally, tablets can be coated by standard aqueous or nonaqueous techniques

[00249] A tablet containing the composition of this invention can be prepared by compression or molding, optionally with one or more accessory ingredients or adjuvants. Compressed tablets can be prepared by compressing, in a suitable machine, the active ingredient in a free-flowing form such as powder or granules, optionally mixed with a binder, lubricant, inert diluent, surface active or dispersing agent. Molded tablets can be made by molding in a suitable machine, a mixture of the powdered compound moistened with an inert liquid diluent.

[00250] The pharmaceutical compositions of the present invention comprise a compound of the invention (or pharmaceutically acceptable salts thereof) as an active ingredient, a pharmaceutically acceptable carrier, and optionally one or more additional therapeutic agents or adjuvants. The instant compositions include compositions suitable for oral, rectal, topical, and parenteral (including subcutaneous, intramuscular, and intravenous) administration, although the most suitable route in any given case will depend on the particular host, and nature and severity of the conditions for which the active ingredient is being administered. The pharmaceutical compositions can be conveniently presented in unit dosage form and prepared by any of the methods well known in the art of pharmacy.

[00251] Pharmaceutical compositions of the present invention suitable for parenteral administration can be prepared as solutions or suspensions of the active compounds in water. A suitable surfactant can be included such as, for example, hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof in oils. Further, a preservative can be included to prevent the detrimental growth of microorganisms.

[00252] Pharmaceutical compositions of the present invention suitable for injectable use include sterile aqueous solutions or dispersions. Furthermore, the compositions can be in the form of sterile powders for the extemporaneous preparation of such sterile injectable solutions or dispersions. In all cases, the final injectable form must be sterile and must be effectively fluid for easy syringability. The pharmaceutical compositions must be stable under the conditions of manufacture and storage; thus, preferably should be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (*e.g.*, glycerol, propylene glycol and liquid polyethylene glycol), vegetable oils, and suitable mixtures thereof.

[00253] Pharmaceutical compositions of the present invention can be in a form suitable for topical use such as, for example, an aerosol, cream, ointment, lotion, dusting powder, mouth

washes, gargles, and the like. Further, the compositions can be in a form suitable for use in transdermal devices. These formulations can be prepared, utilizing a compound of the invention, or pharmaceutically acceptable salts thereof, via conventional processing methods. As an example, a cream or ointment is prepared by mixing hydrophilic material and water, together with about 5 wt% to about 10 wt% of the compound, to produce a cream or ointment having a desired consistency.

[00254] Pharmaceutical compositions of this invention can be in a form suitable for rectal administration wherein the carrier is a solid. It is preferable that the mixture forms unit dose suppositories. Suitable carriers include cocoa butter and other materials commonly used in the art. The suppositories can be conveniently formed by first admixing the composition with the softened or melted carrier(s) followed by chilling and shaping in moulds.

[00255] In addition to the aforementioned carrier ingredients, the pharmaceutical formulations described above can include, as appropriate, one or more additional carrier ingredients such as diluents, buffers, flavoring agents, binders, surface-active agents, thickeners, lubricants, preservatives (including anti-oxidants) and the like. Furthermore, other adjuvants can be included to render the formulation isotonic with the blood of the intended recipient. Compositions containing a compound of the invention, and/or pharmaceutically acceptable salts thereof, can also be prepared in powder or liquid concentrate form.

[00256] In the treatment conditions which require modulation of cellular function related to muscle health, muscle function and/or healthy muscle aging an appropriate dosage level will generally be about 0.01 to 500 mg per kg patient body weight per day and can be administered in single or multiple doses. Preferably, the dosage level will be about 0.1 to about 250 mg/kg per day; more preferably 0.5 to 100 mg/kg per day. A suitable dosage level can be about 0.01 to 250 mg/kg per day, about 0.05 to 100 mg/kg per day, or about 0.1 to 50 mg/kg per day. Within this range the dosage can be 0.05 to 0.5, 0.5 to 5.0 or 5.0 to 50 mg/kg per day. For oral administration, the compositions are preferably provided in the from of tablets containing 1.0 to 1000 milligrams of the active ingredient, particularly 1.0, 5.0, 10, 15, 20, 25, 50, 75, 100, 150, 200, 250, 300, 400, 500, 600, 750, 800, 900 and 1000 milligrams of the active ingredient for the symptomatic adjustment of the dosage of the patient to be treated. The compound can be administered on a regimen of 1 to 4 times per day, preferably once or twice per day. This dosing regimen can be adjusted to provide the optimal therapeutic response.

[00257] It is understood, however, that the specific dose level for any particular patient

will depend upon a variety of factors. Such factors include the age, body weight, general health, sex, and diet of the patient. Other factors include the time and route of administration, rate of excretion, drug combination, and the type and severity of the particular disease undergoing therapy.

[00258] The present invention is further directed to a method for the manufacture of a medicament for modulating cellular activity related to muscle health, muscle function, and/or healthy aging muscles (*e.g.*, treatment of one or more disorders associated with muscle dysfunction or atrophy) in mammals (*e.g.*, humans) comprising combining one or more disclosed compounds, products, or compositions with a pharmaceutically acceptable carrier or diluent. Thus, in one aspect, the invention relates to a method for manufacturing a medicament comprising combining at least one disclosed compound or at least one disclosed product with a pharmaceutically acceptable carrier or diluent.

[00259] The disclosed pharmaceutical compositions can further comprise other therapeutically active compounds, which are usually applied in the treatment of the above mentioned pathological conditions.

[00260] It is understood that the disclosed compositions can be prepared from the disclosed compounds. It is also understood that the disclosed compositions can be employed in the disclosed methods of using.

D. METHODS OF USING THE COMPOUNDS AND COMPOSITIONS

1. MUSCLE ATROPHY

[00261] Muscle atrophy is defined as a decrease in the mass of the muscle; it can be a partial or complete wasting away of muscle. When a muscle atrophies, this leads to muscle weakness, since the ability to exert force is related to mass. Muscle atrophy is a co-morbidity of several common diseases, and patients who have "cachexia" in these disease settings have a poor prognosis.

[00262] Muscle atrophy can also be skeletal muscle loss or weakness caused by malnutrition, aging, muscle disuse (such as voluntary and involuntary bed rest, neurologic disease (such as multiple sclerosis, amyotrophic lateral sclerosis, spinal muscular atrophy, critical illness neuropathy, spinal cord injury, peripheral neuropathy, or peripheral nerve injury), injury to the limbs or joints, casting, other post-surgical forms of limb immobilization, or spaceflight), chronic disease (such as cancer, congestive heart failure, chronic pulmonary disease, chronic renal failure, chronic liver disease, diabetes mellitus, glucocorticoid excess, growth hormone deficiency, IGF-I deficiency, estrogen deficiency, and chronic infections such as HIV/AIDS or tuberculosis), burn injuries, sepsis, other

illnesses requiring mechanical ventiliation, drug-induced muscle disease (such as glucocorticoid-induced myopathy and statin-induced myopathy), genetic diseases that primarily affect skeletal muscle (such as muscular dystrophy, myotonic dystrophy and inclusion body myositis), or autoimmune diseases that affect skeletal muscle (such as polymyositis and dermatomyositis).

[00263] There are many diseases and conditions which cause muscle atrophy, including malnutrition, muscle disuse (secondary to voluntary or involuntary bed rest, neurologic disease (including multiple sclerosis, amyotrophic lateral sclerosis, spinal muscular atrophy, critical illness neuropathy, spinal cord injury or peripheral nerve injury), orthopedic injury, casting, and other post-surgical forms of limb immobilization), chronic disease (including cancer, congestive heart failure, chronic pulmonary disease, chronic renal failure, chronic liver disease, diabetes mellitus, Cushing syndrome, growth hormone deficiency, IGF-I deficiency, estrogen deficiency, and chronic infections such as HIV/AIDS or tuberculosis), burns, sepsis, other illnesses requiring mechanical ventilation, drug-induced muscle disease (such as glucorticoid-induced myopathy and statin-induced myopathy), genetic diseases that primarily affect skeletal muscle (such as muscular dystrophy and myotonic dystrophy), autoimmune diseases that affect skeletal muscle (such as polymyositis and dermatomyositis), spaceflight, and aging.

[00264] Muscle atrophy occurs by a change in the normal balance between protein synthesis and protein degradation. During atrophy, there is a down-regulation of protein synthesis pathways, and an activation of protein breakdown pathways. The particular protein degradation pathway which seems to be responsible for much of the muscle loss seen in a muscle undergoing atrophy is the ATP-dependent, ubiquitin/proteasome pathway. In this system, particular proteins are targeted for destruction by the ligation of at least four copies of a small peptide called ubiquitin onto a substrate protein. When a substrate is thus "poly-ubiquitinated," it is targeted for destruction by the proteasome. Particular enzymes in the ubiquitin/proteasome pathway allow ubiquitination to be directed to some proteins but not others - specificity is gained by coupling targeted proteins to an "E3 ubiquitin ligase." Each E3 ubiquitin ligase binds to a particular set of substrates, causing their ubiquitination. For example, in skeletal muscle, the E3 ubiquitin ligases atrogin-1 and MuRF1 are known to play essential roles protein degradation and muscle atrophy.

[00265] Muscle atrophy can be opposed by the signaling pathways which induce muscle hypertrophy, or an increase in muscle size. Therefore one way in which exercise induces an promote muscle health, promote normal muscle function, and/or promote healthy aging

muscles is to downregulate the pathways which have the opposite effect. One important rehabilitation tool for muscle atrophy includes the use of functional electrical stimulation to stimulate the muscles which has had limited success in the rehabilitation of paraplegic patients.

[00266] In certain aspects, the disclosed compounds can be used as a therapy for illness- and age-related muscle atrophy. It can be useful as a monotherapy or in combination with other strategies that have been considered, such as myostatin inhibition (Zhou, X., *et al.* (2010) Cell 142(4): 531-543). Given its capacity to reduce adiposity, fasting blood glucose and plasma lipid levels, a disclosed compound derivatives can also be used as a therapy for obesity, metabolic syndrome and type 2 diabetes.

[00267] The disclosed compounds can be used as single agents or in combination with one or more other drugs in the treatment, prevention, control, amelioration or reduction of risk of the aforementioned diseases, disorders and conditions for which compounds of formula I or the other drugs have utility, where the combination of drugs together are safer or more effective than either drug alone. The other drug(s) can be administered by a route and in an amount commonly used therefore, contemporaneously or sequentially with a disclosed compound. When a disclosed compound is used contemporaneously with one or more other drugs, a pharmaceutical composition in unit dosage form containing such drugs and the disclosed compound is preferred. However, the combination therapy can also be administered on overlapping schedules. It is also envisioned that the combination of one or more active ingredients and a disclosed compound will be more efficacious than either as a single agent.

[00268] Systemic administration of one or more disclosed compounds (e.g., by parenteral injection or by oral consumption) can be used to promote muscle health, promote normal muscle function, and/or promote healthy aging muscles, and reduce muscle atrophy in all muscles, including those of the limbs and the diaphragm. Local administration of a disclosed compound (by a topical route or localized injection) can be used to promote local muscle health, as can be required following a localized injury or surgery.

[00269] In one aspect, the subject compounds can be coadministered with agents that stimulate insulin signaling, IGFl signaling and/or muscle health including ursolic acid, insulin, insulin analogs, insulin-like growth factor l, metformin, thiazoladinediones, sulfonylureas, meglitinides, leptin, dipeptidyl peptidase-4 inhibitors, glucagon-like peptide-l agonists, tyrosine-protein phosphatase non-receptor type inhibitors, myostatin signaling inhibitors, beta-2 adrenergic agents including clenbuterol, androgens, selective androgen

receptor modulator (such as GTx-024, BMS-564,929, LGD-4033, AC-262,356, JNJ-28330835, LGD-2226, LGD-3303, S-40503, or S-23), aromatase inhibitors (such as anastrozole, letrozole, exemestane, vorozole, formestane, fadrozole, 4-hydroxyandrostenedione, 1,4,6-androstatrien-3,17-dione, and 4-androstene-3,6,17-trione), growth hormone, a growth hormone analog, ghrelin, a ghrelin analog. A disclosed compound or salt thereof can be administered orally, intramuscularly, intravenously or intraarterially. A disclosed compound or salt thereof can be substantially pure. A disclosed compound or salt thereof can be administered at about l0 mg/day to l0 g/day.

[00270] In another aspect, the subject compounds can be administered in combination with agents that stimulate ursolic acid, insulin, insulin analogs, insulin-like growth factor l, metformin, thiazoladinediones, sulfonylureas, meglitinides, leptin, dipeptidyl peptidase-4 inhibitors, glucagon-like peptide-l agonists, tyrosine-protein phosphatase non-receptor type inhibitors, myostatin signaling inhibitors, beta-2 adrenergic agents including clenbuterol, androgens, selective androgen receptor modulator (such as GTx-024, BMS-564,929, LGD-4033, AC-262,356, JNJ-28330835, LGD-2226, LGD-3303, S-40503, or S-23), aromatase inhibitors (such as anastrozole, letrozole, exemestane, vorozole, formestane, fadrozole, 4-hydroxyandrostenedione, 1,4,6-androstatrien-3,17-dione, and 4-androstene-3,6,17-trione), growth hormone, a growth hormone analog, ghrelin, or a ghrelin analog. A disclosed compound or salt thereof can be administered orally, intramuscularly, intravenously or intraarterially. A disclosed compound or salt thereof can be substantially pure. A disclosed compound or salt thereof can be administered at about l0 mg/day to l0 g/day.

[00271] The pharmaceutical compositions and methods of the present invention can further comprise other therapeutically active compounds as noted herein which are usually applied in the treatment of the above mentioned pathological conditions.

2. TREATMENT METHODS

[00272] The compounds disclosed herein are useful for treating, preventing, ameliorating, controlling or reducing the risk of a variety of muscle disorders. Examples of such muscle disorders include, but are not limited to, skeletal muscle atrophy secondary to malnutrition, muscle disuse (secondary to voluntary or involuntary bedrest), neurologic disease (including multiple sclerosis, amyotrophic lateral sclerosis, spinal muscular atrophy, critical illness neuropathy, spinal cord injury or peripheral nerve injury), orthopedic injury, casting, and other post-surgical forms of limb immobilization, chronic disease (including cancer, congestive heart failure, chronic pulmonary disease, chronic renal failure, chronic liver

disease, diabetes mellitus, Cushing syndrome and chronic infections such as HIV/AIDS or tuberculosis), burns, sepsis, other illnesses requiring mechanical ventiliation, drug-induced muscle disease (such as glucorticoid-induced myopathy and statin-induced myopathy), genetic diseases that primarily affect skeletal muscle (such as muscular dystrophy and myotonic dystrophy), autoimmune diseases that affect skeletal muscle (such as polymyositis and dermatomyositis), spaceflight, or age-related sarcopenia. In still further aspects, the invention is related to methods to modulate muscle health, methods to inhibit muscle atrophy.

[00273] Thus, provided is a method for treating or preventing muscle atrophy, comprising: administering to a subject at least one disclosed compound; at least one disclosed pharmaceutical composition; and/or at least one disclosed product in a dosage and amount effective to treat the disorder in the subject.

[00274] Also provided is a method for promoting muscle health, promote normal muscle function, and/or promote healthy aging muscles comprising: administering to a subject at least one disclosed compound; at least one disclosed pharmaceutical composition; and/or at least one disclosed product in a dosage and amount effective to treat the disorder in the subject.

[00275] The compounds disclosed herein are useful for treating, preventing, ameliorating, controlling or reducing the risk of a variety of metabolic disorders. In a further aspect, the disclosed compounds in treating disorders associated with a dysfunction of insulin/IGF-I signaling. Thus, are provided methods to increase insulin/IGF-I signaling, methods to reduce body fat; methods to reduce blood glucose, methods to reduce blood triglycerides, methods to reduce blood cholesterol, methods to reduce obesity, methods to reduce fatty liver disease, and methods to reduce diabetes, and pharmaceutical compositions comprising compounds used in the methods.

a. TREATING MUSCLE ATROPHY

[00276] Disclosed herein is a method of treating muscle atrophy in an animal comprising administering to the animal an effective amount of a compound. The compound can be selected from a tacrine and analogs, naringenin and analogs, allantoin and analogs, conessine and analogs, tomatidine and analogs, ungerine/hippeastrine and analogs, and betulinic acid and analogs, or a mixture thereof. For example, the compound can be a tacrine analog. In another example, the compound can be a naringenin analog. In another example, the compound can be an allantoin analog. In another example, the compound can be a conessine analog. In another example, the compound can be a tomatidine analog. In another example, the compound can be a ungerine/hippeastrine analog. In another example, the compound can

be a betulinic acid analog.

[00277] In one aspect, the compound is administered in an amount between about 0.01 to 500 mg per kg patient body weight per day and can be administered in single or multiple doses. Preferably, the dosage level will be about 0.1 to about 250 mg/kg per day; more preferably 0.5 to 100 mg/kg per day. A suitable dosage level can be about 0.01 to 250 mg/kg per day, about 0.05 to 100 mg/kg per day, or about 0.1 to 50 mg/kg per day. Within this range the dosage can be 0.05 to 0.5, 0.5 to 5.0 or 5.0 to 50 mg/kg per day. For oral administration, the compositions are preferably provided in the from of tablets containing 1.0 to 1000 milligrams of the active ingredient, particularly 1.0, 5.0, 10, 15, 20, 25, 50, 75, 100, 150, 200, 250, 300, 400, 500, 600, 750, 800, 900 and 1000 milligrams of the active ingredient for the symptomatic adjustment of the dosage of the patient to be treated. The compound can be administered on a regimen of 1 to 4 times per day, preferably once or twice per day. This dosing regimen can be adjusted to provide the optimal therapeutic response

[00278] In one aspect, the disclosed compounds inhibit muscle atrophy. In a further aspect, the disclosed compounds promote muscle health, promote normal muscle function, and/or promote healthy aging muscles. In a yet further aspect, the disclosed compounds inhibit of muscle atrophy and promoting muscle health, promoting normal muscle function, and/or promoting healthy aging muscles. In an even further aspect, the disclosed compounds inhibit of muscle atrophy.

[00279] In a further aspect, the compound administered is a disclosed compound or a product of a disclosed method of making a compound. In a yet further aspect, the invention relates to a pharmaceutical composition comprising at least one compound as disclosed herein.

[00280] In a further aspect, the compound is co-administered with an anabolic agent. In a further aspect, wherein the compound is co-administered with ursolic acid or a ursolic acid derivative.

[00281] In a further aspect, the animal is a mammal, fish or bird. In a yet further aspect, the mammal is a primate. In a still further aspect, the mammal is a human. In an even further aspect, the human is a patient.

[00282] In a further aspect, the Muscle Atrophy Signature is Muscle Atrophy Signature 1. In a still further aspect, the Muscle Atrophy Signature is Muscle Atrophy Signature 2.

[00283] In a further aspect, prior to the administering step the mammal has been diagnosed with a need for treatment of a disorder selected muscle atrophy, diabetes, obesity, and fatty liver disease. In a yet further aspect, the disorder is muscle atrophy.

[00284] In a further aspect, prior to the administering step the mammal has been diagnosed with a need for treatment of a disorder associated with a dysfunction in insulin/IGF-I signaling.

[00285] In a further aspect, the treatment of the disorder increases muscle IGF-I signaling. In a still further aspect, the treatment of the disorder increases muscle IGF-I production.

[00286] In a further aspect, prior to the administering step the mammal has been diagnosed with a need for treatment of a disorder associated with circulating levels of leptin. In a still further aspect, the treatment decreases the circulating levels of leptin.

[00287] In a further aspect, administration the methods are promoting muscle health, , promoting normal muscle function, and/or promoting healthy aging muscles in the mammal. In a yet further aspect, administration increases energy expenditure. In a still further aspect, increases brown fat. In an even further aspect, administration increases the ratio of brown fat to white fat. In a still further aspect, administration increases the ratio of skeletal muscle to fat. In a yet further aspect, the compound is co-administered with a disclosed compound or a derivative thereof.

[00288] In a further aspect, the animal is a domesticated animal. In a still further aspect, the domesticated animal is a domesticated fish, domesticated crustacean, or domesticated mollusk. In a yet further aspect, the domesticated animal is poultry. In an even further aspect, the poultry is selected from chicken, turkey, duck, and goose. In a still further aspect, the domesticated animal is livestock. In a yet further aspect, the livestock animal is selected from pig, cow, horse, goat, bison, and sheep.

[00289] In a further aspect, the effective amount is a therapeutically effective amount. In a still further aspect, the effective amount is a prophylactically effective amount. In a yet further aspect, muscle atrophy is prevented by administration of the compound. In an even further aspect, muscle atrophy is treated by administration of the compound. In a still further aspect, the method further comprises the step of identifying the mammal in need of treatment of muscle atrophy. In a yet further aspect, the method further comprises the step of identifying the mammal in a need of prevention of muscle atrophy. In an even further aspect, the mammal has been diagnosed with a need for treatment of muscle atrophy prior to the administering step.

b. PROMOTING MUSCLE HEALTH

[00290] In one aspect, the invention relates to a method for promoting muscle health, promoting normal muscle function, and/or promoting healthy aging muscles in an animal, the method comprising administering to the animal an effective amount of a compound selected

from a tacrine and analogs, naringenin and analogs, allantoin and analogs, conessine and analogs, tomatidine and analogs, ungerine/hippeastrine and analogs, and betulinic acid and analogs, or a mixture thereof, , thereby promoting muscle health in the animal. For example, the compound can be a tacrine analog. In another example, the compound can be a naringenin analog. In another example, the compound can be an allantoin analog. In another example, the compound can be a conessine analog. In another example, the compound can be a tomatidine analog. In another example, the compound can be a ungerine/hippeastrine analog. In another example, the compound can be a betulinic acid analog. In one aspect, the invention relates to a method for promoting muscle health. In another aspect, the invention relates to a method for promoting normal muscle function. In another aspect, the invention relates to a method for promoting healthy aging muscles.

[00291] In one aspect, the invention relates to a method for promoting muscle health, promoting normal muscle function, and/or promoting healthy aging muscles in an animal, the method comprising administering to the animal an effective amount of a compound, wherein the compound down regulates at least one of the induced mRNAs of Muscle Atrophy Signature 1 or Muscle Atrophy Signature 2, compared to expression levels in the same type of the muscle cell in the absence of the compound, and/or wherein the compound up regulates at least one of the repressed mRNAs of Muscle Atrophy Signature 1 or Muscle Atrophy Signature 2, compared to expression levels in the same type of the muscle cell in the absence of the compound, thereby promoting muscle health, promoting normal muscle function, and/or promoting healthy aging muscles in the animal.

[00292] In a further aspect, the animal is a mammal, fish or bird. In a yet further aspect, the mammal is a primate. In a still further aspect, the mammal is a human. In an even further aspect, the human is a patient.

[00293] In a further aspect, the Muscle Atrophy Signature is Muscle Atrophy Signature 1. In a still further aspect, the Muscle Atrophy Signature is Muscle Atrophy Signature 2.

[00294] In a further aspect, prior to the administering step the mammal has been diagnosed with a need for treatment of a disorder selected muscle atrophy, diabetes, obesity, and fatty liver disease. In a yet further aspect, the disorder is muscle atrophy.

[00295] In a further aspect, prior to the administering step the mammal has been diagnosed with a need for treatment of a disorder associated with a dysfunction in insulin/IGF-I signaling.

[00296] In a further aspect, the treatment of the disorder increases muscle IGF-I signaling. In a still further aspect, the treatment of the disorder increases muscle IGF-I production.

[00297] In a further aspect, prior to the administering step the mammal has been diagnosed with a need for treatment of a disorder associated with circulating levels of leptin. In a still further aspect, the treatment decreases the circulating levels of leptin.

[00298] In a further aspect, administration promoting muscle health, promoting normal muscle function, and/or promoting healthy aging muscles in the mammal. In a yet further aspect, administration increases energy expenditure. In a still further aspect, increases brown fat. In an even further aspect, administration increases the ratio of brown fat to white fat. In a still further aspect, administration increases the ratio of skeletal muscle to fat. In a yet further aspect, the compound is co-administered with a disclosed compound or a derivative thereof.

[00299] In a further aspect. the animal is a domesticated animal. In a still further aspect, the domesticated animal is a domesticated fish, domesticated crustacean, or domesticated mollusk. In a yet further aspect, the domesticated animal is poultry. In an even further aspect, the poultry is selected from chicken, turkey, duck, and goose. In a still further aspect, the domesticated animal is livestock. In a yet further aspect, the livestock animal is selected from pig, cow, horse, goat, bison, and sheep.

[00300] In a further aspect, the effective amount is a therapeutically effective amount. In a still further aspect, the effective amount is a prophylactically effective amount. In a yet further aspect, muscle atrophy is prevented by administration of the compound. In an even further aspect, muscle atrophy is treated by administration of the compound. In a still further aspect, the method further comprises the step of identifying the mammal in need of treatment of muscle atrophy. In a yet further aspect, the method further comprises the step of identifying the mammal in a need of prevention of muscle atrophy. In an even further aspect, the mammal has been diagnosed with a need for treatment of muscle atrophy prior to the administering step.

c. Enhancing Muscle Formation

[00301] In one aspect, the invention relates to a method of enhancing muscle formation in a mammal, the method comprising administering to the animal an effective amount of a compound selected from a tacrine and analogs, naringenin and analogs, allantoin and analogs, conessine and analogs, tomatidine and analogs, ungerine/hippeastrine and analogs, and betulinic acid and analogs, or a mixture thereof, thereby promoting muscle health, promoting normal muscle function, and/or promoting healthy aging muscles in the animal. For example, the compound can be a tacrine analog. In another example, the compound can be a naringenin analog. In another example, the compound can be an allantoin analog. In another

example, the compound can be a conessine analog. In another example, the compound can be a tomatidine analog. In another example, the compound can be a ungerine/hippeastrine analog. In another example, the compound can be a betulinic acid analog.

[00302] In a further aspect, the invention relates to a method of enhancing muscle formation in a mammal, the method comprising administering to the animal an effective amount of a compound, wherein the compound down regulates at least one of the induced mRNAs of Muscle Atrophy Signature 1 or Muscle Atrophy Signature 2, compared to expression levels in the same type of the muscle cell in the absence of the compound, and/or wherein the compound up regulates at least one of the repressed mRNAs of Muscle Atrophy Signature 1 or Muscle Atrophy Signature 2, compared to expression levels in the same type of the muscle cell in the absence of the compound, thereby promoting muscle health, promoting normal muscle function, and/or promoting healthy aging muscles in the animal.

[00303] In a further aspect, the mammal is a human. In a still further aspect, the human is a patient. In a yet further aspect, administration of the compound prevents muscle atrophy in the mammal. In an even further aspect, administration of the compound treats muscle atrophy in the mammal. In a still further aspect, administration of the compound promote muscle health, promote normal muscle function, and/or promote healthy aging muscles in the mammal.

[00304] In a further aspect, the compound is administered in an effective amount. In a yet further aspect, the effective amount is a therapeutically effective amount. In a still further aspect, the effective amount is a prophylactically effective amount. In a still further aspect, the method further comprises the step of identifying the mammal in need of treatment of muscle atrophy. In a yet further aspect, the method further comprises the step of identifying the mammal in need of prevention of muscle atrophy. In an even further aspect, the mammal has been diagnosed with a need for treatment of muscle atrophy prior to the administering step.

[00305] In a further aspect. the mammal is a domesticated animal. In a yet further aspect, domesticated animal is livestock. In a yet further aspect, the livestock animal is selected from pig, cow, horse, goat, bison, and sheep.

3. FACILITATING TISSUE FORMATION *IN VITRO*

[00306] In one aspect, the invention relates to a method of enhancing tissue health *in vitro*, the method comprising administering to the tissue an effective amount of a compound wherein the compound down regulates at least one of the induced mRNAs of Muscle Atrophy Signature 1 or Muscle Atrophy Signature 2, compared to expression levels in the same type

of the muscle cell in the absence of the compound, and/or wherein the compound up regulates at least one of the repressed mRNAs of Muscle Atrophy Signature 1 or Muscle Atrophy Signature 2, compared to expression levels in the same type of the muscle cell in the absence of the compound, thereby promoting muscle health, promoting normal muscle function, and/or promoting healthy aging muscles.

[00307] In a further aspect, the compound administered is a disclosed compound. In a further aspect, the compound is selected from a tacrine and analogs, naringenin and analogs, allantoin and analogs, conessine and analogs, tomatidine and analogs, ungerine/hippeastrine and analogs, and betulinic acid and analogs, or a mixture thereof, thereby facilitating tissue formation in vitro. For example, the compound can be a tacrine analog. In another example, the compound can be a naringenin analog. In another example, the compound can be an allantoin analog. In another example, the compound can be a conessine analog. In another example, the compound can be a tomatidine analog. In another example, the compound can be a ungerine/hippeastrine analog. In another example, the compound can be a betulinic acid analog.

[00308] In a further aspect, the tissue comprises animal cells. In a still further aspect, the animal cells are muscle cells. In a yet further aspect, the muscle cells are skeletal muscle stem or progenitor cells. In an even further aspect, the skeletal muscle stem or progenitor cells are grown on a scaffold.

4. MANUFACTURE OF A MEDICAMENT

[00309] In one aspect, the invention relates to a method for the manufacture of a medicament for inhibiting muscle atrophy and for promoting muscle health, promoting normal muscle function, and/or promoting healthy aging muscles in a mammal comprising combining a therapeutically effective amount of a disclosed compound or product of a disclosed method with a pharmaceutically acceptable carrier or diluent.

[00310] In one aspect, the invention relates to a method for manufacturing a medicament associated with muscle atrophy or the need to promote muscle health, promote normal muscle function, and/or promote healthy aging muscles, the method comprising the step of combining an effective amount of one or more of: (a) a compound selected from tacrine analog, naringenin analog, allantoin analog, conessine analog, tomatidine analog, ungerine/hippeastrine analog and betulinic acid analog, or a mixture thereof; (b) a compound that down regulates multiple induced mRNAs of Muscle Atrophy Signature 1, compared to expression levels in the same type of the muscle cell in the absence of the compound; (c) a compound that up multiple repressed mRNAs of Muscle Atrophy Signature 1, compared to

expression levels in the same type of the muscle cell in the absence of the compound; (d) a compound that down regulates multiple induced mRNAs of Muscle Atrophy Signature 2, compared to expression levels in the same type of the muscle cell in the absence of the compound; and/or (e) a compound that up regulates at least one of the repressed mRNAs of Muscle Atrophy Signature 2, compared to expression levels in the same type of the muscle cell in the absence of the compound, with a pharmaceutically acceptable carrier or diluent.

[00311] In a further aspect, the medicament comprises a disclosed compound. In a still further aspect, the compound is selected from a tacrine and analogs, naringenin and analogs, allantoin and analogs, conessine and analogs, tomatidine and analogs, ungerine/hippeastrine and analogs, and betulinic acid and analogs, or a mixture thereof. For example, the compound can be a tacrine analog. In another example, the compound can be a naringenin analog. In another example, the compound can be an allantoin analog. In another example, the compound can be a conessine analog. In another example, the compound can be a tomatidine analog. In another example, the compound can be a ungerine/hippeastrine analog. In another example, the compound can be a betulinic acid analog.

[00312] In a further aspect, the medicament is modulates muscle health. In a still further aspect, the medicament inhibits muscle atrophy. In a yet further aspect, the medicament promote muscle health, promote normal muscle function, and/or promote healthy aging muscles.

5. KITS

[00313] Also disclosed herein are kit comprising a tacrine analog, naringenin analog, allantoin analog, conessine analog, tomatidine analog, ungerine/hippeastrine analog and betulinic acid analog, or a mixture thereof, and one or more of: a) at least one agent known to treat muscle atrophy in an animal; b) at least one agent known to decrease the risk of obtaining muscle atrophy in an animal; c) at least one agent known to have a side effect of muscle atrophy; d) instructions for treating muscle atrophy; or e) at least one anabolic agent.. For example, the compound can be a tacrine analog. In another example, the compound can be a naringenin analog. In another example, the compound can be a allantoin analog. In another example, the compound can be a conessine analog. In another example, the compound can be a tomatidine analog. In another example, the compound can be a ungerine/hippeastrine analog. In another example, the compound can be a betulinic acid analog.

[00314] In one aspect, the kit further comprises at least one agent, wherein the compound and the agent are co-formulated.

[00315] In another aspect, the compound and the agent are co-packaged. The agent can be any agent as disclosed herein, such as anabolic agent, agent known to have a side effect of muscle atrophy, agent known to decrease the risk of obtaining muscle atrophy in an animal, or agent known to treat muscle atrophy in an animal.

[00316] In one aspect, the invention relates to a kit comprising an effective amount of one or more of: (a) a compound selected from a tacrine analog, naringenin analog, allantoin analog, conessine analog, tomatidine analog, ungerine/hippeastrine analog and betulinic acid analog; (b) a compound that down regulates multiple induced mRNAs of Muscle Atrophy Signature 1, compared to expression levels in the same type of the muscle cell in the absence of the compound; (c) a compound that up regulates multiple repressed mRNAs of Muscle Atrophy Signature 1, compared to expression levels in the same type of the muscle cell in the absence of the compound; (d) a compound that down regulates multiple induced mRNAs of Muscle Atrophy Signature 2, compared to expression levels in the same type of the muscle cell in the absence of the compound; and/or (e) a compound that up regulates multiple repressed mRNAs of Muscle Atrophy Signature 2, compared to expression levels in the same type of the muscle cell in the absence of the compound, (f) and one or more of: (i) a protein supplement; (ii) an anabolic agent; (iii) a catabolic agent; (iv) a dietary supplement; (v) at least one agent known to treat a disorder associated with muscle wasting; (vi) instructions for treating a disorder associated with cholinergic activity; or (vii) instructions for using the compound to promote muscle health, promote normal muscle function, and/or promote healthy aging muscles.

[00317] The kits can also comprise compounds and/or products co-packaged, co-formulated, and/or co-delivered with other components. For example, a drug manufacturer, a drug reseller, a physician, a compounding shop, or a pharmacist can provide a kit comprising a disclosed compound and/or product and another component for delivery to a patient.

[00318] It is contemplated that the disclosed kits can be used in connection with the disclosed methods of making, the disclosed methods of using, and/or the disclosed compositions.

6. METHOD OF LOWERING BLOOD GLUCOSE

[00319] In one aspect, the invention relates to a method of lowering blood glucose in an animal comprising administering to the animal an effective amount of a composition comprising ursolic acid and a naringenin analog, thereby lowering the blood glucose in the animal. In one aspect, the naringenin analog can be naringenin. In one aspect, the ursolic acid can be a ursolic acid derivative.

[00320] In another aspect, invention relates to a method of lowering blood glucose in an animal comprising administering to the animal an effective amount of a hippeastrine analog, thereby lowering the blood glucose in the animal. In one aspect, the hippeastrine analog can be hippeastrine.

[00321] In another aspect, invention relates to a method of lowering blood glucose in an animal comprising administering to the animal an effective amount of a conessine analog, thereby lowering the blood glucose in the animal. In one aspect, the conessine analog can be conessine.

[00322] In a further aspect, the animal is a mammal, fish or bird. In a yet further aspect, the mammal is a primate. In a still further aspect, the mammal is a human. In an even further aspect, the human is a patient.

[00323] In a further aspect, prior to the administering step the mammal has been diagnosed with a need for treatment of a disorder associated with the need of lowering blood glucose. .

[00324] In a further aspect, prior to the administering step the mammal has been diagnosed with a need for treatment of a disorder associated with a dysfunction in insulin/IGF-I signaling.

[00325] In a further aspect, the treatment of the disorder increases muscle IGF-I signaling. In a still further aspect, the treatment of the disorder increases muscle IGF-I production.

[00326] In a further aspect, prior to the administering step the mammal has been diagnosed with a need for treatment of a disorder associated with circulating levels of leptin. In a still further aspect, the treatment decreases the circulating levels of leptin.

[00327] In a further aspect. the animal is a domesticated animal. In a still further aspect, the domesticated animal is a domesticated fish, domesticated crustacean, or domesticated mollusk. In a yet further aspect, the domesticated animal is poultry. In an even further aspect, the poultry is selected from chicken, turkey, duck, and goose. In a still further aspect, the domesticated animal is livestock. In a yet further aspect, the livestock animal is selected from pig, cow, horse, goat, bison, and sheep.

[00328] In a further aspect, the effective amount is a therapeutically effective amount. In a still further aspect, the effective amount is a prophylactically effective amount. In a yet further aspect, high blood glucose is prevented by administration of the compound. In a still further aspect, the method further comprises the step of identifying the mammal in need of treatment of lowering of blood glucose. In a yet further aspect, the method further comprises the step of identifying the mammal in a need of prevention the need of lowering blood glucose. In an even further aspect, the mammal has been diagnosed with a need for lowering

of blood glucose prior to the administering step.

7. IDENTIFICATION OF COMPOUNDS THAT INHIBIT MUSCLE ATROPHY

[00329] Also disclosed are methods for identifying a compound that inhibits muscle atrophy when administered in a effective amount to a animal in need of treatment thereof, the method comprising the steps of:(i) selecting a candidate compound; (ii) determining the effect of the candidate compound on a cell's expression levels of a plurality of induced mRNAs and/or repressed mRNAs of a Muscle Atrophy Signature, wherein the candidate compound is identified as suitable for muscle atrophy inhibition if: (a) more than one of the induced mRNAs of the Muscle Atrophy Signature are down regulated, compared to expression levels of the induced mRNAs of the Muscle Atrophy Signature in the same type of cell in the absence of the candidate compound; and/or (b) more than one of the repressed mRNAs of the Muscle Atrophy Signature are up regulated, compared to expression levels of the repressed mRNAs of the Muscle Atrophy Signature in the same type of cell in the absence of the candidate compound. In one aspect, the method further comprises administering the candidate compound to an animal. In yet another aspect, the method further comprises writing a report. In yet another aspect, the method further comprises reporting the results. In yet another aspect, the method further comprises performing further tests on the candidate compound, such as confirmatory tests. In yet another aspect, the method further comprises performing toxicity studies on the candidate compound.

[00330] In a further aspect, the candidate compound comprises a disclosed compound. In a still further aspect, the compound is selected from a tacrine analog, naringenin analog, allantoin analog, conessine analog, tomatidine analog, ungerine/hippeastrine analog and betulinic acid analog, as defined elsewhere herein. For example, the compound can be a tacrine analog. In another example, the compound can be a naringenin analog. In another example, the compound can be an allantoin analog. In another example, the compound can be a conessine analog. In another example, the compound can be a tomatidine analog. In another example, the compound can be a ungerine/hippeastrine analog. In another example, the compound can be a betulinic acid analog.

[00331] In a further aspect, the animal is a mammal, fish or bird. In a yet further aspect, the mammal is a primate. In a still further aspect, the mammal is a human. In an even further aspect, the human is a patient.

[00332] In a further aspect, the Muscle Atrophy Signature is Muscle Atrophy Signature 1. In a still further aspect, the Muscle Atrophy Signature is Muscle Atrophy Signature 2.

[00333] In a further aspect, the Muscle Atrophy Signature is determined according to steps

comprising: a) determining mRNA expression levels in a muscle cell undergoing muscle atrophy, b) determining mRNA expression levels in a muscle cell not undergoing muscle atrophy, wherein an mRNA is determined to be part of the Muscle Atrophy Signature if: (a0 the mRNA is up regulated in the muscle cell undergoing muscle atrophy compared to the muscle cell not undergoing muscle atrophy, or (b) the mRNA is down regulated in the muscle cell undergoing muscle atrophy compared to the muscle cell not undergoing muscle atrophy.

[00334] In one aspect, the muscle cell undergoing atrophy and the muscle cell not undergoing atrophy are harvested from an animal. In another aspect, the muscle cell undergoing atrophy is harvested while the animal is in a state of fasting and the muscle cell not undergoing atrophy is harvested prior to the state of fasting. In yet another aspect, the muscle cell undergoing atrophy is harvested from an immobilized muscle and the muscle cell not undergoing atrophy is harvested from a mobile muscle. In yet another aspect, the muscle cell undergoing atrophy is harvested from an animal with spinal cord injury and the muscle cell not undergoing atrophy is harvested from a muscle that has received electrical stimulation. In yet another aspect, the Muscle Atrophy Signature is determined by selecting mRNAs commonly up regulated or commonly down regulated between two or more of the Muscle Atrophy Signatures of the methods described herein.

[00335] In a further aspect, the invention relates to a method for inhibiting muscle atrophy in a mammal, the method comprising administering to the mammal a therapeutically effective amount of a compound of identified using the method described above.

8. Non-Medical Uses

[00336] Also provided are the uses of the disclosed compounds and products as pharmacological tools in the development and standardization of *in vitro* and *in vivo* test systems for the evaluation of the effects of inhibitors of muscle atrophy related activity in laboratory animals such as cats, dogs, rabbits, monkeys, rats, fish, birds, and mice, as part of the search for new therapeutic agents of promoting muscle health, promoting normal muscle function, and/or promoting healthy aging muscles.

E. Experimental

[00337] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how the compounds, compositions, articles, devices and/or methods claimed herein are made and evaluated, and are intended to be purely exemplary of the invention and are not intended to limit the scope of what the inventors regard as their invention. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are

disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

[00338] Efforts have been made to ensure accuracy with respect to numbers (*e.g.*, amounts, temperature, etc.), but some errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in °C or is at ambient temperature, and pressure is at or near atmospheric.

[00339] Certain materials, reagents and kits were obtained from specific vendors as indicated below, and as appropriate the vendor catalog, part or other number specifying the item are indicated. Vendors indicated below are as follows: "Ambion" is Ambion, a division of Life Technologies Corporation, Austin, Texas, USA; "Applied Biosystems" is Applied Biosystems, a division of Life Technologies Corporation, Carlsbad, California, USA; "Boehringer Mannheim" is Boehringer Mannheim Corporatin, Indiapolis, Indiana, USA; "CardinalHealth" is Cardinal Health, Inc., Dublin, Ohio, USA; "Cell Signaling" is Cell Signaling Technology, Inc., Beverly, Massachussetts, USA; "Columbus Inst" is Columbus Instruments International, Columbus, Ohio, USA; "Harlan" is Harlan Laboratories, Indianapolis, Indiana, USA; "Instrumedics" is Instrumedics, Inc., Richmond, Illinois, USA; "Invitrogen" is Invitrogen Corporation, Carlsbad, California, USA; "Microm" is the Microm division (Walldorf, Germany) of Thermo Fisher Scientific Inc., Rockford, Illinois, USA; "Millipore" is Millipore Corporation, Billerica, Massachussetts, USA; a division of Merck KGaA, Darmstadt, Germany; "Ortho" is Ortho Clinical Diagnostics, Rochester, New York, USA; "Pierce" is Pierce Biotechnology, Inc., Milwaukee, Wisconsin, USA, a division of Thermo Fisher Scientific, Inc.; "R&D Systems" is R&D Systems Inc., Minneapolis, Minnesota, USA; "Roche Diagnostics" is Roche Diagnostics Corporation, Indianapolis, Indiana, USA; "Sakura" is Sakura Finetek USA, Inc., Torrance, California, USA; "Santa Cruz" is Santa Cruz Biotechnology, Inc., Santa Cruz, California, USA; and, "Sigma" is Sigma-Aldrich Corporation, Saint Louis, Missouri, USA.

1. General Methods

a. Human Subject Protocol.

[00340] The study referred to herein was approved by the Institutional Review Board at the University of Iowa, and involved seven healthy adults who gave their informed consent before participating. One week prior to the fasting study, subjects made one visit to the Clinical Research Unit ("CRU") for anthropometric measurements, a dietary interview that established each subject's routine food intake and food preferences, and baseline

determinations of blood hemoglobin ("Hb") A1c turbidimetric immunoinhibition using the BM/Hitachi 911 analyzer (Boehringer Mannheim); plasma triglycerides and plasma free T4 and TSH by electrochemiluminescence immunoassay using the Elecsys® System (Roche Diagnostics); plasma CRP by immuno-turbidimetric assay using the Roche Cobas Integra® high-sensitivity assay (Roche Diagnostics); and, plasma TNF-α levels using the Quantikine® Kit (R&D Systems). To ensure that subjects were eating their routine diet prior to the fasting study, subjects ate only meals prepared by the CRU dietician (based on the dietary interview) for 48 hours before the fasting study. The fasting study began at t = 0 hours, when subjects were admitted to the CRU and began fasting. While fasting, subjects remained in the CRU and were encouraged to maintain their routine physical activities. Water was allowed ad libitum, but caloric intake was not permitted. At about 40 hours, a percutaneous biopsy was taken from the vastus lateralis muscle using a Temno® Biopsy Needle (CardinalHealth; Cat # T1420) under ultrasound guidance. Subjects then ate a CRU-prepared mixed meal, and at t = 46 hours, a muscle biopsy was taken from the contralateral vastus lateralis muscle. Plasma glucose and insulin levels were measured at t = 36, 40, 42 and 46 hours; the Elecsys® system was used to quantitate plasma insulin. Our study protocol of humans with spinal cord injury was described previously (Adams CM, *et al.* (2011) *Muscle Nerve*. 43(1):65-75).

b. MICROARRAY ANALYSIS OF HUMAN SKELETAL MUSCLE MRNA LEVELS.

[00341] Following harvest, skeletal muscle samples were immediately placed in RNAlater (Ambion) and stored at -80 °C until further use. Total RNA was extracted using TRIzol solution (Invitrogen), and microarray hybridizations were performed at the University of Iowa DNA Facility, as described previously (Lamb J, *et al.* (2006) *Science (New York, N.Y* 313(5795):1929-1935). The $\log_2$ hybridization signals as shown herein reflect the mean signal intensity of all exon probes specific for an individual mRNA. To determine which human skeletal muscle mRNAs were significantly altered by fasting ($P \leq 0.02$), paired t-tests were used to compare fasted and fed $\log_2$ signals. To determine which mouse skeletal muscle mRNAs were significantly altered by ursolic acid ($P \leq 0.005$), unpaired t-tests were used to compare $\log_2$ signals in mice fed control diet or diet supplemented with ursolic acid. Highly expressed mRNAs were defined as those significantly altered mRNAs that were repressed from or induced to a $\log_2$ signal > 8. These raw microarray data from humans and mice have been deposited in NCBI's Gene Expression Omnibus ("GEO") and are accessible through GEO Series accession numbers GSE28016 and GSE28017, respectively. Exon array studies

of the effects of fasting on mouse skeletal muscle, and the effects of spinal cord injury on human skeletal muscle were described previously (Adams CM, *et al.* (2011) *Muscle & nerve* 43(1):65-75; Ebert SM, *et al.* (2010) *Molecular Endocrinology* 24(4):790-799).

c. QUANTITATIVE REAL-TIME RT-PCR (QPCR).

[00342] TRIzol-extracted mRNA was treated with DNase I using the Turbo DNA-*free* kit (Ambion). qPCR analysis of human mRNA and mouse *IGF-I* mRNA was performed using TaqMan Gene Expression Assays (Applied Biosystems). First strand cDNA was synthesized from 2 μg of RNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Part No. 4368814). The real time PCR contained, in a final volume of 20 μl, 20 ng of reverse transcribed RNA, 1 μl of 20X TaqMan Gene Expression Assay, and 10 μl of TaqMan Fast Universal PCR Master Mix (Applied Biosystems; Part No. 4352042). qPCR was carried out using a 7500 Fast Real-Time PCR System (Applied Biosystems) in 9600 emulation mode. qPCR analysis of mouse *atrogin-1* and *MuRF1* mRNA levels was performed as previously described (Ebert SM, *et al.* (2010) *Molecular Endocrinology* 24(4):790-799). All qPCR reactions were performed in triplicate and the cycle threshold (Ct) values were averaged to give the final results. To analyze the data, the ΔCt method was used, with the level of *36B4* mRNA serving as the invariant control.

d. MOUSE PROTOCOLS.

[00343] Male C57BL/6 mice, ages 6-8 weeks, were obtained from NCI, housed in colony cages with 12h light/12h dark cycles, and used for experiments within 3 weeks of their arrival. Unless otherwise indicated, mice were maintained on standard chow (Harlan; Teklad Diet, Formula 7013, NIH-31 Modified Open Formula Mouse/Rat Sterilizable Diet). Metformin (Sigma) was dissolved in 0.9% NaCl at a concentration of 250 mg / ml. Ursolic acid (Enzo Life Sciences) was dissolved in corn oil at a concentration of 200 mg / ml (for i.p. injections); alternatively, the ursolic acid was added directly to standard chow (Harlan; Teklad Diet, Formula 7013) or standard high fat diet (Harlan; Teklad Diet, Formula TD.93075) as a customized chow. Oleanolic acid (Sigma) was dissolved in corn oil at a concentration of 200 mg / ml. Mice were fasted by removing food, but not water, for 24 hours. Fasting blood glucose levels were obtained from the tail vein with an ACCU-CHEK® Aviva glucose meter (Roche Diagnostics). Unilateral hindlimb muscle denervation was performed by transsecting the sciatic nerve under anesthesia, and was followed by administration of ursolic acid (200 mg / kg) or vehicle alone (corn oil) via i.p injection twice daily for 7 days. Forelimb grip strength was determined using a grip strength meter equipped with a triangular pull bar (Columbus Inst). Each mouse was subjected to 5 consecutive tests

to obtain the peak value. Plasma IGF-I and leptin levels were measured by RIA at the Vanderbilt University Hormone Assay Core Facility. Plasma cholesterol, triglyceride, creatinine, bilirubin and ALT were measured using the VITROS® 350 Chemistry System (Ortho). All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Iowa.

e. HISTOLOGICAL ANALYSIS.

[00344] Following harvest, tissues were immediately placed in isopentane that had been chilled to -160 ºC with liquid N_2. Muscles were embedded in tissue freezing medium, and 10 μm sections from the mid-belly were prepared using a Microm HM 505 E cryostat equipped with a CryoJane sectioning system (Instrumedics). Adipose tissue was fixed in 10% neutral buffered formalin, embedded in paraffin, and then 4 μm sections were prepared using a Microm HM355 S motorized microtome (Microm). Hematoxylin and eosin stains were performed using a DRS-601 automatic slide stainer (Sakura), and examined on an Olympus IX-71 microscope equipped with a DP-70 camera. Image analysis was performed using ImageJ software (public domain, available from the National Institutes of Health, USA). Muscle fiber diameter was measured using the lesser diameter method, as described elsewhere (Dubowitz V, *et al.* (2007) *Muscle biopsy : a practical approach* (Saunders Elsevier, Philadelphia) 3rd Ed pp XIII, 611 s).

f. ANALYSIS OF IGF-I AND INSULIN-MEDIATED PROTEIN PHOSPHORYLATION.

[00345] Mouse quadriceps muscles were snap frozen in liquid N_2, and Triton-X 100 soluble protein extracts were prepared as described previously (Ebert SM, *et al.* (2010) *Molecular endocrinology* 24(4):790-799). Mouse C2C12 myoblasts were obtained from American Type Culture Collection ("ATCC"), and maintained in Dulbecco's modified Eagle's medium (DMEM; ATCC #30-2002) containing antibiotics (100 units/ml penicillin, 100 μg/ml streptomycin sulfate) and 10% (v/v) fetal bovine serum (FBS). On day 0, myotubes were set-up in 6-well plates at a density of 2.5 X 10^5 cells / well. On day 2, differentiation into myotubes was induced by replacing 10% FBS with 2% horse serum. On day 7, myotubes were serum-starved by washing 2 times with phosphate buffered saline, and then adding fresh serum-free media. After 16 hours of serum-starvation, 10 μM ursolic acid (from a 10 mM stock prepared in DMSO), or an equal volume of DMSO, with or without 10 nM mouse IGF-I (Sigma; Cat. No. I8779) or 10 nM bovine insulin (Sigma; Cat. No. I6634) was directly added to the media. For analysis of Akt, S6K, ERK and FoxO phosphorylation,

myotubes were incubated in the presence or absence of ursolic acid, IGF-I and/or insulin for 20 min, and then harvested into SDS lysis buffer (10 mM Tris-HCl, pH 7.6, 100 mM NaCl, 1% (w/v) SDS, 1 μg/ml pepstatin A, 2 μg/ml aprotonin, 10 μg/ml leupeptin, 200 μM phenylmethylsulfonyl fluoride and a 1:100 dilution of phosphatase inhibitor cocktail 3 (Sigma). An aliquot of each muscle extract or cell lysate was mixed with 0.25 volume of sample buffer (250 mM Tris-HCl, pH 6.8, 10% SDS, 25% glycerol, 0.2% (w/v) bromophenol blue, and 5% (w/v) 2-mercaptoethanol) and heated for 5 min at 95 °C, whereas a separate aliquot was used to determine protein concentration by the BCA kit (Pierce). Samples (25 μg) were subjected to 8% SDS-PAGE, then transferred to Hybond-C extra nitrocellulose filters (Millipore). Immunoblots were performed at 4 °C for 16 h using a 1:2000 dilution of antibodies detecting total Akt, phospho-Akt(Ser473), total S6K, phospho-S6K(T421/S424), total ERK1/2, phospho-ERK(T202/Y204), FoxO3a, or phospho-FoxO1(T24)/FoxO3a(T32) (Cell Signaling). For analysis of IGF-1 receptor or insulin receptor phosphorylation, myotubes were incubated in the presence or absence of ursolic acid, IGF-I and/or insulin for 2 min, and then harvested into RIPA buffer (10 mM Tris-HCL, pH 7.4, 150 mM NaCl, 0.1% (w/v) SDS, 1% (w/v) Triton X-100, 1% Na deoxycholate, 5 mM EDTA, 1mM NaF, 1mM Na orthovanadate, 1 μg/ml pepstatin A, 2 μg/ml aprotonin, 10 μug/ml leupeptin, 200 μM phenylmethylsulfonyl fluoride, 1:100 dilution of phosphatase inhibitor cocktail 2 (Sigma) and a 1:100 dilution of phosphatase inhibitor cocktail 3 (Sigma). The protein concentration was measured using the BCA kit, after which the extract was diluted to a concentration of 1 mg/ml in RIPA buffer (final volume 500 μl). Then 2 μg anti-IGF-1 receptor β antibody (Cell Signaling) or 2 μg anti-insulin receptor β antibody (Santa Cruz) was added with 50 μl protein G plus Sepharose beads (Santa Cruz), and then the samples were rotated at 4 °C for 16 h. Immunoprecipitates were washed three times for 20 min with 1 ml RIPA buffer and then mixed with 100 μl sample buffer (50 mM Tris-HCl (pH 6.8), 2% SDS, 5% glycerol, 0.04% (w/v) bromophenol blue and 5% (w/v) 2-mercaptoethanol), then boiled for 5 min. Immunoprecipitates were subjected to 8% SDS-PAGE. For analysis of total IGF-1 receptor, phospho-insulin receptor and total insulin receptor, proteins were transferred to Hybond-C extra nitrocellulose filters (Millipore). For analysis of phospho-IGF-1 receptor, proteins were transferred to PVDF membranes (Bio-Rad). Immunoblots were performed at room temperature using a 1:2000 dilution of anti-IGF-1 receptor β antibody, 1:5000 dilution of mouse anti-phospho-tyrosine 4G10 monoclonal antibody (Millipore), a 1:2000 dilution of anti-insulin receptor β, or 1:2000 dilution of anti-phospho-insulin receptor β (Y1162/1163)

(Santa Cruz).

g. PTP1B INHIBITION VIA RNA INTERFERENCE.

[00346] The plasmids *pCMV-miR-PTP1B #1* and *pCMV-miR-PTP1B #2* were generated by ligating *PTPN1*-specific oligonucleotide duplexes (Invitrogen) into the *pcDNA6.2GW/EmGFP miR* plasmid (Invitrogen), which contains a CMV promoter driving co-cistronic expression of engineered pre-miRNAs and EmGFP. *pCMV-miR-control* encodes a non-targeting pre-miRNA hairpin sequence (miR-neg control; Invitrogen) in *pcDNA6.2GW/EmGFP miR* plasmid. Male C57BL/6 mice were obtained from NCI at ages 6-8 weeks, and used for experiments within 3 weeks of their arrival. Electroporation of mouse tibialis anterior muscles and isolation of skeletal muscle RNA was performed as described previously (Ebert SM, *et al.* (2010) *Molecular endocrinology* 24(4):790-799). First strand cDNA was synthesized in a 20 μl reaction that contained 2 μg of RNA, random hexamer primers and components of the High Capacity cDNA reverse transcription kit (Applied Biosystems). qPCR analysis of *PTPN1* mRNA levels was performed using a Taqman expression assay as described previously (Ebert SM, *et al.* (2010) *Molecular endocrinology* 24(4):790-799). qPCR was carried out using a 7500 Fast Real-Time PCR System (Applied Biosystems). All qPCR reactions were performed in triplicate and the cycle threshold (Ct) values were averaged to give the final results. Fold changes were determined by the ΔCt method, with level of *36B4* mRNA serving as the invariant control. Skeletal muscle sections were prepared and transfected (EmGFP-positive) muscle fibers were identified and measured as described previously (Ebert SM, *et al.* (2010) *Molecular endocrinology* 24(4):790-799).

h. MEASUREMENT OF SERUM URSOLIC ACID LEVELS.

[00347] Ursolic acid is extracted from serum using a 10:1 mixture of hexane:propanol (recovery > 90%), and then conjugated via its carboxylic acid group to 2-(2,3-naphthalimino)ethyl trifluoromethanesulfonate (Invitrogen; Ne-OTf), a moiety that enhances TUV and fluorescence detection. Derivatized samples are then analyzed on a Waters Acquity UPLC equipped with a 100 X 2.1 mm C18 HSS column with 1.8 μm beads (Waters Part No. 186003533) and a TUV detector.

[00348] .

2. IDENTIFICATION OF THERAPEUTICS TO TREAT MUSCLE ATROPHY

[00349] Skeletal muscle atrophy is common and debilitating condition that lacks a pharmacologic therapy. To identify and develop new therapeutic approaches to this pathophysiological condition (Figure 1), an approach using gene expression signatures to

connect small molecules, genes, and disease was used. Briefly, 63 mRNAs were identified that were regulated by fasting in both human and mouse muscle, and 29 mRNAs that were regulated by both fasting and spinal cord injury in human muscle. These two unbiased mRNA expression signatures of muscle atrophy were used to query the Connectivity Map, an algorithm that allows gene signature datasets to be used to find relationships between small molecules, genes, and disease.

[00350] Three complimentary studies to characterize global atrophy-associated changes in skeletal muscle mRNA levels in humans and mice were carried out. These three studies determined the effects of: A) fasting on human skeletal muscle mRNA levels as described herein, B) spinal cord injury ("SCI") on human skeletal mRNA levels (Adams CM, *et al.* (2011) *Muscle & nerve* 43(1):65-75) and C) fasting on mouse skeletal muscle mRNA levels (Ebert SM, *et al.* (2010) *Molecular endocrinology* 24(4):790-799). In each study, exon expression arrays were used to quantitate levels of more than 16,000 mRNAs. Although there were many significant changes in each study, analysis focused on mRNAs whose levels were similarly altered in at least two atrophy models. Thus, by comparing the effects of fasting on human and mouse skeletal muscle, there were two sets of mRNAs identified: a) 31 mRNAs that were increased by fasting in both species, and b) 32 mRNAs that were decreased by fasting in both species. These evolutionarily conserved, fasting-regulated skeletal muscle mRNAs were termed "muscle atrophy signature-1" (see Figure 2). Next, the effects of fasting and SCI on human skeletal muscle were determined and two sets of mRNAs were identified: a) 18 mRNAs that were increased by fasting and SCI, and b) 17 mRNAs that were decreased by fasting and SCI. This second group of mRNAs was termed "muscle atrophy signature-2" (see Figure 3). Almost all of the mRNAs in muscle atrophy signatures-1 and -2 have previously uncharacterized roles in normal or atrophied skeletal muscle. It was next hypothesized that pharmacologic compounds whose effects on cellular mRNA levels were opposite to muscle atrophy signatures-1 and -2 might inhibit skeletal muscle atrophy. To identify candidate compounds, the Connectivity Map (Lamb J, *et al.* (2006) *Science (New York, N.Y* 313(5795):1929-1935) was used to compare muscle atrophy signatures-1 and -2 to mRNA expression signatures of > 1300 bioactive small molecules. These results identified several predicted inhibitors of human skeletal muscle atrophy, including ursolic acid. The predicted inhibitors of human skeletal muscle atrophy, i.e. compounds with negative connectivity with the muscle atrophy signatures, are shown in Tables 2 and 3 below. Table 2 shows compounds with negative connectivity to human muscle atrophy signature-1 (see Figure 2 for mRNAs in the signature), whereas Table 3 shows compounds with negative

connectivity to human muscle atrophy signature-2 (see Figure 3 for mRNAs in the signature).

[00351] As a proof-of-concept of the utility of muscle atrophy signatures-1 and -2 described herein, the effects of ursolic acid were assessed in mice, and surprisingly it was discovered ursolic acid inhibited muscle atrophy and promoted muscle hypertrophy.

[00352] Table 2. Compounds with negative connectivity to human muscle atrophy signature-1.

Cmap name / cell line	Connectivity score	n	Enrichment	p	Specificity	% Non-null
conessine - HL60	-0.752	1	-0.991	---	---	100
allantoin - HL60	-0.622	1	-0.954	---	---	100
conessine - PC3	-0.598	1	-0.941	---	---	100
tacrine - HL60	-0.551	1	-0.91	---	---	100
tomatidine - HL60	-0.497	1	-0.873	---	---	100
tomatidine - PC3	-0.483	1	-0.861	---	---	100
naringenin - PC3	-0.462	1	-0.846	---	---	100
allantoin - MCF7	-0.347	2	-0.735	0.13873	0.1118	50
tomatidine - MCF7	-0.343	2	-0.78	0.09489	0.2263	50
naringenin - MCF7	-0.219	2	-0.546	0.4127	0.6589	50
allantoin - PC3	-0.077	2	-0.414	0.78446	0.7654	50

[00353] Table 3. Compounds with negative connectivity to human muscle atrophy signature-2.

Cmap name / cell line	Connectivity score	n	Enrichment	p	Specificity	% Non-null
tacrine - HL60	-0.870	1	-0.998	---	---	100
tomatidine - PC3	-0.861	1	-0.998	---	---	100
naringenin - PC3	-0.754	1	-0.990	---	---	100
betulinic acid - HL60	-0.569	1	-0.929	---	---	100
conessine - HL60	-0.543	1	-0.915	---	---	100
allantoin - MCF7	-0.486	2	-0.840	0.05114	0.04710	100
naringenin - MCF7	-0.314	2	-0.460	0.64871	0.84500	50
tomatidine - MCF7	-0.281	2	-0.611	0.30586	0.65260	50

3. EFFECTS OF FASTING ON SKELETAL MUSCLE MRNA EXPRESSION IN HUMANS.

[00354] Prolonged fasting induces muscle atrophy, but its effects on global mRNA expression in human skeletal muscle were not known heretofore. In order to determine the

relationship between global mRNA expression and human skeletal muscle status, seven healthy adult human volunteers (3 male and 4 female) with ages ranging from 25 to 69 years (mean = 46 years) were studied. The overall study design is shown in Figure 4A. The mean body mass index of these subjects (± SEM) was 25 ± 1. Their mean weight was 69.4 ± 4.8 kg. Baseline circulating levels of hemoglobin A1c (HbA1c), triglycerides (TG), thyroid-stimulating hormone (TSH), free thyroxine (free T4), C-reactive protein (CRP) and tumor necrosis factor-α (TNF-α) were within normal limits (Figure 4A). The table (Figure 4A, insert) shows baseline circulating metabolic and inflammatory markers. The graph shows plasma glucose and insulin levels (Figure 4A). Data are means ± SEM from the seven study subjects. In some cases, the error bars are too small to see. While staying in the University of Iowa Clinical Research Unit, the subjects fasted for 40 h by forgoing food but not water. The mean weight loss during the fast was 1.7 ± 0.1 kg (3 ± 0 % of the initial body weight).

[00355] After the 40 h fast, a muscle biopsy was obtained from the subjects' vastus lateralis (VL) muscle. Immediately after the muscle biopsy, the subjects ate a mixed meal. Five hours later (six hours after the first biopsy), a second muscle biopsy from their contralateral VL muscle. Thus, each subject had a muscle biopsy under fasting and nonfasting conditions. As expected, plasma glucose and insulin levels were low at the end of the 40 h fast, rose after the meal, and returned to baseline by the time of the second biopsy (Figure 4A). These data indicate comparable levels of plasma glucose and insulin at the times of the first (fasting) and second (nonfasting) muscle biopsies.

[00356] To determine the effect of fasting on skeletal muscle mRNA expression, RNA was isolated from the paired muscle biopsies and then analyzed it with exon expression arrays. Using $P \leq 0.02$ (by paired t-test) as criteria for statistical significance, it was found that 281 mRNAs were higher in the fasting state and 277 were lower (out of > 17,000 mRNAs measured; see Figure 4B). A complete list of these fasting-responsive mRNAs is shown below in Table X1 ("Change" is the mean $\log_2$ change or difference between fasting and fed states). The data in Table X1 is for all mRNAs in this study whose levels were increased or decreased by fasting ($P \leq 0.02$ by paired t-test).

[00357] Representative fasting-responsive human skeletal muscle mRNAs, and the effect of fasting on their log2 hybridization signals, as assessed by Affymetrix Human Exon 1.0 ST arrays are shown in Figure 4B. In each subject, the fasting signal was normalized to the nonfasting signal from the same subject. Data are means ± SEM from 7 subjects. $P \leq 0.02$ by paired t-test for all mRNAs shown. The complete set of 458 fasting-responsive mRNAs is

shown in Table X1. Most of the differentially expressed mRNAs identified as altered by fasting surprisingly did not have previously known roles in muscle atrophy. However, fasting increased several mRNAs that encode proteins with known roles in catabolic processes such as fat oxidation, reverse cholesterol transport, thermogenesis, inhibition of protein synthesis, autophagy, ubiquitin-mediated proteolysis, glutamine transport and heme catabolism (Figure 4B). Of these, *atrogin-1*, *MuRF1* and *ZFAND5* mRNAs encode proteins known to be required for skeletal muscle atrophy in mice (Bodine SC, *et al.* (2001) *Science (New York, N.Y* 294(5547):1704-1708; Hishiya A, *et al.* (2006) *The EMBO journal* 25(3):554-564). Conversely, fasting significantly decreased several mRNAs encoding proteins with known roles in anabolic processes such as glycogen synthesis, lipid synthesis and uptake, polyamine synthesis, iron uptake, angiogenesis, and mitochondrial biogenesis (Figure 4B). Of these, *PGC-1α* mRNA encodes a protein that inhibits atrophy-associated gene expression and skeletal muscle atrophy in mice (Sandri M, *et al.* (2006) *Proceedings of the National Academy of Sciences of the United States of America* 103(44):16260-16265).

[00358] The results were further validated using qPCR to analyze RNA from paired fed and fasted skeletal muscle biopsy samples obtained from seven healthy human subjects (see Figure 5; data are means ± SEM; * $P \leq 0.01$ by paired t-test.). In each subject, the fasting mRNA level was normalized to the nonfasting level, which was set at 1. The mRNA encoding myostatin (*MSTN*) is a control transcript whose level was not altered by fasting, as assessed by exon expression arrays. Taken together, these data established an mRNA expression signature of fasting in human skeletal muscle.

[00359] Table X1. Fasting-responsive human mRNAs.

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3062082	*PDK4*	NM_002612 // PDK4 // pyruvate dehydrogenase kinase, isozyme 4 // 7q21.3 // 5166	NM_002612	2.15	0.34	0.000
2319340	*SLC25A33*	NM_032315 // SLC25A33 // solute carrier family 25, member 33 // 1p36.22 // 84275	NM_032315	1.42	0.41	0.007

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3165957	*IFNK*	NM_020124 // IFNK // interferon, kappa // --- // 56832 /// ENST00000276943 // IF	NM_020124	0.96	0.28	0.007
3424158	*MYF6*	NM_002469 // MYF6 // myogenic factor 6 (herculin) // 12q21 // 4618 /// ENST00000	NM_002469	0.95	0.12	0.000
3422144	*LGR5*	NM_003667 // LGR5 // leucine-rich repeat-containing G protein-coupled receptor 5	NM_003667	0.88	0.12	0.000
2356115	*TXNIP*	NM_006472 // TXNIP // thioredoxin interacting protein // 1q21.1 // 10628 /// ENS	NM_006472	0.85	0.22	0.004
3233605	*PFKFB3*	NM_004566 // PFKFB3 // 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 //	NM_004566	0.84	0.18	0.002
3151607	*FBXO32*	NM_058229 // FBXO32 // F-box protein 32 // 8q24.13 // 114907 /// NM_148177 // FB	NM_058229	0.82	0.19	0.002
2745547	*GAB1*	NM_207123 // GAB1 // GRB2-associated binding protein 1 // 4q31.21 // 2549 /// NM	NM_207123	0.71	0.08	0.000
3173479	*FOXD4L3*	NM_199135 // FOXD4L3 // forkhead box D4-like 3 // 9q13 // 286380 /// NM_012184 /	NM_199135	0.68	0.25	0.017

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3199500	*CER1*	NM_005454 // CER1 // cerberus 1, cysteine knot superfamily, homolog (Xenopus lae	NM_005454	0.64	0.24	0.019
3444309	*TAS2R9*	NM_023917 // TAS2R9 // taste receptor, type 2, member 9 // 12p13 // 50835 /// EN	NM_023917	0.63	0.22	0.015
3452323	*SLC38A2*	NM_018976 // SLC38A2 // solute carrier family 38, member 2 // 12q // 54407 /// E	NM_018976	0.62	0.13	0.001
3381843	*UCP3*	NM_003356 // UCP3 // uncoupling protein 3 (mitochondrial, proton carrier) // 11q	NM_003356	0.59	0.04	0.000
3147508	*KLF10*	NM_005655 // KLF10 // Kruppel-like factor 10 // 8q22.2 // 7071 /// NM_001032282	NM_005655	0.58	0.11	0.001
3982534	*LPAR4*	NM_005296 // LPAR4 // lysophosphatidic acid receptor 4 // Xq13-q21.1 // 2846 ///	NM_005296	0.57	0.17	0.008
3384321	*RAB30*	NM_014488 // RAB30 // RAB30, member RAS oncogene family // 11q12-q14 // 27314 //	NM_014488	0.56	0.21	0.019
3256192	*C10orf116*	NM_006829 // C10orf116 // chromosome 10 open reading frame 116 // 10q23.2 // 109	NM_006829	0.55	0.19	0.013

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
2705690	*GHSR*	NM_198407 // GHSR // growth hormone secretagogue receptor // 3q26.31 // 2693 ///	NM_198407	0.54	0.20	0.016
3326938	*LOC100130104*	AF274942 // LOC100130104 // PNAS-17 // 11p13 // 100130104	AF274942	0.53	0.16	0.009
2318656	*PER3*	NM_016831 // PER3 // period homolog 3 (Drosophila) // 1p36.23 // 8863 /// ENST00	NM_016831	0.52	0.16	0.009
3209623	*ZFAND5*	NM_001102420 // ZFAND5 // zinc finger, AN1-type domain 5 // 9q13-q21 // 7763 ///	NM_001102420	0.51	0.13	0.005
3741300	*OR1D4*	NM_003552 // OR1D4 // olfactory receptor, family 1, subfamily D, member 4 // 17p	NM_003552	0.50	0.19	0.019
2899176	*HIST1H2BD*	NM_138720 // HIST1H2BD // histone cluster 1, H2bd // 6p21.3 // 3017 /// NM_02106	NM_138720	0.49	0.16	0.010
3439256	*RPS11*	ENST00000270625 // RPS11 // ribosomal protein S11 // 19q13.3 // 6205 /// BC10002	ENST00000270625	0.49	0.11	0.002
2973232	*KIAA0408*	NM_014702 // KIAA0408 // KIAA0408 // 6q22.33 // 9729 /// NM_001012279 // C6orf17	NM_014702	0.49	0.14	0.006

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3291151	*RHOBTB1*	NM_014836 // RHOBTB1 // Rho-related BTB domain containing 1 // 10q21.2 // 9886 /	NM_014836	0.48	0.09	0.001
2358136	*C1orf51*	BC027999 // C1orf51 // chromosome 1 open reading frame 51 // 1q21.2 // 148523 //	BC027999	0.48	0.17	0.016
3948936		---	---	0.47	0.18	0.020
3944129	*HMOX1*	NM_002133 // HMOX1 // heme oxygenase (decycling) 1 // 22q12|22q13.1 // 3162 ///	NM_002133	0.46	0.13	0.006
2968652	*SESN1*	NM_014454 // SESN1 // sestrin 1 // 6q21 // 27244 /// ENST00000302071 // SESN1 //	NM_014454	0.46	0.12	0.004
2951881	*PXT1*	NM_152990 // PXT1 // peroxisomal, testis specific 1 // 6p21.31 // 222659 /// ENS	NM_152990	0.45	0.14	0.008
2819747	*POLR3G*	NM_006467 // POLR3G // polymerase (RNA) III (DNA directed) polypeptide G (32kD)	NM_006467	0.45	0.13	0.007
2957384	*GSTA2*	NM_000846 // GSTA2 // glutathione S-transferase A2 // 6p12.1 // 2939 /// NM_1536	NM_000846	0.44	0.10	0.002

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
4014387	*RPSA*	NM_002295 // RPSA // ribosomal protein SA // 3p22.2 // 3921 /// NM_001012321 //	NM_002295	0.44	0.16	0.018
3021158	*C7orf58*	NM_024913 // C7orf58 // chromosome 7 open reading frame 58 // 7q31.31 // 79974 /	NM_024913	0.44	0.07	0.000
2976155	*OLIG3*	NM_175747 // OLIG3 // oligodendrocyte transcription factor 3 // 6q23.3 // 167826	NM_175747	0.44	0.12	0.006
3261886	*C10orf26*	NM_017787 // C10orf26 // chromosome 10 open reading frame 26 // 10q24.32 // 5483	NM_017787	0.44	0.17	0.019
2489169		---	---	0.42	0.12	0.006
2790062	*TMEM154*	NM_152680 // TMEM154 // transmembrane protein 154 // 4q31.3 // 201799 /// ENST00	NM_152680	0.42	0.14	0.012
3792656	*CCDC102B*	NM_024781 // CCDC102B // coiled-coil domain containing 102B // 18q22.1 // 79839	NM_024781	0.42	0.12	0.007
3554282	*INF2*	NM_022489 // INF2 // inverted formin, FH2 and WH2 domain containing // 14q32.33	NM_022489	0.41	0.14	0.012
2614142	*NR1D2*	NM_005126 // NR1D2 // nuclear receptor subfamily 1, group D, member 2 // 3p24.2	NM_005126	0.39	0.15	0.019

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3404636	*GABARAPL1*	NM_031412 // GABARAPL1 // GABA(A) receptor-associated protein like 1 // 12p13.2	NM_031412	0.39	0.10	0.004
3063856	*tcag7.1177*	ENST00000292369 // tcag7.1177 // opposite strand transcription unit to STAG3 //	ENST00000292369	0.39	0.09	0.003
3461981	*TSPAN8*	NM_004616 // TSPAN8 // tetraspanin 8 // 12q14.1-q21.1 // 7103 /// ENST0000039333	NM_004616	0.39	0.14	0.015
2908154	*C6orf206*	BC029519 // C6orf206 // chromosome 6 open reading frame 206 // 6p21.1 // 221421	BC029519	0.39	0.09	0.003
3415046	*FLJ33996*	AK091315 // FLJ33996 // hypothetical protein FLJ33996 // 12q13.13 // 283401 ///	AK091315	0.39	0.15	0.019
3326400	*CAT*	NM_001752 // CAT // catalase // 11p13 // 847 /// ENST00000241052 // CAT // catal	NM_001752	0.39	0.09	0.003
2390322	*OR2M5*	NM_001004690 // OR2M5 // olfactory receptor, family 2, subfamily M, member 5 //	NM_001004690	0.38	0.12	0.011
2402536	*TRIM63*	NM_032588 // TRIM63 // tripartite motif-containing 63 // 1p34-p33 // 84676 /// E	NM_032588	0.38	0.12	0.009

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
2976768	*CITED2*	NM_006079 // CITED2 // Cbp/p300-interacting transactivator, with Glu/Asp-rich ca	NM_006079	0.37	0.10	0.005
3218528	*ABCA1*	NM_005502 // ABCA1 // ATP-binding cassette, sub-family A (ABC1), member 1 // 9q3	NM_005502	0.37	0.14	0.016
3377861	*DKFZp761E198*	NM_138368 // DKFZp761E198 // DKFZp761E198 protein // 11q13.1 // 91056 /// BC1091	NM_138368	0.37	0.06	0.000
2961347	*FILIP1*	NM_015687 // FILIP1 // filamin A interacting protein 1 // 6q14.1 // 27145 /// EN	NM_015687	0.37	0.10	0.005
3097580	*C8orf22*	NM_001007176 // C8orf22 // chromosome 8 open reading frame 22 // 8q11 // 492307	NM_001007176	0.37	0.08	0.002
3755655	*FBXL20*	NM_032875 // FBXL20 // F-box and leucine-rich repeat protein 20 // 17q12 // 8496	NM_032875	0.35	0.08	0.002
3057505	*CCL26*	NM_006072 // CCL26 // chemokine (C-C motif) ligand 26 // 7q11.23 // 10344 /// EN	NM_006072	0.35	0.12	0.012
3307795	*C10orf118*	NM_018017 // C10orf118 // chromosome 10 open reading frame 118 // 10q25.3 // 550	NM_018017	0.35	0.13	0.020

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3654699	*NUPR1*	NM_001042483 // NUPR1 // nuclear protein 1 // 16p11.2 // 26471 /// NM_012385 //	NM_001042483	0.35	0.10	0.007
3778252	*ANKRD12*	NM_015208 // ANKRD12 // ankyrin repeat domain 12 // 18p11.22 // 23253 /// NM_001	NM_015208	0.34	0.08	0.002
2662560	*C3orf24*	NM_173472 // C3orf24 // chromosome 3 open reading frame 24 // 3p25.3 // 115795 /	NM_173472	0.34	0.08	0.002
3896370	*RP5-1022P6.2*	NM_019593 // RP5-1022P6.2 // hypothetical protein KIAA1434 // 20p12.3 // 56261 /	NM_019593	0.34	0.10	0.007
3389566	*KBTBD3*	NM_198439 // KBTBD3 // kelch repeat and BTB (POZ) domain containing 3 // 11q22.3	NM_198439	0.34	0.08	0.003
3247818	*FAM133B*	NM_152789 // FAM133B // family with sequence similarity 133, member B // 7q21.2	NM_152789	0.34	0.11	0.010
2457988	*ZNF706*	AF275802 // ZNF706 // zinc finger protein 706 // 8q22.3 // 51123 /// BC015925 //	AF275802	0.34	0.12	0.016
3525234	*IRS2*	NM_003749 // IRS2 // insulin receptor substrate 2 // 13q34 // 8660 /// ENST00000	NM_003749	0.34	0.09	0.004

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
2730281	*ODAM*	NM_017855 // ODAM // odontogenic, ameloblast asssociated // 4q13.3 // 54959 ///	NM_017855	0.34	0.12	0.016
3768969	*ABCA5*	NM_018672 // ABCA5 // ATP-binding cassette, sub-family A (ABC1), member 5 // 17q	NM_018672	0.33	0.10	0.008
3687494	*MAPK3*	NM_001040056 // MAPK3 // mitogen-activated protein kinase 3 // 16p11.2 // 5595 /	NM_001040056	0.33	0.09	0.004
3405396	*CREBL2*	NM_001310 // CREBL2 // cAMP responsive element binding protein-like 2 // 12p13 /	NM_001310	0.33	0.07	0.002
3647504	*PMM2*	NM_000303 // PMM2 // phosphomannomutase 2 // 16p13.3-p13.2 // 5373 /// ENST00000	NM_000303	0.33	0.10	0.008
3392840	*BUD13*	NM_032725 // BUD13 // BUD13 homolog (S. cerevisiae) // 11q23.3 // 84811 /// ENST	NM_032725	0.33	0.07	0.002
3453837	*TUBA1A*	NM_006009 // TUBA1A // tubulin, alpha 1a // 12q12-q14.3 // 7846 /// ENST00000301	NM_006009	0.33	0.07	0.002
2409310	*ELOVL1*	NM_022821 // ELOVL1 // elongation of very long chain fatty acids (FEN1/Elo2, SUR	NM_022821	0.32	0.09	0.005

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3837707	*ZNF114*	NM_153608 // ZNF114 // zinc finger protein 114 // 19q13.32 // 163071 /// ENST000	NM_153608	0.31	0.09	0.007
3504434	*XPO4*	NM_022459 // XPO4 // exportin 4 // 13q11 // 64328 /// ENST00000255305 // XPO4 //	NM_022459	0.31	0.10	0.009
2431877		---	---	0.31	0.11	0.017
3837836	*PSCD2*	NM_017457 // PSCD2 // pleckstrin homology, Sec7 and coiled-coil domains 2 (cytoh	NM_017457	0.31	0.05	0.000
3869396	*ZNF432*	NM_014650 // ZNF432 // zinc finger protein 432 // 19q13.33 // 9668 /// ENST00000	NM_014650	0.31	0.09	0.006
3981120	*OGT*	NM_181672 // OGT // O-linked N-acetylglucosamine (GlcNAc) transferase (UDP-N-ace	NM_181672	0.31	0.10	0.013
2622607	*SLC38A3*	NM_006841 // SLC38A3 // solute carrier family 38, member 3 // 3p21.3 // 10991 //	NM_006841	0.30	0.11	0.016
3978812	*FOXR2*	NM_198451 // FOXR2 // forkhead box R2 // Xp11.21 // 139628 /// ENST00000339140 /	NM_198451	0.30	0.09	0.008
3571904	*NPC2*	NM_006432 // NPC2 // Niemann-Pick disease, type C2 // 14q24.3 // 10577 /// NM_00	NM_006432	0.30	0.10	0.011

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
2417945	*PTGER3*	NM_198715 // PTGER3 // prostaglandin E receptor 3 (subtype EP3) // 1p31.2 // 573	NM_198715	0.30	0.11	0.017
3059393	*SEMA3E*	NM_012431 // SEMA3E // sema domain, immunoglobulin domain (Ig), short basic doma	NM_012431	0.30	0.09	0.009
2336456	*MGC52498*	NM_001042693 // MGC52498 // hypothetical protein MGC52498 // 1p32.3 // 348378 //	NM_001042693	0.30	0.10	0.011
3726772	*CROP*	NM_016424 // CROP // cisplatin resistance-associated overexpressed protein // 17	NM_016424	0.30	0.11	0.016
2784265	*IL2*	NM_000586 // IL2 // interleukin 2 // 4q26-q27 // 3558 /// ENST00000226730 // IL2	NM_000586	0.29	0.11	0.019
2495782	*LIPT1*	NM_145197 // LIPT1 // lipoyltransferase 1 // 2q11.2 // 51601 /// NM_145198 // LI	NM_145197	0.29	0.10	0.012
2377094	*PFKFB2*	NM_006212 // PFKFB2 // 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 2 //	NM_006212	0.29	0.10	0.012
2469213	*KLF11*	NM_003597 // KLF11 // Kruppel-like factor 11 // 2p25 // 8462 /// ENST00000305883	NM_003597	0.29	0.10	0.011

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3662387	*HERPUD1*	NM_014685 // HERPUD1 // homocysteine-inducible, endoplasmic reticulum stress-ind	NM_014685	0.29	0.07	0.003
3771215	*ACOX1*	NM_004035 // ACOX1 // acyl-Coenzyme A oxidase 1, palmitoyl // 17q24-q25|17q25.1	NM_004035	0.29	0.10	0.013
3203135	*TOPORS*	NM_005802 // TOPORS // topoisomerase I binding, arginine/serine-rich // 9p21 //	NM_005802	0.28	0.11	0.018
2805482		---	---	0.28	0.09	0.008
3247757	*UBE2D1*	NM_003338 // UBE2D1 // ubiquitin-conjugating enzyme E2D 1 (UBC4/5 homolog, yeast	NM_003338	0.28	0.08	0.007
3444147	*KLRC1*	NM_002259 // KLRC1 // killer cell lectin-like receptor subfamily C, member 1 //	NM_002259	0.28	0.10	0.015
3348891	*C11orf57*	NM_018195 // C11orf57 // chromosome 11 open reading frame 57 // 11q23.1 // 55216	NM_018195	0.28	0.09	0.011
3906942	*SERINC3*	NM_006811 // SERINC3 // serine incorporator 3 // 20q13.1-q13.3 // 10955 /// NM_1	NM_006811	0.28	0.07	0.003
2930418	*UST*	NM_005715 // UST // uronyl-2-sulfotransferase // 6q25.1 // 10090 /// ENST0000036	NM_005715	0.28	0.06	0.002

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3188200	*OR1L1*	NM_001005236 // OR1L1 // olfactory receptor, family 1, subfamily L, member 1 //	NM_001005236	0.28	0.09	0.011
3856075	*ZNF682*	NM_033196 // ZNF682 // zinc finger protein 682 // 19p12 // 91120 /// NM_00107734	NM_033196	0.28	0.10	0.017
3385951	*NOX4*	NM_016931 // NOX4 // NADPH oxidase 4 // 11q14.2-q21 // 50507 /// ENST00000263317	NM_016931	0.28	0.06	0.002
3523881	*KDELC1*	NM_024089 // KDELC1 // KDEL (Lys-Asp-Glu-Leu) containing 1 // 13q33 // 79070 ///	NM_024089	0.28	0.06	0.002
2632778	*EPHA6*	NM_001080448 // EPHA6 // EPH receptor A6 // 3q11.2 // 285220 /// ENST00000389672	NM_001080448	0.28	0.09	0.010
3373272	*OR5W2*	NM_001001960 // OR5W2 // olfactory receptor, family 5, subfamily W, member 2 //	NM_001001960	0.28	0.10	0.015
4017694	*IRS4*	NM_003604 // IRS4 // insulin receptor substrate 4 // Xq22.3 // 8471 /// ENST0000	NM_003604	0.28	0.10	0.016
3545311	*KIAA1737*	NM_033426 // KIAA1737 // KIAA1737 // 14q24.3 // 85457 /// ENST00000361786 // KIA	NM_033426	0.28	0.07	0.003

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3753860	*CCL5*	NM_002985 // CCL5 // chemokine (C-C motif) ligand 5 // 17q11.2-q12 // 6352 /// E	NM_002985	0.28	0.05	0.001
3617312	*SLC12A6*	NM_001042496 // SLC12A6 // solute carrier family 12 (potassium/chloride transpor	NM_001042496	0.27	0.07	0.005
3351315	*UBE4A*	NM_004788 // UBE4A // ubiquitination factor E4A (UFD2 homolog, yeast) // 11q23.3	NM_004788	0.27	0.07	0.004
3755396	*CCDC49*	NM_017748 // CCDC49 // coiled-coil domain containing 49 // 17q12 // 54883 /// EN	NM_017748	0.27	0.09	0.013
2870889	*C5orf13*	NM_004772 // C5orf13 // chromosome 5 open reading frame 13 // 5q22.1 // 9315 ///	NM_004772	0.27	0.09	0.010
2775259	*RASGEF1B*	NM_152545 // RASGEF1B // RasGEF domain family, member 1B // 4q21.21-q21.22 // 15	NM_152545	0.27	0.10	0.015
3165624		---	---	0.27	0.06	0.003
2771654	*CENPC1*	NM_001812 // CENPC1 // centromere protein C 1 // 4q12-q13.3 // 1060 /// ENST0000	NM_001812	0.27	0.09	0.013
3784670	*C18orf21*	NM_031446 // C18orf21 // chromosome 18 open reading frame 21 // 18q12.2 // 83608	NM_031446	0.27	0.08	0.008

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
2364231	*DDR2*	NM_001014796 // DDR2 // discoidin domain receptor tyrosine kinase 2 // 1q23.3 //	NM_001014796	0.26	0.10	0.018
3921442	*SH3BGR*	NM_007341 // SH3BGR // SH3 domain binding glutamic acid-rich protein // 21q22.3	NM_007341	0.26	0.08	0.007
2627368	*C3orf49*	BC015210 // C3orf49 // chromosome 3 open reading frame 49 // 3p14.1 // 132200	BC015210	0.26	0.06	0.003
3250699	*EIF4EBP2*	NM_004096 // EIF4EBP2 // eukaryotic translation initiation factor 4E binding pro	NM_004096	0.26	0.10	0.018
3237788	*PLXDC2*	NM_032812 // PLXDC2 // plexin domain containing 2 // 10p12.32-p12.31 // 84898 //	NM_032812	0.26	0.09	0.013
3285926	*ZNF33B*	NM_006955 // ZNF33B // zinc finger protein 33B // 10q11.2 // 7582 /// ENST000003	NM_006955	0.26	0.10	0.018
3304475	*ARL3*	NM_004311 // ARL3 // ADP-ribosylation factor-like 3 // 10q23.3 // 403 /// ENST00	NM_004311	0.26	0.08	0.008
3364306	*SOX6*	NM_017508 // SOX6 // SRY (sex determining region Y)-box 6 // 11p15.3 // 55553 //	NM_017508	0.26	0.08	0.010

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3185498	*SLC31A2*	NM_001860 // SLC31A2 // solute carrier family 31 (copper transporters), member 2	NM_001860	0.25	0.09	0.015
3998766	*KAL1*	NM_000216 // KAL1 // Kallmann syndrome 1 sequence // Xp22.32 // 3730 /// ENST000	NM_000216	0.25	0.07	0.006
3143266	*PSKH2*	NM_033126 // PSKH2 // protein serine kinase H2 // 8q21.2 // 85481 /// ENST000002	NM_033126	0.25	0.07	0.006
3458911	*CTDSP2*	NM_005730 // CTDSP2 // CTD (carboxy-terminal domain, RNA polymerase II, polypept	NM_005730	0.25	0.06	0.003
3195034	*PTGDS*	NM_000954 // PTGDS // prostaglandin D2 synthase 21kDa (brain) // 9q34.2-q34.3 //	NM_000954	0.25	0.08	0.010
3854066	*C19orf42*	NM_024104 // C19orf42 // chromosome 19 open reading frame 42 // 19p13.11 // 7908	NM_024104	0.25	0.08	0.010
3819474	*ANGPTL4*	NM_139314 // ANGPTL4 // angiopoietin-like 4 // 19p13.3 // 51129 /// NM_001039667	NM_139314	0.25	0.06	0.004
3944084	*TOM1*	NM_005488 // TOM1 // target of myb1 (chicken) // 22q13.1 // 10043 /// ENST000003	NM_005488	0.25	0.07	0.006

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3848243	*INSR*	NM_000208 // INSR // insulin receptor // 19p13.3-p13.2 // 3643 /// NM_001079817	NM_000208	0.24	0.09	0.014
3168415	*CLTA*	NM_007096 // CLTA // clathrin, light chain (Lca) // 9p13 // 1211 /// NM_00107667	NM_007096	0.24	0.08	0.009
2609462	*CAV3*	NM_033337 // CAV3 // caveolin 3 // 3p25 // 859 /// NM_001234 // CAV3 // caveolin	NM_033337	0.24	0.07	0.007
3393834	*C11orf60*	BC022856 // C11orf60 // chromosome 11 open reading frame 60 // 11q23.3 // 56912	BC022856	0.24	0.06	0.003
3755614	*STAC2*	NM_198993 // STAC2 // SH3 and cysteine rich domain 2 // 17q12 // 342667 /// ENST	NM_198993	0.24	0.07	0.009
3627363	*NARG2*	NM_024611 // NARG2 // NMDA receptor regulated 2 // 15q22.2 // 79664 /// NM_00101	NM_024611	0.24	0.06	0.003
3212976	*ZCCHC6*	NM_024617 // ZCCHC6 // zinc finger, CCHC domain containing 6 // 9q21 // 79670 //	NM_024617	0.24	0.08	0.014
3275922	*PRKCQ*	NM_006257 // PRKCQ // protein kinase C, theta // 10p15 // 5588 /// ENST000002631	NM_006257	0.24	0.05	0.002

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3023825	*C7orf45*	BC017587 // C7orf45 // chromosome 7 open reading frame 45 // 7q32.2 // 136263 //	BC017587	0.23	0.09	0.020
3832906	*IL29*	NM_172140 // IL29 // interleukin 29 (interferon, lambda 1) // 19q13.13 // 282618	NM_172140	0.23	0.08	0.015
3529156	*NGDN*	NM_015514 // NGDN // neuroguidin, EIF4E binding protein // 14q11.2 // 25983 ///	NM_015514	0.23	0.08	0.012
2620448	*CLEC3B*	NM_003278 // CLEC3B // C-type lectin domain family 3, member B // 3p22-p21.3 //	NM_003278	0.23	0.08	0.014
3481296	*SGCG*	NM_000231 // SGCG // sarcoglycan, gamma (35kDa dystrophin-associated glycoprotei	NM_000231	0.23	0.09	0.019
3135184	*RB1CC1*	NM_014781 // RB1CC1 // RB1-inducible coiled-coil 1 // 8q11 // 9821 /// NM_001083	NM_014781	0.23	0.07	0.008
2421843	*GBP3*	NM_018284 // GBP3 // guanylate binding protein 3 // 1p22.2 // 2635 /// ENST00000	NM_018284	0.23	0.06	0.004
3385003	*CREBZF*	NM_001039618 // CREBZF // CREB/ATF bZIP transcription factor // 11q14 // 58487 /	NM_001039618	0.23	0.09	0.020

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3610804	*IGF1R*	NM_000875 // IGF1R // insulin-like growth factor 1 receptor // 15q26.3 // 3480 /	NM_000875	0.23	0.08	0.013
3606304	*AKAP13*	NM_006738 // AKAP13 // A kinase (PRKA) anchor protein 13 // 15q24-q25 // 11214 /	NM_006738	0.23	0.04	0.000
2565579	*ANKRD39*	NM_016466 // ANKRD39 // ankyrin repeat domain 39 // 2q11.2 // 51239 /// ENST0000	NM_016466	0.23	0.05	0.003
2722151	*RBPJ*	NM_005349 // RBPJ // recombination signal binding protein for immunoglobulin kap	NM_005349	0.22	0.07	0.008
3031533	*GIMAP4*	NM_018326 // GIMAP4 // GTPase, IMAP family member 4 // 7q36.1 // 55303 /// ENST0	NM_018326	0.22	0.08	0.017
3725481	*UBE2Z*	NM_023079 // UBE2Z // ubiquitin-conjugating enzyme E2Z // 17q21.32 // 65264 ///	NM_023079	0.22	0.06	0.004
3549575	*IFI27*	NM_005532 // IFI27 // interferon, alpha-inducible protein 27 // 14q32 // 3429 //	NM_005532	0.22	0.08	0.016
3725035	*NFE2L1*	NM_003204 // NFE2L1 // nuclear factor (erythroid-derived 2)-like 1 // 17q21.3 //	NM_003204	0.22	0.07	0.011

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3348748	*C11orf1*	NM_022761 // C11orf1 // chromosome 11 open reading frame 1 // 11q13-q22 // 64776	NM_022761	0.22	0.07	0.008
3722039	*RAMP2*	NM_005854 // RAMP2 // receptor (G protein-coupled) activity modifying protein 2	NM_005854	0.22	0.05	0.003
3886704	*STK4*	NM_006282 // STK4 // serine/threonine kinase 4 // 20q11.2-q13.2 // 6789 /// ENST	NM_006282	0.22	0.07	0.012
3645901	*FLJ14154*	NM_024845 // FLJ14154 // hypothetical protein FLJ14154 // 16p13.3 // 79903 /// N	NM_024845	0.22	0.06	0.005
3367673	*MPPED2*	NM_001584 // MPPED2 // metallophosphoesterase domain containing 2 // 11p13 // 74	NM_001584	0.22	0.08	0.017
3219885	*PTPN3*	NM_002829 // PTPN3 // protein tyrosine phosphatase, non-receptor type 3 // 9q31	NM_002829	0.22	0.05	0.003
3791466		---	---	0.22	0.06	0.007
3717635	*ZNF207*	NM_001098507 // ZNF207 // zinc finger protein 207 // 17q11.2 // 7756 /// NM_0034	NM_001098507	0.22	0.08	0.015

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
2648141	*MBNL1*	NM_021038 // MBNL1 // muscleblind-like (Drosophila) // 3q25 // 4154 /// NM_20729	NM_021038	0.22	0.07	0.009
2436938	*PBXIP1*	NM_020524 // PBXIP1 // pre-B-cell leukemia homeobox interacting protein 1 // 1q2	NM_020524	0.21	0.05	0.002
3299705	*PANK1*	NM_148977 // PANK1 // pantothenate kinase 1 // 10q23.31 // 53354 /// NM_148978 /	NM_148977	0.21	0.06	0.007
3628923	*FAM96A*	NM_032231 // FAM96A // family with sequence similarity 96, member A // 15q22.31	NM_032231	0.21	0.05	0.003
2353669	*CD2*	NM_001767 // CD2 // CD2 molecule // 1p13 // 914 /// ENST00000369478 // CD2 // CD	NM_001767	0.21	0.06	0.006
3474450	*PLA2G1B*	NM_000928 // PLA2G1B // phospholipase A2, group IB (pancreas) // 12q23-q24.1 //	NM_000928	0.21	0.08	0.016
3722417	*NBR1*	NM_031858 // NBR1 // neighbor of BRCA1 gene 1 // 17q21.31 // 4077 /// NM_005899	NM_031858	0.21	0.08	0.017
3234760	*CUGBP2*	NM_001025077 // CUGBP2 // CUG triplet repeat, RNA binding protein 2 // 10p13 //	NM_001025077	0.21	0.06	0.004

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3627422	*RORA*	NM_134260 // RORA // RAR-related orphan receptor A // 15q21-q22 // 6095 /// NM_0	NM_134260	0.21	0.06	0.006
3382061	*XRRA1*	NM_182969 // XRRA1 // X-ray radiation resistance associated 1 // 11q13.4 // 1435	NM_182969	0.21	0.08	0.017
3015338	*STAG3*	NM_012447 // STAG3 // stromal antigen 3 // 7q22.1 // 10734 /// ENST00000317296 /	NM_012447	0.21	0.06	0.007
2665720	*ZNF385D*	NM_024697 // ZNF385D // zinc finger protein 385D // 3p24.3 // 79750 /// ENST0000	NM_024697	0.21	0.07	0.013
3154185	*TMEM71*	NM_144649 // TMEM71 // transmembrane protein 71 // 8q24.22 // 137835 /// ENST000	NM_144649	0.21	0.06	0.009
3789947	*NEDD4L*	NM_015277 // NEDD4L // neural precursor cell expressed, developmentally down-reg	NM_015277	0.21	0.08	0.016
2688933	*CD200R2*	ENST00000383679 // CD200R2 // CD200 cell surface glycoprotein receptor isoform 2	ENST00000383679	0.21	0.08	0.016
3379644	*CPT1A*	NM_001876 // CPT1A // carnitine palmitoyltransferase 1A (liver) // 11q13.1-q13.2	NM_001876	0.21	0.04	0.001

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3677795	*CREBBP*	NM_004380 // CREBBP // CREB binding protein (Rubinstein-Taybi syndrome) // 16p13	NM_004380	0.21	0.05	0.004
2358320	*TARS2*	NM_025150 // TARS2 // threonyl-tRNA synthetase 2, mitochondrial (putative) // 1q	NM_025150	0.21	0.06	0.007
3228373	*TSC1*	NM_000368 // TSC1 // tuberous sclerosis 1 // 9q34 // 7248 /// NM_001008567 // TS	NM_000368	0.20	0.06	0.006
3362795	*RNF141*	NM_016422 // RNF141 // ring finger protein 141 // 11p15.4 // 50862 /// ENST00000	NM_016422	0.20	0.08	0.019
3673684	*CDT1*	NM_030928 // CDT1 // chromatin licensing and DNA replication factor 1 // 16q24.3	NM_030928	0.20	0.07	0.015
3042881	*HOXA7*	NM_006896 // HOXA7 // homeobox A7 // 7p15-p14 // 3204 /// ENST00000396347 // HOX	NM_006896	0.20	0.02	0.000
3381817	*UCP2*	NM_003355 // UCP2 // uncoupling protein 2 (mitochondrial, proton carrier) // 11q	NM_003355	0.20	0.05	0.005
3415068	*ANKRD33*	NM_182608 // ANKRD33 // ankyrin repeat domain 33 // 12q13.13 // 341405 /// ENST0	NM_182608	0.20	0.06	0.006

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3633403	*SIN3A*	NM_015477 // SIN3A // SIN3 homolog A, transcription regulator (yeast) // 15q24.2	NM_015477	0.20	0.07	0.014
3380901	*NUMA1*	NM_006185 // NUMA1 // nuclear mitotic apparatus protein 1 // 11q13 // 4926 /// E	NM_006185	0.19	0.04	0.002
2598099	*BARD1*	NM_000465 // BARD1 // BRCA1 associated RING domain 1 // 2q34-q35 // 580 /// ENST	NM_000465	0.19	0.07	0.015
3139722	*NCOA2*	NM_006540 // NCOA2 // nuclear receptor coactivator 2 // 8q13.3 // 10499 /// ENST	NM_006540	0.19	0.06	0.010
3641871	*LINS1*	NM_018148 // LINS1 // lines homolog 1 (Drosophila) // 15q26.3 // 55180 /// NM_00	NM_018148	0.19	0.06	0.013
3401217	*TULP3*	NM_003324 // TULP3 // tubby like protein 3 // 12p13.3 // 7289 /// ENST0000022824	NM_003324	0.19	0.06	0.008
3741997	*ANKFY1*	NM_016376 // ANKFY1 // ankyrin repeat and FYVE domain containing 1 // 17p13.3 //	NM_016376	0.19	0.06	0.008
2622742	*C3orf45*	BC028000 // C3orf45 // chromosome 3 open reading frame 45 // 3p21.31 // 132228 /	BC028000	0.19	0.06	0.013

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3845352	*UQCR*	NM_006830 // UQCR // ubiquinol-cytochrome c reductase, 6.4kDa subunit // 19p13.3	NM_006830	0.19	0.06	0.014
3960356	*BAIAP2L2*	NM_025045 // BAIAP2L2 // BAI1-associated protein 2-like 2 // 22q13.1 // 80115 //	NM_025045	0.19	0.07	0.018
3645947	*CLUAP1*	NM_015041 // CLUAP1 // clusterin associated protein 1 // 16p13.3 // 23059 /// NM	NM_015041	0.19	0.06	0.012
3835544	*ZNF227*	NM_182490 // ZNF227 // zinc finger protein 227 // --- // 7770 /// ENST0000031304	NM_182490	0.18	0.06	0.011
3368748	*FBXO3*	NM_033406 // FBXO3 // F-box protein 3 // 11p13 // 26273 /// NM_012175 // FBXO3 /	NM_033406	0.18	0.07	0.020
3621623	*ELL3*	NM_025165 // ELL3 // elongation factor RNA polymerase II-like 3 // 15q15.3 // 80	NM_025165	0.18	0.05	0.005
3430552	*PWP1*	NM_007062 // PWP1 // PWP1 homolog (S. cerevisiae) // 12q23.3 // 11137 /// ENST00	NM_007062	0.18	0.07	0.016
2844908	*BTNL9*	NM_152547 // BTNL9 // butyrophilin-like 9 // 5q35.3 // 153579 /// ENST0000032770	NM_152547	0.18	0.05	0.005

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
4021508	*ZNF280C*	NM_017666 // ZNF280C // zinc finger protein 280C // Xq25 // 55609 /// ENST000003	NM_017666	0.18	0.07	0.018
2489071	*TET3*	NM_144993 // TET3 // tet oncogene family member 3 // 2p13.1 // 200424 /// ENST00	NM_144993	0.18	0.04	0.003
2516879	*HOXD8*	NM_019558 // HOXD8 // homeobox D8 // 2q31.1 // 3234 /// ENST00000313173 // HOXD8	NM_019558	0.18	0.06	0.015
3740704	*SMYD4*	NM_052928 // SMYD4 // SET and MYND domain containing 4 // 17p13.3 // 114826 ///	NM_052928	0.18	0.06	0.012
3975467	*UTX*	NM_021140 // UTX // ubiquitously transcribed tetratricopeptide repeat, X chromos	NM_021140	0.18	0.06	0.013
3699044	*RFWD3*	NM_018124 // RFWD3 // ring finger and WD repeat domain 3 // 16q22.3 // 55159 ///	NM_018124	0.18	0.06	0.011
3473083	*MED13L*	NM_015335 // MED13L // mediator complex subunit 13-like // 12q24.21 // 23389 ///	NM_015335	0.18	0.02	0.000
2332711	*PPIH*	NM_006347 // PPIH // peptidylprolyl isomerase H (cyclophilin H) // 1p34.1 // 104	NM_006347	0.17	0.06	0.017

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3556990	*JUB*	NM_032876 // JUB // jub, ajuba homolog (Xenopus laevis) // 14q11.2 // 84962 ///	NM_032876	0.17	0.04	0.004
2780143	*BDH2*	NM_020139 // BDH2 // 3-hydroxybutyrate dehydrogenase, type 2 // 4q24 // 56898 //	NM_020139	0.17	0.05	0.006
3899495	*C20orf12*	NM_001099407 // C20orf12 // chromosome 20 open reading frame 12 // 20p11.23 // 5	NM_001099407	0.17	0.05	0.008
3290875	*ANK3*	NM_020987 // ANK3 // ankyrin 3, node of Ranvier (ankyrin G) // 10q21 // 288 ///	NM_020987	0.17	0.03	0.001
3576014	*C14orf102*	NM_017970 // C14orf102 // chromosome 14 open reading frame 102 // 14q32.11 // 55	NM_017970	0.17	0.04	0.002
3644887	*ATP6V0C*	NM_001694 // ATP6V0C // ATPase, H+ transporting, lysosomal 16kDa, V0 subunit c /	NM_001694	0.17	0.06	0.017
2648378	*RAP2B*	NM_002886 // RAP2B // RAP2B, member of RAS oncogene family // 3q25.2 // 5912 ///	NM_002886	0.17	0.06	0.017
2362892	*ATP1A2*	NM_000702 // ATP1A2 // ATPase, Na+/K+ transporting, alpha 2 (+) polypeptide // 1	NM_000702	0.16	0.06	0.015

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
2361488	*RHBG*	NM_020407 // RHBG // Rh family, B glycoprotein // 1q21.3 // 57127 /// ENST000003	NM_020407	0.16	0.06	0.014
3415915	*PFDN5*	NM_002624 // PFDN5 // prefoldin subunit 5 // 12q12 // 5204 /// NM_145897 // PFDN	NM_002624	0.16	0.05	0.011
3433796	*PEBP1*	NM_002567 // PEBP1 // phosphatidylethanolamine binding protein 1 // 12q24.23 //	NM_002567	0.16	0.04	0.004
3788302	*SMAD4*	NM_005359 // SMAD4 // SMAD family member 4 // 18q21.1 // 4089 /// ENST0000039841	NM_005359	0.16	0.05	0.012
3436236	*ZNF664*	NM_152437 // ZNF664 // zinc finger protein 664 // 12q24.31 // 144348 /// ENST000	NM_152437	0.16	0.06	0.016
3441542	*TMEM16B*	NM_020373 // TMEM16B // transmembrane protein 16B // 12p13.3 // 57101 /// ENST00	NM_020373	0.16	0.06	0.018
3456353	*CALCOCO1*	NM_020898 // CALCOCO1 // calcium binding and coiled-coil domain 1 // 12q13.13 //	NM_020898	0.16	0.05	0.010
3888721	*PTPN1*	NM_002827 // PTPN1 // protein tyrosine phosphatase, non-receptor type 1 // 20q13	NM_002827	0.16	0.06	0.020

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3138204	*CYP7B1*	NM_004820 // CYP7B1 // cytochrome P450, family 7, subfamily B, polypeptide 1 //	NM_004820	0.15	0.05	0.014
3278401	*FRMD4A*	NM_018027 // FRMD4A // FERM domain containing 4A // 10p13 // 55691 /// ENST00000	NM_018027	0.15	0.05	0.009
3904226	*RBM39*	NM_184234 // RBM39 // RNA binding motif protein 39 // 20q11.22 // 9584 /// NM_00	NM_184234	0.15	0.05	0.015
3791850	*SERPINB13*	NM_012397 // SERPINB13 // serpin peptidase inhibitor, clade B (ovalbumin), membe	NM_012397	0.15	0.04	0.005
3665603	*CTCF*	NM_006565 // CTCF // CCCTC-binding factor (zinc finger protein) // 16q21-q22.3 /	NM_006565	0.15	0.04	0.004
3969802	*BMX*	NM_203281 // BMX // BMX non-receptor tyrosine kinase // Xp22.2 // 660 /// NM_001	NM_203281	0.15	0.05	0.016
3621276	*HISPPD2A*	NM_014659 // HISPPD2A // histidine acid phosphatase domain containing 2A // 15q1	NM_014659	0.14	0.04	0.005
2325113	*C1orf213*	NM_138479 // C1orf213 // chromosome 1 open reading frame 213 // 1p36.12 // 14889	NM_138479	0.14	0.05	0.012

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3681956	*KIAA0430*	NM_014647 // KIAA0430 // KIAA0430 // 16p13.11 // 9665 /// ENST00000396368 // KIA	NM_014647	0.14	0.05	0.018
3415193	*GRASP*	NM_181711 // GRASP // GRP1 (general receptor for phosphoinositides 1)-associated	NM_181711	0.14	0.05	0.019
3249369	*LRRTM3*	NM_178011 // LRRTM3 // leucine rich repeat transmembrane neuronal 3 // 10q21.3 /	NM_178011	0.14	0.05	0.011
3874023	*PTPRA*	NM_002836 // PTPRA // protein tyrosine phosphatase, receptor type, A // 20p13 //	NM_002836	0.14	0.04	0.004
3809621	*FECH*	NM_001012515 // FECH // ferrochelatase (protoporphyria) // 18q21.3 // 2235 /// N	NM_001012515	0.14	0.04	0.009
3351385	*MLL*	NM_005933 // MLL // myeloid/lymphoid or mixed-lineage leukemia (trithorax homolo	NM_005933	0.14	0.05	0.016
3288707	*ERCC6*	NM_000124 // ERCC6 // excision repair cross-complementing rodent repair deficien	NM_000124	0.14	0.05	0.016

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3624607	*MYO5A*	NM_000259 // MYO5A // myosin VA (heavy chain 12, myoxin) // 15q21 // 4644 /// EN	NM_000259	0.14	0.04	0.006
3353859	*OR4D5*	NM_001001965 // OR4D5 // olfactory receptor, family 4, subfamily D, member 5 //	NM_001001965	0.14	0.05	0.017
2823797	*TSLP*	NM_033035 // TSLP // thymic stromal lymphopoietin // 5q22.1 // 85480 /// NM_1385	NM_033035	0.14	0.05	0.013
2414366	*PPAP2B*	NM_003713 // PPAP2B // phosphatidic acid phosphatase type 2B // 1pter-p22.1 // 8	NM_003713	0.13	0.04	0.007
3878308	*CSRP2BP*	NM_020536 // CSRP2BP // CSRP2 binding protein // 20p11.23 // 57325 /// NM_177926	NM_020536	0.13	0.05	0.019
4025771	*CD99L2*	NM_031462 // CD99L2 // CD99 molecule-like 2 // Xq28 // 83692 /// NM_134446 // CD	NM_031462	0.13	0.04	0.007
3414776	*LETMD1*	NM_015416 // LETMD1 // LETM1 domain containing 1 // 12q13.13 // 25875 /// NM_001	NM_015416	0.13	0.05	0.014
3645253	*SRRM2*	NM_016333 // SRRM2 // serine/arginine repetitive matrix 2 // 16p13.3 // 23524 //	NM_016333	0.13	0.04	0.007

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
2440700	*ADAMTS4*	NM_005099 // ADAMTS4 // ADAM metallopeptidase with thrombospondin type 1 motif,	NM_005099	0.13	0.03	0.005
2609870	*BRPF1*	NM_001003694 // BRPF1 // bromodomain and PHD finger containing, 1 // 3p26-p25 //	NM_001003694	0.13	0.04	0.012
3632298	*ADPGK*	NM_031284 // ADPGK // ADP-dependent glucokinase // 15q24.1 // 83440 /// ENST0000	NM_031284	0.13	0.04	0.007
3184940	*GNG10*	NM_001017998 // GNG10 // guanine nucleotide binding protein (G protein), gamma 1	NM_001017998	0.13	0.04	0.011
3223776	*C5*	NM_001735 // C5 // complement component 5 // 9q33-q34 // 727 /// ENST00000223642	NM_001735	0.13	0.04	0.008
3922100	*MX1*	NM_002462 // MX1 // myxovirus (influenza virus) resistance 1, interferon-inducib	NM_002462	0.12	0.04	0.015
3960478	*CSNK1E*	NM_001894 // CSNK1E // casein kinase 1, epsilon // 22q13.1 // 1454 /// NM_152221	NM_001894	0.12	0.04	0.018
3715703	*SUPT6H*	NM_003170 // SUPT6H // suppressor of Ty 6 homolog (S. cerevisiae) // 17q11.2 //	NM_003170	0.11	0.03	0.005

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
2322818	*PADI3*	NM_016233 // PADI3 // peptidyl arginine deiminase, type III // 1p36.13 // 51702	NM_016233	0.11	0.03	0.006
2393740	*KIAA0562*	NM_014704 // KIAA0562 // KIAA0562 // 1p36.32 // 9731 /// ENST00000378230 // KIAA	NM_014704	0.11	0.03	0.009
3784509	*ZNF271*	NM_001112663 // ZNF271 // zinc finger protein 271 // 18q12 // 10778 /// NM_00662	NM_001112663	0.11	0.04	0.020
3372253	*CUGBP1*	NM_006560 // CUGBP1 // CUG triplet repeat, RNA binding protein 1 // 11p11 // 106	NM_006560	0.11	0.04	0.011
2948259	*TRIM26*	NM_003449 // TRIM26 // tripartite motif-containing 26 // 6p21.3 // 7726 /// ENST	NM_003449	0.11	0.03	0.006
3191900	*NUP214*	NM_005085 // NUP214 // nucleoporin 214kDa // 9q34.1 // 8021 /// ENST00000359428	NM_005085	0.11	0.03	0.003
3105581	*CA3*	NM_005181 // CA3 // carbonic anhydrase III, muscle specific // 8q13-q22 // 761 /	NM_005181	0.11	0.03	0.003
3832457	*RYR1*	NM_000540 // RYR1 // ryanodine receptor 1 (skeletal) // 19q13.1 // 6261 /// NM_0	NM_000540	0.11	0.03	0.006

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3936256	*BCL2L13*	NM_015367 // BCL2L13 // BCL2-like 13 (apoptosis facilitator) // 22q11 // 23786 /	NM_015367	0.10	0.02	0.002
3599280	*PIAS1*	NM_016166 // PIAS1 // protein inhibitor of activated STAT, 1 // 15q // 8554 ///	NM_016166	0.10	0.04	0.017
3755976	*MED24*	NM_014815 // MED24 // mediator complex subunit 24 // 17q21.1 // 9862 /// NM_0010	NM_014815	0.10	0.04	0.019
3656418	*SRCAP*	NM_006662 // SRCAP // Snf2-related CREBBP activator protein // 16p11.2 // 10847	NM_006662	0.10	0.04	0.017
3943101	*DEPDC5*	NM_014662 // DEPDC5 // DEP domain containing 5 // 22q12.3 // 9681 /// NM_0010071	NM_014662	0.09	0.01	0.000
3960685	*DMC1*	NM_007068 // DMC1 // DMC1 dosage suppressor of mck1 homolog, meiosis-specific ho	NM_007068	0.09	0.03	0.013
2434776	*CDC42SE1*	NM_001038707 // CDC42SE1 // CDC42 small effector 1 // 1q21.2 // 56882 /// NM_020	NM_001038707	0.08	0.03	0.014
3438417	*SFRS8*	NM_004592 // SFRS8 // splicing factor, arginine/serine-rich 8 (suppressor-of-whi	NM_004592	0.08	0.03	0.016

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3457696	*PAN2*	NM_014871 // PAN2 // PAN2 polyA specific ribonuclease subunit homolog (S. cerevi	NM_014871	0.08	0.02	0.008
2534615	*SCLY*	NM_016510 // SCLY // selenocysteine lyase // 2q37.3 // 51540 /// ENST00000254663	NM_016510	0.08	0.02	0.004
2765865	*RELL1*	NM_001085400 // RELL1 // RELT-like 1 // 4p14 // 768211 /// NM_001085399 // RELL1	NM_001085400	0.07	0.02	0.002
3765642	*INTS2*	NM_020748 // INTS2 // integrator complex subunit 2 // 17q23.2 // 57508 /// ENST0	NM_020748	0.05	0.01	0.005
2906607	*NFYA*	NM_002505 // NFYA // nuclear transcription factor Y, alpha // 6p21.3 // 4800 ///	NM_002505	-0.07	0.02	0.011
3168102	*CREB3*	NM_006368 // CREB3 // cAMP responsive element binding protein 3 // 9pter-p22.1 /	NM_006368	-0.07	0.02	0.010
3939365	*SMARCB1*	NM_003073 // SMARCB1 // SWI/SNF related, matrix associated, actin dependent regu	NM_003073	-0.07	0.02	0.013
3415229	*NR4A1*	NM_002135 // NR4A1 // nuclear receptor subfamily 4, group A, member 1 // 12q13 /	NM_002135	-0.07	0.03	0.015

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
2437801	*ARHGEF2*	NM_004723 // ARHGEF2 // rho/rac guanine nucleotide exchange factor (GEF) 2 // 1q	NM_004723	-0.09	0.02	0.002
3645565	*THOC6*	NM_024339 // THOC6 // THO complex 6 homolog (Drosophila) // 16p13.3 // 79228 ///	NM_024339	-0.10	0.04	0.018
2406766	*MRPS15*	NM_031280 // MRPS15 // mitochondrial ribosomal protein S15 // 1p35-p34.1 // 6496	NM_031280	-0.11	0.03	0.003
3553141	*KIAA0329*	NM_014844 // KIAA0329 // KIAA0329 // 14q32.31 // 9895 /// ENST00000359520 // KIA	NM_014844	-0.11	0.04	0.018
3297666	*DYDC1*	NM_138812 // DYDC1 // DPY30 domain containing 1 // 10q23.1 // 143241 /// ENST000	NM_138812	-0.11	0.02	0.000
3625674	*RFXDC2*	NM_022841 // RFXDC2 // regulatory factor X domain containing 2 // 15q21.3 // 648	NM_022841	-0.12	0.04	0.012
2926969	*PDE7B*	NM_018945 // PDE7B // phosphodiesterase 7B // 6q23-q24 // 27115 /// ENST00000308	NM_018945	-0.12	0.04	0.013
3525313	*COL4A1*	NM_001845 // COL4A1 // collagen, type IV, alpha 1 // 13q34 // 1282 /// ENST00000	NM_001845	-0.12	0.04	0.014

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
2438892	*FCRL5*	NM_031281 // FCRL5 // Fc receptor-like 5 // 1q21 // 83416 /// ENST00000361835 //	NM_031281	-0.12	0.04	0.009
3220846	*SUSD1*	NM_022486 // SUSD1 // sushi domain containing 1 // 9q31.3-q33.1 // 64420 /// ENS	NM_022486	-0.12	0.03	0.006
3598430	*SLC24A1*	NM_004727 // SLC24A1 // solute carrier family 24 (sodium/potassium/ calcium excha	NM_004727	-0.12	0.05	0.019
3506431	*RNF6*	NM_005977 // RNF6 // ring finger protein (C3H2C3 type) 6 // 13q12.2 // 6049 ///	NM_005977	-0.12	0.04	0.011
3696057	*SLC12A4*	NM_005072 // SLC12A4 // solute carrier family 12 (potassium/chloride transporter	NM_005072	-0.12	0.02	0.001
2519577	*COL3A1*	NM_000090 // COL3A1 // collagen, type III, alpha 1 (Ehlers-Danlos syndrome type	NM_000090	-0.12	0.04	0.012
3734479	*TMEM104*	NM_017728 // TMEM104 // transmembrane protein 104 // 17q25.1 // 54868 /// ENST00	NM_017728	-0.13	0.04	0.015
3345157	*PIWIL4*	NM_152431 // PIWIL4 // piwi-like 4 (Drosophila) // 11q21 // 143689 /// ENST00000	NM_152431	-0.13	0.05	0.015

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
2949471	*NEU1*	NM_000434 // NEU1 // sialidase 1 (lysosomal sialidase) // 6p21.3 // 4758 /// ENS	NM_000434	-0.13	0.04	0.013
2599670	*CRYBA2*	NM_057093 // CRYBA2 // crystallin, beta A2 // 2q34-q36 // 1412 /// NM_005209 //	NM_057093	-0.13	0.04	0.014
3922444	*ABCG1*	NM_207628 // ABCG1 // ATP-binding cassette, sub-family G (WHITE), member 1 // 21	NM_207628	-0.13	0.03	0.003
2760371	*WDR1*	NM_017491 // WDR1 // WD repeat domain 1 // 4p16.1 // 9948 /// NM_005112 // WDR1	NM_017491	-0.14	0.05	0.019
2835440	*TCOF1*	NM_001008656 // TCOF1 // Treacher Collins-Franceschetti syndrome 1 // 5q32-q33.1	NM_001008656	-0.14	0.04	0.007
2451544	*MYOG*	NM_002479 // MYOG // myogenin (myogenic factor 4) // 1q31-q41 // 4656 /// ENST00	NM_002479	-0.14	0.05	0.018
3745504	*SCO1*	NM_004589 // SCO1 // SCO cytochrome oxidase deficient homolog 1 (yeast) // 17p12	NM_004589	-0.14	0.03	0.003
2835213	*PPARGC1B*	NM_133263 // PPARGC1B // peroxisome proliferator-activated receptor gamma, coact	NM_133263	-0.14	0.04	0.006

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3704567	*CBFA2T3*	NM_005187 // CBFA2T3 // core-binding factor, runt domain, alpha subunit 2; trans	NM_005187	-0.14	0.05	0.020
2893562	*RREB1*	NM_002955 // RREB1 // ras responsive element binding protein 1 // 6p25 // 6239 /	NM_002955	-0.14	0.04	0.006
2672712	*SCAP*	NM_012235 // SCAP // SREBF chaperone // 3p21.31 // 22937 /// ENST00000265565 //	NM_012235	-0.14	0.04	0.009
2768197	*CORIN*	NM_006587 // CORIN // corin, serine peptidase // 4p13-p12 // 10699 /// ENST00000	NM_006587	-0.14	0.05	0.011
2495279	*VWA3B*	NM_144992 // VWA3B // von Willebrand factor A domain containing 3B // 2q11.2 //	NM_144992	-0.14	0.04	0.006
2903588	*PFDN6*	NM_014260 // PFDN6 // prefoldin subunit 6 // 6p21.3 // 10471 /// ENST00000399112	NM_014260	-0.14	0.05	0.014
3031383	*REPIN1*	NM_013400 // REPIN1 // replication initiator 1 // 7q36.1 // 29803 /// NM_014374	NM_013400	-0.15	0.05	0.018
3754469	*ACACA*	NM_198839 // ACACA // acetyl-Coenzyme A carboxylase alpha // 17q21 // 31 /// NM_	NM_198839	-0.15	0.05	0.010
3767480	*AXIN2*	NM_004655 // AXIN2 // axin 2 (conductin, axil) // 17q23-q24 // 8313 /// ENST0000	NM_004655	-0.15	0.05	0.013

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
2954506	*CRIP3*	NM_206922 // CRIP3 // cysteine-rich protein 3 // 6p21.1 // 401262 /// ENST000003	NM_206922	-0.15	0.06	0.018
3845263	*ADAMTSL5*	NM_213604 // ADAMTSL5 // ADAMTS-like 5 // 19p13.3 // 339366 /// ENST00000330475	NM_213604	-0.15	0.06	0.016
2565143	*STARD7*	NM_020151 // STARD7 // StAR-related lipid transfer (START) domain containing 7 /	NM_020151	-0.15	0.06	0.016
2321960	*PLEKHM2*	NM_015164 // PLEKHM2 // pleckstrin homology domain containing, family M (with RU	NM_015164	-0.16	0.05	0.009
3829174	*GPATCH1*	NM_018025 // GPATCH1 // G patch domain containing 1 // 19q13.11 // 55094 /// ENS	NM_018025	-0.16	0.03	0.001
2798586	*AHRR*	NM_020731 // AHRR // aryl-hydrocarbon receptor repressor // 5p15.3 // 57491 ///	NM_020731	-0.16	0.05	0.011
2362991	*CASQ1*	NM_001231 // CASQ1 // calsequestrin 1 (fast-twitch, skeletal muscle) // 1q21 //	NM_001231	-0.16	0.06	0.015
3954525	*ZNF280B*	NM_080764 // ZNF280B // zinc finger protein 280B // 22q11.22 // 140883 /// ENST0	NM_080764	-0.16	0.04	0.005

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
4020991	*ACTRT1*	NM_138289 // ACTRT1 // actin-related protein T1 // Xq25 // 139741 /// ENST000003	NM_138289	-0.16	0.05	0.007
3982975	*POU3F4*	NM_000307 // POU3F4 // POU class 3 homeobox 4 // Xq21.1 // 5456 /// ENST00000373	NM_000307	-0.16	0.05	0.013
3963990	*PKDREJ*	NM_006071 // PKDREJ // polycystic kidney disease (polycystin) and REJ homolog (s	NM_006071	-0.16	0.03	0.001
2436401	*JTB*	NM_006694 // JTB // jumping translocation breakpoint // 1q21 // 10899 /// NM_002	NM_006694	-0.16	0.06	0.014
2759654	*ABLIM2*	NM_032432 // ABLIM2 // actin binding LIM protein family, member 2 // 4p16-p15 //	NM_032432	-0.16	0.05	0.007
2437329	*CLK2*	NM_003993 // CLK2 // CDC-like kinase 2 // 1q21 // 1196 /// NR_002711 // CLK2P //	NM_003993	-0.16	0.06	0.016
3401119	*ITFG2*	NM_018463 // ITFG2 // integrin alpha FG-GAP repeat containing 2 // 12p13.33 // 5	NM_018463	-0.16	0.04	0.004
3599709	*GLCE*	NM_015554 // GLCE // glucuronic acid epimerase // 15q23 // 26035 /// ENST0000026	NM_015554	-0.16	0.06	0.014

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3882413	*C20orf114*	NM_033197 // C20orf114 // chromosome 20 open reading frame 114 // 20q11.21 // 92	NM_033197	-0.16	0.06	0.020
3712922	*C17orf39*	NM_024052 // C17orf39 // chromosome 17 open reading frame 39 // 17p11.2 // 79018	NM_024052	-0.16	0.06	0.017
2473376	*EFR3B*	BC049384 // EFR3B // EFR3 homolog B (S. cerevisiae) // 2p23.3 // 22979 /// ENST0	BC049384	-0.17	0.05	0.009
2607262	*STK25*	NM_006374 // STK25 // serine/threonine kinase 25 (STE20 homolog, yeast) // 2q37.	NM_006374	-0.17	0.06	0.015
3755580	*CACNB1*	NM_199247 // CACNB1 // calcium channel, voltage-dependent, beta 1 subunit // 17q	NM_199247	-0.17	0.06	0.013
3402150	*NTF3*	NM_001102654 // NTF3 // neurotrophin 3 // 12p13 // 4908 /// NM_002527 // NTF3 //	NM_001102654	-0.17	0.06	0.020
3014714	*ARPC1B*	NM_005720 // ARPC1B // actin related protein 2/3 complex, subunit 1B, 41kDa // 7	NM_005720	-0.17	0.06	0.020

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3723071	*DBF4B*	NM_145663 // DBF4B // DBF4 homolog B (S. cerevisiae) // 17q21.31|17q21 // 80174	NM_145663	-0.17	0.04	0.002
2371255	*SMG7*	NM_173156 // SMG7 // Smg-7 homolog, nonsense mediated mRNA decay factor (C. eleg	NM_173156	-0.17	0.06	0.014
3217487	*ALG2*	NM_033087 // ALG2 // asparagine-linked glycosylation 2 homolog (S. cerevisiae, a	NM_033087	-0.17	0.06	0.011
3352159	*LOC100130353*	AK130019 // LOC100130353 // hypothetical protein LOC100130353 // 11q23.3 // 1001	AK130019	-0.17	0.06	0.018
3401259	*TEAD4*	NM_003213 // TEAD4 // TEA domain family member 4 // 12p13.3-p13.2 // 7004 /// NM	NM_003213	-0.17	0.07	0.020
3114618	*RNF139*	NM_007218 // RNF139 // ring finger protein 139 // 8q24 // 11236 /// ENST00000303	NM_007218	-0.17	0.06	0.015
2991150	*TSPAN13*	NM_014399 // TSPAN13 // tetraspanin 13 // 7p21.1 // 27075 /// ENST00000262067 //	NM_014399	-0.18	0.05	0.006

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
2875193	*P4HA2*	NM_004199 // P4HA2 // procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline	NM_004199	-0.18	0.05	0.007
4011743	*SLC7A3*	NM_032803 // SLC7A3 // solute carrier family 7 (cationic amino acid transporter,	NM_032803	-0.18	0.06	0.009
3194015	*LCN9*	NM_001001676 // LCN9 // lipocalin 9 // 9q34.3 // 392399 /// ENST00000277526 // L	NM_001001676	-0.18	0.06	0.011
3741040	*MNT*	NM_020310 // MNT // MAX binding protein // 17p13.3 // 4335 /// ENST00000174618 /	NM_020310	-0.18	0.04	0.003
3901851	*ABHD12*	NM_001042472 // ABHD12 // abhydrolase domain containing 12 // 20p11.21 // 26090	NM_001042472	-0.18	0.05	0.004
2324919	*EPHB2*	NM_017449 // EPHB2 // EPH receptor B2 // 1p36.1-p35 // 2048 /// NM_004442 // EPH	NM_017449	-0.18	0.06	0.010
3185976	*COL27A1*	NM_032888 // COL27A1 // collagen, type XXVII, alpha 1 // 9q32 // 85301 /// ENST0	NM_032888	-0.18	0.06	0.009

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
2855434	*C5orf39*	NM_001014279 // C5orf39 // chromosome 5 open reading frame 39 // 5p12 // 389289	NM_001014279	-0.18	0.05	0.007
2334476	*MAST2*	NM_015112 // MAST2 // microtubule associated serine/threonine kinase 2 // 1p34.1	NM_015112	-0.18	0.02	0.000
3962734	*TTLL1*	NM_001008572 // TTLL1 // tubulin tyrosine ligase-like family, member 1 // 22q13.	NM_001008572	-0.18	0.03	0.001
4017538	*COL4A6*	NM_033641 // COL4A6 // collagen, type IV, alpha 6 // Xq22 // 1288 /// NM_001847	NM_033641	-0.18	0.03	0.000
3141589	*IL7*	NM_000880 // IL7 // interleukin 7 // 8q12-q13 // 3574 /// ENST00000263851 // IL7	NM_000880	-0.19	0.05	0.006
2436826	*KCNN3*	NM_002249 // KCNN3 // potassium intermediate/small conductance calcium-activated	NM_002249	-0.19	0.06	0.008
3521174	*ABCC4*	NM_005845 // ABCC4 // ATP-binding cassette, sub-family C (CFTR/MRP), member 4 //	NM_005845	-0.19	0.07	0.017
3768280	*C17orf58*	NM_181656 // C17orf58 // chromosome 17 open reading frame 58 // 17q24.2 // 28401	NM_181656	-0.19	0.07	0.017

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
2363784	*HSPA6*	NM_002155 // HSPA6 // heat shock 70kDa protein 6 (HSP70B') // 1q23 // 3310 /// E	NM_002155	-0.19	0.06	0.011
3928211	*GRIK1*	NM_175611 // GRIK1 // glutamate receptor, ionotropic, kainate 1 // 21q22.11 // 2	NM_175611	-0.19	0.06	0.011
2758978	*EVC2*	NM_147127 // EVC2 // Ellis van Creveld syndrome 2 (limbin) // 4p16.2-p16.1 // 13	NM_147127	-0.19	0.06	0.012
3740664	*C17orf91*	NM_032895 // C17orf91 // chromosome 17 open reading frame 91 // 17p13.3 // 84981	NM_032895	-0.19	0.07	0.015
2782267	*NEUROG2*	NM_024019 // NEUROG2 // neurogenin 2 // 4q25 // 63973 /// ENST00000313341 // NEU	NM_024019	-0.20	0.06	0.010
3826542	*ZNF738*	BC034499 // ZNF738 // zinc finger protein 738 // 19p12 // 148203 /// AK291002 //	BC034499	-0.20	0.05	0.003
3966000	*TYMP*	NM_001113756 // TYMP // thymidine phosphorylase // 22q13|22q13.33 // 1890 /// NM	NM_001113756	-0.20	0.05	0.003
3607447	*ABHD2*	NM_007011 // ABHD2 // abhydrolase domain containing 2 // 15q26.1 // 11057 /// NM	NM_007011	-0.20	0.05	0.005

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3236448	*SUV39H2*	NM_024670 // SUV39H2 // suppressor of variegation 3-9 homolog 2 (Drosophila) //	NM_024670	-0.20	0.07	0.011
2528504	*SPEG*	NM_005876 // SPEG // SPEG complex locus // 2q35 // 10290 /// ENST00000312358 //	NM_005876	-0.20	0.06	0.009
2730746	*SLC4A4*	NM_001098484 // SLC4A4 // solute carrier family 4, sodium bicarbonate cotranspor	NM_001098484	-0.20	0.06	0.007
2544662	*DNMT3A*	NM_175629 // DNMT3A // DNA (cytosine-5-)-methyltransferase 3 alpha // 2p23 // 17	NM_175629	-0.20	0.06	0.007
2937625	*C6orf208*	BC101251 // C6orf208 // chromosome 6 open reading frame 208 // 6q27 // 80069 ///	BC101251	-0.20	0.06	0.007
3233157	*UCN3*	NM_053049 // UCN3 // urocortin 3 (stresscopin) // 10p15.1 // 114131 /// ENST0000	NM_053049	-0.20	0.08	0.017
2548172	*FEZ2*	NM_001042548 // FEZ2 // fasciculation and elongation protein zeta 2 (zygin II) /	NM_001042548	-0.21	0.03	0.000
3877809	*OTOR*	NM_020157 // OTOR // otoraplin // 20p12.1-p11.23 // 56914 /// ENST00000246081 //	NM_020157	-0.21	0.08	0.019

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3839400	*C19orf63*	NM_175063 // C19orf63 // chromosome 19 open reading frame 63 // 19q13.33 // 2843	NM_175063	-0.21	0.04	0.002
3875108	*C20orf196*	AK292708 // C20orf196 // chromosome 20 open reading frame 196 // 20p12.3 // 1498	AK292708	-0.21	0.06	0.006
2970985	*TSPYL4*	NM_021648 // TSPYL4 // TSPY-like 4 // 6q22.1 // 23270 /// ENST00000368611 // TSP	NM_021648	-0.21	0.07	0.011
3189580	*ZBTB43*	NM_014007 // ZBTB43 // zinc finger and BTB domain containing 43 // 9q33-q34 // 2	NM_014007	-0.21	0.08	0.017
3407926	*CMAS*	NM_018686 // CMAS // cytidine monophosphate N-acetylneuraminic acid synthetase /	NM_018686	-0.21	0.03	0.000
3249886	*TET1*	NM_030625 // TET1 // tet oncogene 1 // 10q21 // 80312 /// ENST00000373644 // TET	NM_030625	-0.21	0.06	0.007
3151970	*MTSS1*	NM_014751 // MTSS1 // metastasis suppressor 1 // 8p22 // 9788 /// ENST0000032506	NM_014751	-0.21	0.07	0.009
3937183	*DGCR8*	NM_022720 // DGCR8 // DiGeorge syndrome critical region gene 8 // 22q11.2 // 544	NM_022720	-0.21	0.06	0.008

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3958253	*C22orf28*	BC016707 // C22orf28 // chromosome 22 open reading frame 28 // 22q12 // 51493 //	BC016707	-0.22	0.08	0.019
3607503	*ABHD2*	NM_007011 // ABHD2 // abhydrolase domain containing 2 // 15q26.1 // 11057 /// NM	NM_007011	-0.22	0.07	0.010
2799030	*SLC6A19*	NM_001003841 // SLC6A19 // solute carrier family 6 (neutral amino acid transport	NM_001003841	-0.22	0.06	0.007
3870611	*LILRB3*	NM_001081450 // LILRB3 // leukocyte immunoglobulin-like receptor, subfamily B (w	NM_001081450	-0.22	0.08	0.016
3857811	*C19orf12*	NM_031448 // C19orf12 // chromosome 19 open reading frame 12 // 19q12 // 83636 /	NM_031448	-0.22	0.08	0.019
2500667	*FBLN7*	NM_153214 // FBLN7 // fibulin 7 // 2q13 // 129804 /// ENST00000331203 // FBLN7 /	NM_153214	-0.22	0.08	0.019
3523156	*TMTC4*	NM_032813 // TMTC4 // transmembrane and tetratricopeptide repeat containing 4 //	NM_032813	-0.22	0.07	0.010
2612371	*EAF1*	NM_033083 // EAF1 // ELL associated factor 1 // 3p24.3 // 85403 /// ENST00000396	NM_033083	-0.22	0.07	0.008

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3988638	*LONRF3*	NM_001031855 // LONRF3 // LON peptidase N-terminal domain and ring finger 3 // X	NM_001031855	-0.23	0.08	0.012
3114240	*C8orf32*	BC008781 // C8orf32 // chromosome 8 open reading frame 32 // 8q24.13 // 55093 //	BC008781	-0.23	0.08	0.016
2460368	*TTC13*	NM_024525 // TTC13 // tetratricopeptide repeat domain 13 // 1q42.2 // 79573 ///	NM_024525	-0.23	0.08	0.014
2428425	*PPM1J*	NM_005167 // PPM1J // protein phosphatase 1J (PP2C domain containing) // 1p13.2	NM_005167	-0.23	0.06	0.003
3194986	*LCN12*	NM_178536 // LCN12 // lipocalin 12 // 9q34.3 // 286256 /// ENST00000371633 // LC	NM_178536	-0.23	0.06	0.004
3642875	*RAB11FIP3*	NM_014700 // RAB11FIP3 // RAB11 family interacting protein 3 (class II) // 16p13	NM_014700	-0.23	0.07	0.010
2532378	*CHRND*	NM_000751 // CHRND // cholinergic receptor, nicotinic, delta // 2q33-q34 // 1144	NM_000751	-0.23	0.08	0.018
2995667	*ADCYAP1R1*	NM_001118 // ADCYAP1R1 // adenylate cyclase activating polypeptide 1 (pituitary)	NM_001118	-0.23	0.05	0.002

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3390641	*ARHGAP20*	NM_020809 // ARHGAP20 // Rho GTPase activating protein 20 // 11q22.3-q23.1 // 57	NM_020809	-0.23	0.05	0.003
2830465	*MYOT*	NM_006790 // MYOT // myotilin // 5q31 // 9499 /// ENST00000239926 // MYOT // myo	NM_006790	-0.23	0.07	0.007
2452069	*PIK3C2B*	NM_002646 // PIK3C2B // phosphoinositide-3-kinase, class 2, beta polypeptide //	NM_002646	-0.23	0.02	0.000
3744127	*HES7*	NM_032580 // HES7 // hairy and enhancer of split 7 (Drosophila) // 17p13.1 // 84	NM_032580	-0.23	0.09	0.019
3327057	*FLJ14213*	NM_024841 // FLJ14213 // protor-2 // 11p13-p12 // 79899 /// ENST00000378867 // F	NM_024841	-0.23	0.07	0.007
2664332	*COLQ*	NM_005677 // COLQ // collagen-like tail subunit (single strand of homotrimer) of	NM_005677	-0.23	0.07	0.006
3829160	*C19orf40*	NM_152266 // C19orf40 // chromosome 19 open reading frame 40 // 19q13.11 // 9144	NM_152266	-0.23	0.08	0.012
3708798	*SENP3*	NM_015670 // SENP3 // SUMO1/sentrin/SMT3 specific peptidase 3 // 17p13 // 26168	NM_015670	-0.23	0.06	0.005

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
2358700	*MGC29891*	NM_144618 // MGC29891 // hypothetical protein MGC29891 // 1q21.2 // 126626 /// E	NM_144618	-0.23	0.09	0.019
2755111	*KLKB1*	NM_000892 // KLKB1 // kallikrein B, plasma (Fletcher factor) 1 // 4q34-q35 // 38	NM_000892	-0.24	0.08	0.012
2568968	*UXS1*	NM_025076 // UXS1 // UDP-glucuronate decarboxylase 1 // 2q12.2 // 80146 /// BC00	NM_025076	-0.24	0.08	0.011
2748923	*GUCY1B3*	NM_000857 // GUCY1B3 // guanylate cyclase 1, soluble, beta 3 // 4q31.3-q33 // 29	NM_000857	-0.24	0.07	0.007
3816509	*GADD45B*	NM_015675 // GADD45B // growth arrest and DNA-damage-inducible, beta // 19p13.3	NM_015675	-0.24	0.09	0.016
3376410	*SLC22A24*	BC034394 // SLC22A24 // solute carrier family 22, member 24 // 11q12.3 // 283238	BC034394	-0.24	0.07	0.007
3286393	*ZNF32*	NM_006973 // ZNF32 // zinc finger protein 32 // 10q22-q25 // 7580 /// NM_0010053	NM_006973	-0.24	0.08	0.010
2540157	*ODC1*	NM_002539 // ODC1 // ornithine decarboxylase 1 // 2p25 // 4953 /// ENST000002341	NM_002539	-0.24	0.09	0.020

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
2994835	*CHN2*	NM_004067 // CHN2 // chimerin (chimaerin) 2 // 7p15.3 // 1124 /// NM_001039936 /	NM_004067	-0.24	0.09	0.017
3603199	*IDH3A*	NM_005530 // IDH3A // isocitrate dehydrogenase 3 (NAD+) alpha // 15q25.1-q25.2 /	NM_005530	-0.24	0.05	0.001
3040454	*TWISTNB*	NM_001002926 // TWISTNB // TWIST neighbor // 7p15.3 // 221830 /// ENST0000022256	NM_001002926	-0.24	0.09	0.017
2497301	*TMEM182*	NM_144632 // TMEM182 // transmembrane protein 182 // 2q12.1 // 130827 /// ENST00	NM_144632	-0.24	0.07	0.007
3766716	*TEX2*	NM_018469 // TEX2 // testis expressed 2 // 17q23.3 // 55852 /// ENST00000258991	NM_018469	-0.25	0.07	0.007
3458819	*CYP27B1*	NM_000785 // CYP27B1 // cytochrome P450, family 27, subfamily B, polypeptide 1 /	NM_000785	-0.25	0.08	0.009
3368940	*ABTB2*	NM_145804 // ABTB2 // ankyrin repeat and BTB (POZ) domain containing 2 // 11p13	NM_145804	-0.25	0.08	0.010
3298924	*MMRN2*	NM_024756 // MMRN2 // multimerin 2 // 10q23.2 // 79812 /// ENST00000372027 // MM	NM_024756	-0.25	0.07	0.006

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3529951	*KIAA1305*	NM_025081 // KIAA1305 // KIAA1305 // 14q12 // 57523 /// BC008219 // KIAA1305 //	NM_025081	-0.25	0.08	0.011
3006572	*AUTS2*	NM_015570 // AUTS2 // autism susceptibility candidate 2 // 7q11.22 // 26053 ///	NM_015570	-0.25	0.09	0.017
3025500	*BPGM*	NM_001724 // BPGM // 2,3-bisphosphoglycerate mutase // 7q31-q34 // 669 /// NM_19	NM_001724	-0.25	0.10	0.018
2494709	*CNNM4*	NM_020184 // CNNM4 // cyclin M4 // 2p12-p11.2 // 26504 /// ENST00000377075 // CN	NM_020184	-0.26	0.09	0.016
3329983	*PTPRJ*	NM_002843 // PTPRJ // protein tyrosine phosphatase, receptor type, J // 11p11.2	NM_002843	-0.26	0.08	0.010
2769346	*LNX1*	NM_032622 // LNX1 // ligand of numb-protein X 1 // 4q12 // 84708 /// ENST0000030	NM_032622	-0.26	0.09	0.015
3867195	*FAM83E*	NM_017708 // FAM83E // family with sequence similarity 83, member E // 19q13.32-	NM_017708	-0.26	0.09	0.013
3790529	*GRP*	NM_002091 // GRP // gastrin-releasing peptide // 18q21.1-q21.32 // 2922 /// NM_0	NM_002091	-0.26	0.05	0.001

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3987029	*TMEM164*	NM_032227 // TMEM164 // transmembrane protein 164 // Xq22.3 // 84187 /// ENST000	NM_032227	-0.26	0.10	0.018
3526454	*GRTP1*	NM_024719 // GRTP1 // growth hormone regulated TBC protein 1 // 13q34 // 79774 /	NM_024719	-0.26	0.09	0.015
2438344	*GPATCH4*	NM_182679 // GPATCH4 // G patch domain containing 4 // 1q22 // 54865 /// NM_0155	NM_182679	-0.26	0.07	0.006
3132927	*NKX6-3*	NM_152568 // NKX6-3 // NK6 homeobox 3 // 8p11.21 // 157848 /// ENST00000343444 /	NM_152568	-0.27	0.09	0.014
2672376	*TESSP2*	NM_182702 // TESSP2 // testis serine protease 2 // 3p21.31 // 339906 /// ENST000	NM_182702	-0.27	0.09	0.013
2730347	*C4orf35*	NM_033122 // C4orf35 // chromosome 4 open reading frame 35 // 4q13.3 // 85438 //	NM_033122	-0.27	0.10	0.019
3921068	*ETS2*	NM_005239 // ETS2 // v-ets erythroblastosis virus E26 oncogene homolog 2 (avian)	NM_005239	-0.27	0.03	0.000
2532894	*DGKD*	NM_152879 // DGKD // diacylglycerol kinase, delta 130kDa // 2q37.1 // 8527 /// N	NM_152879	-0.27	0.07	0.003

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
4018454	*AMOT*	NM_133265 // AMOT // angiomotin // Xq23 // 154796 /// NM_001113490 // AMOT // an	NM_133265	-0.27	0.09	0.012
3070507	*RNF148*	NM_198085 // RNF148 // ring finger protein 148 // 7q31.33 // 378925 /// BC029264	NM_198085	-0.27	0.10	0.017
3832256	*SPINT2*	NM_021102 // SPINT2 // serine peptidase inhibitor, Kunitz type, 2 // 19q13.1 //	NM_021102	-0.27	0.10	0.017
3371225	*CHST1*	NM_003654 // CHST1 // carbohydrate (keratan sulfate Gal-6) sulfotransferase 1 //	NM_003654	-0.27	0.07	0.005
3870494	*TFPT*	NM_013342 // TFPT // TCF3 (E2A) fusion partner (in childhood Leukemia) // 19q13	NM_013342	-0.27	0.09	0.010
3863811	*PSG9*	NM_002784 // PSG9 // pregnancy specific beta-1-glycoprotein 9 // 19q13.2 // 5678	NM_002784	-0.28	0.09	0.011
3160175	*VLDLR*	NM_003383 // VLDLR // very low density lipoprotein receptor // 9p24 // 7436 ///	NM_003383	-0.28	0.08	0.007
2794704	*ASB5*	NM_080874 // ASB5 // ankyrin repeat and SOCS box-containing 5 // 4q34.2 // 14045	NM_080874	-0.28	0.11	0.019

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3908901	*KCNB1*	NM_004975 // KCNB1 // potassium voltage-gated channel, Shab-related subfamily, m	NM_004975	-0.28	0.09	0.009
3390852	*FLJ45803*	NM_207429 // FLJ45803 // FLJ45803 protein // 11q23.1 // 399948 /// ENST000003554	NM_207429	-0.28	0.10	0.015
2600689	*EPHA4*	NM_004438 // EPHA4 // EPH receptor A4 // 2q36.1 // 2043 /// ENST00000281821 // E	NM_004438	-0.29	0.07	0.003
3469597	*NUAK1*	NM_014840 // NUAK1 // NUAK family, SNF1-like kinase, 1 // 12q23.3 // 9891 /// EN	NM_014840	-0.29	0.09	0.009
3607232	*ISG20L1*	NM_022767 // ISG20L1 // interferon stimulated exonuclease gene 20kDa-like 1 // 1	NM_022767	-0.29	0.10	0.015
2358426	*ADAMTSL4*	AK023606 // ADAMTSL4 // ADAMTS-like 4 // 1q21.2 // 54507	AK023606	-0.29	0.11	0.016
3853609	*CYP4F2*	NM_001082 // CYP4F2 // cytochrome P450, family 4, subfamily F, polypeptide 2 //	NM_001082	-0.29	0.11	0.016
2936971	*KIF25*	NM_030615 // KIF25 // kinesin family member 25 // 6q27 // 3834 /// NM_005355 //	NM_030615	-0.30	0.09	0.008

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
2997272	*EEPD1*	NM_030636 // EEPD1 // endonuclease/exonuclease/phosphatase family domain contain	NM_030636	-0.30	0.09	0.010
3961253	*RPS19BP1*	NM_194326 // RPS19BP1 // ribosomal protein S19 binding protein 1 // 22q13.1 // 9	NM_194326	-0.30	0.10	0.013
3082373	*VIPR2*	NM_003382 // VIPR2 // vasoactive intestinal peptide receptor 2 // 7q36.3 // 7434	NM_003382	-0.30	0.10	0.011
2340961	*IL12RB2*	NM_001559 // IL12RB2 // interleukin 12 receptor, beta 2 // 1p31.3-p31.2 // 3595	NM_001559	-0.30	0.08	0.005
2736462	*BMPR1B*	NM_001203 // BMPR1B // bone morphogenetic protein receptor, type IB // 4q22-q24	NM_001203	-0.30	0.08	0.004
3774504		---	---	-0.30	0.11	0.016
3395958	*OR8B4*	NM_001005196 // OR8B4 // olfactory receptor, family 8, subfamily B, member 4 //	NM_001005196	-0.30	0.11	0.018
2806231	*BXDC2*	NM_018321 // BXDC2 // brix domain containing 2 // 5p13.2 // 55299 /// ENST000003	NM_018321	-0.31	0.10	0.013
2396858	*NPPB*	NM_002521 // NPPB // natriuretic peptide precursor B // 1p36.2 // 4879 /// ENST0	NM_002521	-0.31	0.11	0.016

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3233322	*C10orf18*	NM_017782 // C10orf18 // chromosome 10 open reading frame 18 // 10p15.1 // 54906	NM_017782	-0.31	0.06	0.001
2439101	*FCRL1*	NM_052938 // FCRL1 // Fc receptor-like 1 // 1q21-q22 // 115350 /// ENST000003681	NM_052938	-0.31	0.06	0.001
2413907	*DHCR24*	NM_014762 // DHCR24 // 24-dehydrocholesterol reductase // 1p33-p31.1 // 1718 ///	NM_014762	-0.31	0.11	0.014
3231186	*C9orf37*	NM_032937 // C9orf37 // chromosome 9 open reading frame 37 // 9q34.3 // 85026 //	NM_032937	-0.31	0.09	0.008
2669955	*XIRP1*	NM_194293 // XIRP1 // xin actin-binding repeat containing 1 // 3p22.2 // 165904	NM_194293	-0.32	0.11	0.013
3345222	*AMOTL1*	NM_130847 // AMOTL1 // angiomotin like 1 // 11q14.3 // 154810 /// ENST0000031782	NM_130847	-0.32	0.11	0.012
2573326	*FLJ14816*	BC112205 // FLJ14816 // hypothetical protein FLJ14816 // 2q14.2 // 84931 /// BC1	BC112205	-0.32	0.11	0.016
3349437	*UNQ2550*	AY358815 // UNQ2550 // SFVP2550 // 11q23.1 // 100130653	AY358815	-0.32	0.09	0.005

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3951117	*ACR*	NM_001097 // ACR // acrosin // 22q13-qter|22q13.33 // 49 /// ENST00000216139 //	NM_001097	-0.32	0.12	0.017
2489140		---	---	-0.32	0.07	0.002
2562115	*LSM3*	CR457185 // LSM3 // LSM3 homolog, U6 small nuclear RNA associated (S. cerevisiae	CR457185	-0.32	0.11	0.011
3572975	*NGB*	NM_021257 // NGB // neuroglobin // 14q24.3 // 58157 /// ENST00000298352 // NGB /	NM_021257	-0.33	0.09	0.004
2439350	*OR6N1*	NM_001005185 // OR6N1 // olfactory receptor, family 6, subfamily N, member 1 //	NM_001005185	-0.33	0.10	0.009
3590275	*CHAC1*	NM_024111 // CHAC1 // ChaC, cation transport regulator homolog 1 (E. coli) // 15	NM_024111	-0.33	0.12	0.014
2397898	*HSPB7*	NM_014424 // HSPB7 // heat shock 27kDa protein family, member 7 (cardiovascular)	NM_014424	-0.33	0.12	0.015
2364677	*PBX1*	NM_002585 // PBX1 // pre-B-cell leukemia homeobox 1 // 1q23 // 5087 /// ENST0000	NM_002585	-0.34	0.07	0.001

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
2474409	*DNAJC5G*	NM_173650 // DNAJC5G // DnaJ (Hsp40) homolog, subfamily C, member 5 gamma // 2p2	NM_173650	-0.34	0.09	0.004
3581373		---	---	-0.34	0.12	0.014
3508330	*HSPH1*	NM_006644 // HSPH1 // heat shock 105kDa/110kDa protein 1 // 13q12.3 // 10808 ///	NM_006644	-0.34	0.13	0.019
3751164	*DHRS13*	NM_144683 // DHRS13 // dehydrogenase/reductase (SDR family) member 13 // 17q11.2	NM_144683	-0.35	0.10	0.006
2908179	*VEGFA*	NM_001025366 // VEGFA // vascular endothelial growth factor A // 6p12 // 7422 //	NM_001025366	-0.35	0.13	0.016
3962448	*dJ222E13.2*	NR_002184 // dJ222E13.2 // similar to CGI-96 // 22q13.2 // 91695 /// BC073834 //	NR_002184	-0.35	0.12	0.014
3747638	*LOC201164*	BC031263 // LOC201164 // similar to CG12314 gene product // 17p11.2 // 201164 //	BC031263	-0.35	0.09	0.004
2821981	*TMEM157*	NM_198507 // TMEM157 // transmembrane protein 157 // 5q21.1 // 345757 /// ENST00	NM_198507	-0.35	0.12	0.015

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3123675	*PPP1R3B*	NM_024607 // PPP1R3B // protein phosphatase 1, regulatory (inhibitor) subunit 3B	NM_024607	-0.35	0.12	0.014
2656837	*ST6GAL1*	NM_173216 // ST6GAL1 // ST6 beta-galactosamide alpha-2,6-sialyltranferase 1 // 3	NM_173216	-0.35	0.13	0.016
3746574	*PMP22*	NM_000304 // PMP22 // peripheral myelin protein 22 // 17p12-p11.2 // 5376 /// NM	NM_000304	-0.36	0.09	0.004
2771342	*EPHA5*	NM_004439 // EPHA5 // EPH receptor A5 // 4q13.1 // 2044 /// NM_182472 // EPHA5 /	NM_004439	-0.36	0.09	0.003
2888674	*MXD3*	NM_031300 // MXD3 // MAX dimerization protein 3 // 5q35.3 // 83463 /// ENST00000	NM_031300	-0.36	0.12	0.012
2353477	*ATP1A1*	NM_000701 // ATP1A1 // ATPase, Na+/K+ transporting, alpha 1 polypeptide // 1p21	NM_000701	-0.36	0.11	0.007
3956984	*ZMAT5*	NM_019103 // ZMAT5 // zinc finger, matrin type 5 // 22cen-q12.3 // 55954 /// NM_	NM_019103	-0.36	0.11	0.009

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
2551651	*ATP6V1E2*	NM_080653 // ATP6V1E2 // ATPase, H+ transporting, lysosomal 31kDa, V1 subunit E2	NM_080653	-0.37	0.13	0.017
3578069	*C14orf139*	BC008299 // C14orf139 // chromosome 14 open reading frame 139 // 14q32.13 // 796	BC008299	-0.37	0.13	0.016
2428501	*SLC16A1*	NM_003051 // SLC16A1 // solute carrier family 16, member 1 (monocarboxylic acid	NM_003051	-0.37	0.14	0.018
3061621	*TFPI2*	NM_006528 // TFPI2 // tissue factor pathway inhibitor 2 // 7q22 // 7980 /// ENST	NM_006528	-0.37	0.09	0.002
3705516	*LOC100131454*	AF229804 // LOC100131454 // similar to hCG1646635 // 17p13.3 // 100131454 /// EN	AF229804	-0.38	0.11	0.008
3306299	*XPNPEP1*	NM_020383 // XPNPEP1 // X-prolyl aminopeptidase (aminopeptidase P) 1, soluble //	NM_020383	-0.38	0.14	0.018
2763550	*PPARGC1A*	NM_013261 // PPARGC1A // peroxisome proliferator-activated receptor gamma, coact	NM_013261	-0.38	0.13	0.012
2769063	*USP46*	NM_022832 // USP46 // ubiquitin specific peptidase 46 // 4q12 // 64854 /// ENST0	NM_022832	-0.38	0.13	0.013

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3806459	*ST8SIA5*	NM_013305 // ST8SIA5 // ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransfera	NM_013305	-0.38	0.10	0.004
3190151	*SLC25A25*	NM_001006641 // SLC25A25 // solute carrier family 25 (mitochondrial carrier; pho	NM_001006641	-0.39	0.09	0.003
2489172	*MTHFD2*	NM_001040409 // MTHFD2 // methylenetetrahydrofolate dehydrogenase (NADP+ depende	NM_001040409	-0.39	0.05	0.000
2952065	*PPIL1*	NM_016059 // PPIL1 // peptidylprolyl isomerase (cyclophilin)-like 1 // 6p21.1 //	NM_016059	-0.39	0.10	0.005
3382015	*CHRDL2*	NM_015424 // CHRDL2 // chordin-like 2 // 11q14 // 25884 /// ENST00000263671 // C	NM_015424	-0.39	0.10	0.003
2711139	*ATP13A5*	NM_198505 // ATP13A5 // ATPase type 13A5 // 3q29 // 344905 /// ENST00000342358 /	NM_198505	-0.40	0.11	0.005
2633917	*RG9MTD1*	NM_017819 // RG9MTD1 // RNA (guanine-9-) methyltransferase domain containing 1 /	NM_017819	-0.41	0.14	0.013
2974671	*C6orf192*	NM_052831 // C6orf192 // chromosome 6 open reading frame 192 // 6q22.3-q23.3 //	NM_052831	-0.41	0.15	0.018

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
2982270	*FLJ27255*	ENST00000355047 // FLJ27255 // hypothetical LOC401281 // 6q25.3 // 401281 /// AK	ENST00000355047	-0.41	0.12	0.007
2778273	*PGDS*	NM_014485 // PGDS // prostaglandin D2 synthase, hematopoietic // 4q22.3 // 27306	NM_014485	-0.41	0.08	0.001
3005332	*RCP9*	NM_014478 // RCP9 // calcitonin gene-related peptide-receptor component protein	NM_014478	-0.41	0.14	0.013
2650393	*PPM1L*	NM_139245 // PPM1L // protein phosphatase 1 (formerly 2C)-like // 3q26.1 // 1517	NM_139245	-0.42	0.12	0.006
3463056	*CSRP2*	NM_001321 // CSRP2 // cysteine and glycine-rich protein 2 // 12q21.1 // 1466 ///	NM_001321	-0.42	0.11	0.005
2459405		---	---	-0.43	0.10	0.003
2570238	*NPHP1*	NM_000272 // NPHP1 // nephronophthisis 1 (juvenile) // 2q13 // 4867 /// NM_20718	NM_000272	-0.43	0.06	0.000
2840616	*NPM1*	NM_002520 // NPM1 // nucleophosmin (nucleolar phosphoprotein B23, numatrin) // 5	NM_002520	-0.43	0.14	0.010
3601051	*NEO1*	NM_002499 // NEO1 // neogenin homolog 1 (chicken) // 15q22.3-q23 // 4756 /// ENS	NM_002499	-0.43	0.09	0.002

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3936515	*TUBA8*	NM_018943 // TUBA8 // tubulin, alpha 8 // 22q11.1 // 51807 /// ENST00000330423 /	NM_018943	-0.43	0.10	0.002
2725013	*UCHL1*	NM_004181 // UCHL1 // ubiquitin carboxyl-terminal esterase L1 (ubiquitin thioles	NM_004181	-0.44	0.11	0.004
2380590	*TGFB2*	NM_003238 // TGFB2 // transforming growth factor, beta 2 // 1q41 // 7042 /// ENS	NM_003238	-0.44	0.16	0.017
2496382	*NPAS2*	NM_002518 // NPAS2 // neuronal PAS domain protein 2 // 2q11.2 // 4862 /// ENST00	NM_002518	-0.46	0.10	0.002
3841574	*LILRB1*	NM_006669 // LILRB1 // leukocyte immunoglobulin-like receptor, subfamily B (with	NM_006669	-0.46	0.16	0.015
3726960	*NME2*	NM_001018137 // NME2 // non-metastatic cells 2, protein (NM23B) expressed in //	NM_001018137	-0.47	0.16	0.013
2649367	*PTX3*	NM_002852 // PTX3 // pentraxin-related gene, rapidly induced by IL-1 beta // 3q2	NM_002852	-0.47	0.11	0.002
2909483	*GPR111*	NM_153839 // GPR111 // G protein-coupled receptor 111 // 6p12.3 // 222611 /// EN	NM_153839	-0.47	0.13	0.006

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
2881950	*SLC36A2*	NM_181776 // SLC36A2 // solute carrier family 36 (proton/amino acid symporter),	NM_181776	-0.48	0.12	0.004
3441190	*FGF6*	NM_020996 // FGF6 // fibroblast growth factor 6 // 12p13 // 2251 /// ENST0000022	NM_020996	-0.48	0.12	0.004
3028911	*C7orf34*	NM_178829 // C7orf34 // chromosome 7 open reading frame 34 // 7q34 // 135927 ///	NM_178829	-0.49	0.18	0.019
2830861	*EGR1*	NM_001964 // EGR1 // early growth response 1 // 5q31.1 // 1958 /// ENST000002399	NM_001964	-0.49	0.19	0.020
3323891	*GAS2*	NM_177553 // GAS2 // growth arrest-specific 2 // 11p14.3-p15.2 // 2620 /// NM_00	NM_177553	-0.49	0.16	0.011
2497252	*SLC9A2*	NM_003048 // SLC9A2 // solute carrier family 9 (sodium/hydrogen exchanger), memb	NM_003048	-0.50	0.11	0.002
3018484	*GPR22*	NM_005295 // GPR22 // G protein-coupled receptor 22 // 7q22-q31.1 // 2845 /// EN	NM_005295	-0.51	0.15	0.008
2712632	*TFRC*	NM_003234 // TFRC // transferrin receptor (p90, CD71) // 3q29 // 7037 /// ENST00	NM_003234	-0.51	0.12	0.003
3214451	*NFIL3*	NM_005384 // NFIL3 // nuclear factor, interleukin 3 regulated // 9q22 // 4783 //	NM_005384	-0.53	0.14	0.004

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
2435981	*S100A12*	NM_005621 // S100A12 // S100 calcium binding protein A12 // 1q21 // 6283 /// ENS	NM_005621	-0.54	0.19	0.014
3320675	*RIG*	U32331 // RIG // regulated in glioma // 11p15.1 // 10530	U32331	-0.54	0.10	0.001
3290746	*SLC16A9*	NM_194298 // SLC16A9 // solute carrier family 16, member 9 (monocarboxylic acid	NM_194298	-0.54	0.15	0.006
3055703	*NSUN5C*	NM_032158 // NSUN5C // NOL1/NOP2/Sun domain family, member 5C // 7q11.23 // 2602	NM_032158	-0.57	0.17	0.008
3265494	*TRUB1*	NM_139169 // TRUB1 // TruB pseudouridine (psi) synthase homolog 1 (E. coli) // 1	NM_139169	-0.57	0.17	0.008
3374213	*OR1S2*	NM_001004459 // OR1S2 // olfactory receptor, family 1, subfamily S, member 2 //	NM_001004459	-0.58	0.20	0.013
3318253	*OR51L1*	NM_001004755 // OR51L1 // olfactory receptor, family 51, subfamily L, member 1 /	NM_001004755	-0.59	0.18	0.009
3294280	*DNAJC9*	NM_015190 // DNAJC9 // DnaJ (Hsp40) homolog, subfamily C, member 9 // 10q22.2 //	NM_015190	-0.59	0.22	0.018

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
2899095	*HIST1H4A*	NM_003538 // HIST1H4A // histone cluster 1, H4a // 6p21.3 // 8359 /// ENST000003	NM_003538	-0.60	0.16	0.005
2378068	*G0S2*	NM_015714 // G0S2 // G0/G1switch 2 // 1q32.2-q41 // 50486 /// ENST00000367029 //	NM_015714	-0.63	0.22	0.016
3737677	*LOC100129503*	AF218021 // LOC100129503 // hypothetical protein LOC100129503 // 17q25.3 // 1001	AF218021	-0.64	0.19	0.007
3300115	*PPP1R3C*	NM_005398 // PPP1R3C // protein phosphatase 1, regulatory (inhibitor) subunit 3C	NM_005398	-0.69	0.26	0.020
3279058	*ACBD7*	NM_001039844 // ACBD7 // acyl-Coenzyme A binding domain containing 7 // 10p13 //	NM_001039844	-0.69	0.13	0.001
4031156	*RPS4Y2*	NM_001039567 // RPS4Y2 // ribosomal protein S4, Y-linked 2 // Yq11.223 // 140032	NM_001039567	-0.71	0.17	0.003
2979246	*RAET1L*	NM_130900 // RAET1L // retinoic acid early transcript 1L // 6q25.1 // 154064 ///	NM_130900	-0.75	0.26	0.013
3321150	*ARNTL*	NM_001178 // ARNTL // aryl hydrocarbon receptor nuclear translocator-like // 11p	NM_001178	-0.80	0.20	0.004

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3862873	*CYP2A6*	NM_000762 // CYP2A6 // cytochrome P450, family 2, subfamily A, polypeptide 6 //	NM_000762	-1.12	0.34	0.009

[00360]

4. IDENTIFICATION OF URSOLIC ACID AS AN INHIBITOR OF FASTING-INDUCED MUSCLE ATROPHY.

[00361] The Connectivity Map describes the effects of > 1300 bioactive small molecules on global mRNA expression in several cultured cell lines, and contains search algorithms that permit comparisons between compound-specific mRNA expression signatures and mRNA expression signatures of interest (Lamb J, *et al.* (2006) *Science (New York, N.Y* 313(5795):1929-1935). It was hypothesized herein that querying the Connectivity Map with the mRNA expression signature of fasting (muscle atrophy signature-1) would identify inhibitors of atrophy-associated gene expression and thus, potential inhibitors of muscle atrophy. It was also reasoned herein that increasing the specificity of the query would enhance the output. To this end, as described herein, an evolutionarily conserved mRNA expression signature of fasting was discovered by comparing the effect of fasting on human skeletal muscle to the effect of a 24 h fast on mouse skeletal muscle. The mouse studies were described previously (Ebert SM, *et al.* (2010) *Molecular endocrinology* 24(4):790-799). Altogether, 35 mRNAs that were increased by fasting and 40 mRNAs that were decreased by fasting were identified in both human and mouse skeletal muscle (Table X2; the data in column labeled "Change" show mean changes in $\log_2$ hybridization signals between fasting and fed states for the species indicated, [Mean $\log_2$ mRNA levels for fasted] minus [Mean $\log_2$ mRNA levels in unfasted]; *P*-values were determined with paired t-tests). The data shown in Table X2 includes all mRNAs whose levels were increased by fasting in human muscle ($P \leq 0.02$) and in mouse muscle ($P \leq 0.05$), and all mRNAs whose levels were decreased by fasting in human muscle ($P \leq 0.02$) and in mouse muscle ($P \leq 0.05$). Of the mRNAs shown in Table X2, 63 mRNAs were represented on the HG-U133A arrays used in the Connectivity Map (Figure 6A). These mRNAs (31 increased by fasting and 32 decreased by fasting) were used to query the Connectivity Map for candidate small molecule inhibitors of muscle atrophy.

[00362] Table X2. Fasting-regulated mRNAs common to human and mouse skeletal

muscle.

mRNA	Protein	Human		Mouse	
		Mean Log2 Change (Fasting - Fed)	*P*	Mean Log2 Change (Fasting - Fed)	*P*
PDK4	pyruvate dehydrogenase kinase, isozyme 4	2.15	0.000	1.91	0.000
TXNIP	thioredoxin interacting protein	0.85	0.004	0.60	0.038
FBXO32	F-box protein 32	0.82	0.002	2.13	0.000
SLC38A2	solute carrier family 38, member 2	0.62	0.001	0.33	0.036
UCP3	uncoupling protein 3 (mitochondrial, proton carrier)	0.59	0.000	1.02	0.001
ZFAND5	zinc finger, AN1-type domain 5	0.51	0.005	0.57	0.001
HMOX1	heme oxygenase (decycling) 1	0.46	0.006	0.17	0.035
SESN1	sestrin 1	0.46	0.004	1.51	0.001
GABARAPL1	GABA(A) receptor-associated protein like 1	0.39	0.004	1.18	0.000
CAT	catalase	0.39	0.003	0.85	0.001
CITED2	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain	0.37	0.005	0.29	0.010
ABCA1	ATP-binding cassette, sub-family A (ABC1), member 1	0.37	0.016	0.26	0.018
FBXL20	F-box and leucine-rich repeat protein 20	0.35	0.002	0.46	0.001
XPO4	exportin 4	0.31	0.009	0.22	0.022
HERPUD1	homocysteine-inducible, endoplasmic reticulum stress-inducible, ubiquitin-like domain 1	0.29	0.003	0.27	0.029
ACOX1	acyl-Coenzyme A oxidase 1, palmitoyl	0.29	0.013	0.53	0.006
NOX4	NADPH oxidase 4	0.28	0.002	0.41	0.018
UBE4A	ubiquitination factor E4A (UFD2 homolog, yeast)	0.27	0.004	1.08	0.010
INSR	insulin receptor	0.24	0.014	0.58	0.003
IGF1R	insulin-like growth factor 1 receptor	0.23	0.013	0.40	0.001
PANK1	pantothenate kinase 1	0.21	0.007	0.78	0.000
NBR1	neighbor of BRCA1 gene 1	0.21	0.017	0.39	0.009

mRNA	Protein	Human		Mouse	
		Mean Log2 Change (Fasting - Fed)	*P*	Mean Log2 Change (Fasting - Fed)	*P*
RORA	RAR-related orphan receptor A	0.21	0.006	0.39	0.006
TMEM71	transmembrane protein 71	0.21	0.009	0.40	0.008
CPT1A	carnitine palmitoyltransferase 1A (liver)	0.21	0.001	0.21	0.020
UCP2	uncoupling protein 2 (mitochondrial, proton carrier)	0.20	0.005	0.33	0.024
TULP3	tubby like protein 3	0.19	0.008	0.22	0.008
MED13L	mediator complex subunit 13-like	0.18	0.000	0.23	0.011
CALCOCO1	calcium binding and coiled coil domain 1	0.16	0.010	0.31	0.028
MYO5A	myosin VA (heavy chain 12, myoxin)	0.14	0.006	0.36	0.012
PPAP2B	phosphatidic acid phosphatase type 2B	0.13	0.007	0.09	0.029
SRRM2	serine/arginine repetitive matrix 2	0.13	0.007	0.24	0.040
ADPGK	ADP-dependent glucokinase	0.13	0.007	0.16	0.009
SUPT6H	suppressor of Ty 6 homolog (S. cerevisiae)	0.11	0.005	0.26	0.036
SFRS8	splicing factor, arginine/serine-rich 8	0.08	0.016	0.13	0.011
NFYA	nuclear transcription factor Y, alpha	-0.07	0.011	-0.31	0.045
MRPS15	mitochondrial ribosomal protein S15	-0.11	0.003	-0.25	0.001
PDE7B	phosphodiesterase 7B	-0.12	0.013	-0.51	0.011
WDR1	WD repeat domain 1	-0.14	0.019	-0.21	0.047
ACACA	acetyl-Coenzyme A carboxylase alpha	-0.15	0.010	-0.22	0.041
AXIN2	axin 2 (conductin, axil)	-0.15	0.013	-0.12	0.046
CASQ1	calsequestrin 1 (fast-twitch, skeletal muscle)	-0.16	0.015	-0.26	0.015
ZNF280B	zinc finger protein 280B	-0.16	0.005	-0.34	0.046
JTB	jumping translocation breakpoint	-0.16	0.014	-0.42	0.030
CACNB1	calcium channel, voltage-dependent, beta 1 subunit	-0.17	0.013	-0.43	0.003

mRNA	Protein	Human		Mouse	
		Mean Log2 Change (Fasting - Fed)	*P*	Mean Log2 Change (Fasting - Fed)	*P*
ALG2	asparagine-linked glycosylation 2 homolog	-0.17	0.011	-0.39	0.019
TSPAN13	tetraspanin 13	-0.18	0.006	-0.30	0.028
P4HA2	procollagen-proline, 2-oxoglutarate 4-dioxygenase, alpha II polypeptide	-0.18	0.007	-0.12	0.012
TTLL1	tubulin tyrosine ligase-like family, member 1	-0.18	0.001	-0.29	0.043
SUV39H2	suppressor of variegation 3-9 homolog 2 (Drosophila)	-0.20	0.011	-0.26	0.014
SLC4A4	solute carrier family 4, sodium bicarbonate cotransporter, member 4	-0.20	0.007	-0.69	0.003
DNMT3A	DNA (cytosine-5-)-methyltransferase 3 alpha	-0.20	0.007	-0.48	0.000
FEZ2	fasciculation and elongation protein zeta 2 (zygin II)	-0.21	0.000	-0.50	0.019
MTSS1	metastasis suppressor 1	-0.21	0.009	-0.22	0.033
TMTC4	transmembrane and tetratricopeptide repeat containing 4	-0.22	0.010	-0.17	0.035
PPM1J	protein phosphatase 1J (PP2C domain containing)	-0.23	0.003	-0.30	0.012
ARHGAP20	Rho GTPase activating protein 20	-0.23	0.003	-0.22	0.013
ABTB2	ankyrin repeat and BTB (POZ) domain containing 2	-0.25	0.010	-0.18	0.005
CNNM4	cyclin M4	-0.26	0.016	-0.27	0.005
GRTP1	growth hormone regulated TBC protein 1	-0.26	0.015	-0.54	0.002
RNF148	ring finger protein 148	-0.27	0.017	-0.35	0.014
SPINT2	serine peptidase inhibitor, Kunitz type, 2	-0.27	0.017	-0.23	0.026
PBX1	pre-B-cell leukemia homeobox 1	-0.34	0.001	-0.22	0.000
HSPH1	heat shock 105kDa/110kDa protein 1	-0.34	0.019	-0.20	0.043

mRNA	Protein	Human		Mouse	
		Mean Log2 Change (Fasting - Fed)	*P*	Mean Log2 Change (Fasting - Fed)	*P*
VEGFA	vascular endothelial growth factor A	-0.35	0.016	-0.26	0.002
PMP22	peripheral myelin protein 22	-0.36	0.004	-0.13	0.012
PPARGC1A	peroxisome proliferative activated receptor, gamma, coactivator 1 alpha	-0.38	0.012	-0.39	0.030
ST8SIA5	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 5	-0.38	0.004	-0.48	0.011
PPIL1	peptidylprolyl isomerase (cyclophilin)-like 1	-0.39	0.005	-0.52	0.016
PPM1L	protein phosphatase 1 (formerly 2C)-like	-0.42	0.006	-0.46	0.000
NEO1	neogenin homolog 1 (chicken)	-0.43	0.002	-0.31	0.037
TGFB2	transforming growth factor, beta 2	-0.44	0.017	-0.30	0.003
PTX3	pentraxin-related gene, rapidly induced by IL-1 beta	-0.47	0.002	-0.48	0.000
GAS2	growth arrest-specific 2	-0.49	0.011	-0.23	0.044
TFRC	transferrin receptor (p90, CD71)	-0.51	0.003	-1.37	0.011

[00363] The left side of Figure 6B shows the 10 Connectivity Map instances (or data sets) with the most significant positive correlations ($P < 0.004$) to the effect of fasting in skeletal muscle. The connectivity score, represented on the y-axis, is a measure of the strength of the correlation (Lamb J, *et al.* (2006) *Science (New York, N.Y* 313(5795):1929-1935); the compound and cell-line is shown below the bar representing the Connectivity Score. Of these, 6 involved wortmannin or LY-294002 (inhibitors of phosphoinositide 3-kinase (PI3K)) or rapamycin (an inhibitor of the mammalian target of rapamycin complex 1 (mTORC1)). Since PI3K and mTORC1 mediate effects of insulin and IGF-I, and since insulin/IGF-I signaling inhibits muscle atrophy and atrophy-associated changes in skeletal muscle mRNA expression (Bodine SC, *et al.* (2001) *Nat Cell Biol* 3(11):1014-1019; Sandri M, *et al.* (2004) *Cell* 117(3):399-412), these results lent confidence that the Connectivity Map might be used to identify potential inhibitors of muscle atrophy. The right side of Figure 6B shows the 10

Connectivity Map instances with the most significant negative correlations ($P < 0.004$) to the effect of fasting in skeletal muscle. These compounds, whose effects on cultured cell lines were opposite to the effect of fasting on muscle, included metformin (an insulin-sensitizing agent widely used to treat type 2 diabetes), as well as ursolic acid. Further experiments focused on metformin and ursolic acid. To test the hypothesis that metformin and ursolic acid might reduce fasting-induced muscle atrophy, each compound was administered, or vehicle alone, via i.p. injection to C57BL/6 mice. The mice were then fasted, and after 12 hours of fasting, the mice received a second dose of the compound or vehicle. After 24 hours of fasting, the blood glucose was measured and muscles were harvested. The data shown in Figures 4C-4H are means ± SEM from 16 mice. Both metformin (250 mg / kg) and ursolic acid (200 mg / kg) significantly reduced fasting blood glucose (Figures 4C and 4D). The effects of metformin and ursolic acid on fasting-induced muscle atrophy were also examined, i.e. the effect of 24 h fast (relative to ad lib feeding) on wet weight of lower hindlimb skeletal muscle (bilateral tibialis anterior ("TA" muscle), gastrocnemius, and soleus; see Figures 4E-4G). In the absence of metformin and ursolic acid, fasting reduced muscle weight by 9 % (Figure 6E). Although metformin did not alter muscle weight in fasted mice (Figure 6F), ursolic acid increased it by 7 ± 2 % (Figure 6G). Moreover, consistent with the predicted inhibitory effect on fasting-induced gene expression described herein, ursolic acid reduced fasting levels of *atrogin-1* and *MuRF1* mRNA levels in the TA muscles of fasted mice (Figure 6H; the data shown are normalized to the levels in vehicle-treated mice, which were set at 1). In Figures 4E-4H, each data point represents one mouse and the horizontal bars denote the means. In Figures 4C-4H, *P*-values were determined using unpaired t-tests. Thus, ursolic acid, but not metformin, decreased fasting-induced muscle atrophy.

5. URSOLIC ACID REDUCES DENERVATION-INDUCED MUSCLE ATROPHY.

[00364] The Connectivity Map was queried with a second mRNA expression signature, muscle atrophy signature-2 (described above), to determine if this muscle atrophy signature would also correlate with ursolic acid, among other compounds. As described above, muscle atrophy signature-2 was an mRNA expression signature identified as described herein for human skeletal muscle mRNAs that were induced or repressed by fasting and also by spinal cord injury ("SCI"). The studies of the effects of SCI on human skeletal muscle gene expression were described previously (Adams CM, *et al.* (2011) *Muscle Nerve*. 43(1):65-75). Using this approach with the muscle atrophy expression signatures described herein, there were 18 human mRNAs that were increased by fasting and SCI, and 17 human mRNAs that were decreased by fasting and SCI, and are shown in Table X3 ("Change" represents mean

changes in $\log_2$ hybridization signals for pairs as indicated, e.g. fasting and fed states for column labeled "(Fasting - Fed)" or untrained and trained for the column labeled "(Untrained - Trained)"). The data in Table X3 include all mRNAs whose levels were increased by fasting ($P \leq 0.02$) and by SCI ($P \leq 0.05$), and all mRNAs whose levels were decreased by fasting ($P \leq 0.02$) and by SCI ($P \leq 0.05$). *P*-values in Table X3 were determined with paired t-tests.

[00365] Table X3. Human skeletal muscle mRNAs induced or repressed by fasting and SCI.

mRNA	Protein	EFFECT OF FASTING		EFFECT OF SCI	
		Change (Fasting – Fed)	*P*	Change (Untrained – Trained)	*P*
OR1D4	olfactory receptor, family 1, subfamily D, member 4	0.50	0.019	0.65	0.030
RHOBTB 1	Rho-related BTB domain containing 1	0.48	0.001	0.71	0.032
TSPAN8	tetraspanin 8	0.39	0.015	1.79	0.023
FLJ33996	hypothetical protein FLJ33996	0.39	0.019	0.68	0.020
NUPR1	nuclear protein 1	0.35	0.007	0.65	0.030
IRS2	insulin receptor substrate 2	0.34	0.004	0.21	0.035
NPC2	Niemann-Pick disease, type C2	0.30	0.011	0.39	0.042
KLF11	Kruppel-like factor 11	0.29	0.011	0.22	0.034
ZNF682	zinc finger protein 682	0.28	0.017	0.72	0.013
NOX4	NADPH oxidase 4	0.28	0.002	0.56	0.007
PLXDC2	plexin domain containing 2	0.26	0.013	0.38	0.022
CTDSP2	CTD small phosphatase 2	0.25	0.003	0.34	0.021
CAV3	caveolin 3	0.24	0.007	0.56	0.020
IGF1R	insulin-like growth factor 1 receptor	0.23	0.013	0.63	0.040
FLJ14154	hypothetical protein FLJ14154	0.22	0.005	0.30	0.021
CUGBP2	CUG triplet repeat, RNA binding protein 2	0.21	0.004	0.14	0.034
MLL	myeloid/lymphoid or mixed-lineage leukemia	0.14	0.016	0.30	0.040
SUPT6H	suppressor of Ty 6 homolog	0.11	0.005	0.19	0.024
MRPS15	mitochondrial ribosomal protein S15	-0.11	0.003	-0.33	0.001
RFXDC2	regulatory factor X domain containing 2	-0.12	0.012	-0.10	0.037
PDE7B	phosphodiesterase 7B	-0.12	0.013	-0.39	0.011
PFDN6	prefoldin subunit 6	-0.14	0.014	-0.42	0.021

mRNA	Protein	EFFECT OF FASTING		EFFECT OF SCI	
		Change (Fasting – Fed)	*P*	Change (Untrained – Trained)	*P*
ZNF280B	zinc finger protein 280B	-0.16	0.005	-0.30	0.028
TSPAN13	tetraspanin 13	-0.18	0.006	-0.56	0.023
TTLL1	tubulin tyrosine ligase-like family, member 1	-0.18	0.001	-0.37	0.020
CMAS	cytidine monophosphate N-acetylneuraminic acid synthetase	-0.21	0.000	-0.22	0.025
C8orf32	chromosome 8 open reading frame 32	-0.23	0.016	-0.11	0.049
GUCY1B3	guanylate cyclase 1, soluble, beta 3	-0.24	0.007	-0.24	0.008
ZNF32	zinc finger protein 32	-0.24	0.010	-0.21	0.030
VLDLR	very low density lipoprotein receptor	-0.28	0.007	-0.16	0.015
HSPB7	heat shock 27kDa protein family, member 7 (cardiovascular)	-0.33	0.015	-0.77	0.032
VEGFA	vascular endothelial growth factor A	-0.35	0.016	-0.43	0.020
SLC16A1	solute carrier family 16, member 1	-0.37	0.018	-0.94	0.015
PPARGC1A	peroxisome proliferative activated receptor, gamma, coactivator 1 alpha	-0.38	0.012	-0.74	0.001
C6orf192	chromosome 6 open reading frame 192	-0.41	0.018	-0.39	0.042

[00366] Of the mRNAs listed in Table X3, 29 were represented on the HG-U133A arrays used in the Connectivity Map (Figure 7A), but only 10 were common to the 63 mRNAs used in the first Connectivity Map query described above for muscle atrophy signature-1 (*IGF-IR, NOX4, SUPT6H, MRPS15, PDE7B, PGC-1a, TSPAN13, TTLL1, VEGFA* and *ZNF280B*). The mRNAs listed in Figure 7A represent human muscle atrophy signature-2: mRNAs altered by both fasting and SCI in human muscle. These mRNAs, as described above, were used to query the Connectivity Map. Inclusion criteria were: $P \leq 0.02$ in fasted human muscle (by t-test), $P \leq 0.05$ in untrained, paralyzed muscle (by t-test), and the existence of complimentary probes on HG-U133A arrays. Connectivity Map instances with the most significant positive and negative correlations to the effect of fasting and SCI in human muscle. $P < 0.005$ for all compounds are shown in Figure 7B. The results partially

overlapped with the results of the first search: both search strategies identified LY-294002, wortmannin and rapamycin as predicted mimics of atrophy-inducing stress, and ursolic acid (but not metformin) as a predicted inhibitor (Figure 7B).

[00367] Because muscle atrophy signature-2 utilized data from SCI subjects, it was hypothesized that ursolic acid might reduce denervation-induced muscle atrophy. To test this, the left hindlimb muscles a denervation-induced skeletal muscle atrophy model in mouse was used. Briefly, on day 0, the left hindlimbs of C57BL/6 mice were denervated by transsecting the left sciatic nerve. This approach allowed the right hindlimb to serve as an intra-subject control. Mice were then administered ursolic acid (200 mg/kg) or an equivalent volume of vehicle alone (corn oil) via i.p. injection twice daily for seven days. During this time, mice continued to have ad libitum access to food. On day 7, muscle tissues were harvested for analysis, and the left (denervated) and right (innervated) hindlimb muscles in both groups (ursolic acid vs. vehicle administration) were compared. Ursolic acid significantly decreased denervation-induced muscle loss (Figure 7C). In Figure 7C, weights of the left (denervated) lower hindlimb muscles were normalized to weights of the right (innervated) lower hindlimb muscles from the same mouse. Each data point represents one mouse, and horizontal bars denote the means and the *P*-value was determined using an unpaired t-test. Histologically, this effect of ursolic acid was reflected as an increase in the size of denervated skeletal muscle fiber diameter in denervated gastrocnemius (D) and TA (E) muscles (Figures 5D and 5E, respectivel). The data shown in Figures 5D and 5E are from > 2500 muscle fibers per condition; $P < 0.0001$ by unpaired t-test. Thus, ursolic acid reduced denervation-induced muscle atrophy.

6. URSOLIC ACID INDUCES SKELETAL MUSCLE HYPERTROPHY.

[00368] The results from the denervation-induced muscle atrophy model suggested that ursolic acid reduced muscle atrophy, thus the hypothesis that ursolic acid might promote muscle hypertrophy in the absence of an atrophy-inducing stress was reasonable. Mice were provided ad lib access to either standard chow (control diet) or standard chow supplemented with 0.27% ursolic acid (ursolic acid diet) for 5 weeks before grip strength was measured and tissues were harvested. After five weeks, mice administered ursolic had increased lower hindlimb muscle weight (Figure 8A), quadriceps weight (Figure 8B), and upper forelimb muscle (triceps and biceps) weight (Figure 8C). Each data point in Figures 6A-6C represents one mouse, and horizontal bars denote the means. The effect of ursolic acid in this study on skeletal muscle fiber size distribution is shown in Figure 8D. Each distribution represents measurements of > 800 triceps muscle fibers from 7 animals (> 100 measurements / animal);

$P < 0.0001$. The effect of ursolic acid on peak grip strength (normalized to body weight) is shown in Figure 8E. Each data point represents one mouse, and horizontal bars denote the means. Non-normalized grip strength data were 157 ± 9 g (control diet) and 181 ± 6 g (ursolic acid diet) ($P = 0.04$).

[00369] Moreover, dietary ursolic acid increased the specific force generated by muscles ex vivo (Figure 9). Briefly, six-week old male C57BL/6 mice were provided either standard diet or diet containing 0.27% ursolic acid for 16 weeks before being euthanized. The lower hindlimb was removed (by transsecting the upper hindlimb mid-way through the femur), and placed in Krebs solution aerated with 95% O2 and 5% CO2. The gastrocnemius, soleus and tibialis anterior muscles, as well as the distal half of the tibia and fibula were then removed and discarded, leaving the extensor digitorum longus and peroneus muscles with their origins and insertions intact. A suture was placed through the proximal tendon and secured to the distal femur fragment. This ex vivo preparation was then mounted vertically in a water jacket bath (Aurora Scientific 1200A Intact Muscle Test System, filled with aerated Krebs solution) by attaching the suture to a servo-controlled lever (superiorly) and clamping the metatarsals (inferiorly). Passive muscle force was adjusted to a baseline of 1 g, and then muscles were stimulated with supramaximal voltage (80 V) at 100 Hz. The mean time from euthanasia to maximal force measurements was 10 min. After force measurements, muscles were removed and weighed in order to calculate specific titanic force. Maximal tetanic force and muscle weight did not differ between the two groups ($P = 0.20$ and 0.26, respectively). Data are means ± SEM from 5-6 mice per diet. *P*-values were determined with a t-test. Together, the data in Figures 6 and 7 provide morphological and functional evidence that ursolic acid induced skeletal muscle hypertrophy.

7. URSOLIC ACID INDUCES TROPHIC CHANGES IN SKELETAL MUSCLE GENE EXPRESSION.

[00370] The foregoing results suggested that ursolic acid might alter skeletal muscle gene expression. To test this hypothesis, an unbiased approach was used, specifically exon expression arrays were used to analyze gastrocnemius muscle mRNA expression in mice that had been fed diets lacking or containing ursolic acid for 5 weeks. Mice were provided ad lib access to either standard chow (control diet) or standard chow supplemented with 0.27% ursolic acid (ursolic acid diet) for 5 weeks before gastrocnemius muscle RNA was harvested and analyzed by Affymetrix Mouse Exon 1.0 ST arrays (n = 4 arrays per diet). Each array assessed pooled gastrocnemius RNA from two mice. Stringent criteria were used for ursolic acid-induced effects on mRNA levels ($P < 0.005$), and mRNAs with low levels of expression

were disregarded (i.e. only transcripts that were increased to a mean $\log_2$ hybridization signal ≥ 8, or repressed from a mean $\log_2$ hybridization signal ≥ 8 were included). The results were that ursolic acid decreased 18 mRNAs and increased 51 mRNAs (out of > 16,000 mRNAs analyzed. The results are shown in Table X4 ("Change" is the meang $\log_2$ change or difference between mice on ursolic acid diet and control diet, i.e. [Mean $\log_2$ mRNA levels in ursolic acid diet] minus [Mean $\log_2$ mRNA levels in control diet]).

[00371] Table X4. Mouse skeletal muscle mRNAs induced or repressed by ursolic acid.

mRNA	Protein	Change	*P*
Smox	spermine oxidase	0.81	0.001
Lyz2	lysozyme 2	0.71	0.001
C3	complement component 3	0.70	0.000
Tyrobp	TYRO protein tyrosine kinase binding protein	0.69	0.001
Lum	lumican	0.61	0.001
Igf1	insulin-like growth factor 1	0.56	0.005
Fmo1	flavin containing monooxygenase 1	0.47	0.000
Ostn	osteocrin	0.43	0.001
Nampt	nicotinamide phosphoribosyltransferase	0.41	0.003
H19	H19 fetal liver mRNA	0.39	0.004
Hipk2	homeodomain interacting protein kinase 2	0.38	0.002
Fbp2	fructose bisphosphatase 2	0.37	0.003
Gpx1	glutathione peroxidase 1	0.36	0.001
Sepp1	selenoprotein P, plasma, 1	0.35	0.004
Parp3	poly (ADP-ribose) polymerase family, member 3	0.32	0.001
Hspb8	heat shock protein 8	0.32	0.000
Musk	muscle, skeletal, receptor tyrosine kinase	0.31	0.004
Fhl3	four and a half LIM domains 3	0.31	0.005
Hsph1	heat shock 105kDa/110kDa protein 1	0.30	0.001
Arfgap2	ADP-ribosylation factor GTPase activating protein 2	0.30	0.001
Cd24a	CD24a antigen	0.28	0.002
Sepx1	selenoprotein X 1	0.28	0.003
Hk2	hexokinase 2	0.26	0.003
Ggct	gamma-glutamyl cyclotransferase	0.24	0.005
Trip10	thyroid hormone receptor interactor 10	0.23	0.000
Npc1	Niemann Pick type C1	0.22	0.001
Asb5	ankyrin repeat and SOCs box-containing 5	0.21	0.001
Vps29	vacuolar protein sorting 29 (S. pombe)	0.20	0.000
Ahsa2	AHA1, activator of heat shock protein ATPase homolog 2	0.18	0.001
Lsm14a	LSM14 homolog A (SCD6, S. cerevisiae)	0.18	0.004
Pdha1	pyruvate dehydrogenase E1 alpha 1	0.18	0.001
Trappc2l	trafficking protein particle complex 2-like	0.16	0.004
Ube2l3	ubiquitin-conjugating enzyme E2L 3	0.16	0.003
Ctsb	cathepsin B	0.16	0.003

mRNA	Protein	Change	*P*
D0H4S114	DNA segment, human D4S114	0.15	0.004
Psma2	proteasome (prosome, macropain) subunit, alpha type 2	0.15	0.005
Mrpl46	mitochondrial ribosomal protein L46	0.15	0.001
Eef1e1	eukaryotic translation elongation factor 1 epsilon 1	0.15	0.002
Krr1	KRR1, small subunit (SSU) processome component, homolog	0.15	0.005
Ndufaf4	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, assembly factor 4	0.14	0.005
Ndufs2	NADH dehydrogenase (ubiquinone) Fe-S protein 2	0.14	0.002
2610507B 11Rik	RIKEN cDNA 2610507B11 gene	0.14	0.000
Ssr4	signal sequence receptor, delta	0.14	0.000
Ndufs4	NADH dehydrogenase (ubiquinone) Fe-S protein 4	0.14	0.003
Sqstm1	sequestosome 1	0.12	0.001
Gfm1	G elongation factor, mitochondrial 1	0.12	0.003
2310016M 24Rik	RIKEN cDNA 2310016M24 gene	0.12	0.004
Sod2	superoxide dismutase 2, mitochondrial	0.12	0.001
Prdx5	peroxiredoxin 5	0.10	0.005
BC004004	cDNA sequence BC004004	0.06	0.001
Ghitm	growth hormone inducible transmembrane protein	0.05	0.005
Foxn3	forkhead box N3	-0.09	0.000
Klhl31	kelch-like 31 (Drosophila)	-0.09	0.001
Acadm	acyl-Coenzyme A dehydrogenase, medium chain	-0.11	0.001
Eif4g3	eukaryotic translation initiation factor 4 gamma, 3	-0.12	0.005
Nrap	nebulin-related anchoring protein	-0.14	0.003
Golga4	golgi autoantigen, golgin subfamily a, 4	-0.14	0.003
Paip2b	poly(A) binding protein interacting protein 2B	-0.16	0.000
Pde4dip	phosphodiesterase 4D interacting protein (myomegalin)	-0.18	0.001
Sfpq	splicing factor proline/glutamine rich	-0.18	0.005
Pnn	pinin	-0.18	0.002
D4Wsu53e	DNA segment, Chr 4, Wayne State University 53, expressed	-0.18	0.003
Mlec	malectin	-0.19	0.003
Cacna1s	calcium channel, voltage-dependent, L type, alpha 1S	-0.22	0.001
Sfrs5	splicing factor, arginine/serine-rich 5 (SRp40, HRS)	-0.22	0.005
Nnt	nicotinamide nucleotide transhydrogenase	-0.24	0.002

mRNA	Protein	Change	*P*
Adprhl1	ADP-ribosylhydrolase like 1	-0.26	0.002
Ddit4l	DNA-damage-inducible transcript 4-like	-0.32	0.000
Fbxo32	F-box protein 32 (Atrogin-1)	-0.35	0.001

[00372] As discussed above, *atrogin-1* and *MuRF1* are transcriptionally up-regulated by atrophy-inducing stresses (see Figure 4B and Sacheck JM, *et al.* (2007) *Faseb J* 21(1):140-155), and they are required for muscle atrophy (Bodine SC, *et al.* (2001) *Science (New York, N.Y* 294(5547):1704-1708). Moreover, in the studies of fasted mice as described herein above, ursolic acid reduced *atrogin-1* and *MuRF1* mRNAs (Figure 6H). Consistent with that finding, the arrays indicated that dietary ursolic acid reduced *atrogin-1* mRNA, which was the most highly repressed mRNA (Figure 10A). The results shown in Figure 10A represent a subset of the mRNAs from Table X4 which had the greatest increase or decrease in expression level in response to ursolic acid. Although *MuRF1* mRNA was not measured by the arrays used in these experiments, qPCR analysis confirmed that dietary ursolic acid repressed both *atrogin-1* and *MuRF1* mRNAs (Figure 10B; data are means ± SEM). Interestingly, one of the most highly up-regulated muscle mRNAs was *IGF1* (Figures 8A and 8B), which encodes insulin-like growth factor-I (IGF-I), a locally generated autocrine/paracrine hormone. *IGF1* mRNA is known to be transcriptionally induced in hypertrophic muscle (Hameed M, *et al.* (2004) *The Journal of physiology* 555(Pt 1):231-240; Adams GR & Haddad F (1996) *J Appl Physiol* 81(6):2509-2516; Gentile MA, *et al.* (2010) *Journal of molecular endocrinology* 44(1):55-73). In addition, increased skeletal muscle *IGF1* expression reduces denervation-induced muscle atrophy (Shavlakadze T, *et al.* (2005) *Neuromuscul Disord* 15(2):139-146), and stimulates muscle hypertrophy (Barton-Davis ER, *et al.* (1998) *Proceedings of the National Academy of Sciences of the United States of America* 95(26):15603-15607; Musarò A, *et al.* (2001) *Nature Genetics* 27(2):195-200). Moreover, by stimulating skeletal muscle insulin/IGF-I signaling, IGF-I represses *atrogin-1* and *MuRF* mRNAs (Sacheck JM, *et al.* (2004) *Am J Physiol Endocrinol Metab* 287(4):E591-601; Frost RA, *et al.* (2009) *J Cell Biochem* 108(5):1192-1202.), as well as *DDIT4L* mRNA (*ibid*), which, after *atrogin-1* mRNA, was the second most highly repressed mRNA in muscle from ursolic acid-treated mice (Figure 10A). Thus, 5 weeks of dietary ursolic acid altered skeletal muscle gene expression in a manner known to reduce atrophy and promote hypertrophy, and muscle-specific *IGF1* induction emerged as a likely contributing mechanism in ursolic acid-induced muscle hypertrophy. The effect of ursolic acid on plasma

IGF-I levels was also determined, which primarily reflect growth hormone-mediated hepatic IGF-I production (Yakar S, *et al.* (1999) *Proceedings of the National Academy of Sciences of the United States of America* 96(13):7324-7329). Although diets containing 0.14% or 0.27% ursolic acid increased muscle mass (described in greater detail below; Figure 12A), neither increased plasma IGF-I (Figure 10C). For the data in Figure 10C, mice were provided ad lib access to either standard chow (control diet) or standard chow supplemented with the indicated concentration of ursolic acid for 7 weeks before plasma IGF-I levels were measured. Each data point represents one mouse, and horizontal bars denote the means. *P*-values were determined by one-way ANOVA with Dunnett's post-test. Of note, exon expression arrays indicated that ursolic acid increased levels of all measured *IGF1* exons (exons 2-6; Figure 11A). The data in Figure 11A are mean exon-specific $\log_2$ hybridization signals from the arrays described in Table X2. However, ursolic acid did not alter levels of mRNAs encoding myostatin (which reduces muscle mass, for example see Lee SJ (2004) *Annu Rev Cell Dev Biol* 20:61-86), or twist or myogenin (which are induced by IGF-I during development, for example see Dupont J, *et al.* (2001) *The Journal of biological chemistry* 276(28):26699-26707; Tureckova J, *et al.* (2001) *The Journal of biological chemistry* 276(42):39264-39270). Moreover, ursolic acid did not alter the amount of *IGF1* mRNA in adipose tissue (Figure 11B). Briefly, the data shown in Figure 11B were obtained as follows: mice were provided ad lib access to either standard chow (control diet) or standard chow supplemented with 0.27% ursolic acid (ursolic acid diet) for 7 weeks before retroperitoneal adipose tissue was harvested for qPCR quantification of *IGF1* mRNA. The data shown are means ± SEM from 5 mice per group. Without wishing to be bound by a particular theory, ursolic acid-mediated *IGF1* induction may be localized to skeletal muscle.

8. URSOLIC ACID ENHANCES SKELETAL MUSCLE IGF-I SIGNALING.

[00373] Although muscle-specific *IGF1* induction is characteristic of, and contributes to, muscle hypertrophy, it may be a relatively late event that promotes hypertrophy after it has been initiated by other stimuli (Adams GR, *et al.* (1999) *J Appl Physiol* 87(5):1705-1712). Without wishing to be bound by a particular theory, it is possible that ursolic acid might have a more proximal effect on insulin/IGF-I signaling. In a previous study of non-muscle cell lines (CHO/IR and 3T3-L1 cells), ursolic acid enhanced insulin-mediated Akt activation (Jung SH, *et al.* (2007) *The Biochemical journal* 403(2):243-250). To determine whether ursolic acid might have a similar effect in skeletal muscle, the level of phosphorylated Akt was assessed in quadriceps muscles of mice fed diets lacking or containing ursolic acid. Briefly, mice were provided ad lib access to either standard chow (control diet) or standard

chow supplemented with 0.27% ursolic acid for 16 weeks. Total protein extracts from quadriceps muscles were subjected to SDS-PAGE, followed by immunoblot analysis for phosphorylated and total Akt, as indicated. A representative immunoblot is shown in Figure 10D. Immunoblot data were quantitated as follows: in each mouse, the level of phospho-Akt was normalized to the level of total Akt; these ratios were then normalized to the average phospho-Akt/total Akt ratio from control mice and the results are shown in Figure 10E (data are means ± SEM from 9 mice per diet. *P*-value was determined by unpaired t-test). The data show that in quadriceps, ursolic acid increased Akt phosphorylation by 1.8-fold.

[00374] The effect of ursolic acid on Akt activation was examined in C2C12 skeletal myotubes, a well-established in vitro model of skeletal muscle (Sandri M, *et al.* (2004) *Cell* 117(3):399-412; Stitt TN, *et al.* (2004) *Mol Cell* 14(3):395-403). Use of an *in vitro* system, such as C2C12 skeletal myotubes, circumvented potentially confounding effects from non-muscle tissues, and enabled a determination of whether IGF-I or insulin was required for ursolic acid's effect. The latter consideration was important because circulating IGF-I and insulin are always present in healthy animals. Use of an *in vitro* system also allowed testing of a clearly defined concentration of ursolic acid (10 μM, similar what was used in the Connectivity Map (8.8 μM)) for a clearly defined time of incubation (20 min). These considerations were important because the in vivo pharmacokinetic properties of ursolic acid are not yet known.

[00375] For the data shown in Figures 8F-8K, serum-starved C2C12 myotubes were treated in the absence or presence of ursolic acid (10 μM) and/or IGF-I (10 nM), as indicated. For studies of the IGF-I receptor, cells were harvested 2 min later, and protein extracts were subjected to immunoprecipitation with anti-IGF-I receptor β antibody, followed by immunoblot analysis with anti-phospho-tyrosine or anti-IGF-I receptor β antibodies to assess phospho- and total IGF-I receptor, respectively. For other studies, cells were harvested 20 min after addition of ursolic acid and/or IGF-I, and immunoblot analyses were performed using total cellular protein extracts and antibodies specific for the phosphorylated or total proteins indicated. Representative immunoblots showing effect of ursolic acid on IGF-I-mediated phosphorylation of Akt (Figure 10F), S6K (Figure 10G) and IGF-I receptor (Figure 10H). Data from immunoblots was quantitated as follows: levels in the presence of ursolic acid and IGF-I were normalized to levels in the presence of IGF-I alone, which were set at 1 and are indicated by the dashed line. The data shown in Figure 10I are means ± SEM from ≥ 3 experiments.

[00376] For the data shown in Figures 9C – 9F, serum-starved C2C12 myotubes were treated in the absence or presence of ursolic acid (10 μM), insulin (10 nM) and/or IGF-I (10 nM), as indicated. For studies of the insulin receptor, cells were harvested 2 min later, and protein extracts were subjected to immunoprecipitation with anti-insulin receptor β antibody, followed by immunoblot analysis with anti-phospho-insulin receptor β (Y1162/1163) or anti-insulin receptor β antibodies to assess phospho- and total insulin receptor, respectively. For other studies, cells were harvested 20 min after addition of ursolic acid, insulin and/or IGF-I, and immunoblot analyses were performed using total cellular protein extracts and antibodies specific for the phosphorylated or total proteins indicated.

[00377] When serum-starved myotubes were treated with ursolic acid alone, Akt phosphorylation did not increase (Figure 10F). However, in the presence of IGF-I, ursolic acid increased Akt phosphorylation by 1.9-fold (Figures 8F and 8I). Ursolic acid also increased Akt phosphorylation in the presence of insulin (Figure 11C). Thus, ursolic acid enhanced IGF-I-mediated and insulin-mediated Akt phosphorylation. The finding that ursolic acid enhanced muscle Akt activity in vivo and in vitro was consistent with the finding that ursolic acid's mRNA expression signature negatively correlated with the mRNA expression signatures of LY-294002 and wortmannin (Figures 4B and 5B), which inhibit insulin/IGF-I signaling upstream of Akt. However, ursolic acid's signature also negatively correlated with the signature of rapamycin, which inhibits insulin/IGF-I signaling downstream of Akt.

[00378] Although ursolic acid alone did not increase S6K phosphorylation (Figure 11D), it enhanced IGF-I-mediated and insulin-mediated S6K phosphorylation (Figures 8G, 8I and 9D). To further investigate the mechanism, the effect of ursolic acid on the IGF-I receptor was examined. Ursolic acid increased IGF-I receptor phsophorylation in the presence but not the absence of IGF-I (Figures 8H and 8I). Similarly, ursolic acid increased insulin receptor phosphorylation in the presence but not the absence of insulin (Figure 11E). Both of these effects were rapid, occurring within 2 minutes after the addition of ursolic acid and either IGF-I or insulin. Consistent with enhanced signaling at the level of the IGF-I and insulin receptors, ursolic acid also enhanced IGF-I-mediated and insulin-mediated ERK phosphorylation (Figures 8J and 9F). Moreover, ursolic acid enhanced IGF-I-mediated phosphorylation (inhibition) of FoxO transcription factors, which activate transcription of *atrogin-1* and *MuRF1* mRNAs (Figure 10K; Sandri M, *et al.* (2004) *Cell* 117(3):399-412; Stitt TN, *et al.* (2004) *Mol Cell* 14(3):395-403.). Without wishing to be bound by a particular

theory, ursolic acid represses atrophy-associated gene expression and promotes muscle hypertrophy by increasing activity of the IGF-I and insulin receptors.

9. URSOLIC ACID REDUCES ADIPOSITY.

[00379] Mice were provided ad lib access to standard chow supplemented with the indicated concentration (weight percent in chow, either 0.14% or 0.28% as indicated in Figure 12) of ursolic acid for 7 weeks before tissues were harvested for analysis. Data are means ± SEM from 10 mice per diet. Data for the effects of ursolic acid on weights of skeletal muscle (quadriceps + triceps), epididymal fat, retroperitoneal fat and heart are shown in Figure 12A. The *P*-values, determined by one-way ANOVA with post-test for linear trend, were < 0.001 for muscle; 0.01 and 0.04 for epididymal and retroperitoneal fat, respectively; and 0.46 for heart. The data show that 7 weeks of dietary ursolic acid increased skeletal muscle weight in a dose-dependent manner, with a peak effect at 0.14% ursolic acid. Interestingly, although ursolic acid increased muscle weight, it did not increase total body weight (Figure 12B; *P*-values were 0.71 and 0.80 for initial and final weights, respectively).

[00380] The data in Figure 12A also show that 7 weeks of dietary ursolic acid reduced the weight of epididymal and retroperitoneal fat depots, with a peak effect at 0.14%. In another study, mice were provided ad lib access to either standard chow (control diet) or standard chow supplemented with 0.27% ursolic acid (ursolic acid diet) for 5 weeks. The relationship between skeletal muscle weight (quadriceps, triceps, biceps, TA, gastrocnemius and soleus) and retroperitoneal adipose weight is shown in Figure 12C. Each data point in Figure 12C represents one mouse; $P < 0.001$ for both muscle and adipose by unpaired t-test. The data show that 5 weeks of ursolic acid administration (0.14%) also reduced adipose weight. Thus, muscle and fat weights were inversely related. Without wishing to be bound by a particular theory, ursolic acid-treated mice contain less fat because, in part, ursolic acid increases Akt activity (see Figures 8 and 9), and muscle-specific increases in Akt activity reduce adiposity as a secondary consequence of muscle hypertrophy (Lai KM, *et al.* (2004) *Molecular and cellular biology* 24(21):9295-9304; Izumiya Y, *et al.* (2008) *Cell metabolism* 7(2):159-172).

[00381] Ursolic acid reduced adipose weight by reducing adipocyte size as shown by data in Figures 10D - 10F. Figure 12D shows a representative H&E stain of retroperitoneal fat for animals feed a control data or a chow with 0.27% ursolic acid as indicated. The data in Figure 12D are shown quantitatively in Figure 12E in terms of adipocyte diameter, where data point represents the average diameter of ≥ 125 retroperitoneal adipocytes from one mouse. The retroperitoneal adipocyte size distribution. Each distribution represents combined adipocyte measurements (> 1000 per diet) from Figure 12E.

[00382] The changes in adipocyte size were accompanied by a significant reduction in plasma leptin levels, which correlated closely with adipose weight (see Figures 10G and 10H). In Figure 12G, each data point represents one mouse, and horizontal bars denote the means. P-values were determined by t-test. In Figure 12H, each data point represents one mouse. Importantly, ursolic acid also significantly reduced plasma triglyceride (Figure 12I) and cholesterol (Figure 12J). In Figures 10I and 10J, each data point represents one mouse, and horizontal bars denote the means. P-values were determined by unpaired t-test. Although ursolic acid reduced leptin, it did not alter food intake (Figure 13A). In this study, mice were provided ad lib access to either standard chow (control diet) or standard chow supplemented with 0.27% ursolic acid (ursolic acid diet) for 4 weeks. Mice were then moved to a comprehensive animal metabolic monitoring system (CLAMS; Columbus Instruments, Columbus, OH) and provided with ad lib access to the same diets. Food consumption was measured for 48 hours. Data are means ± SEM from 6 mice per group. However, ursolic acid did not alter weights of heart (Figure 12A), liver or kidney (Figures 11B and 11C), nor did it elevate plasma markers of hepatotoxicity or nephrotoxicity (alanine aminotransferase, bilirubin and creatinine; see Figures 11D – 11F). The data in Figures 11B – 11F were obtained as follows: mice were provided ad lib access to either standard chow (control diet) or standard chow supplemented with 0.27% ursolic acid (ursolic acid diet) for 5 weeks before tissues and plasma were harvested for the indicated measurements; each data point represents one mouse, and horizontal bars denote the means. For Figure 13, *P*-values were determined with unpaired t-tests. Thus, dietary ursolic acid had two major effects: skeletal muscle hypertrophy and reduced adiposity.

10. URSOLIC ACID REDUCES WEIGHT GAIN AND WHITE ADIPOSE TISSUE.

[00383] The findings that ursolic acid increased skeletal muscle and decreased adiposity suggested that ursolic acid might increase energy expenditure, which would lead to obesity resistance. To test this, C57BL/6 mice were given ad libitum access to a high fat diet (HFD; Teklad TD.93075; 55% calories from fat) lacking or containing 0.27% ursolic acid. After 7 weeks, mice from each group were studied for three days in comprehensive lab animal monitoring systems ("CLAMS"; Columbus Instruments). In the CLAMS, mice were maintained on the same diet they had been eating since the beginning of the experiment. Following CLAMS, tissues were harvested for analysis. In high fat-fed mice, ursolic acid dramatically reduced weight gain, and this effect was apparent within one week (Figure 14A). As previously observed in mice fed ursolic acid and standard chow (Figure 8), ursolic acid increased grip strength and muscle mass (Figures 12B and 12C). Moreover, ursolic acid

reduced retroperitoneal and epididymal fat (Figures 12D and 12E). Interestingly, in the scapular fat pad, which contains a mixture of white and thermogenic brown fat, ursolic acid reduced white fat (Figure 14F), but increased brown fat (Figure 14G). Importantly, increased skeletal muscle and brown adipose tissue would be predicted to increase energy expenditure. Indeed, CLAMS revealed that ursolic acid increased energy expenditure (Figure 14H), providing an explanation for how ursolic acid reduces adiposity and obesity. Remarkably, CLAMS analysis revealed that ursolic acid-treated mice consumed more food (Figure 14I), even though they gained less weight (Figure 14A). For the data shown in Figure 14A, data are means ± SEM from 12 control mice and 15 treated mice, but it should be noted that some error bars are too small to see; $P < 0.01$ at 1 wk and each subsequent time point. In Figures 12B – 12I, each data point represents one mouse and horizontal bars denote the means. *P*-values were determined with unpaired t-tests.

11. URSOLIC ACID REDUCES OBESITY-RELATED PRE-DIABETES, DIABETES, FATTY LIVER DISEASE AND HYPERCHOLESTEROLEMIA.

[00384] The study was carried out as follows: C57BL/6 mice were given ad libitum access to a high fat diet ("HFD"; Teklad TD.93075; 55% calories from fat) lacking or containing 0.27% ursolic acid. After 5 weeks, mice were fasted for 16 h before blood glucose was measured via the tail vein (Figure 15A). Normal fasting blood glucose: ≤ 100 mg/dl. (B-I) After 7 weeks, liver and plasma were harvested for analysis (Figures 13B – 13I). The data shown in Figure 15A suggest that most mice fed HFD without ursolic acid for 6 weeks developed impaired fasting glucose (pre-diabetes) or diabetes. Importantly, this was prevented by ursolic acid (Figure 15A). In addition, mice fed HFD without ursolic acid developed fatty liver disease, as evidenced by increased liver weight (>30% increase above normal mouse liver weight of 1500 mg; Figure 15B), hepatocellular lipid accumulation (Figure 15C, H&E stain at 20X magnification; Figure 15D, lipid-staining osmium at 10X magnification), and elevated plasma liver function tests (Figure 15E, AST; 13F, ALT; 13G, alkaline phosphatase (labeled as "Alk. Phos. in figure); and, 13H, cholesterol). However, ursolic acid prevented all of these hepatic changes (Figure 15B – 13G). In addition, ursolic acid reduced obesity-related hypercholesterolemia (Figure 15H). In Figures 13A, 13B, and 13E-13H, each data point represents one mouse and horizontal bars denote the means.

12. OLEANOLIC ACID DOES NOT INCREASE SKELETAL MUSCLE MASS.

[00385] The effect of ursolic acid on skeletal muscle weight and liver weight was compared to the effects by oleanolic acid and metformin. Metformin was a compound identified from muscle atrophy signature-1, but not muscle atrophy signature-2. Oleanolic

acid, like ursolic acid is a pentacyclic acid triterpane. This is a structurally similar compound to ursolic acid. However, the two compounds are distinct: oleanolic acid has two methyl groups at position 20, whereas ursolic acid has a single methyl group at each of positions 19 and 20 (compare Figures 14A and 14D). Both ursolic acid and oleanolic acid reduce blood glucose, adiposity and hepatic steatosis (Wang ZH, *et al.* (2010) *European journal of pharmacology* 628(1-3):255-260; Jayaprakasam B, *et al.* (2006) *J Agric Food Chem* 54(1):243-248; de Melo CL, *et al.* (2010) *Chem Biol Interact* 185(1):59-65). In addition, both ursolic acid and oleanolic acid possess a large number of cellular effects and biochemical targets, including nearly equivalent inhibition of protein tyrosine phosphatases ("PTPs"; see Zhang W, *et al.* (2006) *Biochimica et biophysica acta* 1760(10):1505-1512; Qian S, *et al.* (2010) *J Nat Prod* 73(11):1743-1750; Zhang YN, *et al.* (2008) *Bioorg Med Chem* 16(18):8697-8705). However, the effects of these compounds on skeletal muscle mass were not known.

[00386] Because some PTPs (particularly PTP1B) dephosphorylate (inactivate) the insulin receptor, PTP inhibition represented a potential mechanism to explain ursolic acid-mediated enhancement of insulin signaling. Thus, because oleanolic acid and ursolic acid inhibit PTP1B and other PTPs with similar efficacy and potency in vitro (Qian S, *et al.* (2010) *J Nat Prod* 73(11):1743-1750; Zhang YN, *et al.* (2008) *Bioorg Med Chem* 16(18):8697-8705), testing oleanolic acid's effects on skeletal mass tests the potential role of PTP inhibition. It should be noted that neither ursolic acid nor oleanolic acid is known to inhibit PTPs in vivo, and neither of these compounds are known to enhance IGF-I signaling. Moreover, ursolic acid's capacity to inhibit PTPs has been disputed based on ursolic acid's failure to delay insulin receptor de-phosphorylation in cultured cells (Jung SH, *et al.* (2007) *The Biochemical journal* 403(2):243-250), and ursolic acid's capacity to act as an insulin mimetic (Jung SH, *et al.* (2007) *The Biochemical journal* 403(2):243-250). In addition, global and muscle-specific PTP1B knockout mice do not possess increased muscle mass, although they are resistant to obesity and obesity-related disorders(Delibegovic M, *et al.* (2007) *Molecular and cellular biology* 27(21):7727-7734; Klaman LD, *et al.* (2000) *Molecular and cellular biology* 20(15):5479-5489). Furthermore, ursolic acid increases pancreatic beta cell mass and serum insulin levels in vivo, perhaps via its anti-inflammatory effects (Wang ZH, *et al.* (2010) *European journal of pharmacology* 628(1-3):255-260; Jayaprakasam B, *et al.* (2006) *J Agric Food Chem* 54(1):243-248; de Melo CL, *et al.* (2010) *Chem Biol Interact* 185(1):59-65).. Importantly, inflammation is now recognized as a central pathogenic mechanism in muscle atrophy, metabolic syndrome, obesity, fatty liver disease and type 2 diabetes. Thus, the

existing data suggest at least four mechanisms to explain ursolic acid's capacity to increase insulin signaling in vivo: PTP inhibition, direct stimulation of the insulin receptor, increased insulin production, and reduced inflammation. Of these four potential mechanisms, only the latter three have been demonstrated in vivo.

[00387] To compare the effects of ursolic acid and oleanolic acid on skeletal muscle and liver weight, C57BL/6 mice were administered ursolic acid (200 mg / kg), oleanolic acid (200 mg / kg), or vehicle alone (corn oil) via i.p. injection. Mice were then fasted, and after 12 hours of fasting, mice received a second dose of ursolic acid, oleanolic acid, or vehicle. After 24 hours of fasting, lower hindlimb skeletal muscles and liver were harvested and weighed. As shown previously, ursolic acid increased skeletal muscle weight (Figure 16B), but not liver weight (Figure 16C). In contrast, oleanolic acid increased liver weight (FIG. 14F), but not skeletal muscle weight (Figure 16E). Interestingly, metformin (250 mg / kg) resembled oleanolic acid in biological effect: it increased liver weight (Figure 16I), but not muscle weight (Figure 16H). Without wishing to be bound by a particular theory, ursolic acid increases skeletal muscle and inhibit muscle atrophy by a pathway that does not involve PTP inhibition.

13. TARGETED INHIBITION OF PTP1B DOES NOT INDUCE SKELETAL MUSCLE HYPERTROPHY.

[00388] To further rule out the potential role of PTP1B inhibition in skeletal muscle hypertrophy, PTP1B expression was specifically reduced in mouse skeletal muscle by transfecting plasmid DNA constructed to express RNA interference constructs. Briefly, C57BL/6 mouse tibialis anterior muscles were transfected with 20 μg *pCMV-miR-control* (control plasmid transfected in the left TA) or either 20 μg *pCMV-miR-PTP1B #1* (encoding miR-PTP1B #1; transfected in the right TA) or 20 μg *pCMV-miR-PTP1B #2* (encoding miR-PTP1B #2; transfected in the right TA). miR-PTP1B #1 and miR-PTP1B #2 encode two distinct RNA interference (RNAi) constructs targeting distinct regions of *PTP1B* mRNA. Tissue was harvested 10 days following transfection.

[00389] Of note with regard to Figure 17A, mRNA measurements were taken from the entire TA muscle. Because electroporation transfects only a portion of muscle fibers, the data underestimate *PTP1B* knockdown in transfected muscle fibers. In Figure 17A, mRNA levels in the right TA were normalized to levels in the left TA, which were set at 1; data are means ± SEM from 3 mice. In Figure 17B, in each TA muscle, the mean diameter of > 300 transfected fibers was determined; data are means ± SEM from 3 TA muscles per condition.

For both Figures 15A and 15B, *P*-values were determined with one-tailed paired t-tests.

[00390] Although both miR-PTP1B constructs reduced *PTP1B* mRNA (Figure 17A), neither increased skeletal muscle fiber diameter (Figure 17B). These data demonstrate that targeted PTP1B inhibition does not cause muscle fiber hypertrophy. Without wishing to be bound by a particular theory, ursolic acid does not increase skeletal muscle by inhibiting PTP1B.

14. URSOLIC ACID SERUM LEVELS ASSOCIATED WITH INCREASED MUSCLE MASS AND DECREASED ADIPOSITY.

[00391] To determine the dose-response relationship between dietary ursolic acid and muscle and adipose weight, C57BL/6 mice were fed standard chow containing varying amounts of ursolic acid for 7 weeks. Serum ursolic acid levels from mice were determined as described above. As shown previously in Figure 12A, ursolic acid increased skeletal muscle weight and decreased weight of retroperitoneal and epididymal fat pads in a dose-dependent manner, but did not alter heart weight (Figure 18A; data are means ± SEM). These effects of ursolic acid were discernable at 0.035% ursolic acid and were maximal at doses ≥ 0.14% ursolic acid. Serum was collected from these same mice at the time of necropsy, and then measured random serum ursolic acid levels via ultra high performance liquid chromatography (UPLC). The data indicate that ursolic acid serum levels in the range of 0.25 – 0.5 μg / ml are sufficient to increase muscle mass and decrease adiposity (Figure 18B; data are means ± SEM). Of note, 0.5 μg / ml equals 1.1 μM ursolic acid, close to the dose used in the Connectivity Map (8.8 μM) and in the C2C12 experiments (10 μM) described above.

[00392] The data described herein indicate that ursolic acid reduced muscle atrophy and stimulated muscle hypertrophy in mice. Importantly, ursolic acid's effects on muscle were accompanied by reductions in adiposity, fasting blood glucose and plasma leptin, cholesterol and triglycerides, as well as increases in the ratio of skeletal muscle to fat, the amount of brown fat, the ratio of brown fat to white fat, and increased energy expenditure. Without wishing to be bound by a particular theory, ursolic acid reduced muscle atrophy and stimulated muscle hypertrophy by enhancing skeletal muscle IGF-I expression and IGF-I signaling, and inhibiting atrophy-associated skeletal muscle mRNA expression.

[00393] All of the compositions and/or methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. It will be apparent to those skilled in the art that various modifications and variations can be made in the present invention without departing from the scope or spirit of the invention. More

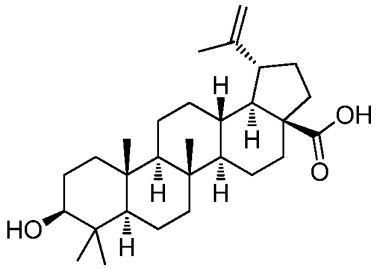
specifically, certain agents which are both chemically and physiologically related can be substituted for the agents described herein while the same or similar results can be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims. Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the invention being indicated by the following claims.

15. Treatment of Muscle Atrophy

[00394] Several compounds have been shown to treat muscle atrophy as shown below.

a. Betulinic Acid

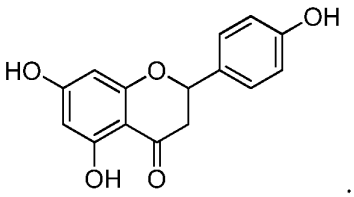
[00395] Betulinic acid has the following structure:



[00396] The mRNA expression signature of betulinic acid negatively correlated to human muscle atrophy signature-2. Therefore betulinic acid, like ursolic acid, could inhibit skeletal muscle atrophy. To test this, a mouse model of immobilization-induced skeletal muscle atrophy was used: mice were administered vehicle (corn oil) or varying doses of ursolic acid (positive control) or betulinic acid via intraperitoneal injection twice a day for two days. One tibialis anterior (TA) muscle was immobilized with a surgical staple, leaving the contralateral mobile TA as an intra-subject control. The vehicle or the same dose of ursolic acid or betulinic acid was continuously administered via i.p. injection twice daily for six days before comparing weights of the immobile and mobile TAs. As expected, immobilization caused muscle atrophy, and ursolic acid reduced muscle atrophy in a dose-dependent manner, with maximal inhibition at 200 mg/kg (Figure 19A). Betulinic acid also reduced muscle atrophy in a dose-dependent manner, with maximal inhibition at $\leq$ 50 mg/kg (Figure 19B). These data indicate that betulinic acid reduces immobilization-induced muscle atrophy, and it is more potent than ursolic acid.

b. Naringenin

[00397] Naringenin has the following structure:



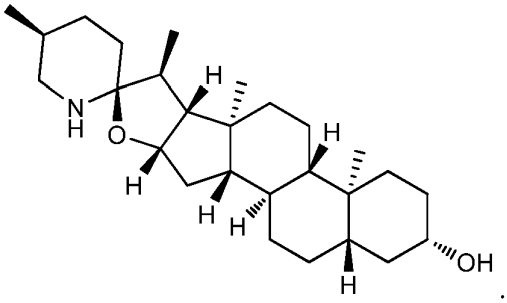
[00398] The mRNA expression signature of naringenin negatively correlated to human muscle atrophy signatures-1 and -2. Therefore naringenin could inhibit skeletal muscle atrophy. To test this, mice were administered vehicle (corn oil), ursolic acid (200 mg/kg), naringenin (200 mg/kg), or the combination of ursolic acid and naringenin (each at 200 mg/kg) via i.p injection twice a day for two days. One tibialis anterior (TA) muscle was immobilized with a surgical staple, leaving the contralateral mobile TA as an intrasubject control. Vehicle or the same doses of ursolic acid and/or naringenin was continuously administered via i.p. injection twice daily for six days before comparing weights of the immobile and mobile TAs. Like ursolic acid, naringenin reduced muscle atrophy (Figure 20). The combination of ursolic acid and naringenin also reduced muscle atrophy, but not more than either compound alone (Figure 20). These data indicate that naringenin reduces skeletal muscle atrophy.

[00399] Like ursolic acid, naringenin reduces blood glucose, as well as obesity and fatty liver disease. Therefore ursolic acid and naringenin could have additive effects. To determine this, weight-matched mice were provided ad libitum access to standard (Harlan Teklad formula 7013), high fat diet (HFD; Harlan Teklad formula TD93075), or HFD containing varying concentrations of ursolic acid (0.15%) and/or naringenin (0.5% or 1.5%). After the mice consumed these diets for 5 weeks, fasting blood glucose, total body weight, fat mass, liver weight, grip strength, and skeletal muscle weight was measured. As expected, HFD increased blood glucose, and this increase in blood glucose was partially prevented by ursolic acid and naringenin (Figure 21A). The combination of ursolic acid plus either dose of naringenin reduced blood glucose more than either compound alone, and it restored blood glucose to normal levels (Figure 21A). Importantly, ursolic acid and naringenin did not have additive effects on total body weight (Figure 21B), fat mass (Figure 21C), liver weight (Figure 21D), grip strength (Figure 21E), or skeletal muscle weight (Figure 21F). In addition, ursolic acid increased strength to a greater extent than naringenin (Figure 21E), and ursolic acid, but not naringenin, increased muscle weight (Figure 21F). These differences between ursolic acid and naringenin in high fat fed mice indicates that ursolic acid and naringenin have differences in their mechanisms of action, which could explain their additive effects on

fasting blood glucose.

c. TOMATIDINE

[00400] Tomatidine has the following structure:

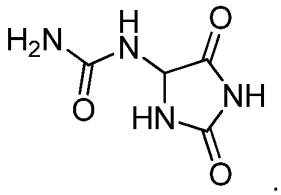


[00401] The mRNA expression signature of tomatidine negatively correlated to human muscle atrophy signatures-1 and -2. Therefore tomatidine could inhibit skeletal muscle atrophy. To test this, mice were administered vehicle (corn oil) or tomatidine (50, 100 or 200 mg/kg) via i.p injection twice a day for two days. One tibialis anterior (TA) muscle was immobilized with a surgical staple, leaving the contralateral mobile TA as an intrasubject control. Vehicle or the same doses of tomatidine was administered via i.p. injection twice daily for six days before comparing weights of the immobile and mobile TAs. All 3 doses of tomatidine reduced muscle atrophy, and the effect was maximal at 50 mg/kg (Figure 22A). The same protocol was used to compare the effects of vehicle (corn oil) and tomatidine (5, 15 or 50 mg/kg) on immobilization-induced muscle atrophy. Tomatidine reduced muscle atrophy in dose-dependent manner, with maximal effect at 50 mg/kg and EC50 < 5 mg/kg (Figure 22B). These data indicate that tomatidine reduces immobilization-induced muscle atrophy, and it is more potent than ursolic acid.

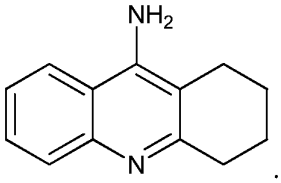
[00402] Tomatidine could also inhibit skeletal muscle atrophy induced by fasting. To test this, food was withdrawn from mice, and then vehicle, ursolic acid (200 mg/kg) or tomatidine (50 mg/kg) were administered by i.p. injection. Twelve hours later, mice received another i.p. injection of vehicle or the same dose of ursolic acid or tomatidine. Twelve hours later, skeletal muscles were harvested and weighed. Both ursolic acid and tomatidine increased skeletal muscle, indicating decreased fasting-induced skeletal muscle atrophy (Figure 23A). We next used the same protocol to compare the effects of vehicle (corn oil) and tomatidine (5, 15 and 50 mg/kg). Tomatidine reduced muscle atrophy in dose-dependent manner, with maximal effect at 50 mg/kg and EC50 between 5 and 15 mg/kg (Figure 23B).

d. ALLANTOIN, TACRINE, UNGERINE, HIPPEASTRINE AND CONESSINE

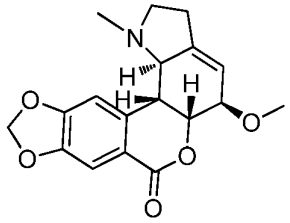
[00403] Allantoin has the following structure:



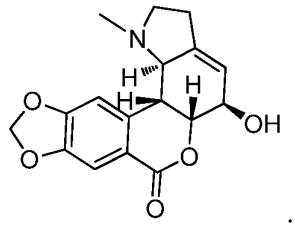
[00404] Tacrine has the following structure:



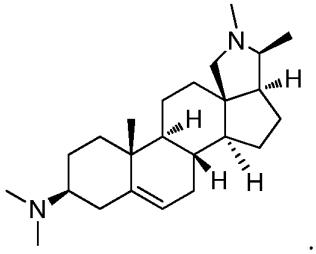
[00405] Ungerine has the following structure:



[00406] Hippeastrine has the following structure:



[00407] Conessine has the following structure:



[00408] The mRNA expression signatures of allantoin, tacrine, ungerine (Prestwick-689), hippeastrine (Prestwick-675) and conessine also negatively correlated to human muscle atrophy signatures-1 and -2. Therefore these compounds could inhibit skeletal muscle atrophy. To test this, the fasting-induced muscle atrophy model described above was used to compare the effects of ursolic acid (200 mg/kg), tomatidine (50 mg/kg), allantoin (2 mg/kg), tacrine (4 mg/kg), ungerine (2 mg/kg), hippeastrine (2 mg/kg) and conessine (2 mg/kg). Like ursolic acid and tomatidine, allantoin, tacrine, ungerine, hippeastrine and conessine increased

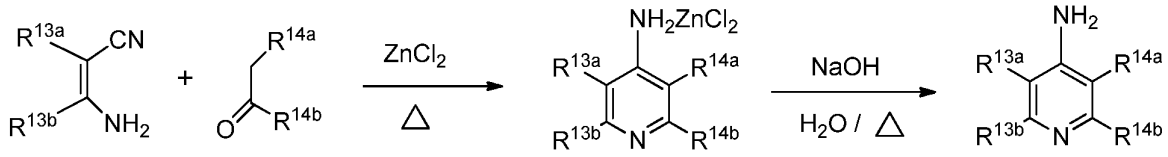
muscle weight in fasted mice (Figure 24), indicating that these compounds decrease skeletal muscle atrophy.

[00409] Since ursolic acid and naringenin reduced fasting blood glucose, hippeastrine (2 mg/kg) and conessine (2 mg/kg) could have a similar effect. Hippeastrine and conessine reduced fasting blood glucose (Figure 25).

16. PROPHETIC SYNTHESIS OF TACRINE AND ANALOGS

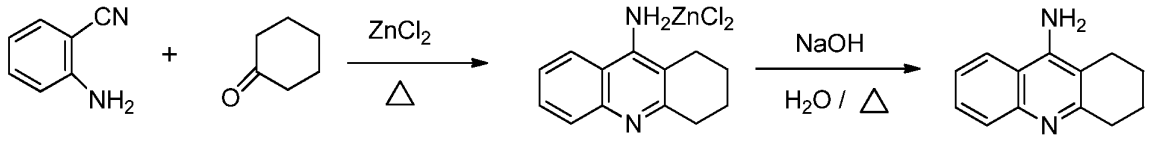
[00410] The formulas disclosed herein could be synthesized by reacting an anthranilonitrile derivative with a cyclohexanone derivative in the presence of zinc chloride (Proctor et al., *Curr Medici. Chem.*, 2000, 7, 295-302). Such reaction is shown in Scheme 1A.

SCHEME 1A



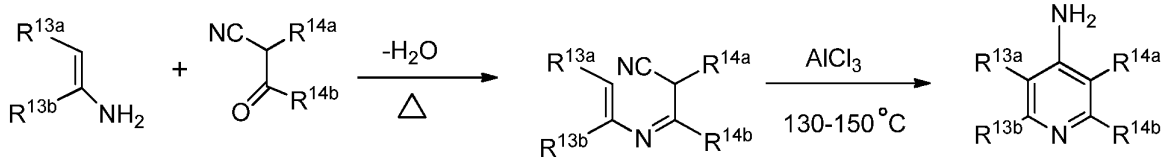
[00411] Thus, tacrine can be synthesized as shown in scheme 1B.

SCHEME 1B



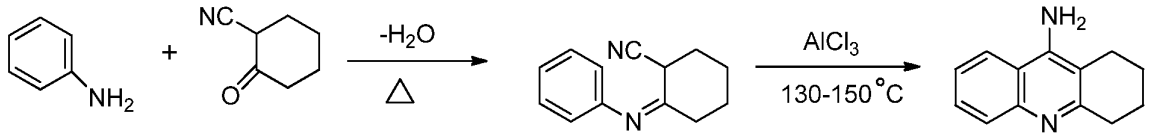
[00412] The formulas disclosed herein could also be synthesized by reacting an α-cyanocyclonones with a vide variety of anilines using either $TiCl_4$ or $AlCl_3$ as reagents (Proctor et al., *Curr Medici. Chem.*, 2000, 7, 295-302). An example of such reaction is shown in Scheme 1C.

SCHEME 1C



[00413] Thus, tacrine could be synthesized as shown in scheme 1D.

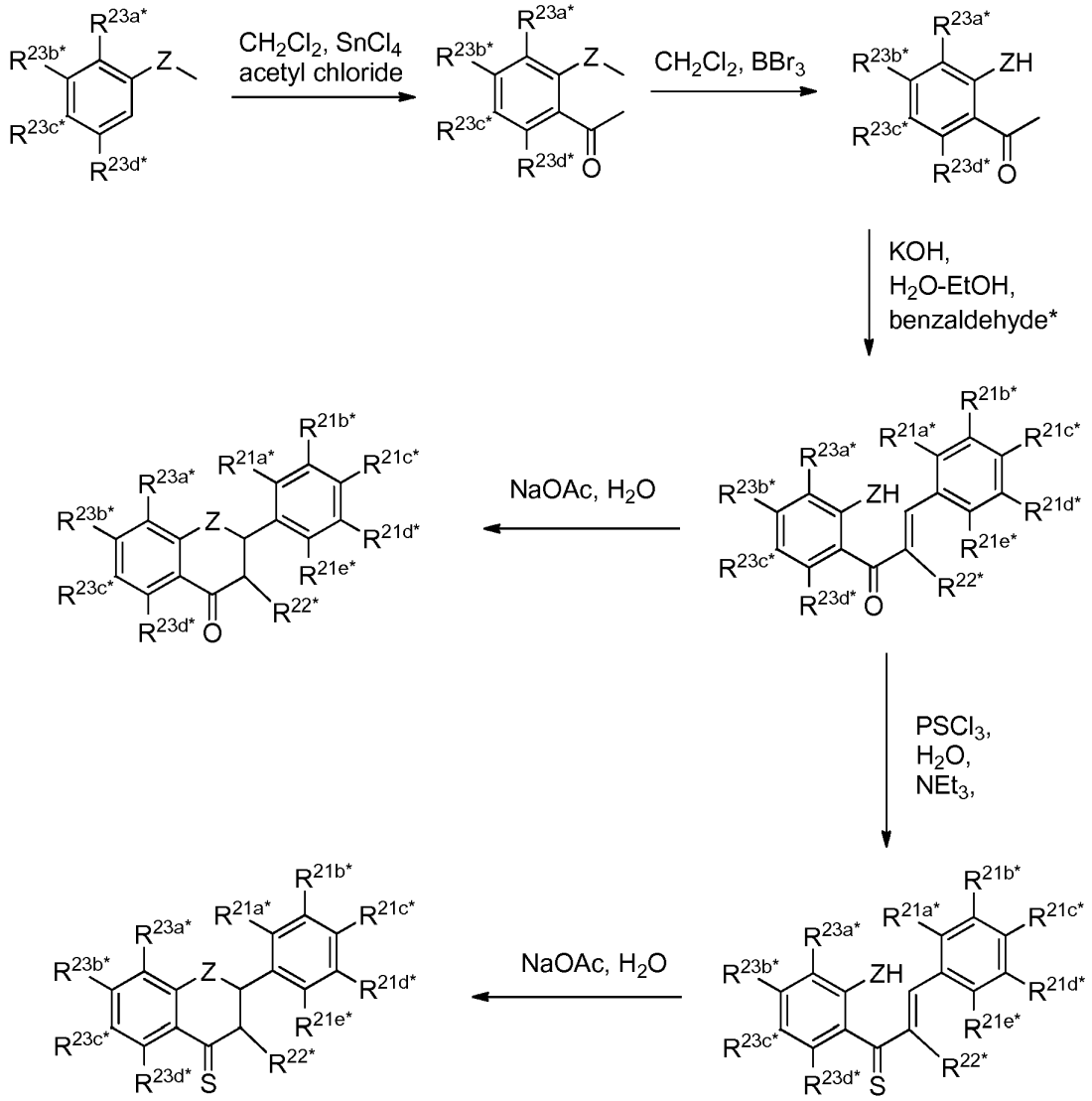
SCHEME 1D



17. PROPHETIC SYNTHESIS OF NARINGENIN AND ANALOGS

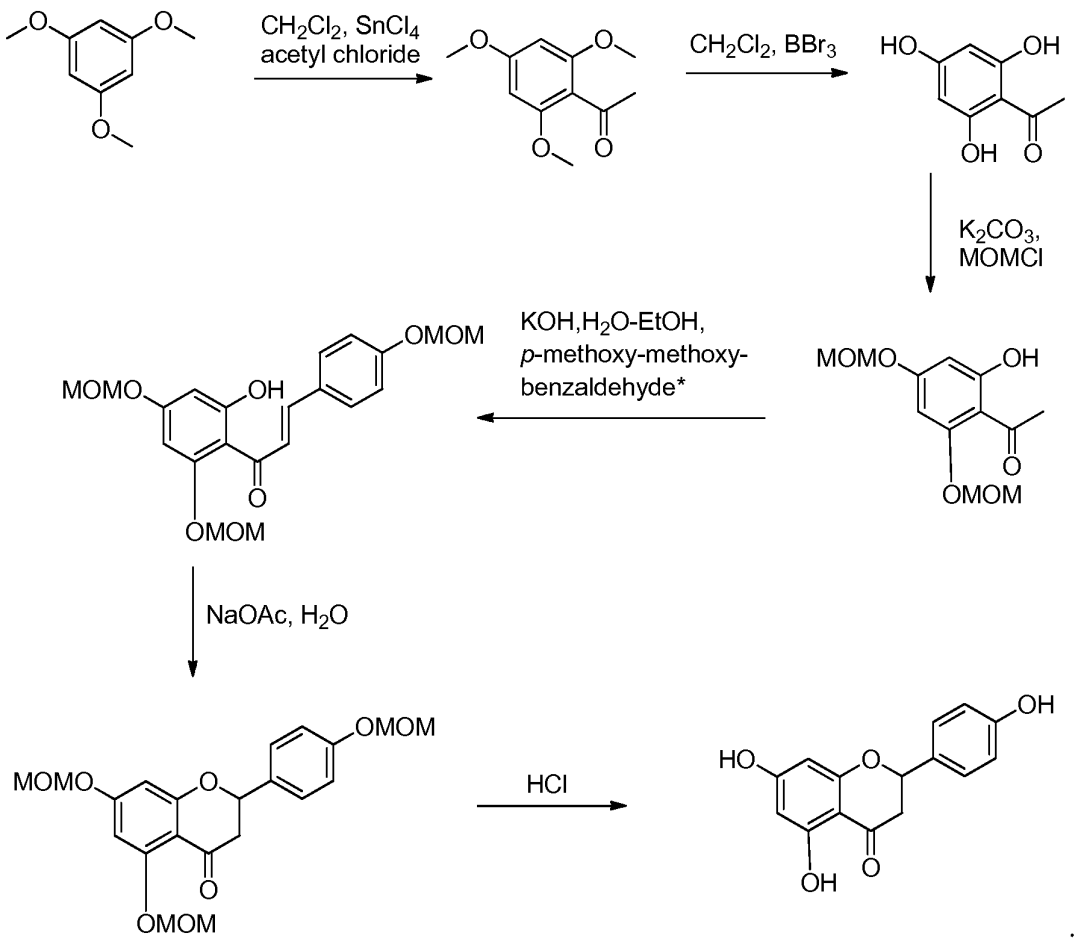
[00414] The disclosed formulas could be synthesized as described in PCT application WO 2007/053915 by De Keukkeleire et al. which is hereby incorporated in its entirety by reference. In another example, Glucoyl substituted naringenin could be extracted as described in U.S. Patent 6,770,630 by Kashiwaba et al. which is hereby incorporated in its entirety by reference. As described by De Keukkeleire et al. the disclosed formulas could be synthesized as shown in Scheme 2A:

SCHEME 2A



[00415] The formation of the thioketone was described by Pathak, et al. (*J. Org. Chem.*, 2008, *73*, 2890-2893). The * in the scheme denotes moieties that is or can be converted, using known chemistry, into the disclosed R moieties. For example, the synthesis of naringenin is shown in Scheme 2B.

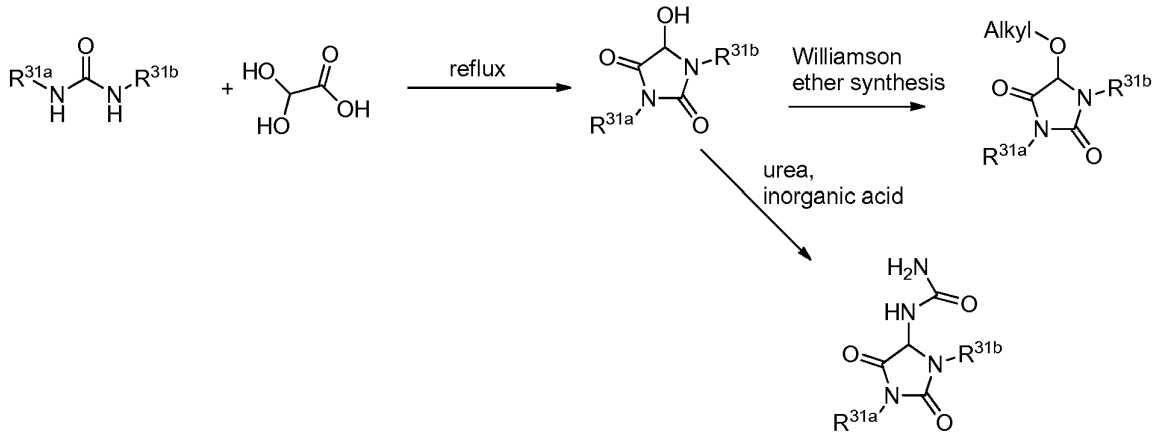
SCHEME 2B



18. PROPHETIC SYNTHESIS OF ALLANTOIN AND ANALOGS

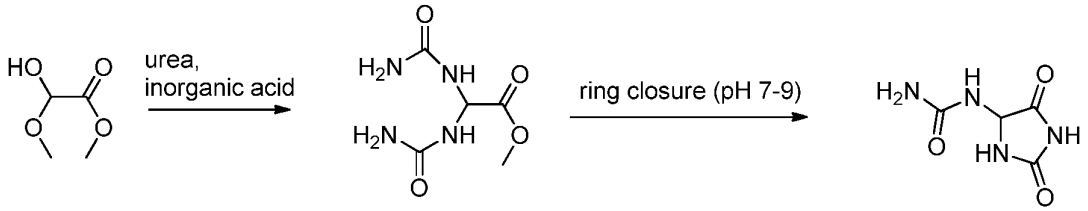
[00416] The disclosed formulas could be made using a variety of chemistry known in the art. For example, one set of the disclosed formulas could be made as shown in Scheme 3A and as described in U.S. Patent 4,647,574 by Ineaga et al, which is hereby incorporated herein by reference in its entirety.

SCHEME 3A



[00417] Allantoin could be prepared as described in U.S. Patent 5,196,545 by Schermanz, which is hereby incorporated herein by reference in its entirety, and as shown in Scheme 3B.

SCHEME 3B

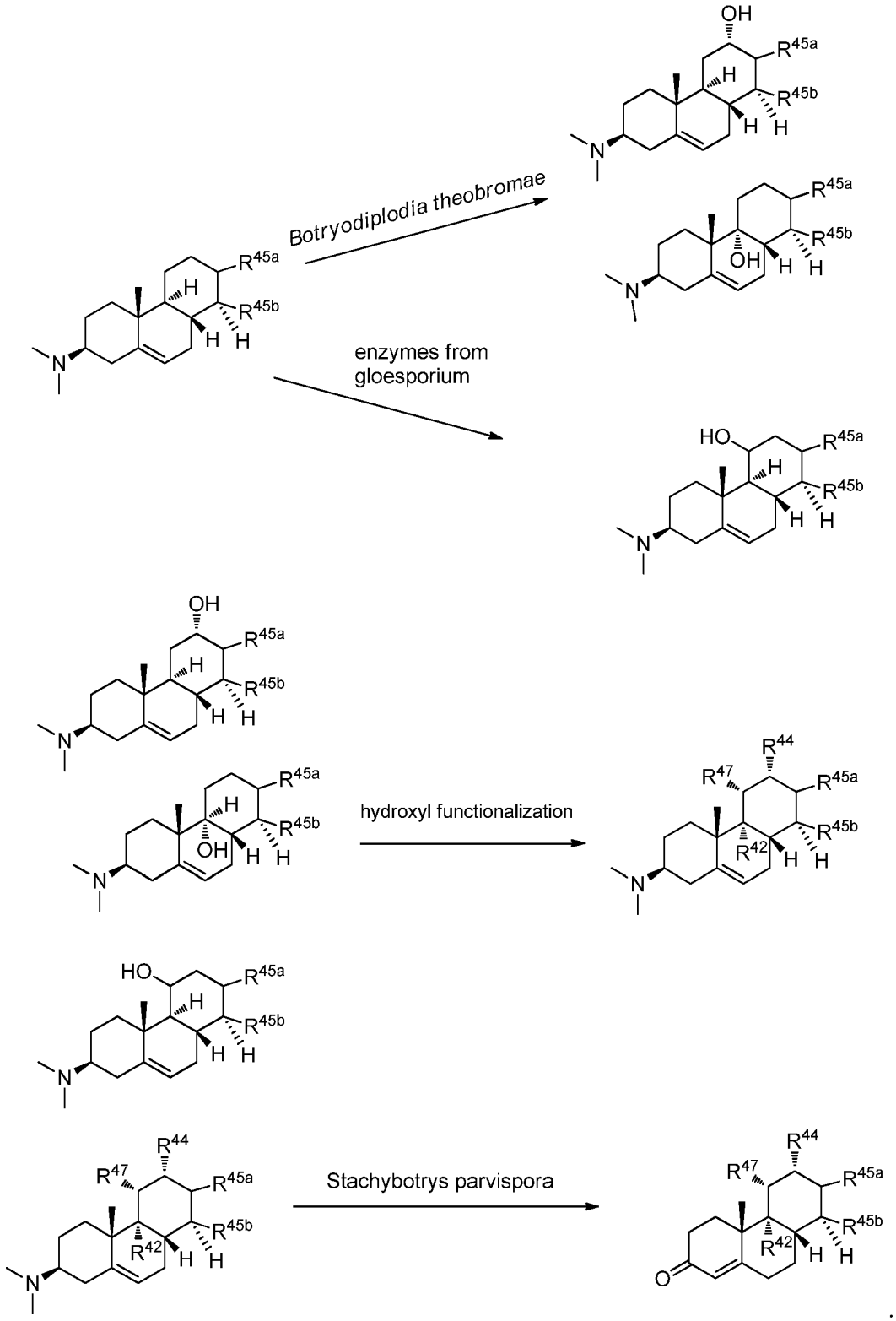


[00418] A comprehensive guide for how to make the disclosed formulas can be found in Kirk-Othmer Encyclopedia of Chemical Technology under the chapter *Hydantoin and Its Derivatives* by Avendaño et al (2000), which is hereby incorporated herein by reference in its entirety.

19. PROPHETIC SYNTHESIS OF CONESSINE AND ANALOGS

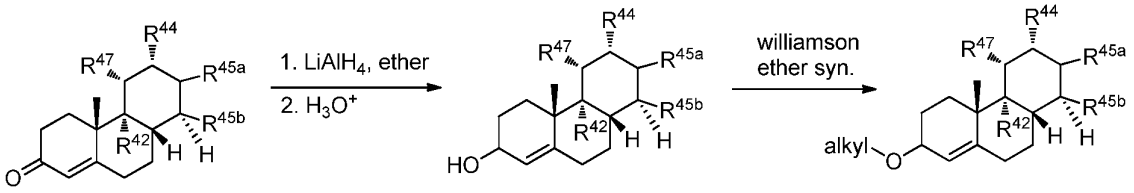
[00419] Conessine is a steroid alkaloid found in plant species from the *Apocynaceae* family, for example in *Holarrhena floribunda*. Conessine derivatives could be prepared as described in U.S. patents 3,539,449, 3,466,279, and 3,485,825 by Marx, which are hereby incorporated by reference in their entirety. As described in U.S. patents 3,539,449, 3,466,279, and 3,485,825 by Marx, conessine derivatives could be prepared using micro-organisms such as the fungus *Stachybotrys parvispora* and enzymes from *Gloeosporium, Colletotrichum,* and *Myrothecium*. For example, see Scheme 4A.

SCHEME 4A



[00420] The conessine oxo derivatives could be further modified via a reduction and subsequent chemistry known to one skilled in the art, as shown in Scheme 4B.

SCHEME 4B



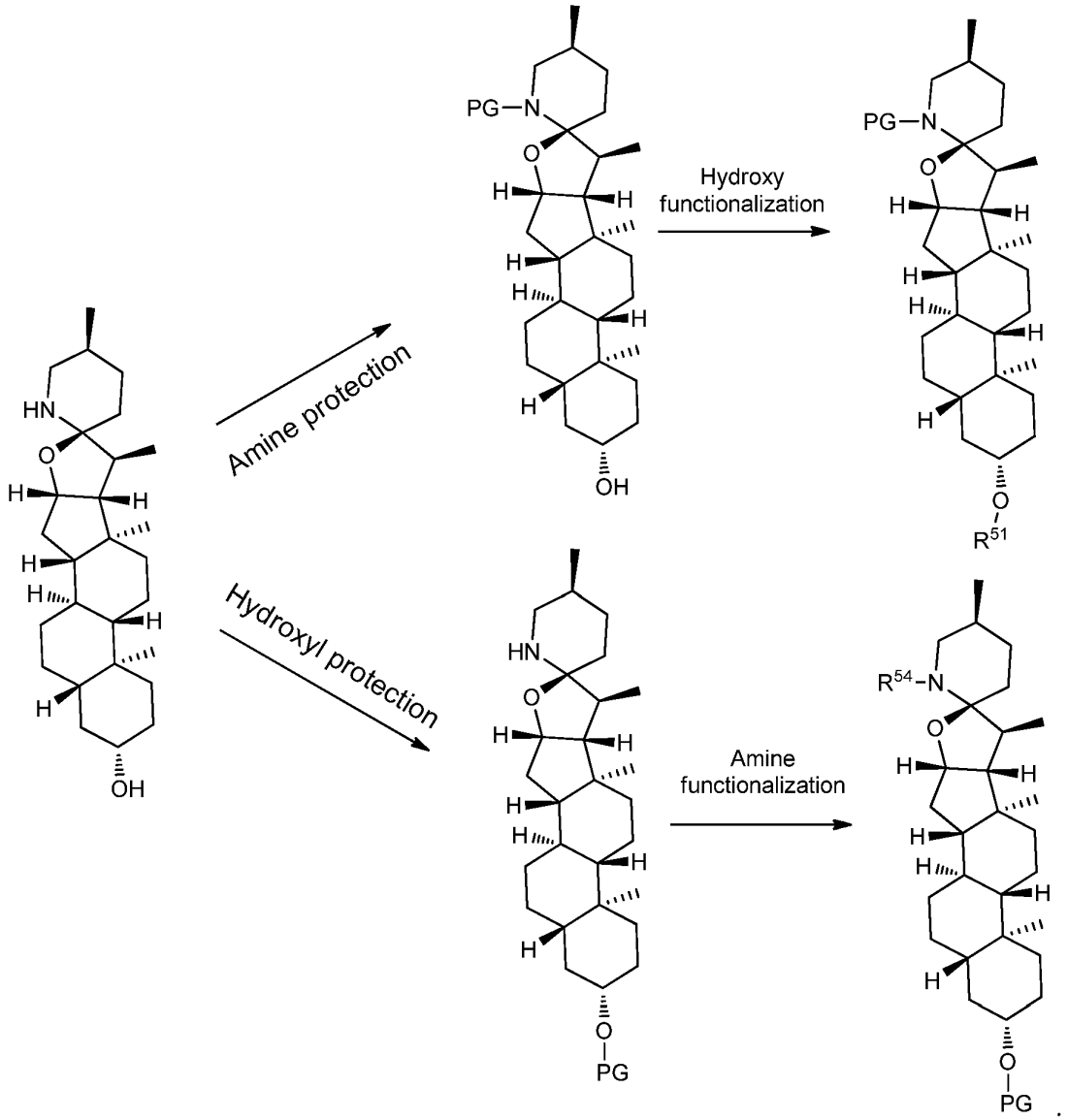
[00421] The hydroxyl functionality could undergo a number of chemical reactions known in the art. One example, as shown in Scheme 4B, is a Williamson ether synthesis.

[00422] Conessine derivatives could be prepared synthetically as described in U.S. patent 2,910,470, which is hereby incorporated by reference in its entirety. Conessine derivatives are also described in WO 2011/046978 by Orlow, which is hereby incorporated by reference in its entirety. Synthesis of the disclosed formulas is also described in U.S. Patent 3,625,941 by Pappo, which is hereby incorporated in its entirety by reference.

20. PROPHETIC SYNTHESIS OF TOMATIDINE AND ANALOGS

[00423] The formulas disclosed herein could be synthesized by the method disclosed by Uhle, and Moore, *J. Am. Chem. Soc.* **76,** 6412 (1954); Uhle, *J. Am. Chem. Soc.* **83,** 1460 (1961); and Kessar *et al., Tetrahedron* **27,** 2869 (1971), which are all hereby incorporated by reference in their entirety. The disclosed compounds can also be made as shown in Scheme 5A.

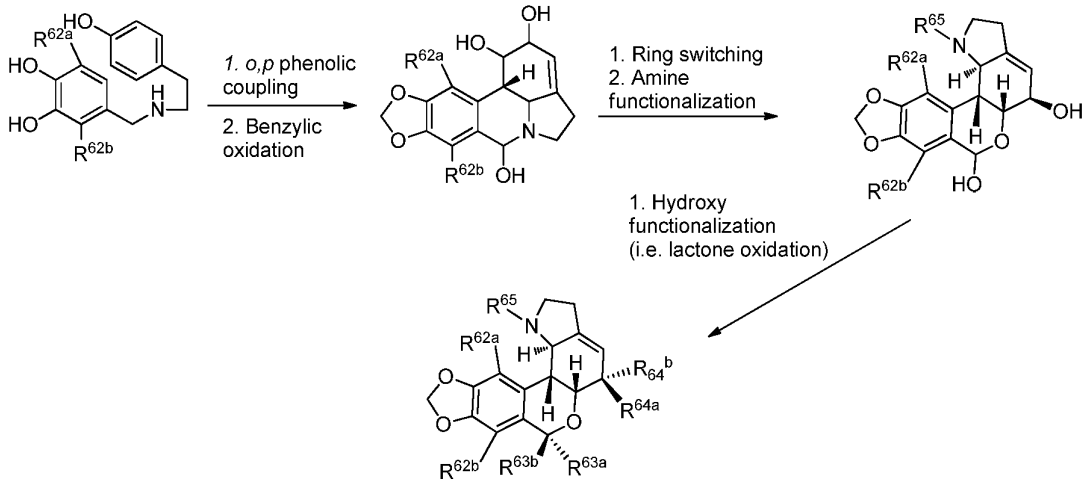
SCHEME 5A



21. PROPHETIC SYNTHESIS OF HIPPEASTRINE/UNGERINE AND ANALOGS

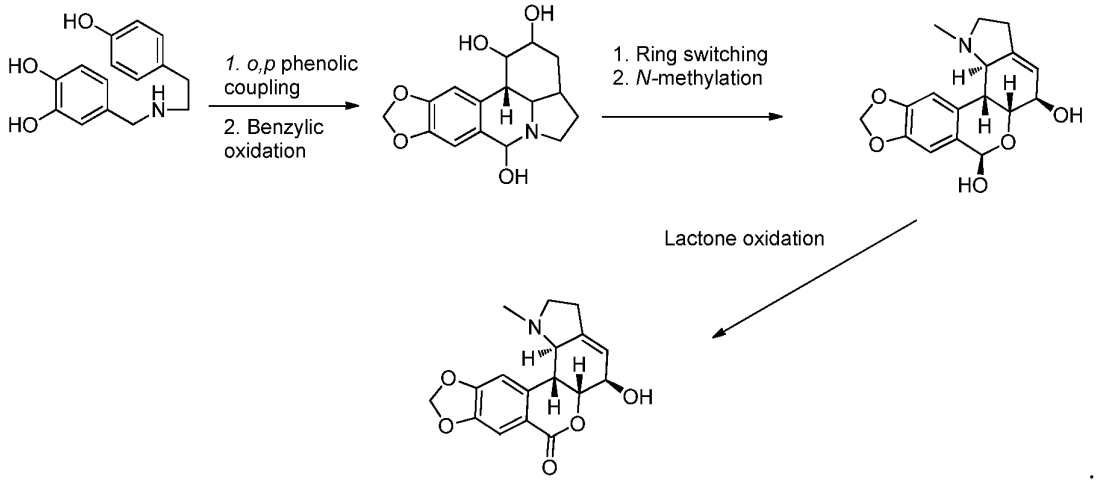
[00424] The disclosed formulas can be synthesized by method disclosed by Mañas et al. (*J. Am. Chem. Soc.* 2010, 132, 5176-78), which is hereby incorporated by reference in its entirety. Thus, disclosed formulas can be synthesized as shown in Scheme 6A.

SCHEME 6A



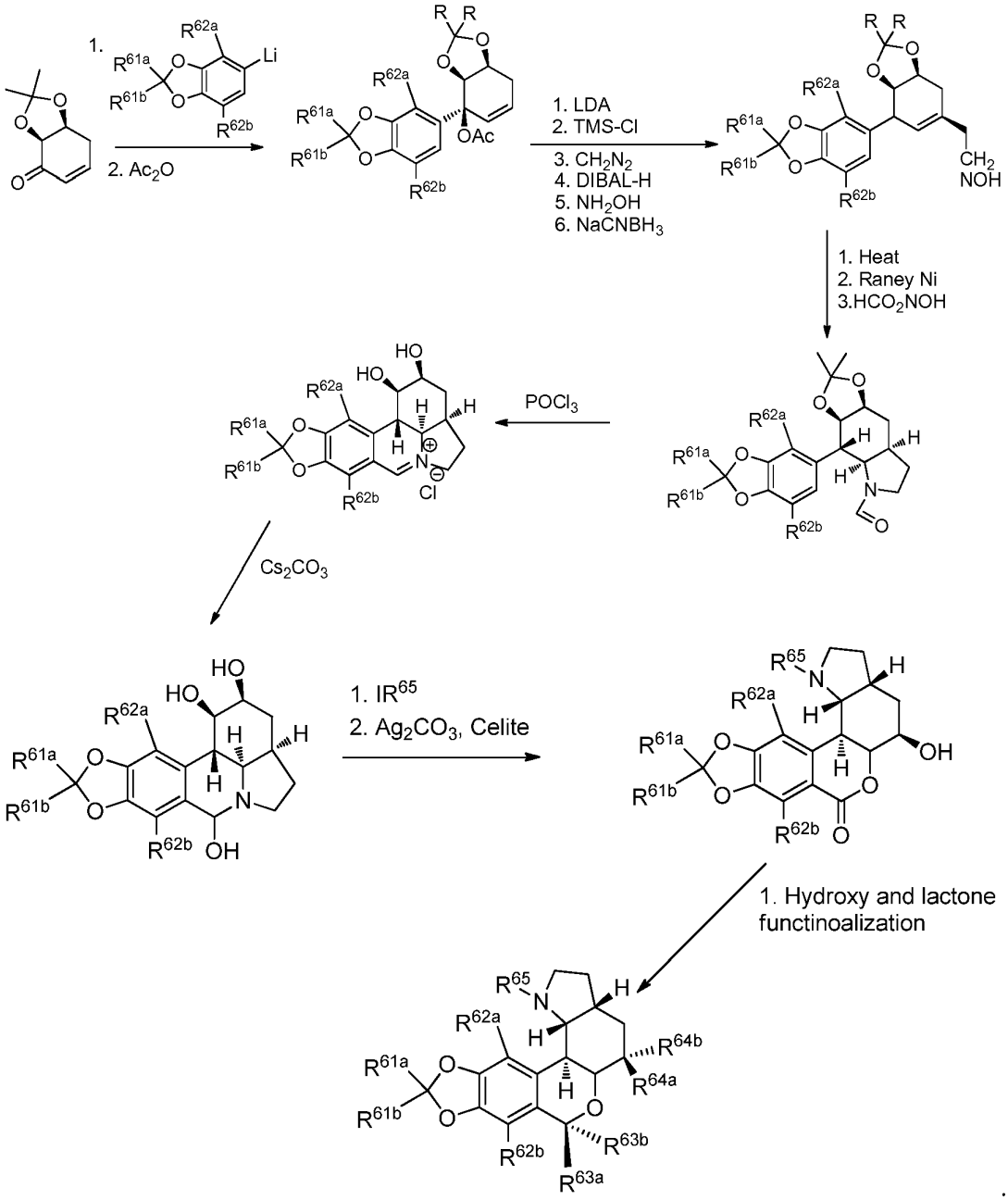
Thus, for example, Hippeastrine can be made as shown in Scheme 6B.

SCHEME 6B



[00425] Another route to make the disclosed formulas is shown in Scheme 6C, as demonstrated by Mañas et al..

SCHEME 6B



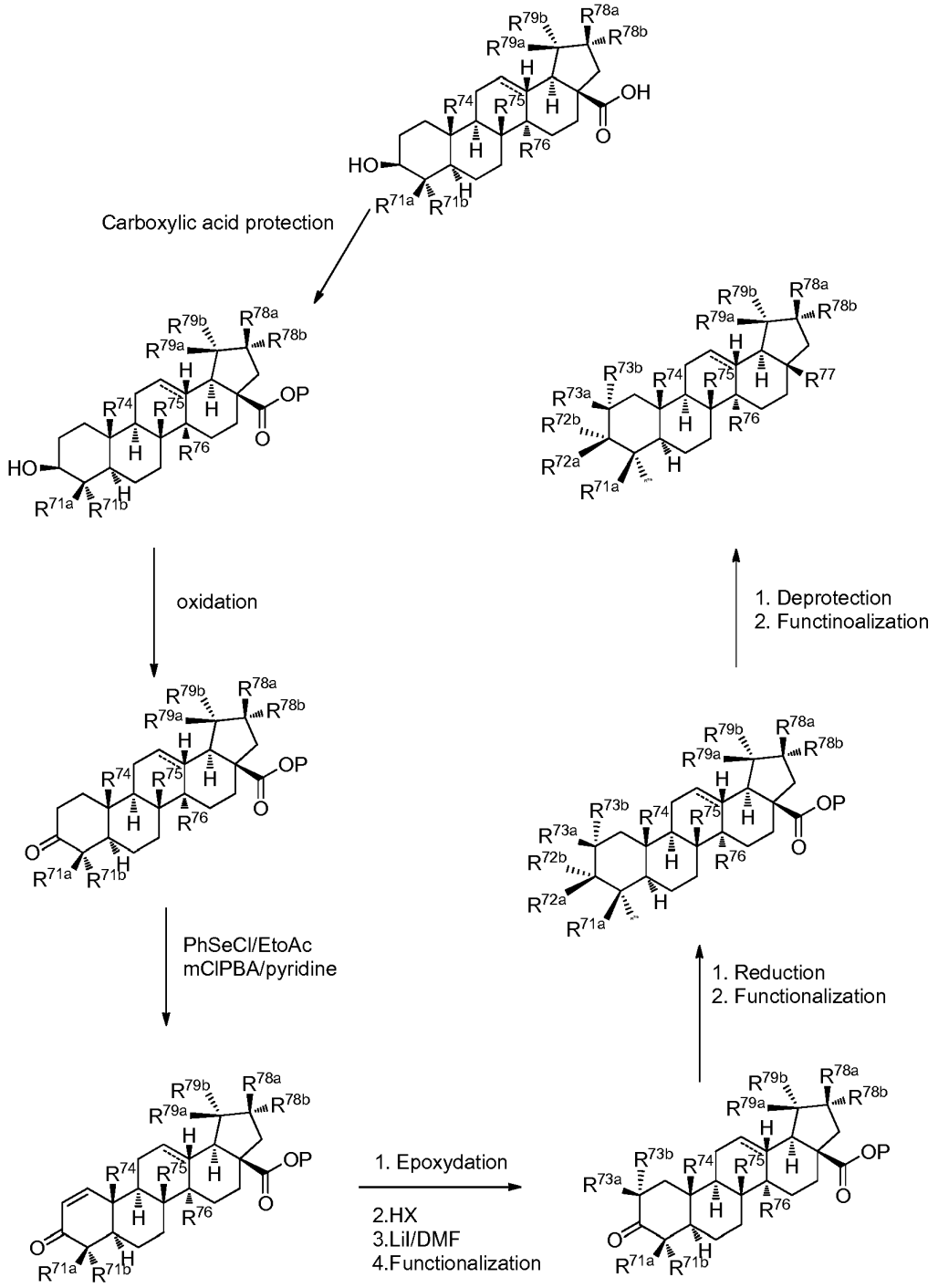
[00426] The disclosed derivatives can also be made using methods disclosed by Haning et al (*Org. Biomolec.Chem.* 2011, 9, 2809-2820).

22. PROPHETIC SYNTHESIS OF BETULINIC ACID AND ANALOGS

[00427] Betulininc acid analogs are also described in International Published application WO 2011/153315 by Regueiro-Ren et al. and in International Published application WO 2008/063318 by Safe et al. which are hereby incorporated by reference in its entirety. Betulinic acid analogs of the present invention of the present invention could be prepared

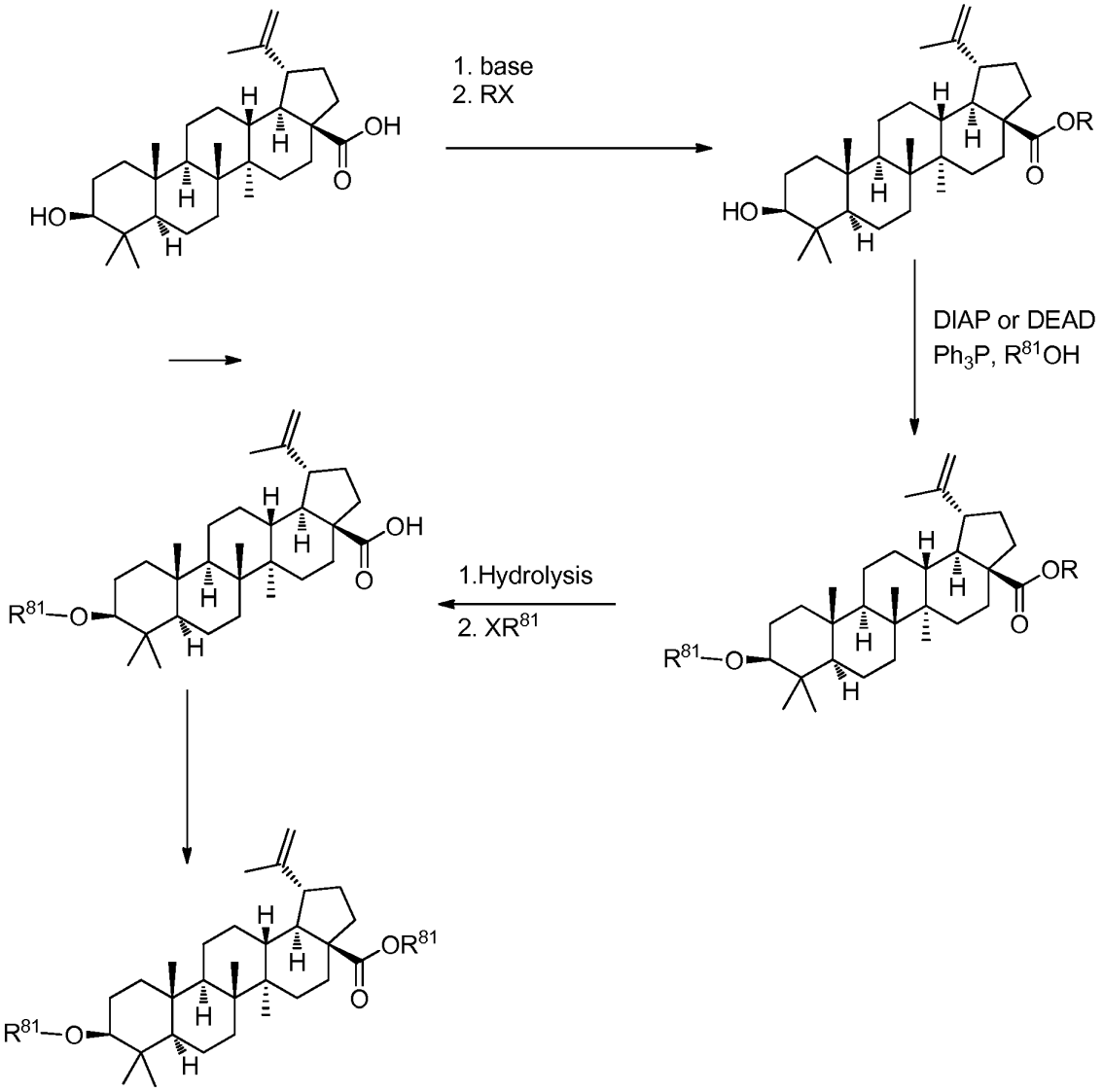
generically as shown below in scheme 7A. The starting materials could be made with methods known in the art.

SCHEME 7A



[00428] Compounds are represented in generic form, with substituents as noted in compound descriptions elsewhere herein. A more specific example is set forth below in scheme 7B.

SCHEME 7B



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23. MUSCLE ATROPHY SIGNATURE – 3

[00429] Induced and repressed mRNA were evaluated for muscle atrophy signature-3. The statisitical significance for the identified mRNAs was defined as $P \leq 0.01$.

[00430] For induced mRNAs: mouse tibialis anterior mRNAs significantly induced by 1 week of denervation and significantly induced by 24 h fasting.

[00431] For repressed mRNAs: mouse tibialis anterior mRNAs significantly repressed by 1 week of denervation and significantly repressed by 24 h fasting.

[00432] The identified induced mRNAs included 1200011I18Rik, 2310004I24Rik, Akap8l, Als2, Anapc7, Apod, Arrdc3, Atp6v1h, BC027231,Bsdc1, Ccdc77, Cd68, Cdkn1a, Ctps2, Ctsl, D930016D06Rik, Ddx21, Depdc7, Dido1, nttip2, Ece1, Eda2r, Egln3, Elk4,

Erbb2ip, Errfi1, Fbxo30, Fbxo32, Fip1l1, Frg1, Gabarapl1, Gadd45a, Gnl2, Gnl3, Herpud2, Hpgd, Hspb7, Htatip2, Impact, Kdm3a, Klhl5, Lpin2, Med12, Mfap1b, Mgea5, Mknk2, Nmd3, Nup93, ORF19, Pacrgl, Parp4, Pdk4, Phc3, Plaa, Ppfibp1, Psma2, Ranbp10, Ranbp9, Rassf4, Riok1, Rlim, Sf3b1, Sik1, Slc20a1, Sln, Spag5, Srsf2ip, Syf2, Tbc1d15, Tbk1, Tekt1, Tgif1, Tmem140, Tmem71, Tnks, Trim25, Trmt1, Tspyl2, Tsr1, Tulp3, Txlng, Ubfd1, Ubxn4, Utp14a, Wdr3, and Xpo4.

[00433] The identified repressed mRNAs included 1600014C10Rik, 1700021F05Rik, 2310003L22Rik, 2310010M20Rik, 2310028O11Rik, 2310061C15Rik, 2610528E23Rik, 2810432L12Rik, Abcd2, Acvr1, Aimp2, Ank1, Aqp4, Arl3, Asb10, Aurka, Bhlhe41, Bpnt1, Camk2a, Cby1, Cc2d2a, Cdc14a, Cdc42ep2, Clcn1, Cntfr, Col15a1, Col6a3, Cox11, Cox7b, Crhr2, D0H4S114, Ddit3, Deb1, Dexi, Dhrs7c, Eif4e, Endog, Epha7, Exd2, Fam69a, Fhod3, Fn3k, Fndc5, Fsd2, Gcom1, Gdap1, Gm4841, Gm5105, Gm9909, Gnb5, Gpd2, Grtp1, Heatr5a, Hlf, Homer1, Ikzf2, Inppl1, Irx3, Itgb6, Jarid2, Jph2, Khdrbs3, Klf7, Klhl23, Ky, Lrp2bp, Lrrfip1, Map2k6, Map3k4, Mat2a, Mkks, Mkl1, Mrc2, Mreg, Mrpl39, Narf, Ntf5, Nudt3, Osbpl6, Ostc, Parp8, Pkia, Plcd4, Podxl, Polk, Polr3k, Ppm1l, Pppde2, Prss23, Psd3, Psph, Ptpmt1, Ptx3, Qrsl1, Rasgrp3, Rhobtb3, Ric8b, Rnf150, Rsph1, Rundc1, Rxrg, Sel1l3, Sema3a, Sgcd, Shisa2, Sirt5, Slc25a19, Slc41a3, Slc4a4, Slco5a1, Snrnp35, Stac3, Ston2, Stradb, Stxbp4, Tfrc, Tmc7, Tmem218, Tmtc1, Tnfaip2, Tob1, Trim35, Ttl, Vegfa, and Vgll4.

24. Muscle Atrophy Signature – 4

[00434] Induced and repressed mRNA were evaluated for muscle atrophy signature-4. The statisitical significance for the identified mRNAs was defined as $P \leq 0.01$.

[00435] For induced mRNAs: mouse tibialis anterior mRNAs significantly induced by 1 week of denervation and significantly induced by 1 week of Gadd45a overexpression.

[00436] For repressed mRNAs: mouse tibialis anterior mRNAs significantly repressed by 1 week of denervation and significantly repressed by 1 week of Gadd45a overexpression.

[00437] The identified induced mRNAs included 2410089E03Rik, 6720456H20Rik, Abca1, Abhd2, Abr, Aif1l, Akap6, Alg8, Alox5ap, mpd3, Ankrd1, Anxa4, Aoah, App, Araf, Arfgap3, Arhgef2, Arpc3, Arpp21, Atf7ip, Atp6ap2, Atp6v1h, Atp7a, Atp8b1, B4galt5, Bax, Baz2a, Bhlhb9, Bmp2k, C3ar1, Canx, Casp3, Ccdc111, Ccdc122, Ccdc93, Ccndbp1, Cct4, Cd68, Cd82, Cdkn1a, Cep192, Cgref1, Chd4, Chrna1, Chrnb1, Chrng, Chuk, Clec12a, Clec4a3, Col19a1, Copb2, Cpne2, Cstb, Ctnna1, Ctps2, Ctsd, Ctsl, Ctss, Ctsz, Cyb5r3, Cybb, Cyr61, D10Wsu52e, D930016D06Rik, Dcaf13, Dclre1c, Dctn5, Ddb1, Ddhd1, Decr2, Derl1, Dhx9, Dido1, Dnajc1, Eda2r, Eef1b2, Eef2, Emr1, Epb4.1l3, Erbb2ipm, Erlin1, Esyt1,

Fam108c, Fam115a, Fbxo30, Frrs1, Fst, Fubp1, Fyb, Gab2, Gabarap, Gadd45a, Galc, Galnt7, Ganab, Gigyf2, Gm3435, Gnb2l1, Gng2, Gnl2, Gnl3, Gprasp1, Gpsm2, Gramd1b, H19, H2-Aa, Hmgn3, Hn1, Hnrnpu, Hprt, Hsp90ab1, Hsp90b1, Hspa2, Hspa4, Hspb8, Htatip2, Id2, Ifi30, Igbp1, Igdcc4, Ilf3, Imp4, Impact, Irak4, Itm2b, Ivns1abp, Kcnn3, Kdm3a, Khdrbs1, Kif5b, Klhl5, Krt18, Lbh, Lgals3, Lgmn, Lpar6, Lpin2, Lyz2, Macf1, Map1lc3a, Map3k1, Map4k4, Marveld2, Matr3, Mcm6, Mdm2, Mdm4, Me2, Med12, Mgea5, Micall1, Mpp1, Mrc1, Mtap1b, Myf6, Myl4, Myo5a, Ncam1, Nip7, Nln, Nop58, Nrcam, Nup93, Nvl, Obfc2a, Osbpl8, Palm2, Parp4, Pcbd1, Pcgf3, Pdlim3, Pfn1, Pgd,Pik3r3, Plaa, Plekha5, Plxdc2, Plxna1, Polr2a, Polr3b, Ppfibp1, Ppib, Prep, Prkdc, Prmt1, Prss48, Prune2, Psmb1, Psmd5, Rad50, Rassf4, Rb1, Rbm45, Reep5, Rgs2, Riok3, Rlim, Rnasel, Rpl31, Rps3, Rps9, Rrad, Rras2, Rspry1, Runx1, Sap30bp, Sema4d, Sema6a, Serf1, Serpinb6a, Sesn3, Sf3b1, Sf3b3, Sgpl1, Sh3d19, Sh3pxd2a, Sh3rf1, Sik1, Sirpa, Slc20a1, Slc25a24, Slc9a7, Slc9a9, Sln, Smarcad1, Smc1a, Smc5, Snd1, Snx5, Spin1, Srp14, Ssu72, Stam, Supt5h, Tbc1d8, Tbcd, Tbxas1, Tec, Tgfbr1, Tgs1, Thoc5, Thumpd3, Tiam2, Tlr4, Tlr6, Tmeff1, Tmem176b, Tmem179b, Tmem209, Tmem38b, Tnc, Tnfrsf22, Tnfrsf23, Tnnt2, Trim25, Trp63, Tubb5, Tubb6, Tyrobp, Uchl1, Ugcg, Usp11, Usp5, Wasf2, Wbp5, Wbscr27, Wdr36, Wdr61, Wdr67, Wdr77, Wdyhv1, Wsb1, Ylpm1, Ypel2, Ywhab, Zfp280d, Zfp318, Zfp346, Zfp36l1, and Zmynd8.

[00438] The identified repressed mRNAs included 0610012G03Rik, 1110001J03Rik, 1110067D22Rik, 2010106G01Rik, 2310002L09Rik, 2310003L22Rik, 2310010M20Rik, 2610507B11Rik, 2610528E23Rik, 2810407C02Rik, 4931409K22Rik, 4933403F05Rik, 5730437N04Rik, 9630033F20Rik, A2ld1, A930018M24Rik, Abcb1a, Abcb4, Abcd2, Abi3bp, Acaa2, Acadm, Acadvl, Acat1, Acot13, Adal, Adcy10, Adk, Adssl1, Aes, AI317395, Aimp2, Ak1, Alas2, Aldh1a1, Ank, Ank1, Ankrd9, Ano2, Ano5, Aplp2, Apobec2, Aqp4, Ar, Arhgap19, Arhgap20, Arhgap31, Arl3, Asb10, Asb11, Asb12, Asb14, Asb15, Atp11a, Atp13a5, Atp1b1, Atp5a1, Atp5e, Atp8a1, Atxn1, B4galt4, Bckdk, Bhlhe41, Bpgm, Bpil1, Brp44, Btbd1, C2cd2, Camk2a, Camk2g, Capn5, Car8 ,Cast ,Cc2d2a, Ccng1, Ccnk mCd34, Cd36 ,Cdc14a ,Cdc42ep3, Cdh5, Cdnf, Ces1d, Chchd10, Chchd3, Cib2, Ckm, Clcn1, Clic5, Cmbl, Cntfr, Col11a1 ,Coq9, Cox11, Cox6a2, Cox8b, Cpt1b, Csrp2bp, Cuedc1, Cyb5b, Cyyr1, D0H4S114, D1Ertd622e, Dab2ip, Dcun1d2, Deb1, Decr1, Dgkb, Dhrs7c, Dlat, Dlc1, Dlg1, Dlst, Dnajb5, Dusp28, Ecsit, Eef1a2, Eepd1, Efcab2, Eif4e, Endog, Eno3, Epas1, Epha7, Etfb, Exd2, Eya1, Fam132a, Fastkd3, Fbp2, Fbxo3, Fdx1, Fez2, Fgfbp1, Fh1, Fitm2, Flt1, Fmo5, Fsd2, Fxyd1, Fzd4, G3bp2, Ganc, Gbas, Gcom1, Gdap1, Ghr, Gjc3, Glb1l2, Gm4841, Gm4861, Gm4951, Gm5105, Gmpr, Gpcpd1, Gpd1, Gpd2, Gpt2, Grsf1,

Gucy1a3, Gys1, Hadh, Hfe2, Hivep2, Hk2, Hlf, Homer1, Hsdl2, Idh3a, Idh3g, Il15ra, Inpp5a, Irx3, Jak2, Jam2, Jph1, Kcna7, Kcnj2, Kcnn2, Kdr, Khdrbs3, Kif1b, Kif1c, Kitl, Klf12, Klhl23, Klhl31, Klhl31, Klhl7, Ky, Ldb3, Lifr, Lmbr1, Lphn1, Lpin1, Lpl, Lrig1, Lrrc39, Lynx1, Man2a2, Maob, Map2k6, Map2k7, Map3k4, Mapkapk2, Mbnl1, Mccc1, Mdh1, Mdh2, Me3, Mfn1, Mfn2, Mgst3, Mlf1, Mpnd, Mpz, Mr1, Mreg, Mtus1, Mybpc2, Myo5c, Myom2, Myoz1, Narf, Ndrg2, Ndufa3, Ndufa5, Ndufa8, Ndufb8, Ndufb9, Ndufs1, Ndufs2, Ndufs6, Ndufs8, Ndufv1, Nf2, Nos1, Nr1d1, Nudt3, Oat, Ociad2, Ocrl, Osbpl6, Osgepl1, Ostn, Paqr9, Parp3, Pcmtd1, Pcnt, Pcnx, Pdgfd, Pdha1, Pdpr, Pfkfb3, Pfkm, Pfn2, Pgam2, Phb, Phka1, Phkg1, Phtf2, Phyh, Pitpnc1, Pkdcc, Pkia, Pla2g12a, Pla2g4e, Plcb1, Plcd4, Pln, Pmp22, Ppara, Ppargc1a, Ppat, Ppm1a, Ppm1l, Ppp1cb, Ppp1r1a, Ppp2r2a, Ppp3cb, Prelp, Prkab2, Prkca, Prkg1, Ptp4a3, Ptprb, Pttg1, Pxmp2, Pygm, Rab28, Rasgrp3, Rcan2, Rgs5, Rhot2, Rnf123, Rpa1, Rpl3l, Rtn4ip1, Samd12, Samd5, Satb1, Scn4a, Scn4b, Sdha, Sdhb, Sdr39u1, Sel1l3, Sema6c, Serpine2, Shisa2, Slc15a5, Slc16a3, Slc19a2, Slc24a2, Slc25a11, Slc25a12, Slc25a3, Slc25a4, Slc2a12, Slc2a4, Slc35f1, Slc37a4, Slc43a3, Slc4a4, Slc6a13, Slc6a8, Slc9a3r2, Slco3a1, Smarca1, Smox, Smyd1, Snrk, Sorbs2, Spop, Srl, St3gal3, St3gal6, St6galnac6, Stk38l, Stradb, Strbp, Strbp, Stxbp4, Suclg1, Tab2, Taf3, Tarsl2, Tcea3, Thra, Tiam1, Timp4, Tln2, Tmem126a, Tmem126b, Tmem65, Tnfaip2, Tnmd, Tnnc2, Tnni2, Tnxb, Tomm40l, Trak1, Trak2, Trim24, Trpc3, Tuba8, Txlnb, Txnip, U05342, Uaca, Ulk2, Uqcrc1, Uqcrfs1, Uqcrq, Vamp5, Vdac1, Vegfa, Vegfb, Xpr1, Yipf7, Zfand5, Zfp191, and Zfp238.

F. REFERENCES

[00439] The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

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[00502] It is to be understood that, if any prior art publication is referred to herein, such reference does not constitute an admission that the publication forms a part of the common general knowledge in the art, in Australia or any other country.

[00502] In the claims which follow and in the preceding description of the invention, except where the context requires otherwise due to express language or necessary implication, the word "comprise" or variations such as "comprises" or "comprising" is used in an inclusive sense, i.e. to specify the presence of the stated features but not to preclude the presence or addition of further features in various embodiments of the invention.

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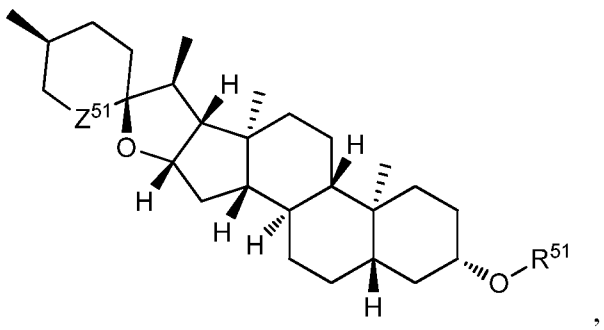
CLAIMS

What is claimed is:

1. Use of a compound having a structure represented by a formula E1:



E1

or a tautomer, solvate, or pharmaceutically acceptable salt thereof, in the manufacture of a medicament for: (a) increasing skeletal muscle mass, (b) reducing skeletal muscle atrophy, (c) increasing strength, (d) increasing energy expenditure, (e) increasing the ratio of skeletal muscle to fat, (f) reducing obesity, (g) promoting normal muscle function, (h) improving muscle function, or (i) promoting healthy aging muscles, wherein

R^{51} is selected from H, unsubstituted linear or branched C1-C6 alkyl, unsubstituted cycloalkyl, and COR^{53};

R^{53} is unsubstituted linear or branched C1-C6 alkyl or unsubstituted cycloalkyl,;

Z^{51} is NR^{54}; and

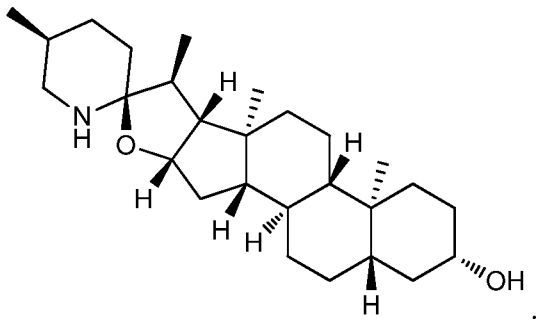
R^{54} is selected from H, unsubstituted linear or branched C1-C6 alkyl and unsubstituted cycloalkyl.

2. Use according to claim 1, wherein R^{51} is selected from H, unsubstituted linear or branched C1-C6 alkyl and unsubstituted cycloalkyl.

3. Use according to claim 1 or 2, wherein R^{51} is H.

4. Use according to any one of claims 1 to 3, wherein R^{54} is H.

5. Use according to claim 1, wherein the compound has the structure represented by the formula:



6. Use according to any one of claims 1 to 5, in the manufacture of a medicament for the treatment of muscle atrophy in an animal.

7. Use according to any one of claims 1 to 5, in the manufacture of a medicament for increasing muscle mass in an animal.

8. Use according to any one of claims 1 to 5, in the manufacture of a medicament for enhancing muscle formation in an animal.

9. Use according to any one of claims 1 to 5, in the manufacture of a medicament for promoting normal muscle function in an animal.

10. Use according to any one of claims 1 to 5, in the manufacture of a medicament for promoting healthy aging muscles in an animal.

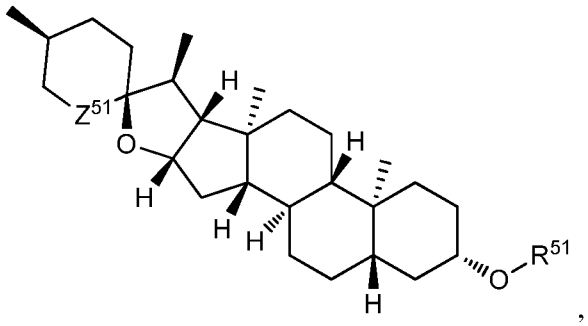
11. Use according to any one of claims 1 to 5, in the manufacture of a medicament for increasing strength in an animal.

12. Use according to any one of claims 1 to 5, in the manufacture of a medicament for increasing energy expenditure in an animal.

13. Use according to any one of claims 1 to 5, in the manufacture of a medicament for increasing the ratio of skeletal muscle to fat in an animal.

14. Use according to any one of claims 1 to 5, in the manufacture of a medicament for reducing obesity in an animal.

15. A method of (a) increasing skeletal muscle mass, (b) reducing skeletal muscle atrophy, (c) increasing strength, (d) increasing energy expenditure, (e) increasing the ratio of skeletal muscle to fat, (f) reducing obesity, (g) promoting normal muscle function, (h) improving muscle function, or (i) promoting healthy aging muscles comprising administering a compound of the formula E1:



E1

or a tautomer, solvate, or pharmaceutically acceptable salt thereof, or a composition that provides a compound of formula E1 or a tautomer, solvate, or pharmaceutically acceptable salt thereof,

wherein

R^{51} is selected from H, unsubstituted linear or branched C1-C6 alkyl, unsubstituted cycloalkyl and COR^{53};

R^{53} is unsubstituted linear or branched C1-C6 alkyl or unsubstituted cycloalkyl;

Z^{51} is NR^{54}; and

R^{54} is selected from H, unsubstituted linear or branched C1-C6 alkyl and unsubstituted cycloalkyl.

16. A method according to claim 15, wherein R^{51} is selected from H, unsubstituted linear or branched C1-C6 alkyl and unsubstituted cycloalkyl.

17. A method according to either one of claims 15 or 16, wherein R^{51} is H.

18. A method according to any one of claims 15 to 17, wherein R^{54} is H.

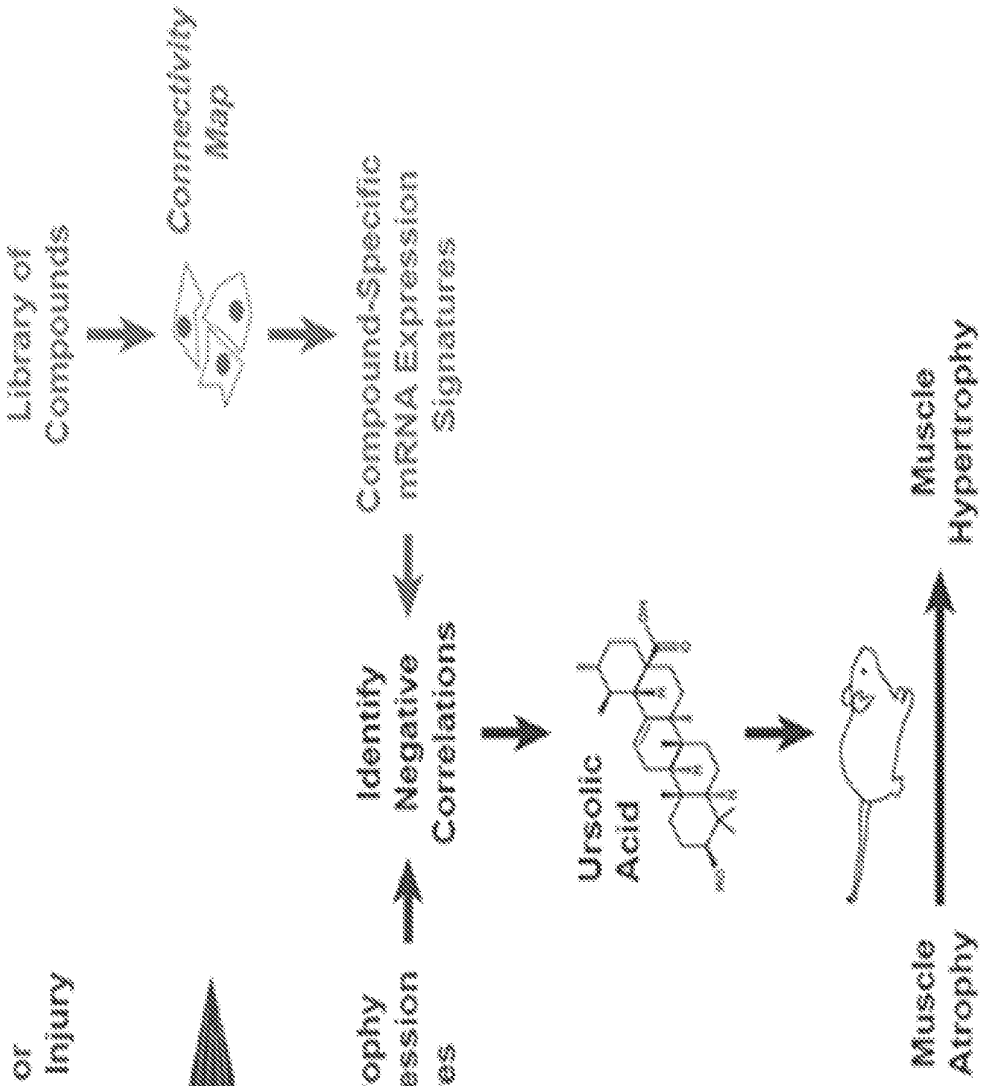

Fasting or Spinal Cord Injury
Human Muscle
Muscle Atrophy mRNA Expression Signatures
Identify Negative Correlations
Library of Compounds
Connectivity Map
Compound-Specific mRNA Expression Signatures
Ursolic Acid
Muscle Atrophy
Muscle Hypertrophy

Figure 1

Human Muscle Atrophy Signature-1

Conserved Effects of Fasting on Human and Mouse Skeletal Muscle	
Induced mRNAs	Repressed mRNAs
ABCA1	*ACACA*
ACOX1	*BPGM*
ADPGK	*CACNB1*
CALCOCO1	*CASQ1*
CAT	*CNNM4*
CITED2	*DNMT3A*
CPT1A	*FEZ2*
GABARAPL1	*GAS2*
HERPUD1	*GRTP1*
HMOX1	*HSPH1*
IGF1R	*JTB*
INSR	*MRPS15*
MED13L	*MTSS1*
MYO5A	*NEO1*
NBR1	*NFYA*
NOX4	*P4HA2*
PDK4	*PBX1*
PPAP2B	*PDE7B*
RORA	*PMP22*
SESN1	*PGC-1α*
SFRS8	*PTX3*
SLC38A2	*SLC4A4*
SRRM2	*SPINT2*
SUPT6H	*ST8SIA5*
TULP3	*SUV39H2*
TXNIP	*TFRC*
UBE4A	*TGFB2*
UCP2	*TSPAN13*
UCP3	*TTLL1*
XPO4	*VEGFA*
ZFAND5	*WDR1*
	ZNF280B

Figure 2

Human Muscle Atrophy Signature-2

Conserved Effects of Fasting and SCI on Human Skeletal Muscle	
Induced mRNAs	Repressed mRNAs
CAV3	*CMAS*
CTDSP2	*GUCY1B3*
CUGBP2	*HSPB7*
IGF1R	*MRPS15*
IRS2	*PDE7B*
KLF11	*PFDN6*
MLL	*PGC-1α*
NOX4	*SLC16A1*
NPC2	*TSPAN13*
NUPR1	*TTLL1*
OR1D4	*VEGFA*
RHOBTB1	*VLDLR*
SUPT6H	*ZNF280B*
TSPAN8	*ZNF32*
ZNF682	

Figure 3

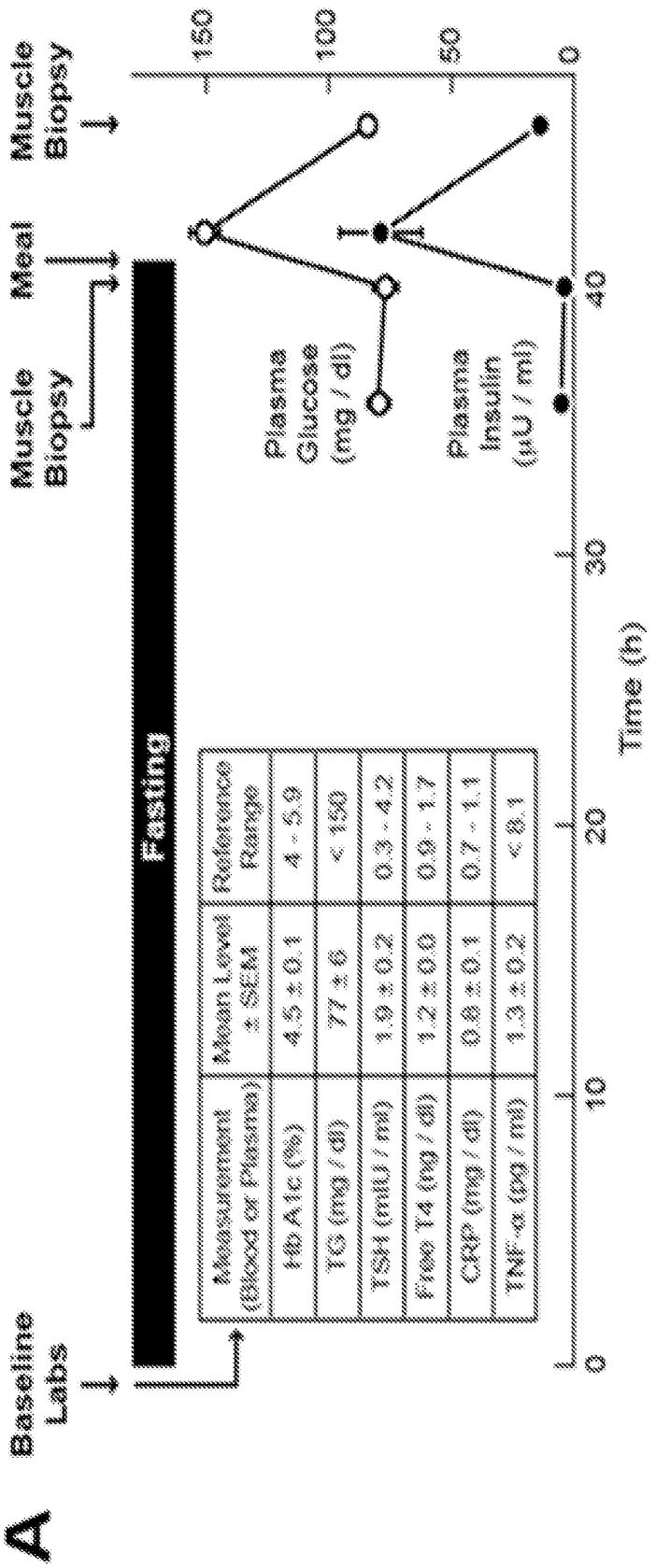

A
Baseline Labs
Fasting
Muscle Biopsy
Meal
Muscle Biopsy
Plasma Glucose (mg / dl)
Plasma Insulin (µU / ml)
150
100
50
0
0
10
20
30
40
Time (h)
Measurement (Blood or Plasma)
Mean Level ± SEM
Reference Range
Hb A1c (%)
4.5 ± 0.1
4 - 5.9
TG (mg / dl)
77 ± 6
< 150
TSH (mIU / ml)
1.9 ± 0.2
0.3 - 4.2
Free T4 (ng / dl)
1.2 ± 0.0
0.9 - 1.7
CRP (mg / dl)
0.8 ± 0.1
0.7 - 1.1
TNF-α (pg / ml)
1.3 ± 0.2
< 8.1

FIGURE 4A

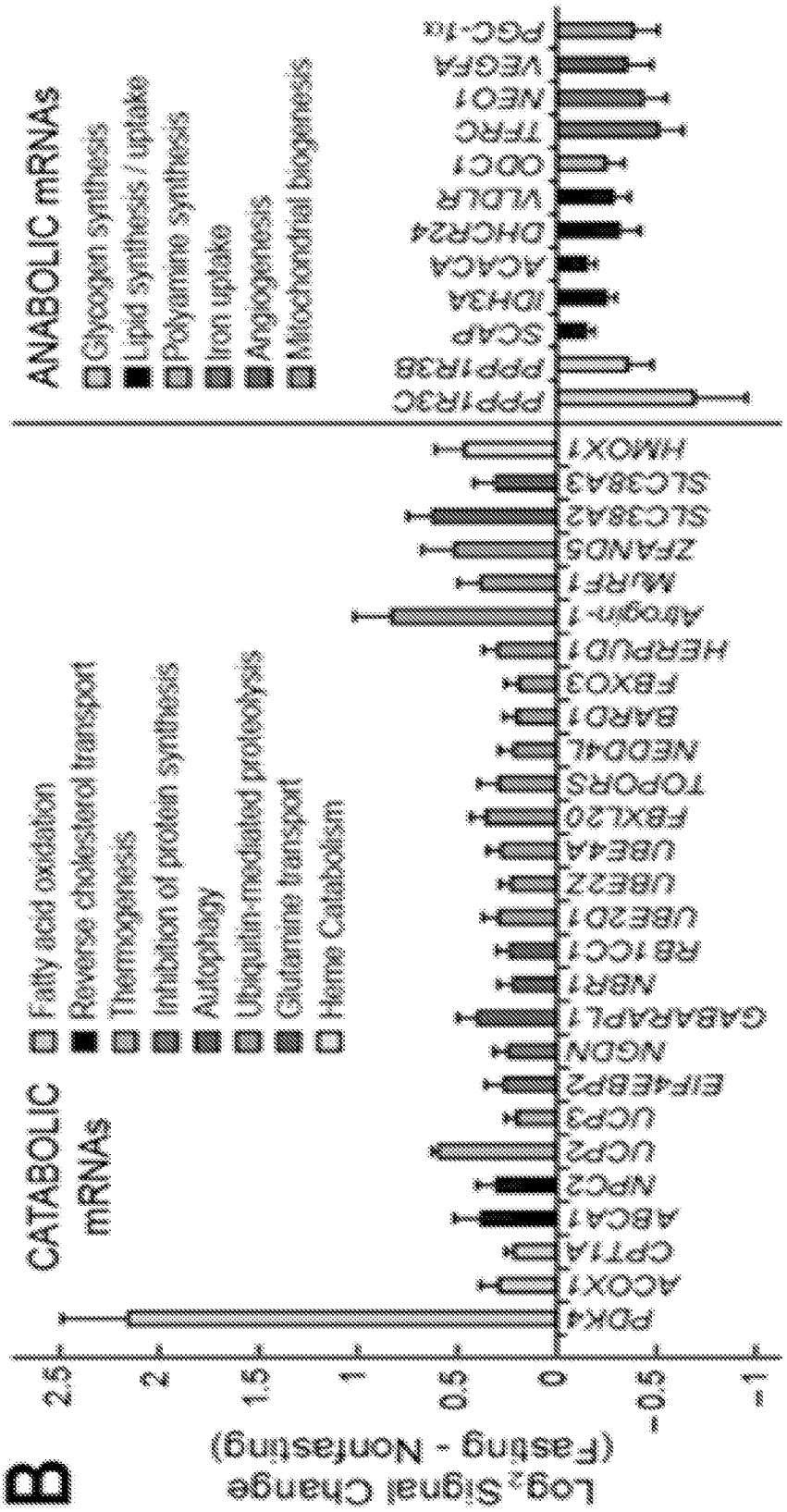

B
Log2 Signal Change (Fasting - Nonfasting)
2.5
2
1.5
1
0.5
0
-0.5
-1
CATABOLIC mRNAs
Fatty acid oxidation
Reverse cholesterol transport
Thermogenesis
Inhibition of protein synthesis
Autophagy
Ubiquitin-mediated proteolysis
Glutamine transport
Heme Catabolism
PDK4
ACOX1
CPT1A
ABCA1
NPC2
UCP2
UCP3
EIF4EBP2
NCDN
GABARAPL1
NBR1
RB1CC1
UBE2D1
UBE2Z
UBE4A
FBXL20
TOPORS
NEDD4L
BARD1
FBXO3
HERPUD1
Atrogin-1
MuRF1
ZFAND5
SLC38A2
SLC38A3
HMOX1
ANABOLIC mRNAs
Glycogen synthesis
Lipid synthesis / uptake
Polyamine synthesis
Iron uptake
Angiogenesis
Mitochondrial biogenesis
PPP1R3C
PPP1R3B
SCAP
IDH3A
ACACA
DHCR24
VLDLR
CDC1
TFRC
NEO1
VEGFA
PGC-1α

FIGURE 4B

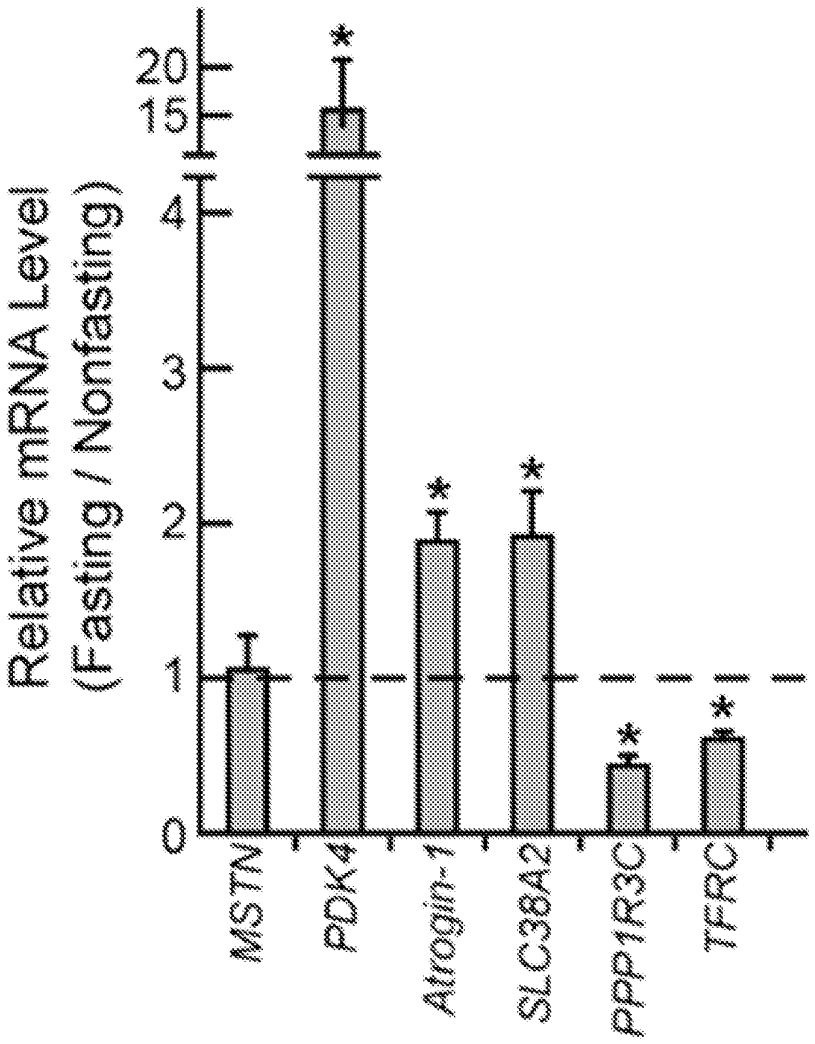
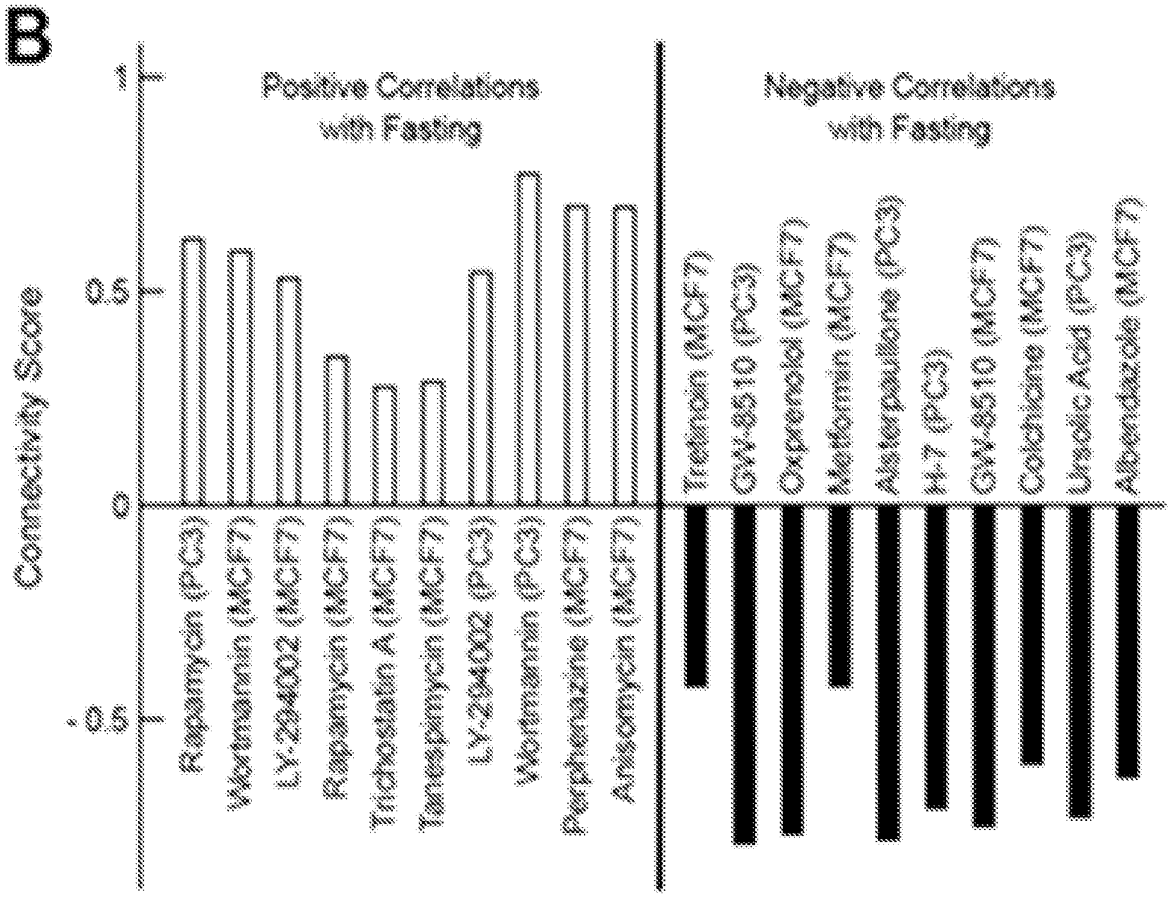

Relative mRNA Level
(Fasting / Nonfasting)
20
15
4
3
2
1
0
*
*
*
*
*
MSTN
PDK4
Atrogin-1
SLC38A2
PPP1R3C
TFRC

Figure 5

A

Conserved Effects of Fasting on Human and Mouse Skeletal Muscle	
Induced mRNAs	Repressed mRNAs
ABCA1	*ACACA*
ACOX1	*BPGM*
ADPGK	*CACNB1*
CALCOCO1	*CASQ1*
CAT	*CNNM4*
CITED2	*DNMT3A*
CPT1A	*FEZ2*
GABARAPL1	*GAS2*
HERPUD1	*GRTP1*
HMOX1	*HSPH1*
IGF1R	*JTB*
INSR	*MRPS15*
MED13L	*MTSS1*
MYO5A	*NEO1*
NBR1	*NFYA*
NOX4	*P4HA2*
PDK4	*PBX1*
PPAP2B	*PDE7B*
RORA	*PMP22*
SESN1	*PGC-1α*
SFRS8	*PTX3*
SLC38A2	*SLC4A4*
SRRM2	*SPINT2*
SUPT6H	*ST8SIA5*
TULP3	*SUV39H2*
TXNIP	*TFRC*
UBE4A	*TGFB2*
UCP2	*TSPAN13*
UCP3	*TTLL1*
XPO4	*VEGFA*
ZFAND5	*WDR1*
	ZNF280B

FIGURE 6A



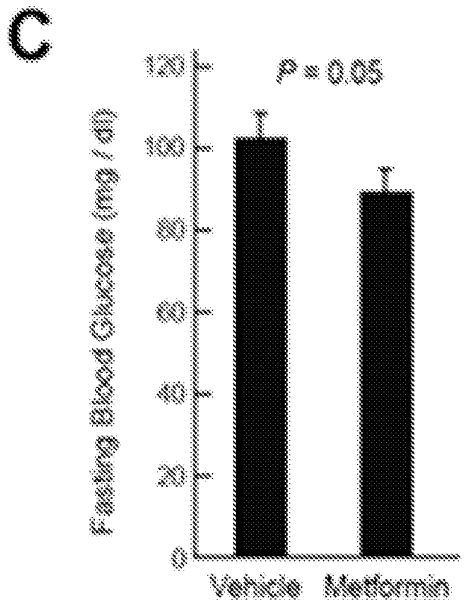


FIGURE 6B and 6C

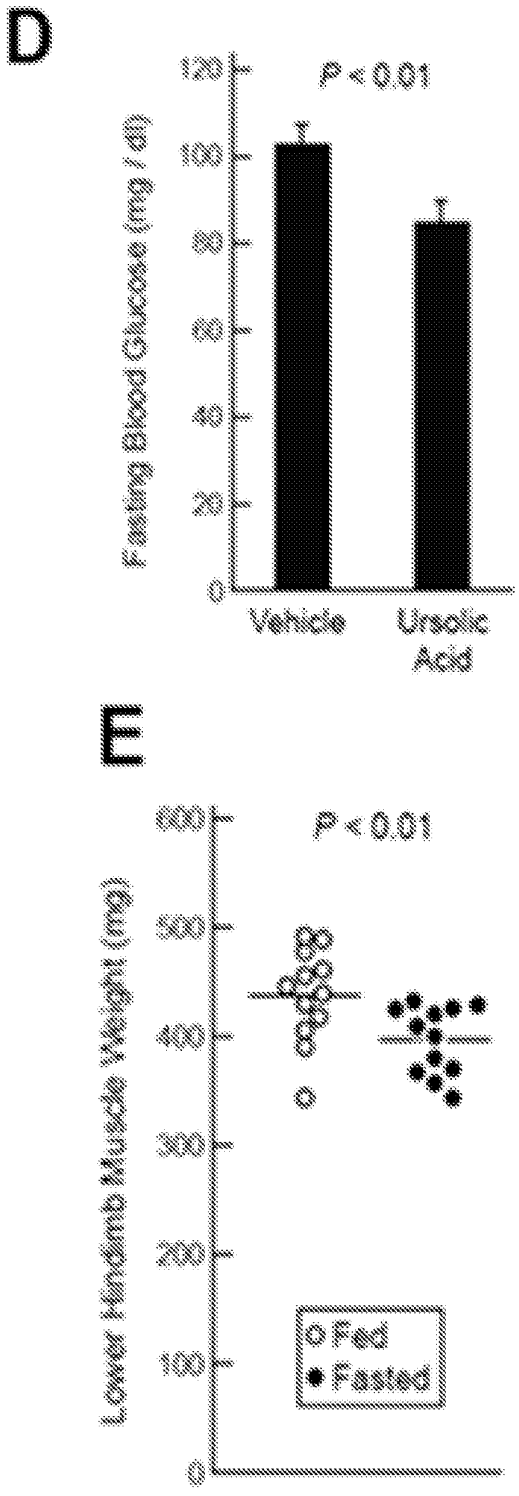


FIGURE 6D and 6E

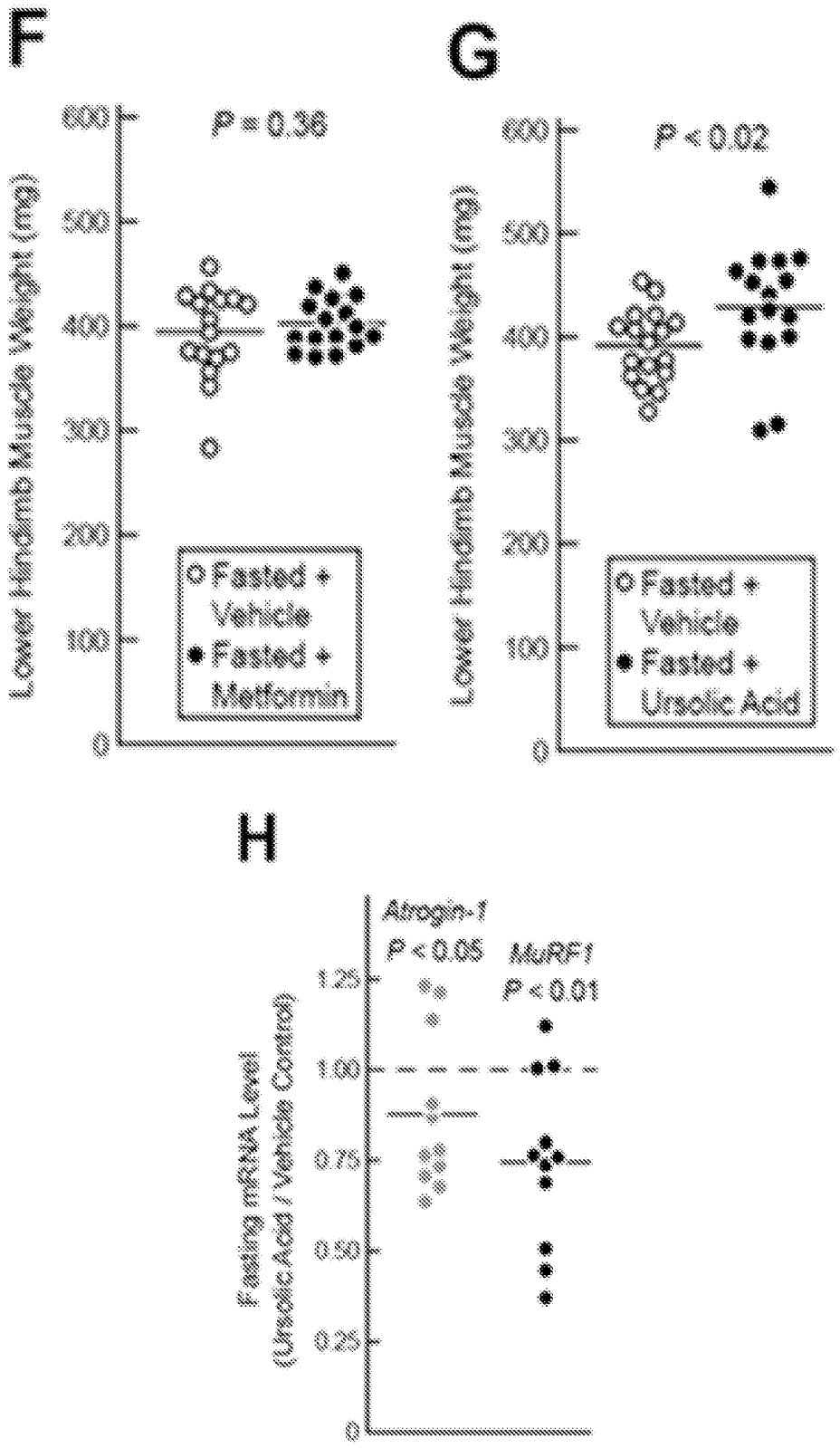

F
P = 0.36
Lower Hindlimb Muscle Weight (mg)
600
500
400
300
200
100
0
Fasted + Vehicle
Fasted + Metformin
G
P < 0.02
Lower Hindlimb Muscle Weight (mg)
600
500
400
300
200
100
0
Fasted + Vehicle
Fasted + Ursolic Acid
H
Atrogin-1
P < 0.05
MuRF1
P < 0.01
Fasting mRNA Level (Ursolic Acid / Vehicle Control)
1.25
1.00
0.75
0.50
0.25
0

FIGURE 6F-6G

A

Conserved Effects of Fasting and SCI on Human Skeletal Muscle	
Induced mRNAs	Repressed mRNAs
CAV3	*CMAS*
CTDSP2	*GUCY1B3*
CUGBP2	*HSPB7*
IGF1R	*MRPS15*
IRS2	*PDE7B*
KLF11	*PFDN6*
MLL	*PGC-1α*
NOX4	*SLC16A1*
NPC2	*TSPAN13*
NUPR1	*TTLL1*
OR1D4	*VEGFA*
RHOBTB1	*VLDLR*
SUPT6H	*ZNF280B*
TSPAN8	*ZNF32*
ZNF682	

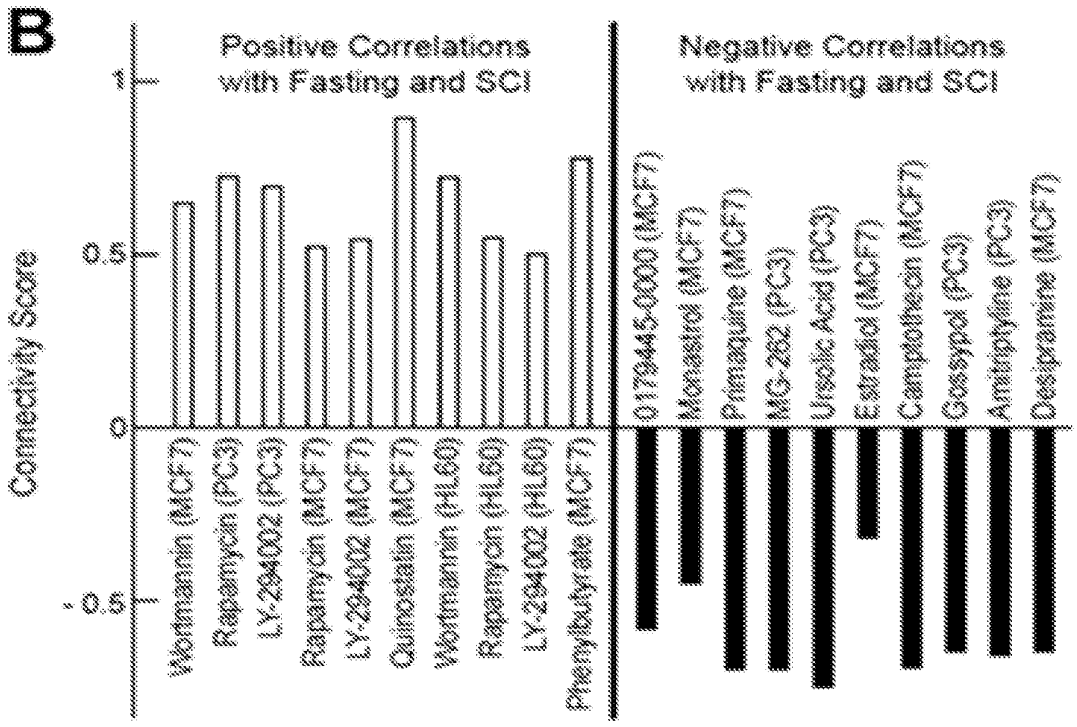


FIGURE 7A and 7B

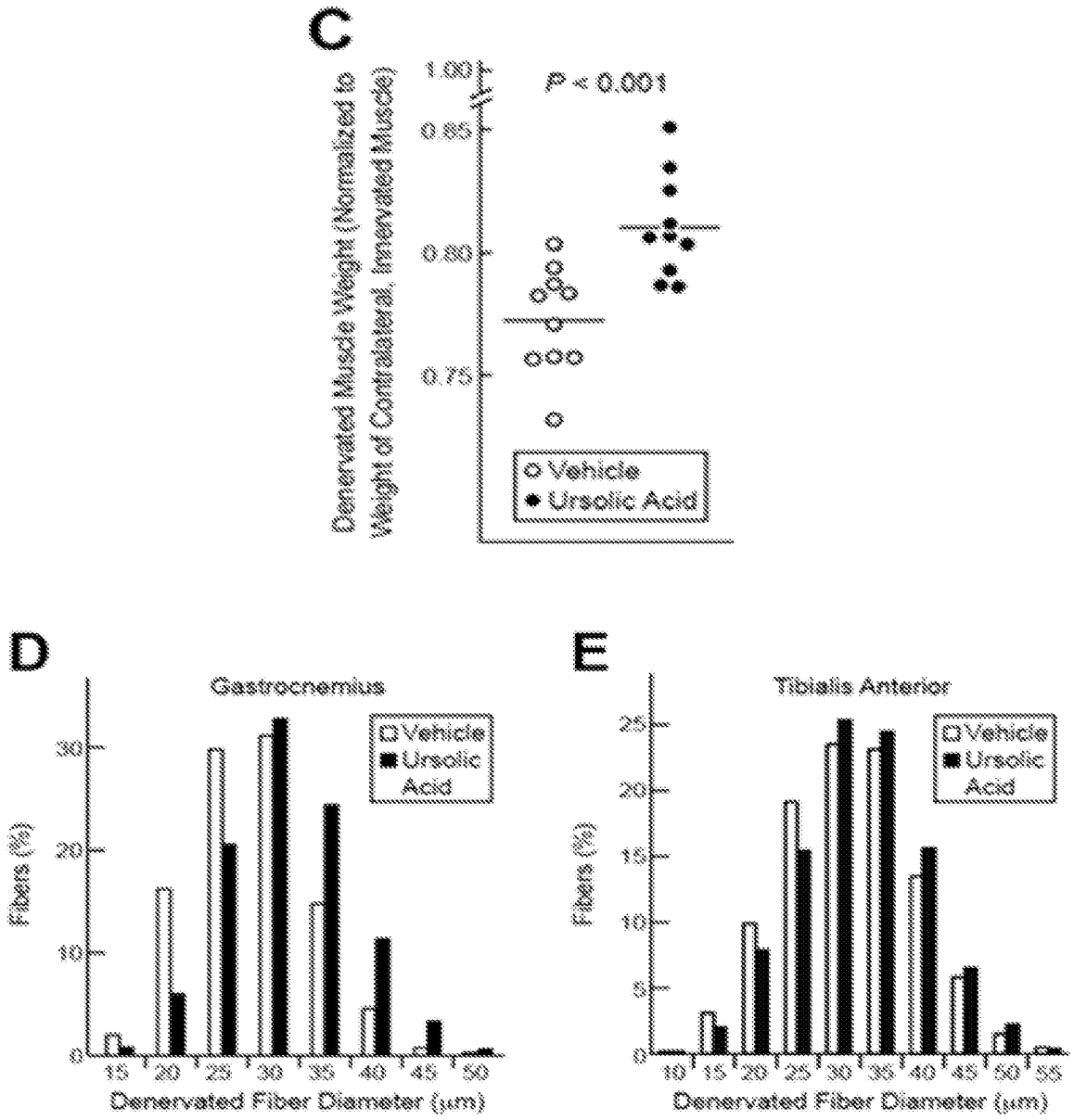

C
Denervated Muscle Weight (Normalized to Weight of Contralateral, Innervated Muscle)
1.00
0.85
0.80
0.75
P < 0.001
Vehicle
Ursolic Acid
D
Gastrocnemius
Fibers (%)
30
20
10
0
Vehicle
Ursolic Acid
15 20 25 30 35 40 45 50
Denervated Fiber Diameter (μm)
E
Tibialis Anterior
Fibers (%)
25
20
15
10
5
0
Vehicle
Ursolic Acid
10 15 20 25 30 35 40 45 50 55
Denervated Fiber Diameter (μm)

FIGURE 7C-7E

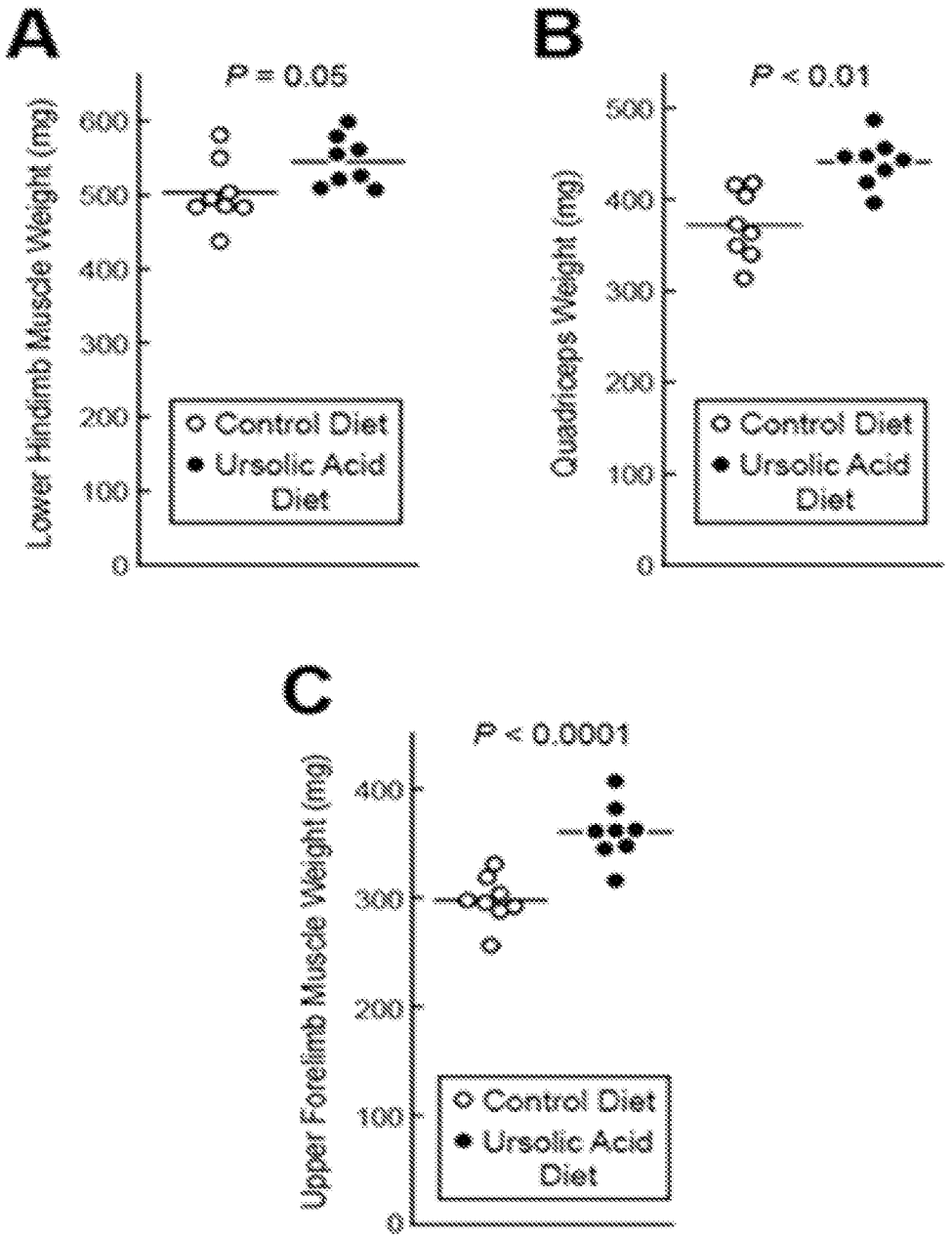

A
P = 0.05
Lower Hindlimb Muscle Weight (mg)
600
500
400
300
200
100
0
Control Diet
Ursolic Acid Diet
B
P < 0.01
Quadriceps Weight (mg)
500
400
300
200
100
0
Control Diet
Ursolic Acid Diet
C
P < 0.0001
Upper Forelimb Muscle Weight (mg)
400
300
200
100
0
Control Diet
Ursolic Acid Diet

FIGURE 8A-8C

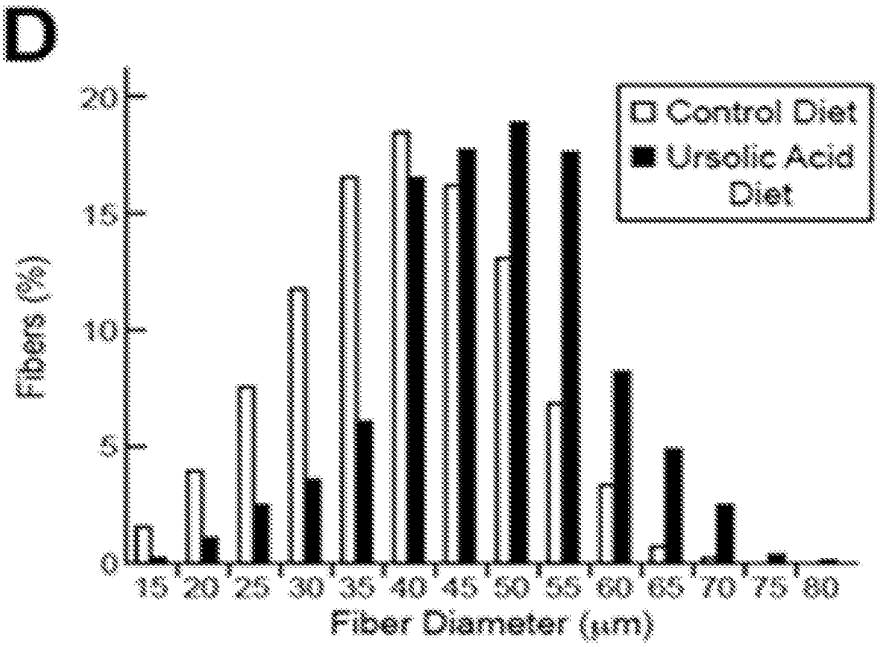


FIGURE 8D and 8E

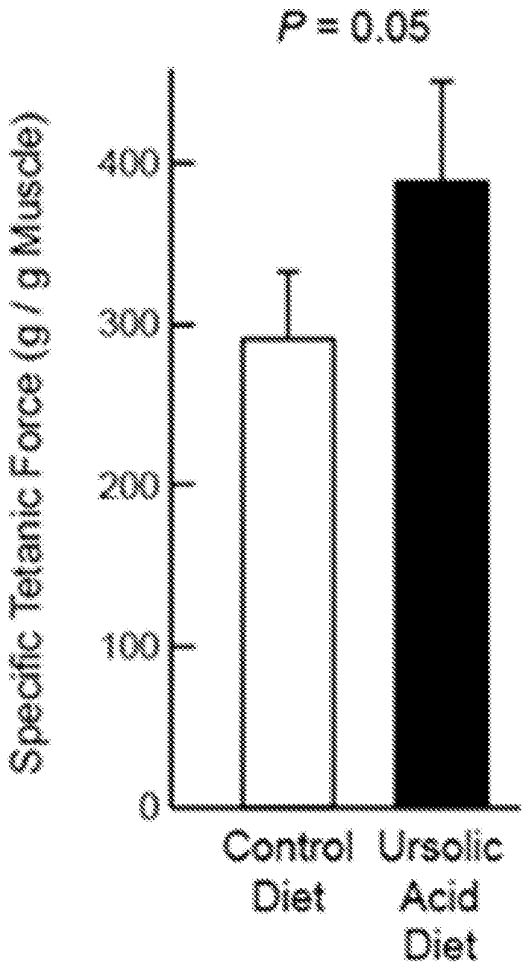
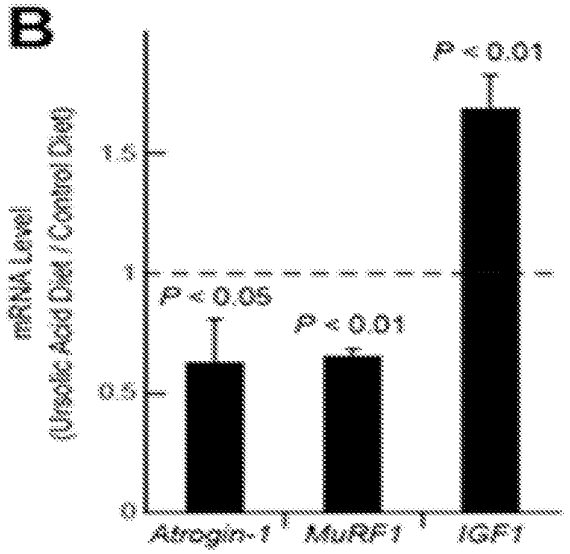

P = 0.05
Specific Tetanic Force (g / g Muscle)
400
300
200
100
0
Control Diet
Ursolic Acid Diet

Figure 9

A

	Rank	mRNA	Log_2 Signal (Ursolic Acid - Control)
Repressed	1	*Atrogin-1*	-0.35
	2	*DDIT4L*	-0.32
	3	*AOPRHL1*	-0.26
	4	*NNT*	-0.23
	5	*SFRS5*	-0.22
	6	*CACNA1S*	-0.22
Induced	1	*SMOX*	0.81
	2	*LYZ2*	0.71
	3	*C3*	0.70
	4	*TYROBP*	0.69
	5	*LUM*	0.61
	6	*IGF1*	0.56

B



C

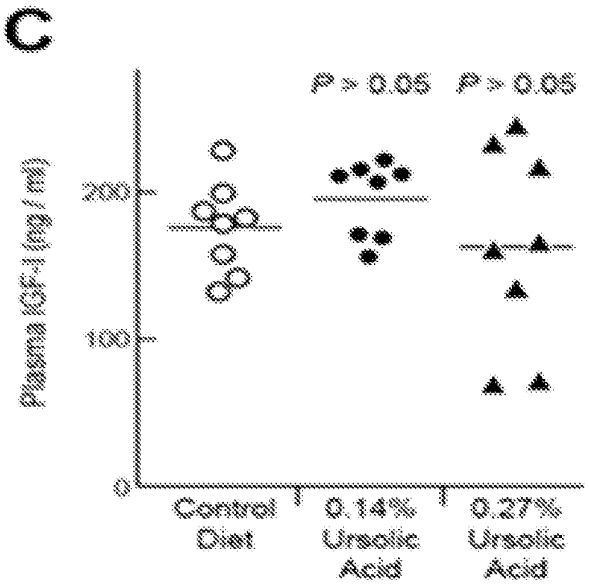


FIGURE 10A-10C

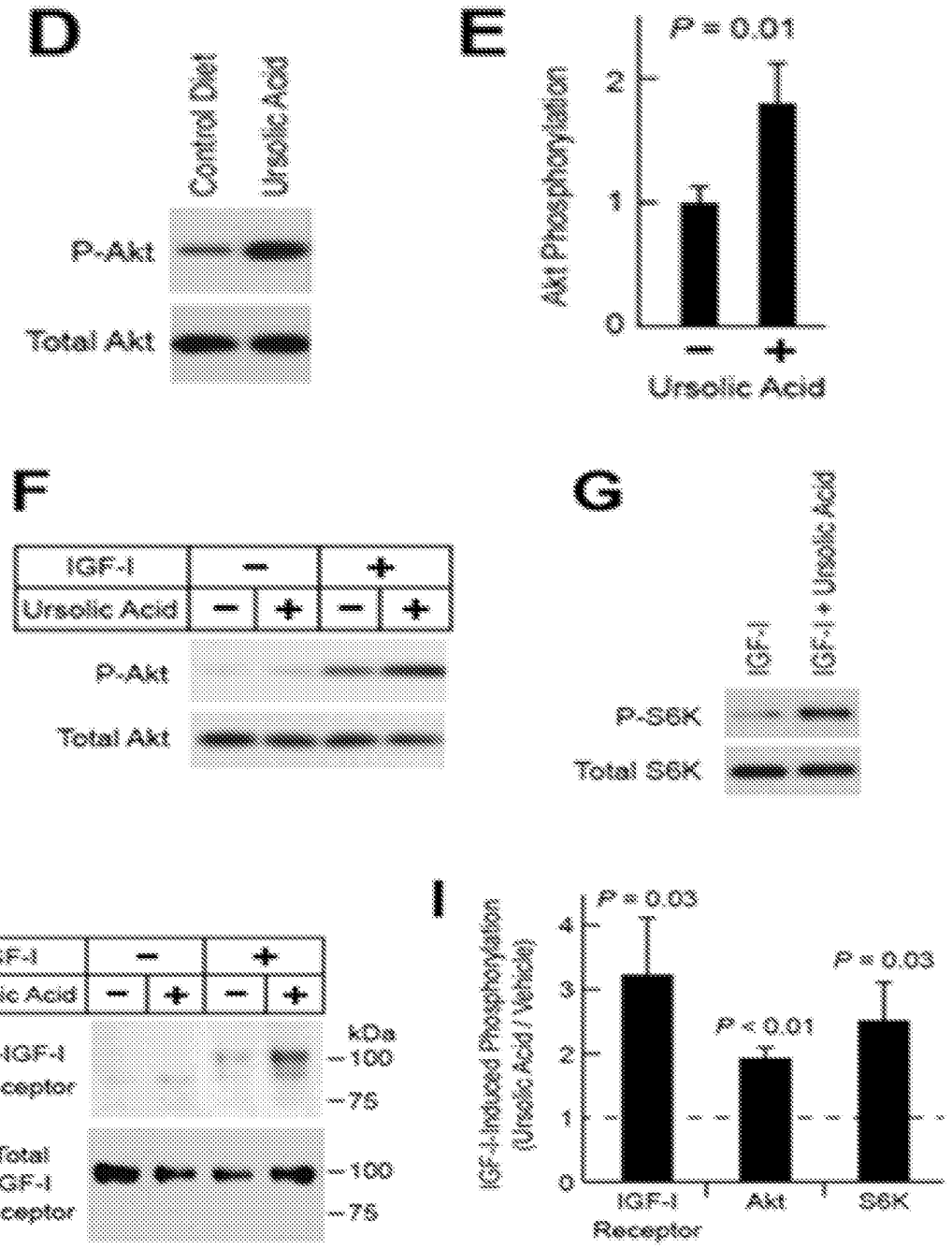


FIGURE 10D-10I

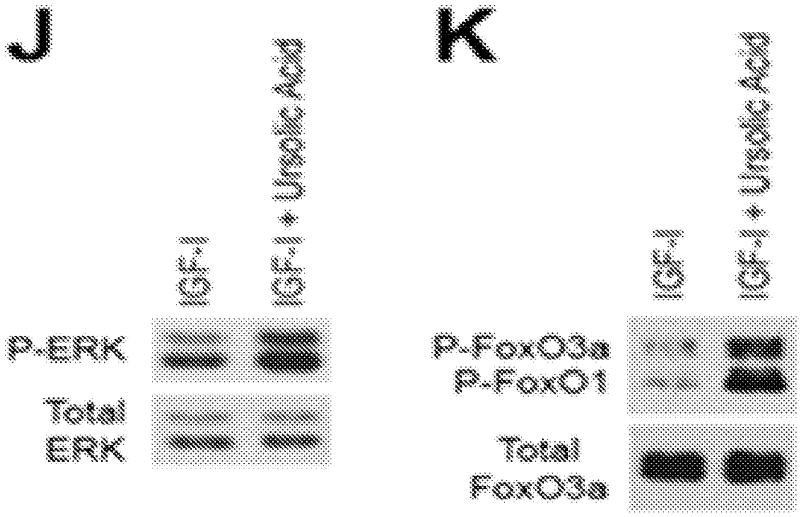


FIGURE 10J and 10K

A

Probe ID	Probe Position	IGF1 Exon	Log2 Hybridization Signals			p
			Control	Ursolic Acid	Ursolic - Control	
4000042	chr 10: 87322047 - 87323726	2	5.65	5.93	0.28	0.14
4714835	chr 10: 87324043 - 87324110	2	7.28	7.92	0.65	0.00
5307208	chr 10: 87327403 - 87327614	3	8.32	8.72	0.40	0.01
5201482	chr 10: 87376384 - 87376503	4	9.73	10.28	0.54	0.01
5254483	chr 10: 87378043 - 87378085	5	5.73	6.73	1.00	0.02
5200449	chr 10: 87378100 - 87378172	5	5.77	6.17	0.40	0.20
5015224	chr 10: 87378301 - 87378413	5	7.61	8.53	0.93	0.04
5467760	chr 10: 87383446 - 87383470	6	9.65	9.90	0.25	0.16

FIGURE 11A

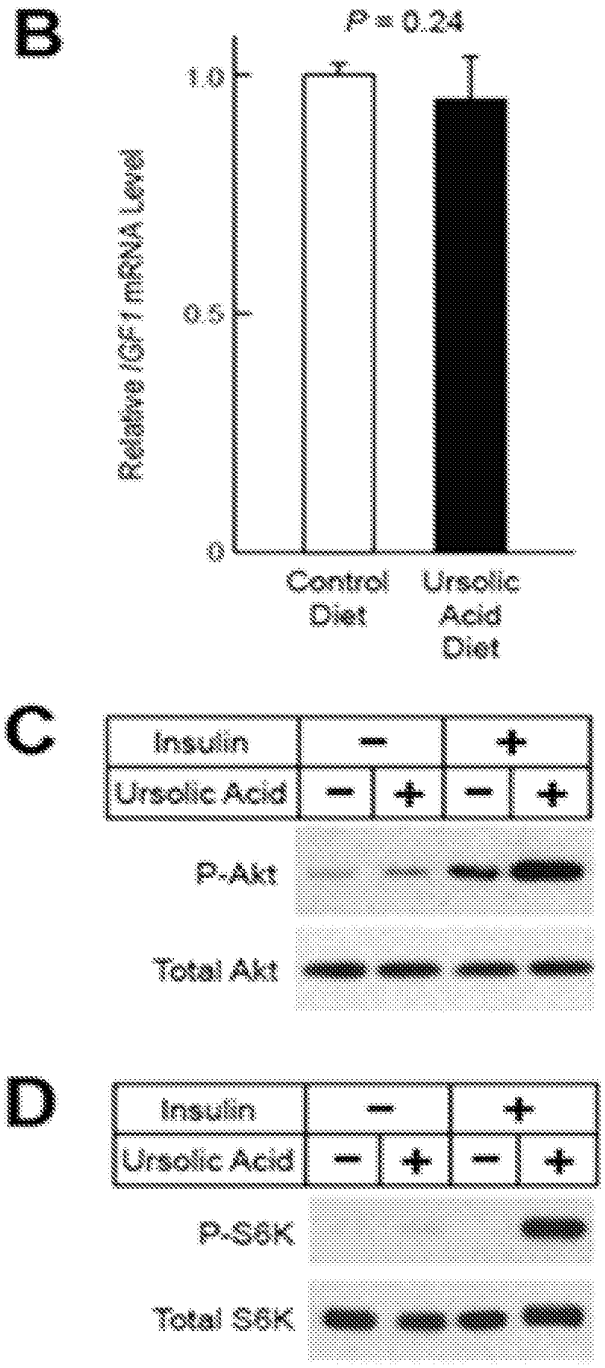


FIGURE 11B-11D

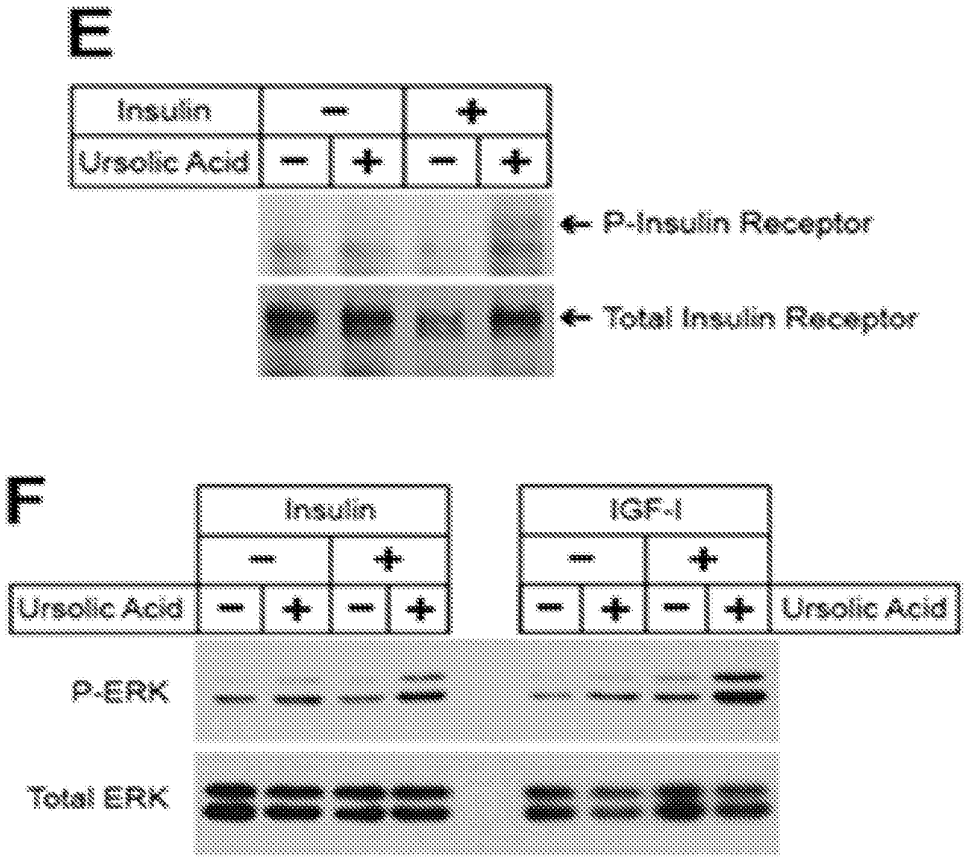


FIGURE 11E and 11F

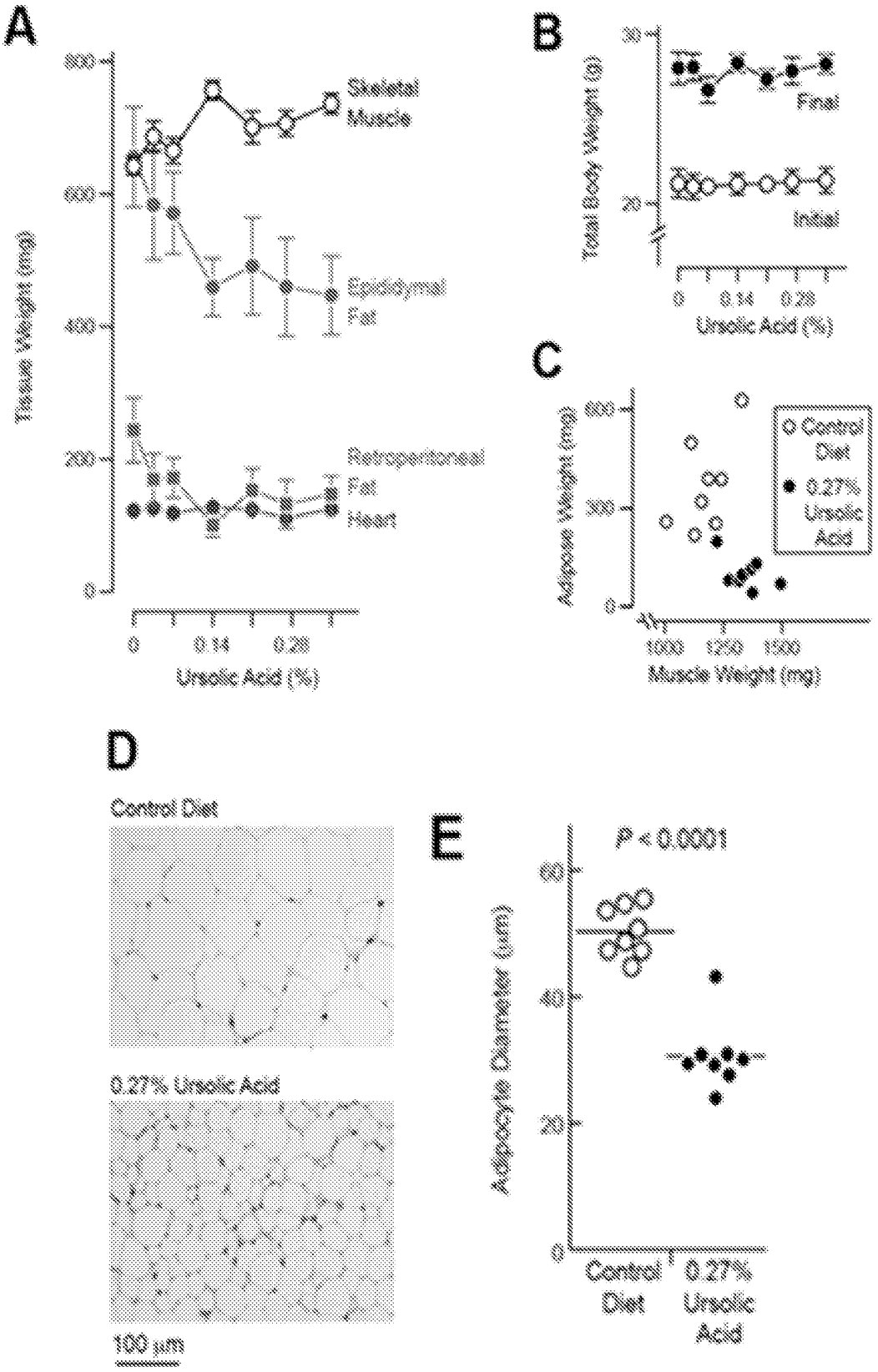
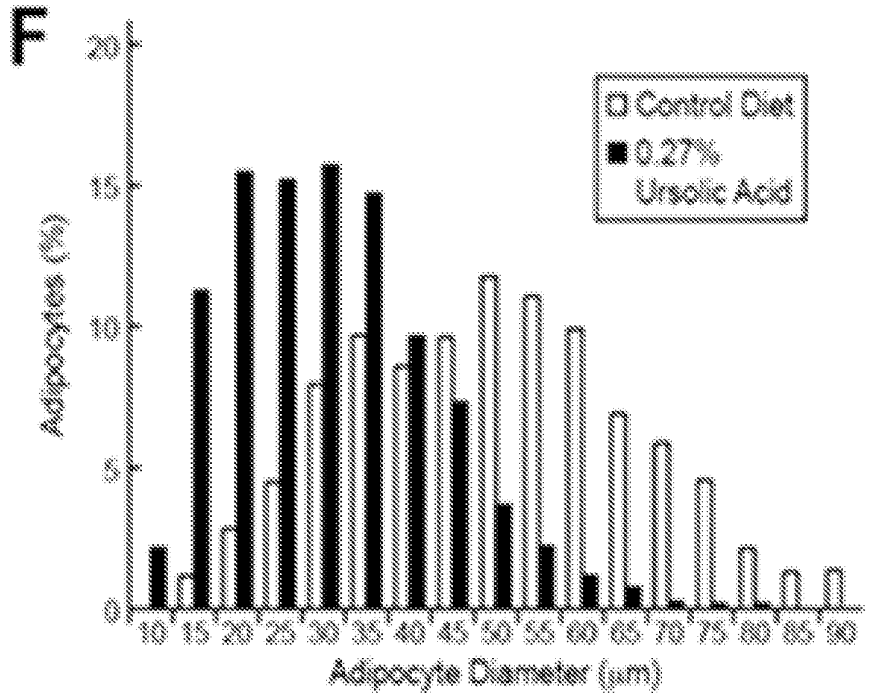


FIGURE 12A-12E



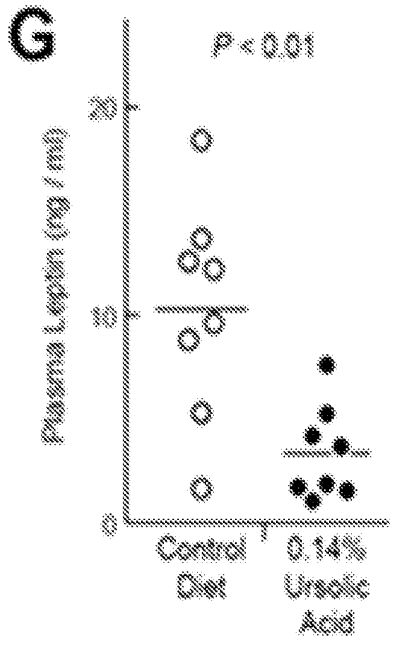


FIGURE 12F and 12G

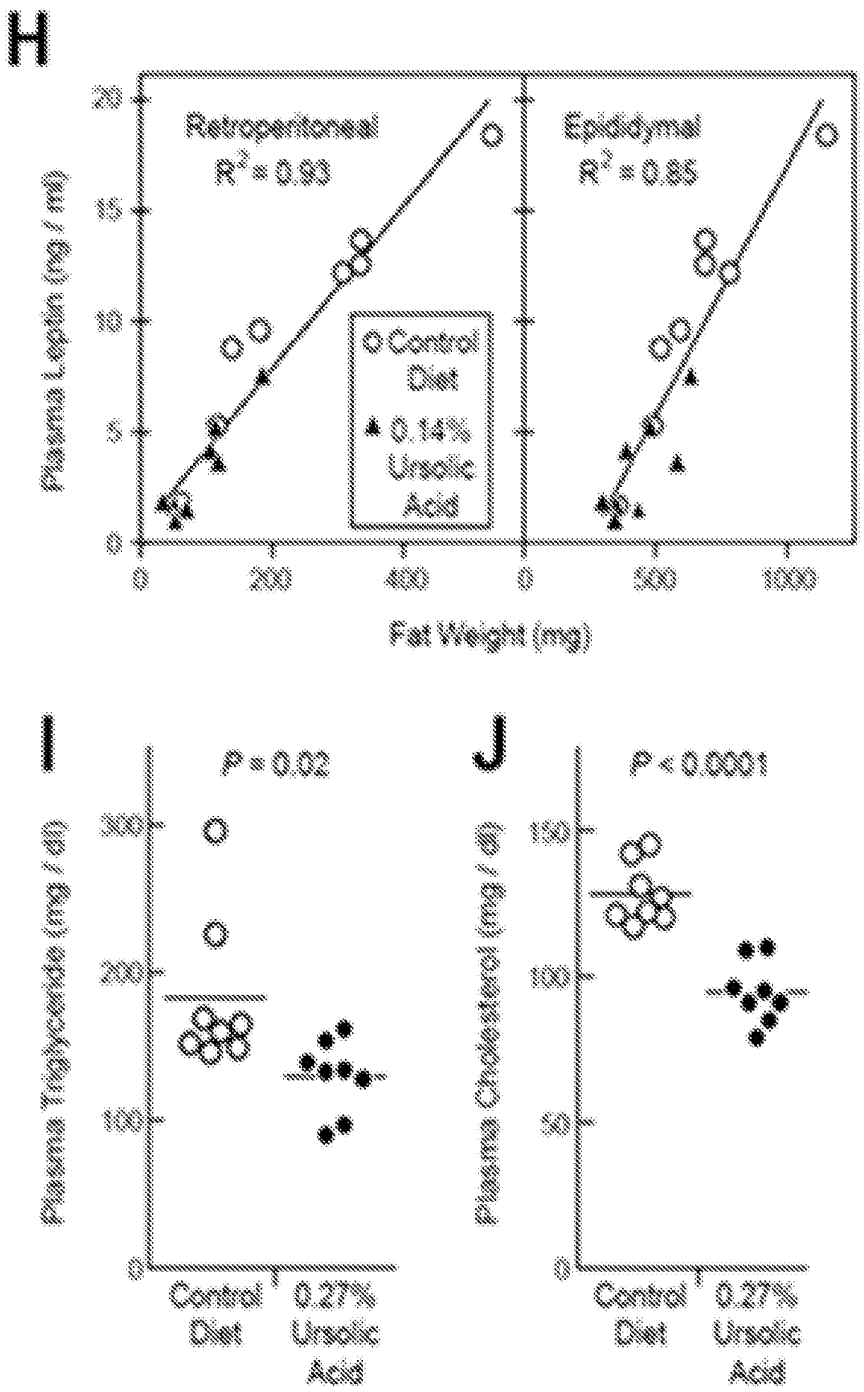


FIGURE 12H-12J

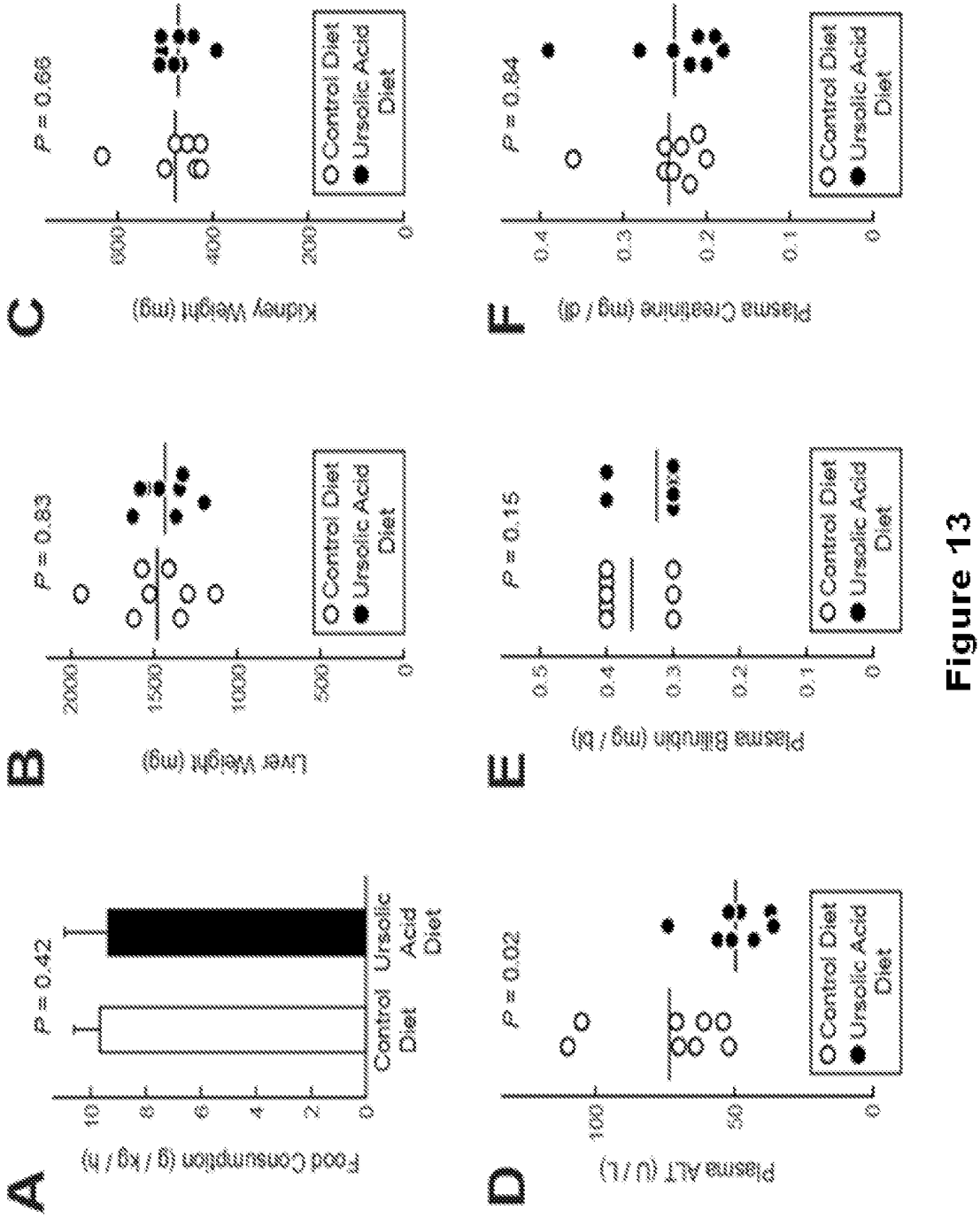

A
Food Consumption (g / kg / h)
P = 0.42
Control Diet
Ursolic Acid Diet
B
Liver Weight (mg)
P = 0.83
C
Kidney Weight (mg)
P = 0.68
D
Plasma ALT (U / L)
P = 0.02
E
Plasma Bilirubin (mg / dl)
P = 0.15
F
Plasma Creatinine (mg / dl)
P = 0.84
Control Diet
Ursolic Acid Diet

Figure 13

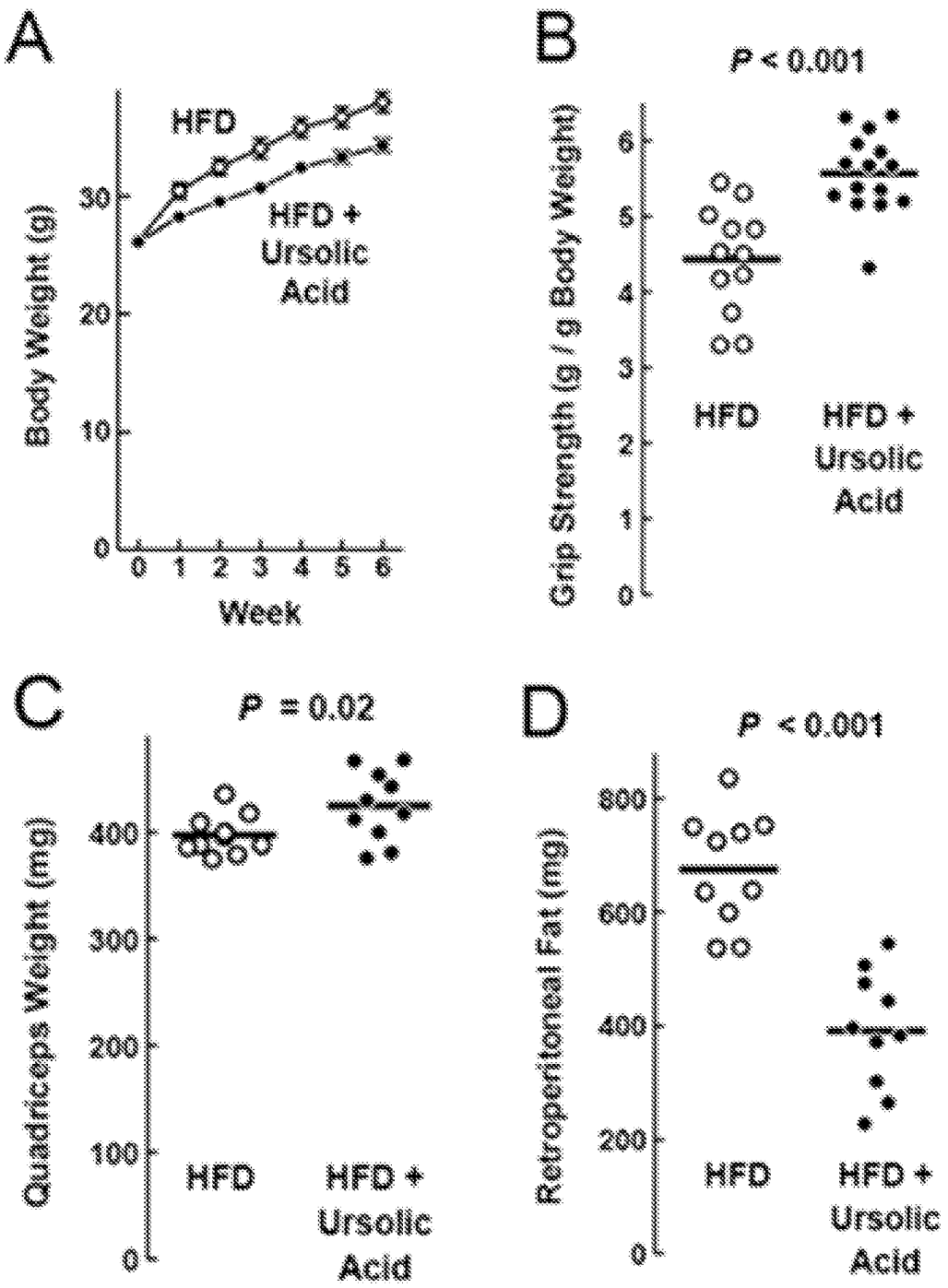


FIGURE 14A-14D

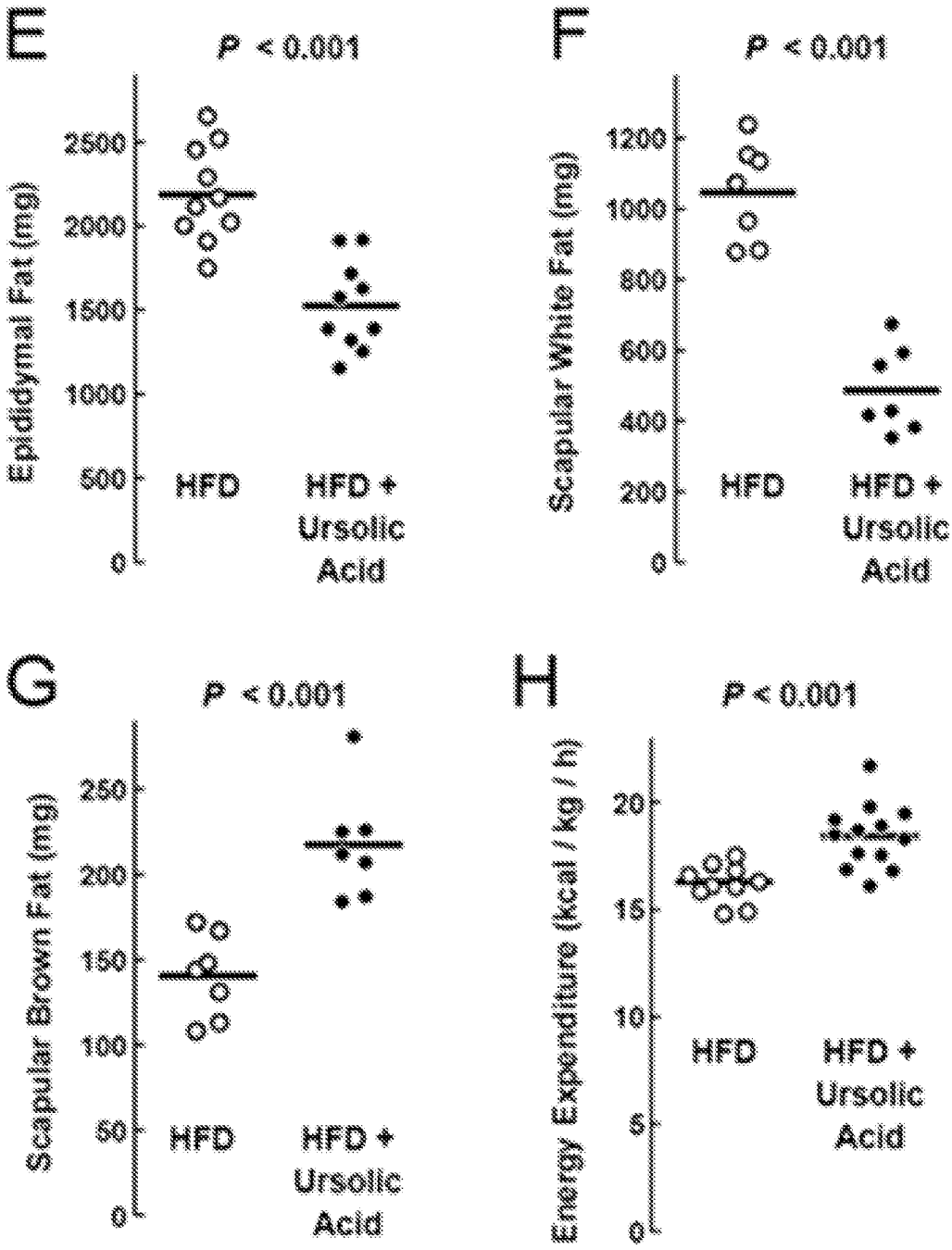


FIGURE 14E-14H

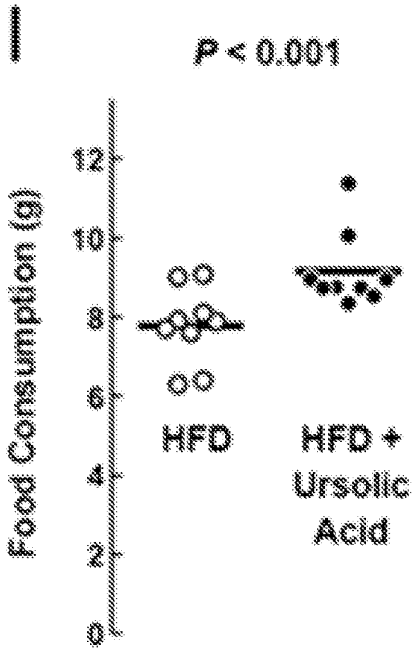


FIGURE 14I

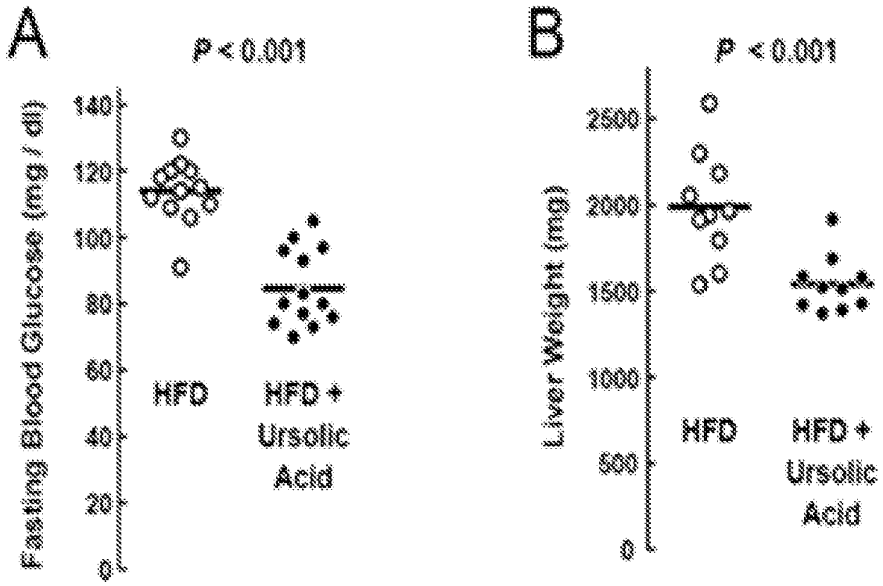


FIGURE 15A and 15B

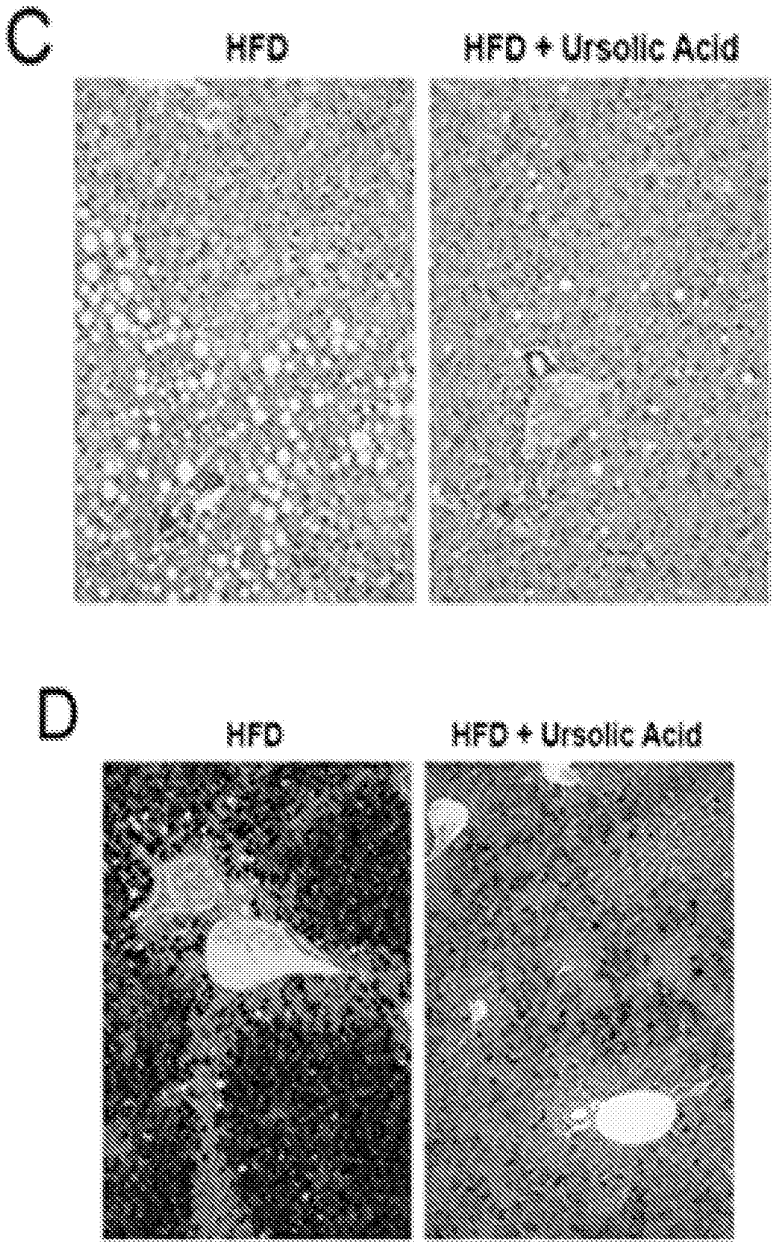


FIGURE 15C and 15D

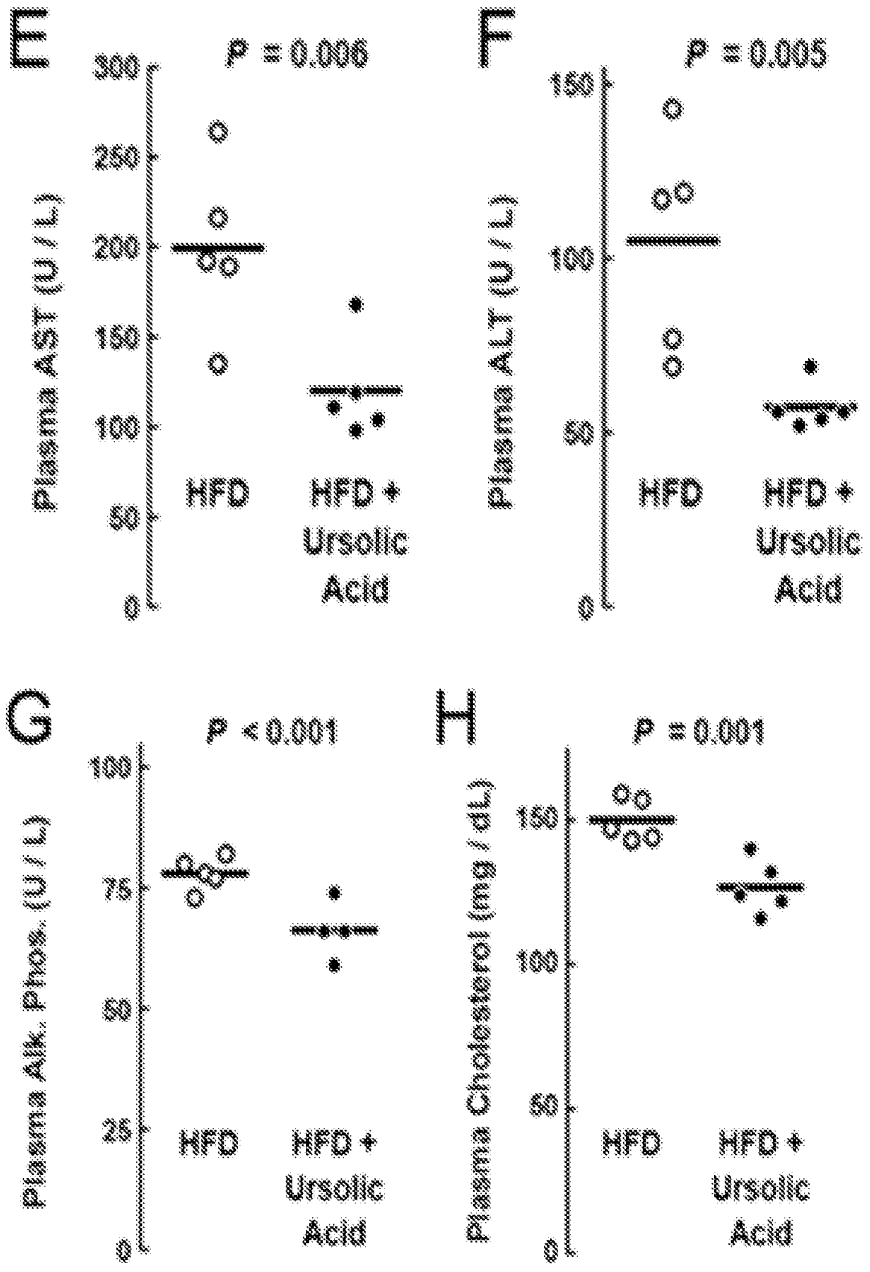


FIGURE 15E-15H

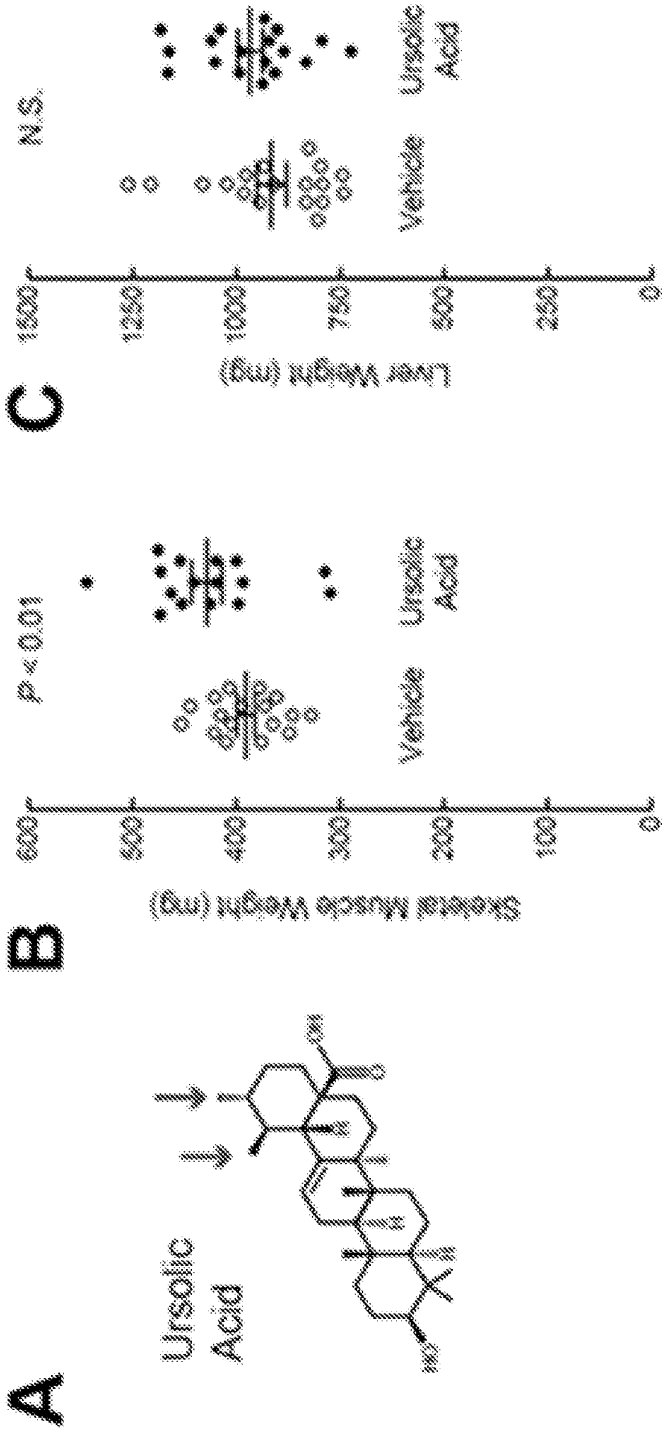


FIGURE 16A-16C

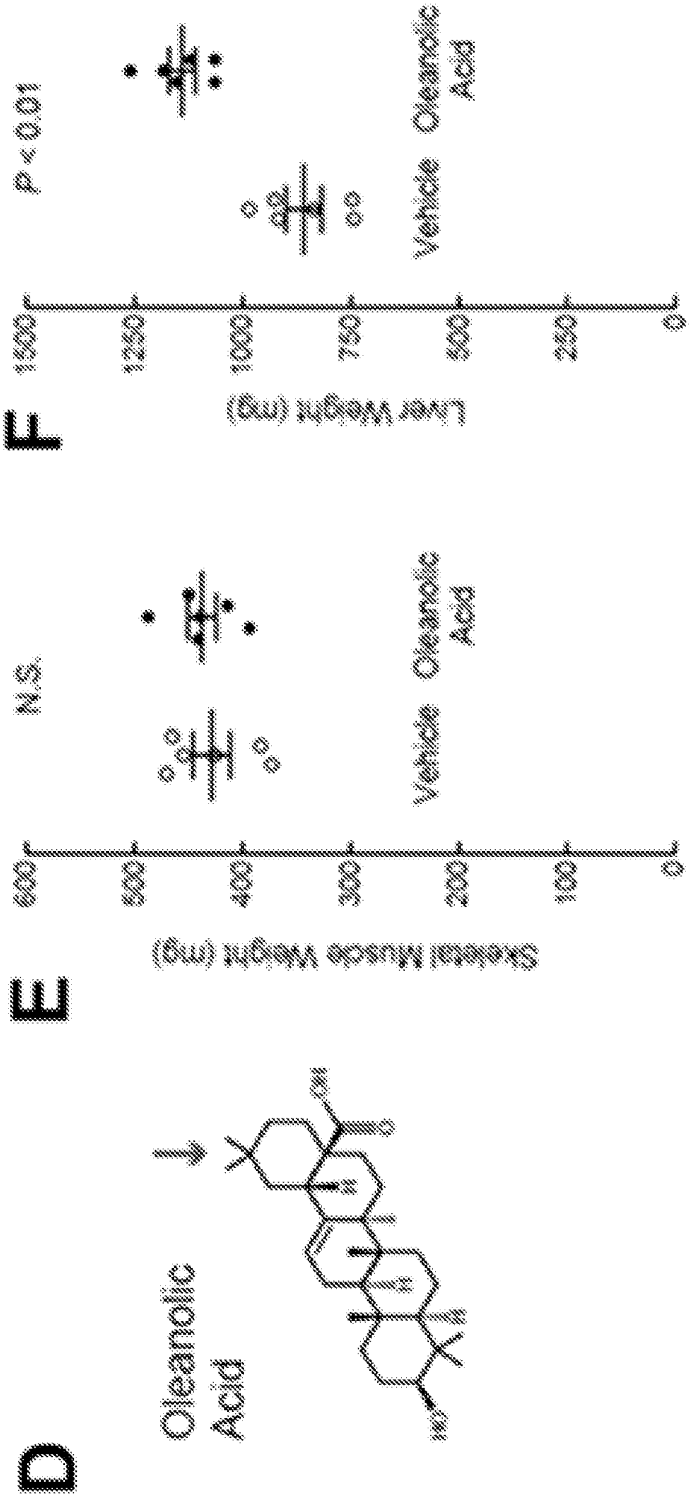


FIGURE 16D-16F

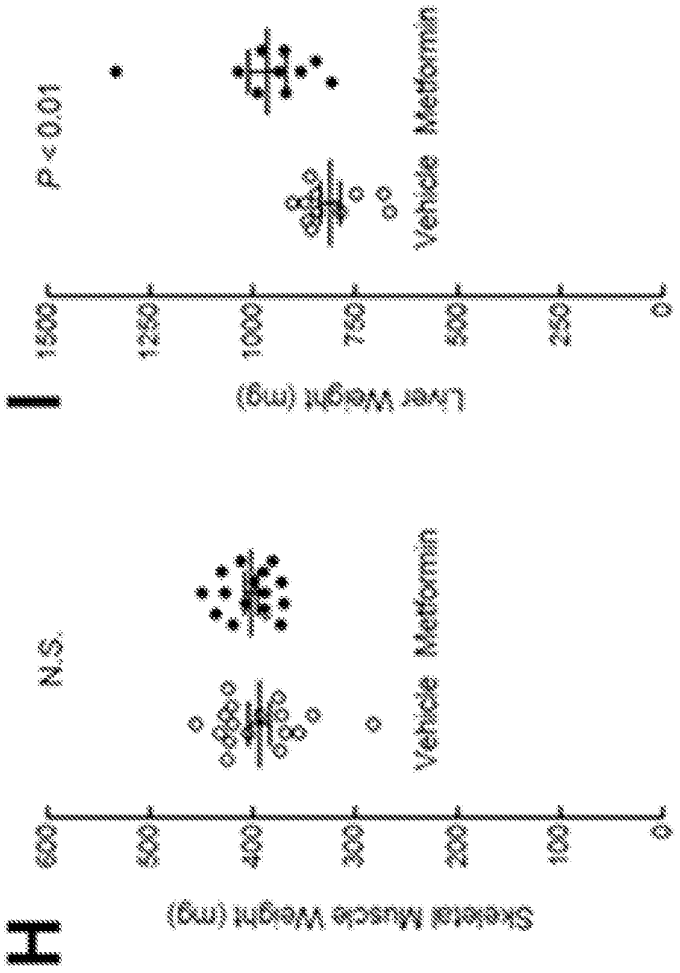


FIGURE 16G – 16I

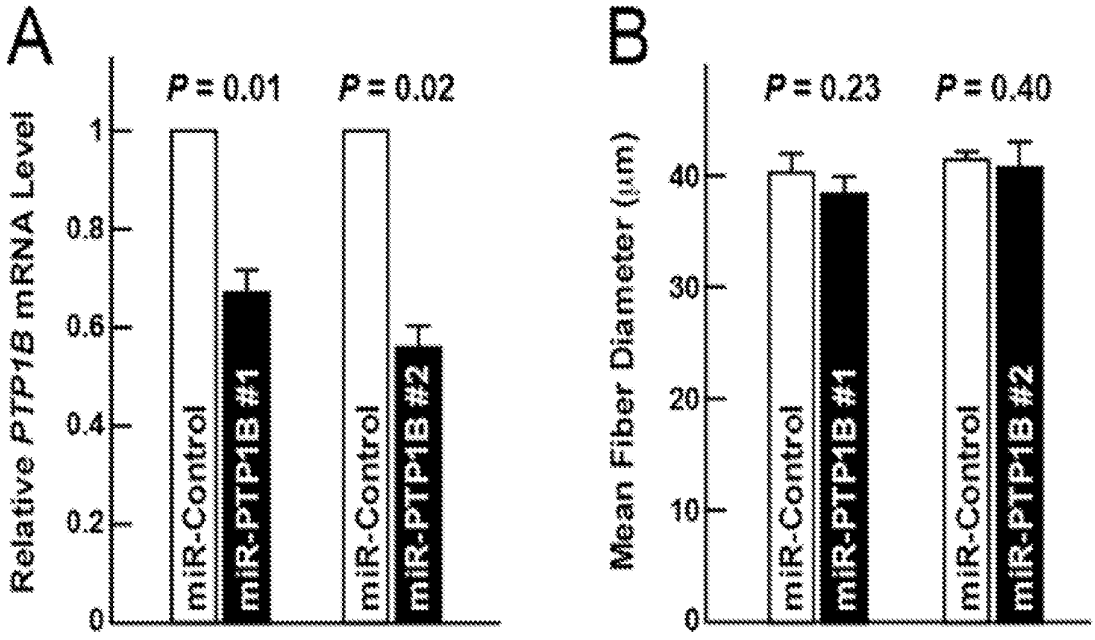


Figure 17

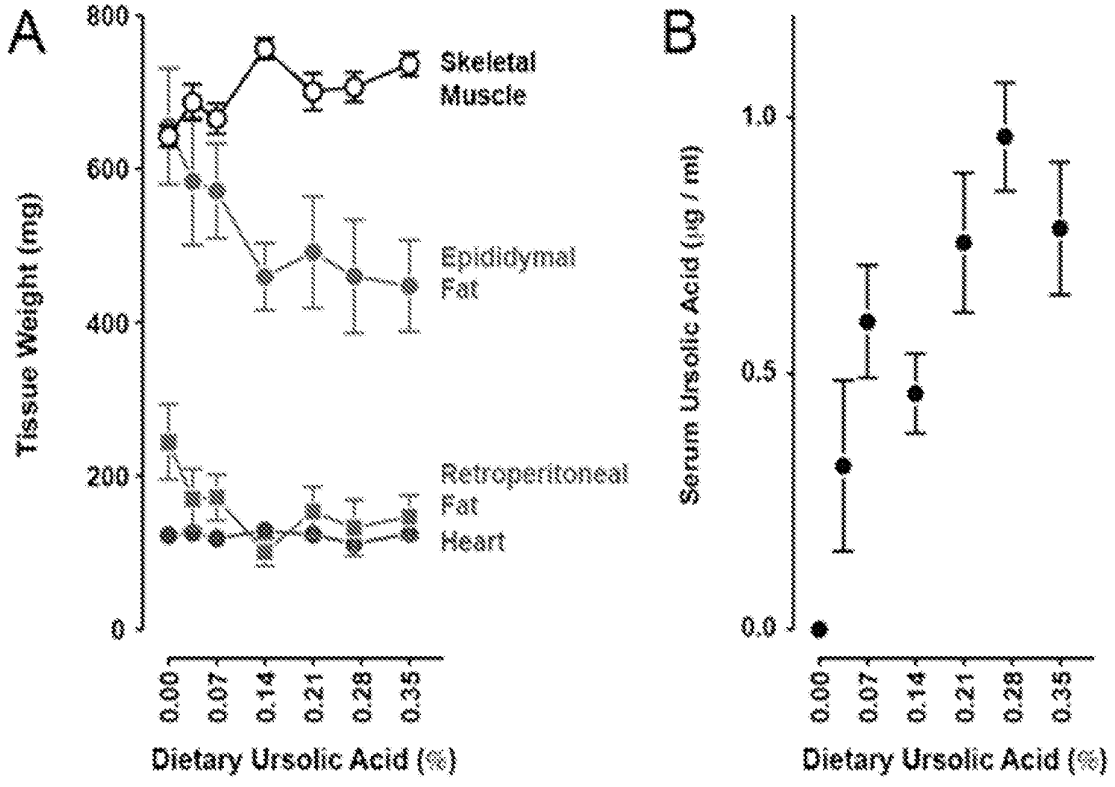
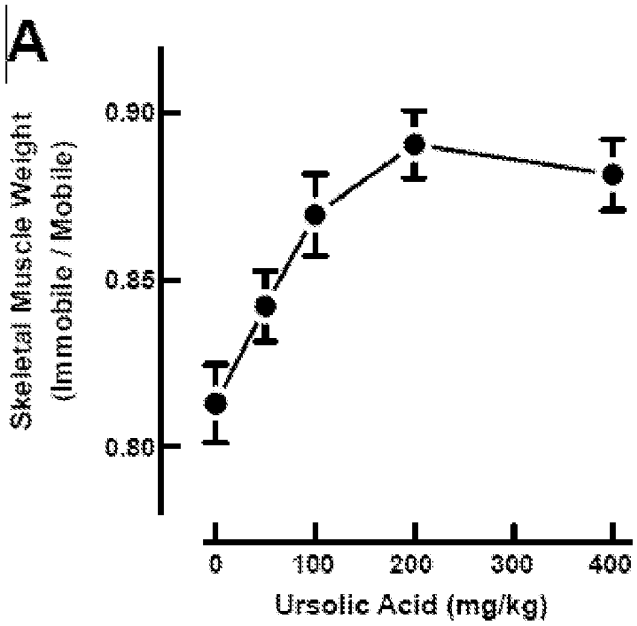


Figure 18


A
Skeletal Muscle Weight
(Immobile / Mobile)
0.90
0.85
0.80
0
100
200
300
400
Ursolic Acid (mg/kg)

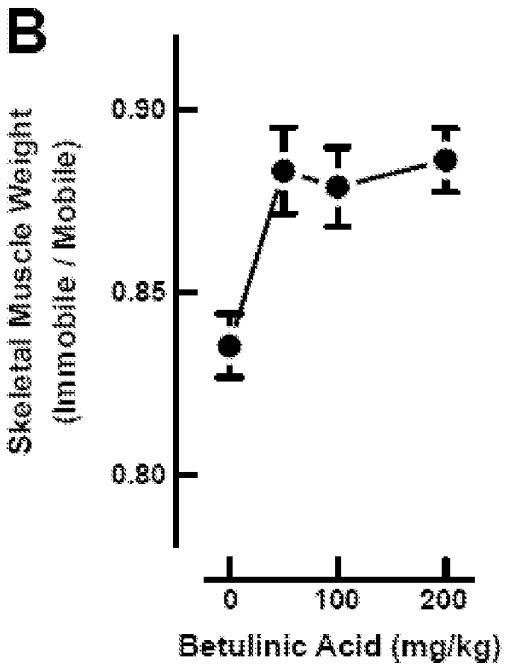

B
Skeletal Muscle Weight
(Immobile / Mobile)
0.90
0.85
0.80
0
100
200
Betulinic Acid (mg/kg)

FIGURE 19

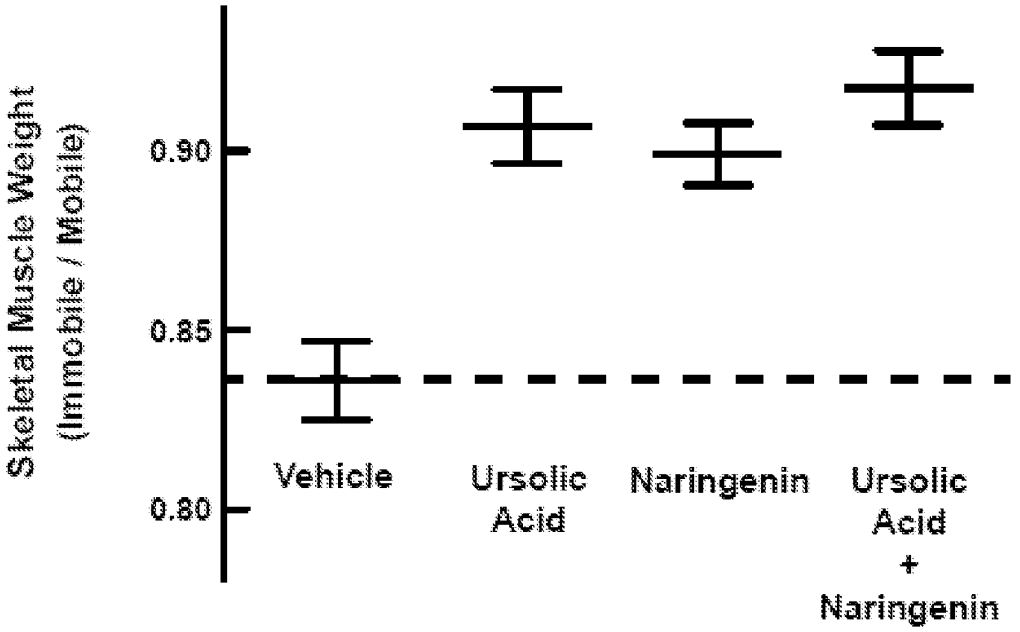


FIGURE 20

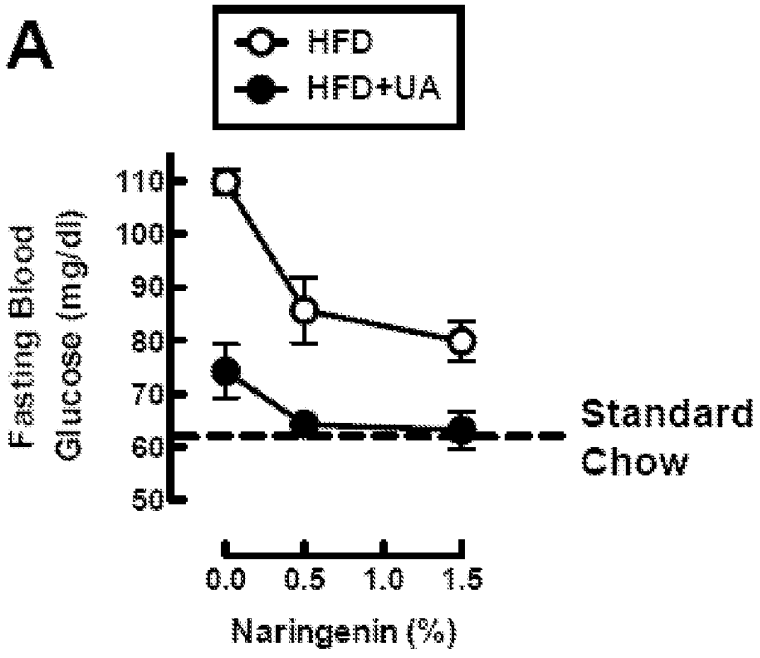
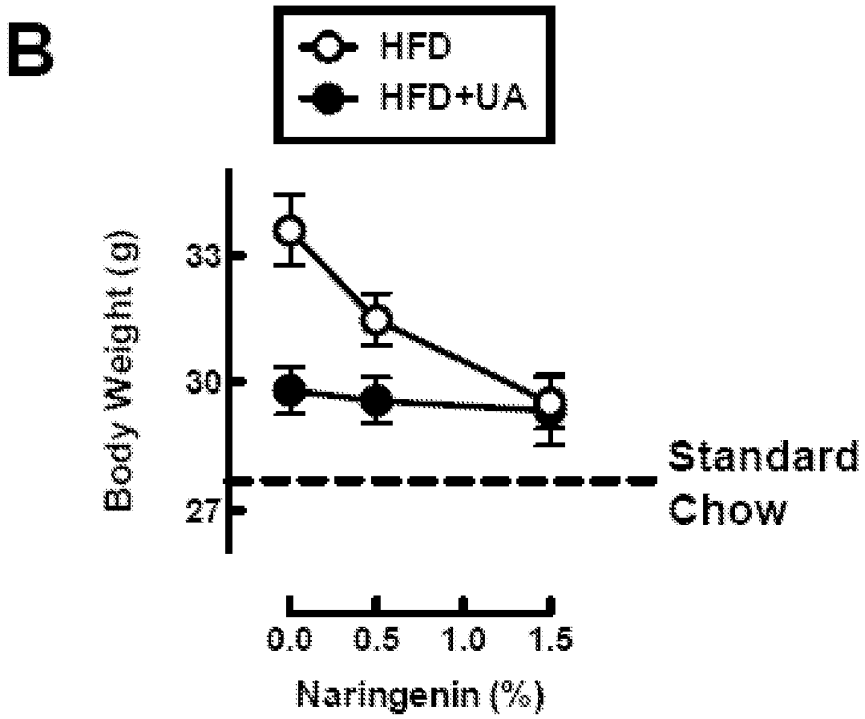


FIGURE 21A



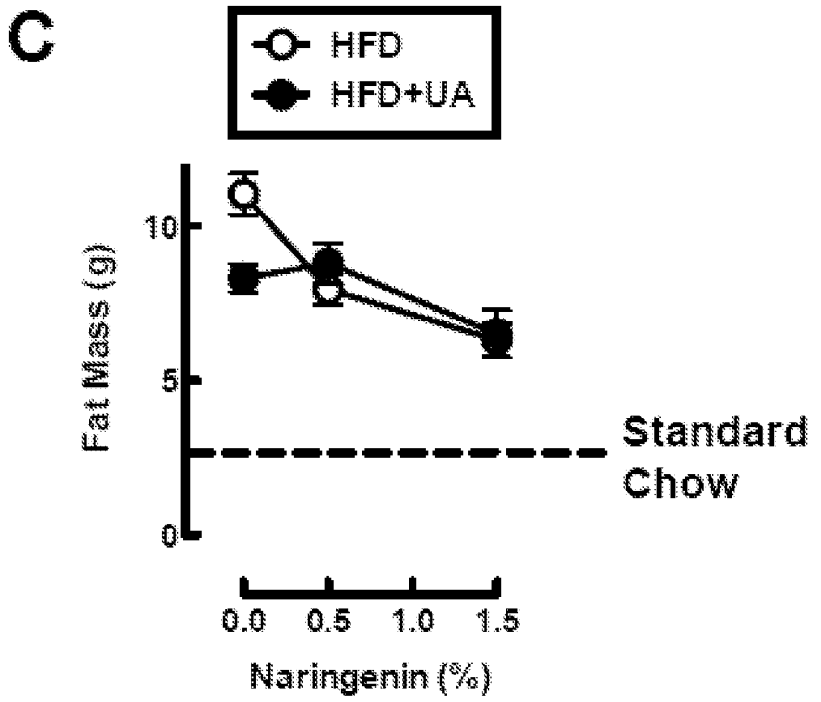


FIGURE 21B and 21C

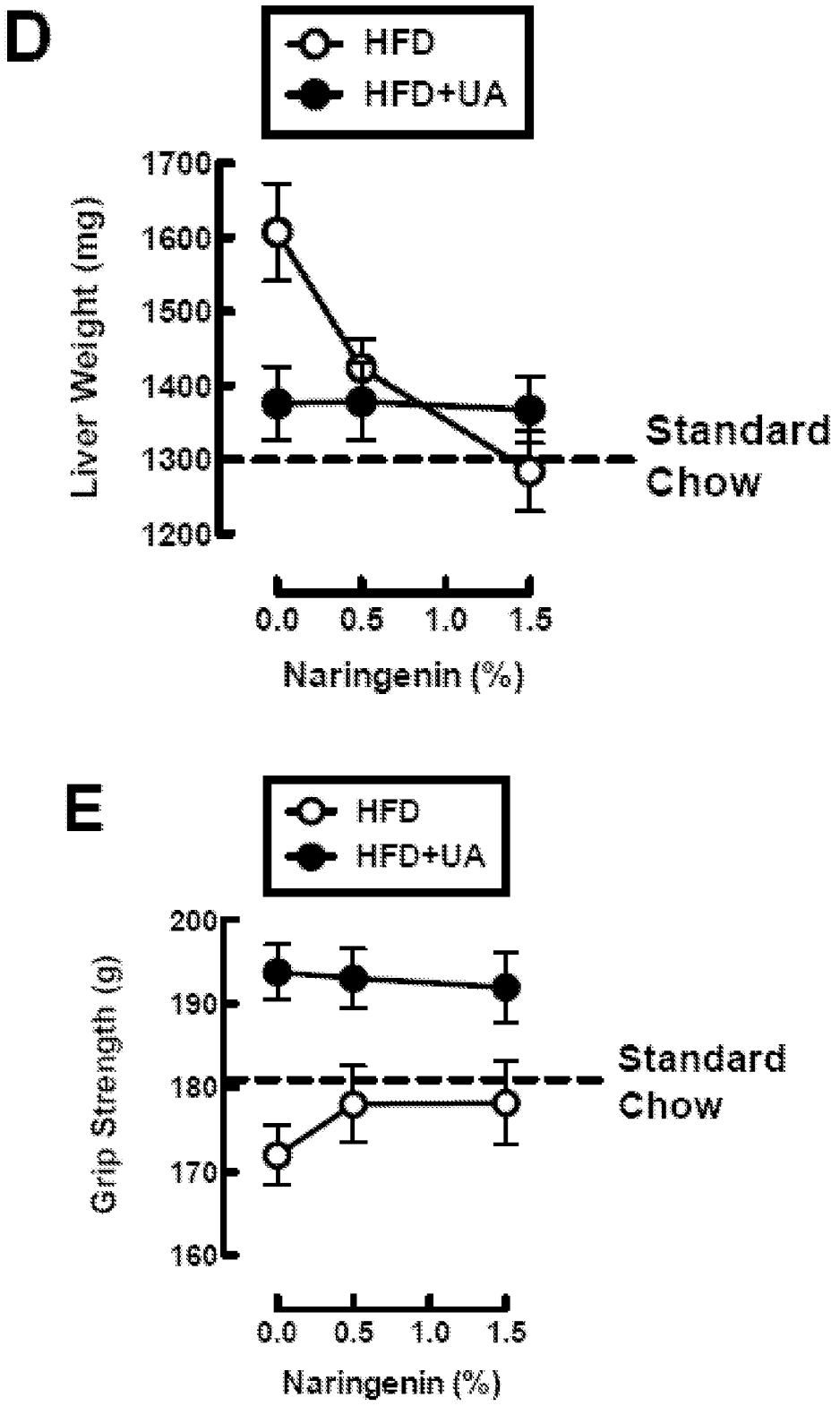


FIGURE 21D and 21E

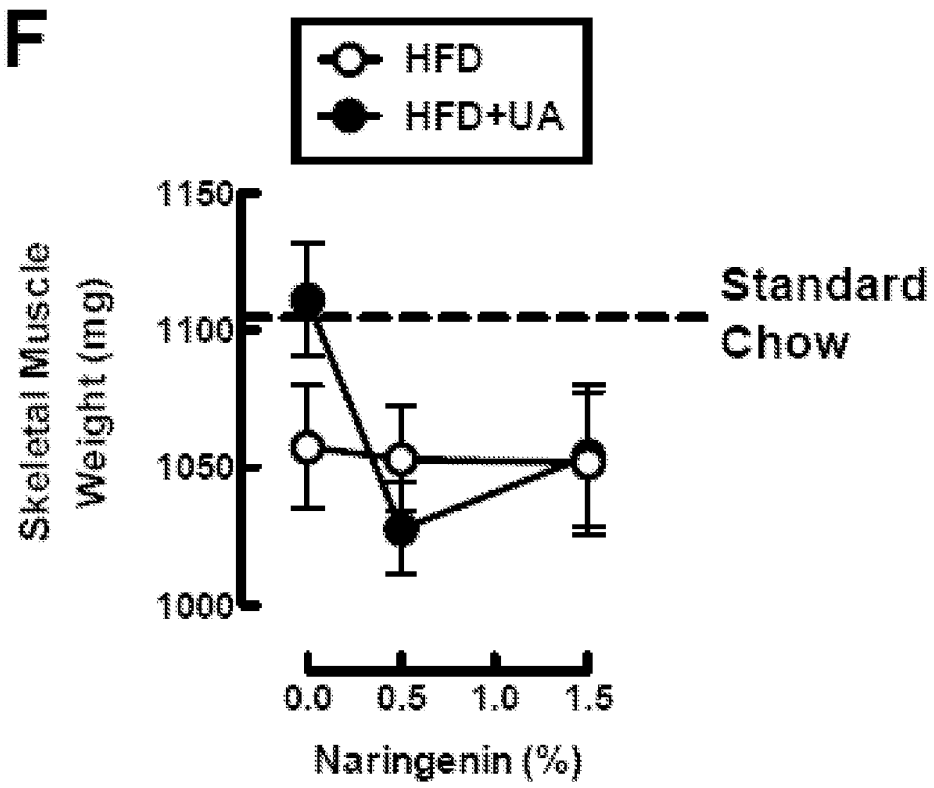


FIGURE 21F

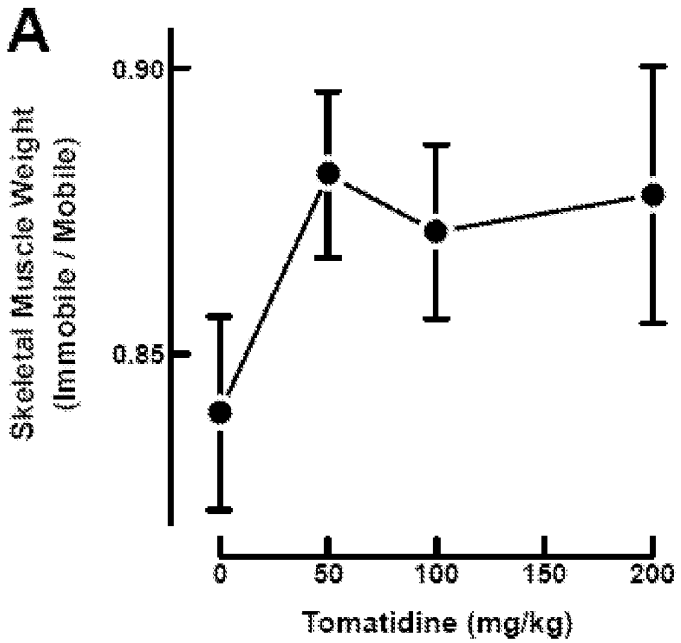


FIGURE 22A

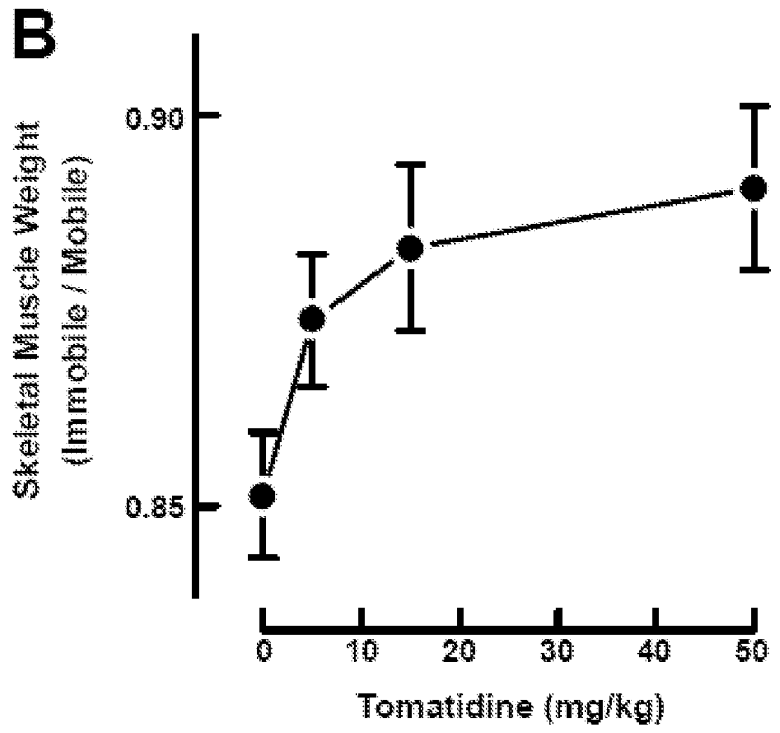


FIGURE 22B

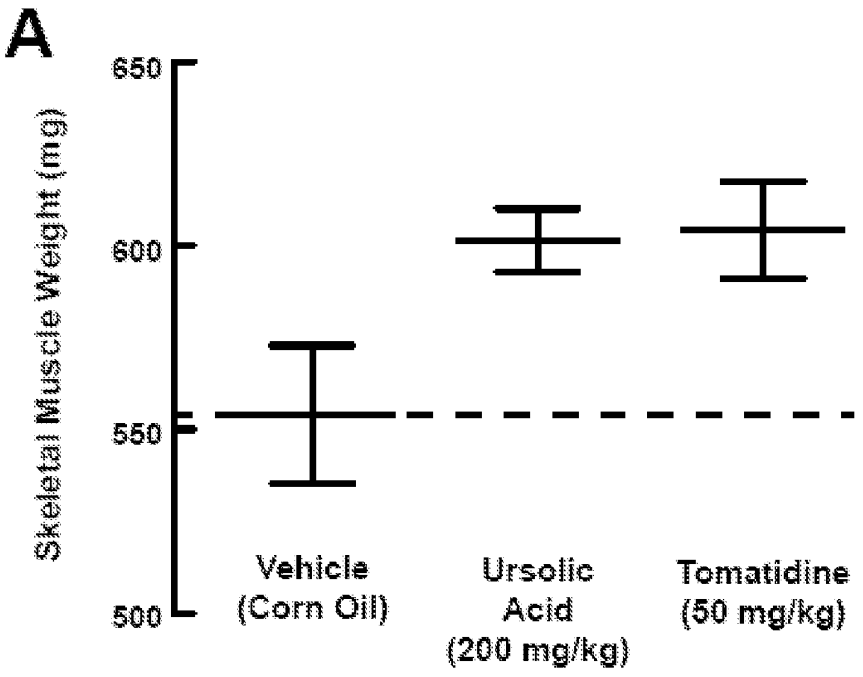


FIGURE 23A

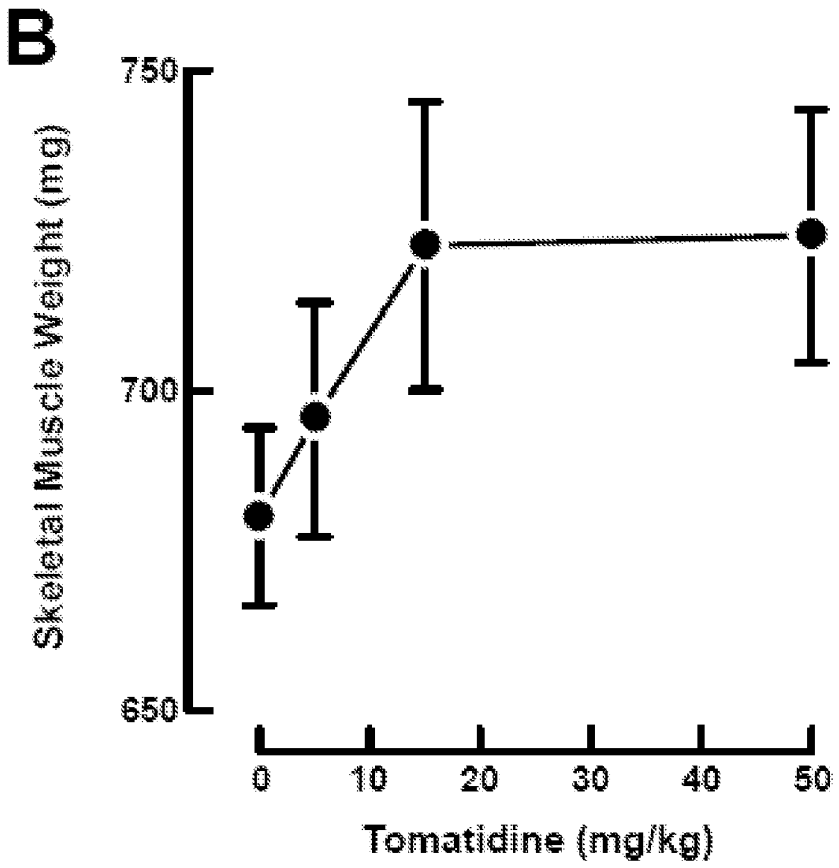

B
750
700
650
Skeletal Muscle Weight (mg)
0
10
20
30
40
50
Tomatidine (mg/kg)

FIGURE 23B

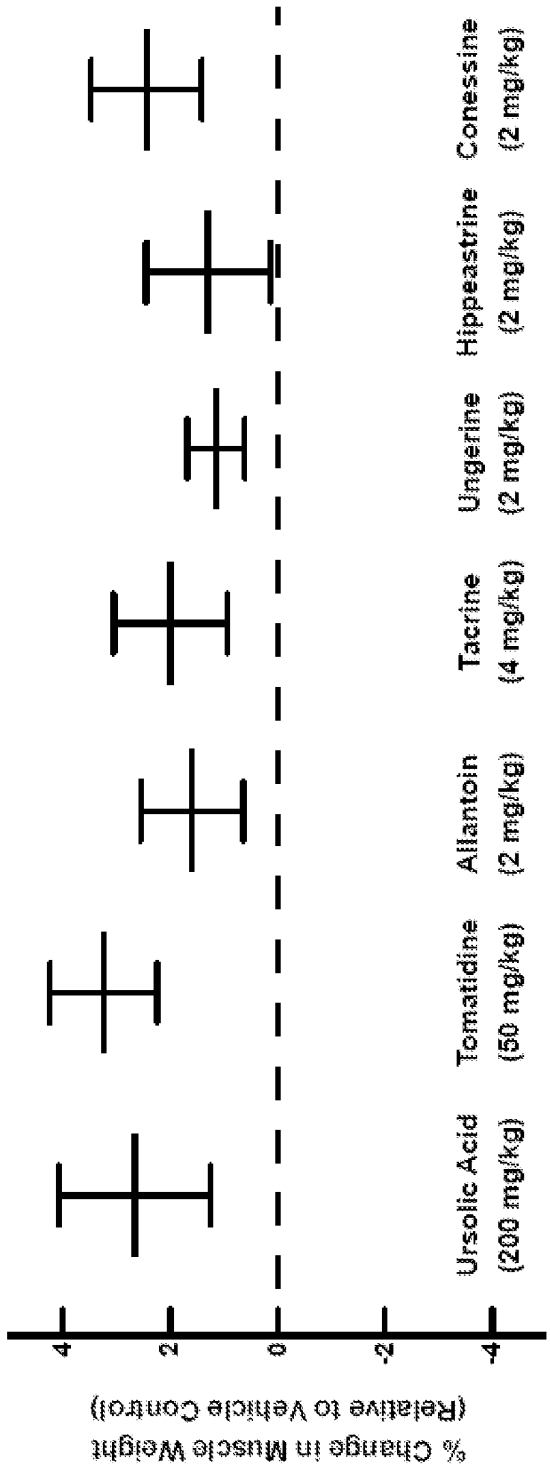

% Change in Muscle Weight
(Relative to Vehicle Control)
4
2
0
-2
-4
Ursolic Acid (200 mg/kg)
Tomatidine (50 mg/kg)
Allantoin (2 mg/kg)
Tacrine (4 mg/kg)
Ungerine (2 mg/kg)
Hippeastrine (2 mg/kg)
Conessine (2 mg/kg)

FIGURE 24

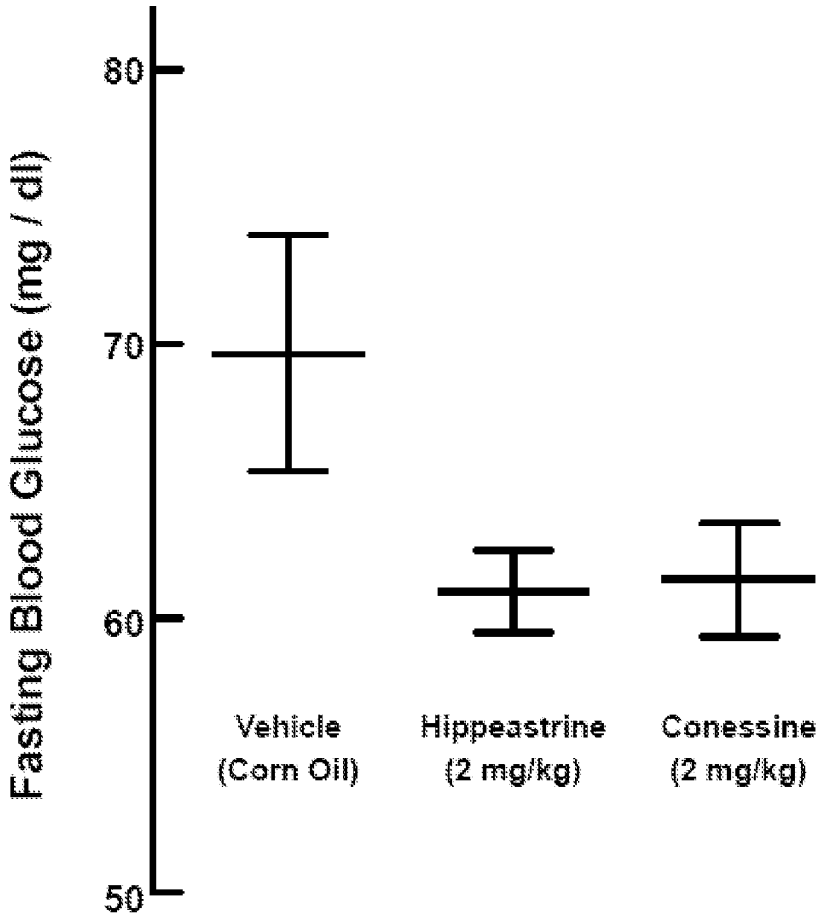

Fasting Blood Glucose (mg / dl)
80
70
60
50
Vehicle
(Corn Oil)
Hippeastrine
(2 mg/kg)
Conessine
(2 mg/kg)

FIGURE 25