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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
9 July 2009 (09.07.2009)

PCT

(10) International Publication Number
WO 2009/086526 A2

(71) Applicant (for all designated States except US): THE SALK INSTITUTE FOR BIOLOGICAL STUDIES [US/US]; Office of Technology Management, 10010 N. Torrey Pines Road, La Jolla, CA 92037-1099 (US).
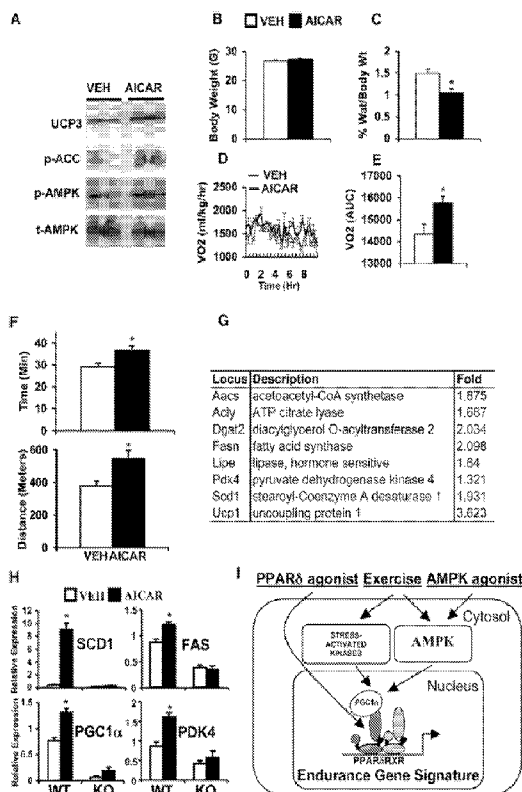
(72) Inventors; and
(75) Inventors/Applicants (for US only): EVANS, Ronald, M. [US/US]; 1471 Cottontail Lane, La Jolla, CA 92037 (US). NARKAR, Vihang, A. [IN/US]; 3762 Mykonos Lane #89, San Diego, CA 92130 (US). SHAW, Reuben, J. [US/US]; 12636 Ragweed Street, San Diego, CA 92129 (US). DOWNES, Michael [AU/US]; 2730 Havasupai Avenue, San Diego, CA 92117 (US). YU, Ruth, T. [US/US]; 1250 Cave Street, Unit 4, La Jolla, CA 92037 (US).

(74) Agent: BAKER, Joseph, R., Jr.; Gavrilovich, Dodd & Lindsey LLP, 4660 La Jolla Village Drive, Suite 750, San Diego, CA 92122 (US).

(54) Title: METHODS FOR ENHANCING MUSCLE PERFORMANCE AND TONE

FIGS. 11A-I

(57) Abstract: This disclosure concerns the use of agonists of AMP-activated protein kinase (AMPK) for improving exercise and modifying energy metabolism in a subject. The disclosure also relates to a combination of AMPK and peroxisome proliferator-activated receptor (PPAR) δ agonists for improving exercise performance in a subject, methods for identifying substance-enhanced exercise performance in a subject, and methods for identifying compounds that affect the interaction of PPARδ with exercise-induced kinases.

WO 2009/086526 A2

RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),

European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report*

METHODS FOR ENHANCING MUSCLE PERFORMANCE AND TONE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001]   This application claims the benefit of US Provisional Application No. 61/080,841, filed July 15, 2008, herein incorporated by reference. This application is a continuation-in-part of and claims priority to U.S. Application No. 11/966,851, and International Application No. PCT/US2007/089124, both filed December 28, 2008, the disclosures of which is incorporated herein by reference.

ACKNOWLEDGEMENT OF GOVERNMENT SUPPORT

FIELD OF THE INVENTION

[0003]   This disclosure concerns the use of agonists of AMP-activated protein kinase (AMPK) for improving exercise and modifying energy metabolism in a subject. The disclosure also provides methods of treating muscle wasting diseases and disorders and promoting muscle tone in sedentary subjects. The disclosure also relates to a combination of AMPK and peroxisome proliferator-activated receptor (PPAR)δ agonists for improving exercise performance in a subject, and methods for identifying compounds that modulate gene expression profiles associated with muscle tone, endurance or performance.

BACKGROUND

[0004]   Skeletal muscle is an adaptive tissue composed of multiple myofibers that differ in their metabolic and contractile properties including oxidative slow-twitch (type I), mixed oxidative/glycolytic fast- twitch (type Ha) and glycolytic fast-twitch (type Hb) myofibers (Fluck *et al.*, Rev. Physiol. Biochem. Pharmacol., 146:159-216, 2003; Pette and Staron, Microsc. Res. Tech., 50:500-509, 2000). Type I muscle fibers preferentially express enzymes that oxidize fatty acids, contain slow isoforms of contractile proteins and are more resistant to fatigue than are

glycolytic muscle fibers (Fluck *et al.*, Rev. Physiol. Biochem.
Pharmacol., 146:159-216, 2003; Pette and Staron, Microsc. Res.
Tech., 50:500-509, 2000). Type II fibers preferentially metabolize
glucose and express the fast isoforms of contractile proteins
(Fluck *et al.*, Rev. Physiol. Biochem. Pharmacol, 146:159-216, 2003;
Pette and Staron, Microsc. Res. Tech., 50:500-509, 2000).

[0005]   Endurance exercise training triggers a complex
remodeling program in skeletal muscle that progressively enhances
performance in athletes such as marathon runners, mountain climbers
and cyclists. This involves changes in metabolic programs and
structural proteins within the myofibers that alter the energy
substrate utilization and contractile properties that act to reduce
muscle fatigue (Fluck *et al.*, Rev. Physiol. Biochem. Pharmacol,
146:159-216, 2003; Pette and Staron, Microsc. Res. Tech., 50:500-
509, 2000). Training based adaptations in the muscle are linked to
increases in the expression of genes involved in the slow-twitch
contractile apparatus, mitochondrial respiration and fatty acid
oxidation (Holloszy and Coyle, J. Appl. Physiol. , 56:831-838,
1984; Booth and Thomason, Physiol. Rev. , 71:541-585, 1991; Schmitt
et al., Physiol. Genomics, 15:148-157, 2003; Yoshioka *et al.*, FASEB
J., 17:1812-1819, 2003; Mahoney *et al.*, FASEB J., 19:1498-1500,
2005; Mahoney and Tarnopolsky, Phys. Med. Rehabil Clin. N. Am.,
16:859-873, 2005; Siu *et al.*, J. Appl Physiol, 97:277-285, 2004;
Gamier *et al.*, FASEB J., 19:43-52, 2005; Short *et al.*, J. Appl
Physiol, 99:95-102, 2005; Timmons *et al.*, FASEB J., 19:750-760,
2005). Such exercise training-related adaptations can improve
performance and protect against obesity and related metabolic
disorders (Wang *et al.*, P. Biol, 2:e294, 2004; Koves *et al.*, J.
Biol. Chem., 280:33588-33598, 2005). Moreover, skeletal muscles
rich in oxidative slow-twitch fibers are resistant to muscle
wasting (Minnaard *et al.*, Muscle Nerve. 31: 339-48, 2005).

SUMMARY

[0006]   The disclosure also provide a method of treating a
muscle wasting disease, muscle atrophy or aging comprising
administering to a subject a composition comprising an AMPK
agonist, wherein the muscle tone, mass or endurance is promoted.

In one embodiment, the method comprises treating a subject that may
have an immobilized limb or which may be immobilized due to other
medical treatments to promote or maintain muscle tone in the
subject.

[0007]   The disclosure demonstrates that unexpected finding that
orally active AMPK agonists are sufficient as a single agent to
improve exercise endurance by nearly 45% in non-exercised subjects.

[0008]   The disclosure provides a method for enhancing an
exercise effect in a subject, comprising administering to a subject
an AMP kinase (AMPK) agonist wherein an exercise effect is
enhanced. The AMPK agonist can be any AMPK agonist, derivatives,
salts or esters thereof. In one embodiment, the AMPK agonist is
AICAR. The method can further comprise administering to the
subject an effective amount of a PPARδ agonist (*e.g.*, GW1516),
thereby further enhancing the exercise effect in the subject. The
subject can be a racing animal including a human, equine, or
canine.

[0009]   The disclosure further comprises a method for
identifying the use of performance-enhancing substances in an
exercise-trained subject comprising determining in a biological
sample taken from an exercise-trained subject the presence of an
AMPK agonist and/or expression of one or more molecules listed in
Tables 2, 4 or 6.

[0010]   The disclosure also provides a composition comprising an
AMPK agonist and a PPARδ agonist in a pharmaceutically acceptable
carrier. The composition may be an energy supplement, beverage,
food product or pharmaceutical. The AMPK agonist or PPAR agonist
can be a salt, ester, prodrug, precursor or derivative thereof.

[0011]   This disclosure illustrates that, despite expectations
to the contrary, pharmacological activation of AMPK or endogenous
PPARδ in adults, promote remodeling of skeletal muscle to an
oxidative phenotype or increase running endurance in such subjects.
In addition, agonist-induced activation of endogenous PPARδ in
combination with exercise led to a unique "gene expression
signature" in skeletal muscle, which was distinct from the gene
expression profile obtained by either exercise or drug intake

alone, and revealed direct interactions between PPARδ and exercise-
induced kinases (such as AMPK α1 and/or AMPK 2).

[0012]   These and other discoveries described herein serve as
the basis for disclosed methods. For example, it can now be
appreciated that PPARδ agonists (e.g., GW1516) used in combination
with exercise can enhance exercise-induced effects, such as to
improve exercise endurance (e.g., running endurance) even more than
may be achieved by exercise alone. In another example, the
expression of one or more genes and/or proteins that are uniquely
regulated by the combination of exercise and PPARδ agonist
administration can be used to identify subjects using drugs to
enhance exercise performance. In still other examples, the newly
identified protein complexes, including PPARδ and exercise-induced
kinases (such as AMPK α1 and/or AMPK cc2), can be used to identify
agents that have potential to affect PPARδ-regulated gene networks
and the corresponding downstream biochemical and/or physiological
effects.

[0013]   The foregoing and other features will become more
apparent from the following detailed description of several
embodiments, which proceeds with reference to the accompanying
figures.

BRIEF DESCRIPTION OF THE FIGURES

[0014]   FIG. 1A is a series of bar graphs showing the effects of
orally administered PPARδ agonist (GW1516) on mRNA expression
levels of three biomarkers of fatty acid oxidation, uncoupling
protein 3 (UCP3), carnitine palmitoyltransferase I (mCPT I), and
pyruvate dehydrogenase kinase, isoenzyme 4 (PDK4), in quadriceps
muscle isolated from sedentary vehicle-treated (V), sedentary
GW1516-treated (GW), sedentary VP16-PPAR5 transgenic (TG), and
sedentary wild-type littermates of VP16-PPAR5 transgenic mice (WT).
Data are presented as mean ± SEM of N=4-9 mice each analyzed in
triplicate. * Represents a statistically significant difference
between V and GW1516 groups (p<0.05, unpaired student's t-test), or
TG and WT groups (p<0.05, unpaired student's t-test).

[0015]   FIG. 1B-D are a series of bar graphs showing the
regulation of oxidative genes UCP3, mCPT I, and PDK4 by GW1516 (GW)

in wild-type (WT) and PPARδ null (KO) primary muscle cells. *
represents statistical significance between V and indicated groups
(p<0.05, One Way ANOVA; post hoc: Dunnett's Multiple Comparison
Test).

[0016]   FIG. 1E is a series of bar graphs showing running
endurance of vehicle-treated sedentary (V; open bars) and GW1516-
treated sedentary (GW; black bars) mice before (Week 0) and after
(Week 5) treatment. Running endurance is quantified by the amount
of time for which (left panel) or the distance (right panel)
animals in each group ran on the treadmill. Data is represented as
mean ± SD values from N=6 mice.

[0017]   FIGS. 2A-C show the effects of administration of a PPARδ
agonist, GW1516, on the gastrocnemius muscle of sedentary (V or GW)
or trained (Tr or Tr+GW) mice. FIG. 2A shows digital images of
representative meta-chromatically stained frozen cross-sections of
gastrocnemius muscle from vehicle-treated, sedentary (V), GW1516-
treated, sedentary (GW), vehicle-treated, exercised (Tr) and
GW1516-treated, exercised (Tr+GW) mice. Type I (slow oxidative)
fibers are darkly stained. FIG. 2B is a bar graph showing the
percentage of type I fibers (as a percentage of the total fibers)
in V, GW, Tr, and Tr+GW gastrocnemius (N=3). FIG. 2C is a bar graph
showing the fold change in mitochondrial DNA to nuclear DNA ratio
in V (left bar), GW (left center bar), Tr (right center bar), and
Tr+GW (right bar) groups of mice (N=9). Data in (B) and (C) are
presented as mean ± SEM. In each bar graph, * represents a
statistical difference between V and the group(s) indicated by
asterisk (p<0.05, One-Way ANOVA; post hoc: Dunnett's Multiple
Comparison Test).

[0018]   FIGS. 3A-D are a series of bar graphs showing gene
expression in quadriceps muscle isolated from V, GW, Tr and Tr+GW
groups. FIG. 3A shows the relative gene expression levels of
biomarkers for fatty acid oxidation (UCP3, mCPT I, PDK4; from left
to right). (B) shows the relative gene expression levels of
biomarkers for fatty acid storage (SCD1, FAS, SREBPIc). (C) shows
the relative gene expression levels of biomarkers for fatty acid
uptake (FAT/CD36, LPL). Data is presented as mean ± SEM of N=9

mice, each analyzed in triplicate. * represents statistically significant difference between V and the group(s) indicated by asterisk (p<0.05, One Way ANOVA; post hoc: Dunnett's Multiple Comparison Test). (D) shows images of Western blots illustrating protein expression levels of oxidative biomarkers (myoglobin, UCP3, CYCS, SCD1) and loading control (tubulin) in protein lysates prepared from quadriceps (N=3).

[0019]   FIG. 4 shows a graph of muscle triglyceride levels in gastrocnemius muscle of V, GW, Tr and Tr+GW mice. Data is presented as mean ± SEM of N=9 mice, each analyzed in triplicate. * represents statistical significance between V and group(s) indicated by asterisk (*p<0.05, One Way ANOVA; post hoc:Dunnett's Multiple Comparison Test).

[0020]   FIGS. 5A-C are bar graphs showing the effects of GW1516 treatment on running endurance in exercise-trained mice. Bar graphs of the (A) time and (B) distance that vehicle- (V; open bars) and GW1516-treated (GW; black bars) mice ran on a treadmill before (Week 0) and after (Week 5) exercise training. Data is represented as mean ± SD of N=6 mice. *** represents statistically significant difference between V and GW groups (p<0.001; One Way ANOVA; post hoc:Tukey's Multiple Comparison Test). (C) is a bar graph showing epididymal white adipose to body weight ratio in V, GW, Tr and Tr+GW mice. Data is presented as mean ± SEM of N=9 mice, each analyzed in triplicate. * represents statistical significance between V and group(s) indicated by asterisk (*p<0.05, One Way ANOVA; post hoc:Dunnett's Multiple Comparison Test).

[0021]   FIG. 5D shows digital images of H&E-stained cross-sections of epididymal white adipose from V, GW, Tr and Tr+GW mice. Similar results were obtained from N=3 mice. * represents statistical significance between V and group(s) indicated by asterisk (*p<0.05, One Way ANOVA; post hoc:Dunnett's Multiple Comparison Test).

[0022]   FIG. 6 shows a Venn diagram comparing GW, Tr and Tr+GW target genes identified in microarray analysis of quadriceps. Also shown are a classification of target genes in Tr+GW mice and relative expression of 48 unique TR+GW target genes in GW, TR,

TR+GW, and VP16-PPARdelta muscles. Data is an average of N=3
samples in each group. The selection criteria used a p<0.05 on
Bonferroni's multiple comparison test and a fold change greater
than 1.5.

[0023]   FIG. 7A is a series of Western blot images showing AMPK
activation by exercise. The levels of phospho-AMPK (phospho-AMPK)
and total- AMPK in quadriceps muscle of sedentary (Sed/C57B1) and
exercise-trained (Tr/C57B1) mice (N=5-7) are shown.

[0024]   FIG. 7B is a series of Western blot images showing AMPK
activation by VP16-PPARd over-expression. The levels of phospho-
AMPK (phospho-AMPK) and total- AMPK in quadriceps muscle of
sedentary wild-type or transgenic mice (Sed/WT or Sed/TG) are
shown.

[0025]   FIGS. 8A-C show the synergistic regulation of muscle
gene expression by PPARδ and AMPK. (A) Venn diagram comparing GW,
AI, and AI+GW target genes identified in microarray analysis of
quadriceps. Data is an average of N=3 samples in each group. The
selection criteria used a p<0.05 on Bonferroni's multiple
comparison test and fold change greater than 1.5. (B) Comparison of
Tr+GW and AI+GW dependent gene signatures identified in quadriceps.
Data is an average of N=3 samples in each group. The selection
criteria used is similar to one used in FIG. 8A. (C) Classification
of 52 targets that were common to Tr+GW and AI+GW gene signatures.

[0026]   FIGS. 9A-H show the expression of (A) UCP3, (B) mCPT I,
(C) PDK4, (D) SCD1, (E) ATP citrate lyase, (F) HSL, (G) mFABP, and
(H) LPL transcripts in quadriceps of mice treated with vehicle (V),
GW 1516 (GW), AICAR (AI) and the combination of the two drugs (GW+
AI) for 6 days. Data is presented as mean ± SEM of N=6 mice in each
group, analyzed in triplicate. * Indicates statistically
significant difference between V and indicated groups (p<0.05, One
Way ANOVA; post hoc: Dunnett's Multiple Comparison Test).

[0027]   FIGS. 10A-L demonstrate the AMPK-PPARδ interaction. (A-
D) show the expression of metabolic genes in wild type and PPARδ
null (KO) primary muscle cells treated with V, GW, AI and GW+AI
(bars from left to right) for 24 hours. In (E-F, J), AD293 cells
were transfected with PPARδ+RXRα+Tk-PPRE along with control vector,

AMPK α1, cc2 and/or PGC 1α as indicated above. (E) Induction of basal PPARδ transcriptional activity by AMPK α1 or cc2. (F) Dose-dependent induction of PPARδ transcriptional activity is enhanced by AMPKα1 (closed circle) or AMPK cc2 (closed square) compared to control (open triangle). In (G-I, K), AD293 cells were transfected and processed as indicated. (G-H) Representative blot showing co-immunoprecipitation of transfected (G) or endogenous (H) AMPK with Flag-PPARδ. (I) Metabolic p32 labeling of PPARδ in AD293 cells transfected as described. (J) Synergistic regulation of basal (V) and ligand (GW) dependent PPARδ transcriptional activity by AMPK cc2 subunit and PGC 1α. (K) Co- immunoprecipitation of PPARδ but not AMPK α2 subunit with Flag-PGC1α. (L) Model depicting exercise-PPARδ interaction in re -programming muscle genome.

[0028]   Figure 11A-I shows that AICAR increases running endurance. (A-F) C57Bl/6J mice were treated with vehicle (open bars or thin lines) or AICAR (500 mg/kg/day, 4 weeks) (closed bars or thick lines). (A) Representative immunoblots showing levels of UCP3, phospho-acetyl CoA carboxylase (ACC), phospho-AMPK, and total-AMPK in quadriceps. (B) Average body weight. (C) Percent epididymal fat mass to body weight ratio. (D) Oxygen consumption rates (mg/kg/hr) measured over 12 hr period. (E) Data in (D) represented as AUC. (F) Running endurance measured as a function of time (upper panel) and distance (lower panel). (G) Representative oxidative genes induced by AICAR treatment (250 mg/kg/day, 6 days). (H) Expression of oxidative biomarkers (Scd1, Fasn, Ppargc1a, Pdk4) in wild-type and PPARd null primary myoblast treated with vehicle (open bars) or AICAR (closed bars) for 72 hr. (I) Model depicting the interaction between exercise and AMPK-PPARd in reprogramming muscle genome. Data in (B) and (C) (n = 10), (D) and (E) (n = 4), (F) (n =15-20), and (H) (n = 9) are presented as mean ±SEM, and * indicates statistical significance (p <0.05, unpaired student's t test).

SEQUENCE INFORMATION

[0029]   Nucleic acid and amino acid sequences may be referred to herein by GenBank accession number. It is understood that the

sequences given such GenBank accession numbers are incorporated by reference as they existed and were known as of December 29, 2007.

DETAILED DESCRIPTION

[0030]   Unless specifically noted otherwise herein, the definitions of the terms used are standard definitions used in the art of pharmaceutical sciences. 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 pharmaceutical carrier" includes mixtures of two or more such carriers, and the like.

[0031]   Also, the use of "or" means "and/or" unless stated otherwise. Similarly, "comprise," "comprises," "comprising" "include," "includes," and "including" are interchangeable and not intended to be limiting.

[0032]   It is to be further understood that where descriptions of various embodiments use the term "comprising," those skilled in the art would understand that in some specific instances, an embodiment can be alternatively described using language "consisting essentially of" or "consisting of."

[0033]   Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this disclosure belongs. Although any methods and reagents similar or equivalent to those described herein can be used in the practice of the disclosed methods and compositions, the exemplary methods and materials are now described.

[0034]   All publications mentioned herein are incorporated herein by reference in full for the purpose of describing and disclosing the methodologies, which are described in the publications, which might be used in connection with the description herein. The publications discussed above and throughout the text are provided solely for their disclosure prior to the filing date of the disclosure. Nothing herein is to be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior disclosure.

[0035]   Given the numerous benefits of exercise on general
health, identification of orally active agents that mimic or
potentiate the genetic effects of endurance exercise is a long-
standing, albeit elusive, medical goal. High doses of certain
natural extracts such as resveratrol can improve endurance (Lagouge
et al., 2006). The aerobic effects of resveratrol are thought to
depend on activation of SIRT1-PGC1a coactivator complex in skeletal
muscle. However, the downstream transcriptional factor(s) targeted
by SIRT1/PGC1a in mediating these effects are not known. More
importantly, both SIRT1/PGC1a and resveratrol activate multiple
targets, and thus whether there is a specific signaling pathway
that can be selectively activated by a synthetic drug to improve
endurance is not known.

[0036]   Exercise training activates a number of transcriptional
regulators and serine-threonine kinases in skeletal muscles that
contribute to metabolic reprogramming (Bassel-Duby and Olson,
2006). Overexpression of a constitutively active PPARδ (VP16-PPARδ)
in skeletal muscles of transgenic mice preprograms an increase in
oxidative muscle fibers, enhancing running endurance by nearly 100%
in untrained adult mice (Wang *et al.*, 2004). One of the best
understood serine-threonine kinases is AMP-activated protein kinase
(AMPK), a master regulator of cellular and organism metabolism.

[0037]   AMP kinase agonists such as AICAR have been studied for
insulin regulation, diabetes and obesity. However, AMP kinases
have not previously been demonstrated to promote muscle tone or to
improve endurance or exercise. The disclosure provides that AMPK
agonists provide a beneficial effect for muscle wasting diseases
and disorders, benefits to sedentary subject or immobilized limbs
and in combination with PPARd agonist an unexpected synergistic
effect.

[0038]   The disclosure demonstrates that PPARδ agonist such as,
for example, GW1516 (shown to be bioactive in humans) enables mice
to run 60%-75% longer and further than the nontreated controls
only; however, such an effect is only seen when administration of a
PPARδ agonist is combined with exercise training. This "super-
endurance phenotype" is linked to a transcriptional boost provided

by exercise-activated AMPK resulting in a novel endurance gene
signature (see, *e.g.*, Figure 10L)

[0039]   The disclosure also demonstrated that this super-
endurance phenotype can be obtained, in the absence of exercise
training, by orally active AMPK agonist and that such an AMPK
agonist is sufficient as a single agent to improve running
endurance by nearly 45% in nonexercised subjects.

[0040]   AMP-activated protein kinase (AMPK) and AMPK kinase
(AMPKK) comprise a protein kinase cascade. The AMPK cascade
regulates fuel production and utilization intracellularly. For
example, low cellular fuel (*e.g.*, an increase in AMP concentration)
increase AMPK activity. Once activated, AMPK functions either to
conserve ATP or to promote alternative methods of ATP generation.

[0041]   5'AMP-activated protein kinase or AMPK consists of three
proteins (subunits) that together make a functional enzyme that
plays a role in cellular energy homeostasis. It is expressed in a
number of tissues, including the liver, brain, and skeletal muscle.
Activation of AMPK has been shown to activate hepatic fatty acid
oxidation and ketogenesis, inhibit cholesterol synthesis,
lipogenesis, and triglyceride synthesis, inhibit adipocyte
lipolysis and lipogenesis, stimulate skeletal muscle fatty acid
oxidation and muscle glucose uptake, and modulate insulin secretion
by pancreatic beta-cells.

[0042]   Triggering the activation of AMPK can be carried out
with increasing concentrations of AMP. The γ subunit of AMPK
undergoes a conformational change so as to expose the active site
(Thr-172) on the α subunit. The conformational change of the γ
subunit of AMPK can be accomplished under increased concentrations
of AMP. Increased concentrations of AMP will give rise to the
conformational change on the γ subunit of AMPK as two AMP bind the
two Bateman domains located on that subunit. This role of AMP is
demonstrated in experiments that show AMPK activation via an AMP
analogue 5-amino-4-imidazolecarboxamide ribotide (ZMP) which is
derived from 5-amino-4-imidazolecarboxamide riboside (AICAR).

[0043]   As muscles contract, ATP is hydrolyzed, forming ADP. ADP
then helps to replenish cellular ATP by donating a phosphate group

to another ADP, forming an ATP and an AMP. As more AMP is produced
during muscle contraction, the AMP:ATP ratio dramatically
increases, leading to the allosteric activation of AMPK.

[0044]   Various AMPK agonist are known in the art. Methods and
compositions comprising such AMPK agonist are provided herein. The
use of such AMPK agonist improves muscle tone and muscle mass, as
well as improve endurance compared to subjects not receiving such
AMPK agonists. Various AMPK agonists are described herein and are
known in the art. In one embodiment, the AMPK agonist comprises an
AICAR compound. Other compounds useful in the method of the
disclosure include analogs of AICAR (such as those disclosed in U.
S. Patent No. 5,777,100, hereby incorporated by reference herein)
and prodrugs or precursors of AICAR (such as those disclosed in U.
S. Patent No. 5,082,829, hereby incorporated by reference herein),
which increase the bioavailability of AICAR, all of which are well-
known to those of ordinary skill in the art. Other activators of
AMPK include those described in U.S. Patent Publication No.
20060287356 to Iyengar et al. (the disclosure of which is
incorporated herein by reference). Conventionally known AMPK-
activating compounds include, in addition to the aforementioned
leptin, adiponectin, and metformin, AICAR (5-aminoimidazole-4-
carboxamide). Other AMPK agonists include, but are not limited to,
DRL-16536 (Dr. Reddy's/Perlecan Pharma), BG800 compounds
(Betagenon), furan-2-carboxylic acid derivative (Hanall, KR; see
also Int'l. Application Publ. WO/2008/016278, incorporated herein
by reference), A-769662 (Abbott) (structure I; see also, Cool et
al., Cell Metabol. 3:403-416, 2006); AMPK agonist under development
by Metabasis as set forth in Int'l. Publication No. WO/2006/033709;
MT-39 series of compounds (Mercury Therapeutics); and AMPK agonist
under development by TransTech Pharma.
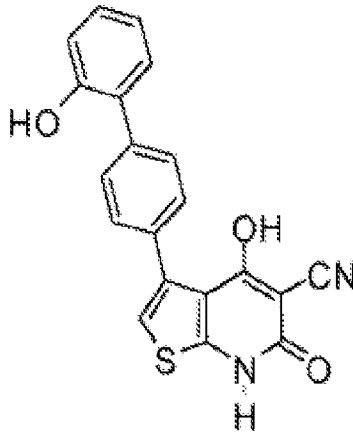
(I)

[0045]   AICAR, for example, is taken into the cell and converted
to ZMP, an AMP analog that has been shown to activate AMPK. ZMP
acts as an intracellular AMP mimic, and, when accumulated to high
enough levels, is able to stimulate AMPK activity (Corton, J. M.
et.al. Eur. J. Biochem. 229: 558 (1995)). However, ZMP also acts as
an AMP mimic in the regulation of other enzymes, and is therefore
not a specific AMPK activator (Musi, N. and Goodyear, L. J. Current
Drug Targets--Immune, Endocrine and Metabolic Disorders 2:119
(2002)).

[0046]   The disclosure provides methods for stimulating an
"exercise conditioned state" in a subject. The method includes
administering to a subject an AMPK agonist in an amount sufficient
to simulate an energy deficient state in a subject. By "energy
deficient state" refers to a state in which the γ subunit of AMPK
undergoes a conformation change, there is increased catabolism of
fat stores in a subject or there is conservation of ATP energy
stores or a metabolic state found in an exercising individual. The
exercise conditioned state can be accomplished in the absence of
exercise using the AMPK agonist of the disclosure (an "exercise-
free conditioning"). However, it will be recognized, that although
administration of an AMPK agonist can promote an exercise
conditioned state, it may be desirable or appropriate for a subject
to perform exercise conditioning even with the administration of an
AMPK agonist.

[0047]   Stimulating and exercise conditioned states not only has benefits to athletic training, but also provides benefits to subject who, do to injury, disease or disorder, are unable to exercise a limb/muscle or where the subject is sedentary or immobilized. By stimulating an exercise conditioned state the subject can maintain muscle tone and/or mass in the limb or muscle and promote health or recovery.

[0048]   Exercise is known to have many effects on subjects that perform it. Exercise effects at the molecular, biochemical, and/or cellular levels (e.g., modified regulation of genes and/or gene networks and corresponding proteins involved in energy substrate utilization and contractile properties of muscle) form the basis of physiological effects that are observed at the tissue, organ, and/or whole body levels (e.g., increased cardiorespiratory endurance, muscular strength, muscular endurance, and/or flexibility, and/or improvements in body appearance).

[0049]   In general terms, exercise is the performance of some physical activity. A single episode (also referred to as a bout) of physical activity is performed for a particular duration and at a particular intensity. If more than one bout of exercise is performed, separate bouts of exercise may have the same or different durations and/or the same or different intensities.

[0050]   In some embodiments, a single bout of exercise may last for up to 30 minutes, up to 45 minutes, up to 60 minutes, up to 90 minutes, up to 2 hours, up to 2.5 hours, up to 3 hours, or even longer. Typically, in the absence of a prior exercise history, repeated episodes of physical activity are needed to achieve an exercise-induced effect (such as, increased aerobic capacity or increase running endurance) for a PPARδ agonist to be effective. However, the administration of an AMPK agonist eliminates that need for exercise to see an effective exercise-induced or promoting effect.

[0051]   Thus, in some disclosed methods, no exercise is needed when an AMPK agonist is taken alone or prior to an PPARδ agonist. However, in some instances bouts of physical activity may be repeated within a single day; for instance, up to 2 bouts of

exercise per day, up to 3 bouts of exercise per day, up to 4 bouts of exercise per day, up to 5 bouts of exercise per day, or even more bouts per day. Some professional athletes or racing mammals may exercise in repeated bouts for a total of 8 hours or more a day. In other method embodiments, bouts (or repeated bouts) of exercise are performed on a daily basis, 6 times per week, 5 times per week, 4 times per week or 3 times per week. In at least some of the disclosed methods, exercise may continue for at least 2 weeks, for at least 4 weeks, for at least 6 weeks, for at least 3 months, for at least 6 months, for at least 1 year, for at least 3 years, or indefinitely (for the lifetime of the subject).

[0052]   Exercise generally is performed at an intensity that is more than the usual (e.g., average, median, normal standard, or normoactive) activity for a subject, and/or at or less than the maximum activity achievable by a subject performing a particular exercise. Any known indicator of physical performance can be used to determine whether a subject is performing more than a usual amount of activity, including, for instance, measuring heart rate, repetition rate (e.g., revolutions per second, minutes per mile, lifts per minute, and many others), and/or force output. In some methods, a bout of exercise is performed at sub-maximal intensity; for instance, at about 10% maximal intensity, 25% maximal intensity, 50% maximal intensity, or 75% maximal intensity. In other methods, a bout of exercise is performed at 40%-50% maximal heart rate, 50%-60% maximal heart rate, 60%-70% maximal heart rate, or 75%-80% maximal heart rate, where maximum heart rate for a human subject is calculated as: 220 bps - (age of the subject).

[0053]   Exercise is generally grouped into three types: (i) flexibility exercise (such as, stretching), which is believed to, at least, improve the range of motion of muscles and joints; (ii) aerobic exercise; and (iii) anaerobic exercise (such as, weight training, functional training or sprinting) which is believed to, at least, increase muscle strength and mass.

[0054]   Aerobic exercise refers to a physical activity in which oxidative or aerobic metabolism (as compared to glycolytic or anaerobic metabolism) substantially predominates in exercised

15

skeletal muscles. In particular method embodiments, a subject performs one or more aerobic exercises. Exemplary aerobic exercises include, without limitation, aerobics, calisthenics, cycling, dancing, exercise machines (rowing machine, cycling machine (e.g., inclined or upright), climbing machine, elliptical trainers, and/or skiing machines), basketball, football, baseball, soccer, footbag, housework, jogging, martial arts, massage, pilates, rowing, running, skipping, swimming, walking, yoga, boxing, gymnastics, badminton, cricket, track and field, golf, ice hockey, lacrosse, rugby, tennis, or combinations thereof.

[0055]   The disclosed methods contemplate enhancing any known or observable effect of exercise (such as an aerobic exercise, like walking or running). In particular methods, running endurance (e.g., running distance and/or running time) is enhanced. In another embodiment, the methods and compositions are useful for treating a subject having muscle immobility, muscle wasting disease or disorder or a sedentary subject. In one such embodiment, muscle tone or mass is improved or maintained in a subject having muscle immobility, muscle wasting disease or disorder or a sedentary activity compared to a subject that does not receive a composition of the disclosure (e.g., an AMPK agonist, or a combination of an AMPK agonist or PPAR agonist). In one embodiment, the compositions and methods of the disclosure can reduce muscle loss or the rate of muscle loss in a subject having a muscle wasting disease by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more compared to a subject not receiving a composition of the disclosure.

[0056]   Muscle weakness, tone, and atrophy result from a number of diseases and disorders including denervation or prolonged muscle disuse. When deprived of regular exercise, muscle fibers lose both bulk and length, producing a visible loss of muscle size and contour and apparent emaciation or deformity in the affected area. Even slight atrophy usually causes some loss of motion or power. Atrophy usually results from neuromuscular disease or injury. However, muscle tone, atrophy and weakness may also stem from certain metabolic, cardiovascular or endocrine disorders and prolonged immobility. Some muscle atrophy also occurs with aging.

The compositions of the disclosure are useful for treating such muscle weakness, tone and atrophy comprising administering an AMPK agonist alone or in combination with a PPARd agonist.

[0057]   Muscle wasting disease includes muscle weakness and atrophy that typically begin in a limb (*e.g.*, hand, arm, or leg). Eventually, weakness and atrophy spread to the trunk, neck, tongue, larynx, pharynx, and legs; progressive respiratory muscle weakness leads to respiratory insufficiency. Other findings include muscle flaccidity, fasciculations, hyperactive deep tendon reflexes, slight leg muscle spasticity, dysphagia, impaired speech, excessive drooling, and depression.

[0058]   Fibrous scar tissue formation, pain, and loss of serum proteins from severe burns can limit muscle movement, resulting in atrophy. Muscle atrophy is a late sign of irreversible ischemia, along with contractures, paralysis, and loss of pulses. Herniated disk can cause muscle weakness, disuse, and ultimately, atrophy. Hypercortisolism may cause limb weakness and eventually atrophy. Hypothyroidism can cause weakness and atrophy of proximal limb muscles. Injuries that result in immobilization of a limb can lead to muscle atropy and muscle wasting including, but not limited to, meniscal tears or broken bones or other cartilage injuries resulting from prolonged knee or limb immobility. Multiple sclerosis is a degenerative disease that cause arm and leg atrophy as a result of chronic progressive weakness; spasticity and contractures may also develop. Osteoarthritis eventually causes atrophy proximal to involved joints as a result of progressive weakness and disuse. Parkinson's disease causes muscle rigidity, weakness, and disuse producing muscle atrophy. Peripheral nerve trauma or injury to or prolonged pressure on a peripheral nerve leads to muscle weakness and atrophy. Peripheral neuropathy can lead to muscle weakness that progresses slowly to flaccid paralysis and eventually atrophy. Distal extremity muscles are generally affected first. Associated findings include loss of vibration sense; paresthesia, hyperesthesia, or anesthesia in the hands and feet; mild to sharp, burning pain; anhidrosis; glossy red skin; and diminished or absent deep tendon reflexes. Damaged spinal nerve

roots can cause muscle atrophy as well as weakness. Rheumatoid arthritis causes muscle atrophy in the late stages of this disorder, as joint pain and stiffness decrease range of motion and discourage muscle use. Spinal cord injury or trauma can produce severe muscle weakness and flaccid, then spastic, paralysis, eventually leading to atrophy. Stroke may produce contralateral or bilateral weakness and eventually atrophy of the arms, legs, face, and tongue. Associated signs and symptoms depend on the site and extent of vascular damage and may include dysarthria, aphasia, ataxia, apraxia, agnosia, and ipsilateral paresthesia or sensory loss. Prolonged steroid therapy can interfere with muscle metabolism and can lead to atrophy, most prominently in the limbs. As mentioned above, prolonged immobilization from bed rest, casts, splints, or traction may cause muscle weakness and atrophy. Any of these diseases or disorder can be treated with a composition or combination of compositions of the disclosure. Other muscle wasting disease or disorders are recognized in the art.

[0059]   The disclosure provides compositions and methods useful for treating such disease and disorders by promoting or maintaining muscle mass or tone. For example, administering an AMPK agonist such as AICAR alone or in combination with a PPARd agonist can promote muscle tone or mass. In healthy subjects this can contribute to an enhanced exercise effect. In subjects with a potential for losing muscle mass or having a muscle wasting disease or disorder the compositions and methods of the disclosure can slow or eliminate the rate of such muscle atrophy.

[0060]   Enhancing an exercise effect (such as running endurance) means that such effect is improved in a subject more than would have occurred by exercise alone. In some method embodiments, an enhanced exercise effect is determined by discontinuing administration of an AMPK agonist or a PPARδ agonist in the subject and observing (e.g., qualitatively or quantitatively) a reduction in the exercise effect of interest (e.g., aerobic endurance, such as running endurance). In some instances, an exercise effect of interest such as a AMPK-agonist induced effect or the PPARδ-enhanced portion of which is lost upon discontinuance of an AMPK

agonist or PPARδ agonist administration, will be reduced by at least about 5%, by at least about 10%, by at least about 20%, by at least about 30%, or by at least about 50% as compared to the magnitude of the effect with exercise alone.

[0061]   The disclosed methods can be performed in any subject capable of performing physical activity (e.g., aerobic exercise). In some method embodiments, a subject is a living multi-cellular vertebrate organism (e.g., human and/or non-human animals). In other exemplary methods, a subject is a mammal (including humans and/or non-human mammals such as veterinary or laboratory mammals) or, in more particular examples, a racing mammal (such as a horse, a dog, or a human). In still other methods, a subject is an adult, an exercise-trained subject, or a healthy subject. Some representative adult, human subjects are 16 years old or older, 18 years old or older, or 21 years old or older. In some embodiment, the subject does not perform any routine exercise. In some embodiment, some representative exercised- trained subjects have performed physical activity (such described in detail above) for at least 4 weeks, for at least 6 weeks, for at least 3 months, or for at least 6 months. In some examples the subject is healthy, for example, is a subject in which no known disease or disorder has been diagnosed or would be apparent after reasonable inquiry to an ordinarily skilled physician in the field to which the disease or disorder pertains.

[0062]   As described more fully below, the AMPK agonist may be administered orally, parenterally, intramuscularly, intravascularly or by any appropriate route. A subject can be any mammalian subject (e.g., equine, canine, or human). An AMPK agonist is particularly useful in combination with agents that promote muscle fiber development and growth. Examples of such agents include agonists of the PPAR family of proteins.

[0063]   PPARs are members of the nuclear receptor superfamily of ligand-inducible transcription factors. They form heterodimers with retinoid X receptors (RXRs) and bind to consensus DNA sites composed of direct repeats of hexameric DNA sequences separated by 1 bp. In the absence of ligand, PPAR-RXR heterodimers recruit

corepressors and associated histone deacetylases and chromatin-
modifying enzymes, silencing transcription by so-called active
repression (Ordentlich *et al.*, Curr. Top. Microbiol. Immunol,
254:101-116, 2001; Jepsen and Rosenfeld, J. Cell Sci, 115:689-698,
2002; Privalsky, Ann. Rev. Physiol, 66:315-360, 2004). Ligand
binding induces a conformational change in PPAR-RXR complexes,
releasing repressors in exchange for coactivators. Ligand-activated
complexes recruit the basal transcriptional machinery, resulting in
enhanced gene expression. PPARs bind to lower-affinity ligands
generated from dietary fat or intracellular metabolism. In keeping
with their roles as lipid sensors, ligand-activated PPARs turn on
feed-forward metabolic cascades to regulate lipid homeostasis via
the transcription of genes involved in lipid metabolism, storage,
and transport.

[0064]   Three PPAR isotypes exist in mammals: α (also known as
NR1C1), γ (also known as NR1C3), and δ (also known as β or NR1C2).
PPARδ is expressed in most cell types with relative abundance
(Smith, Biochem. Soc. Trans., 30(6): 1086- 1090, 2002), which led
to early speculation that it may serve a "general housekeeping
role" (Kliewer *et al.*, Proc. Natl. Acad. Sci U. S. A, 91:7355-7359,
1994). More recently, PPARδ transgenic mouse models and discoveries
aided by the development of high-affinity PPARδ agonists have
revealed PPARδ as a key transcriptional regulator with effects in
diverse tissues including fat, skeletal muscle, and the heart (for
review see, *e.g.*, Barish *et al.*, J. Clin. Invest., 116(3):590-597,
2006).

[0065]   Targeted expression of a constitutively active PPARδ
receptor (VP16-PPARδ) transgene in rodent skeletal muscle promoted
remodeling of skeletal muscle to an oxidative phenotype and
increased running endurance in unexercised adult mice (PLoC Biol,
2:e294, 2004). The observed PPARδ-mediated reprogramming of muscle
fibers involved the increased expression of genes related to fatty
acid oxidation, mitochondrial respiration, oxidative metabolism,
and slow-twitch contractile apparatus (Wang *et al.*, PLoC. Biol.,
2:e294, 2004). These VP16-PPARδ transgenic mice, who had a
phenotype similar to endurance-trained athletes, but who had had no

exercise training, suggest that pharmacological activation of
endogenous PPARδ in an adult, sedentary subject might provide an
exercise effect without the actual exercise. Given the numerous
benefits of exercise on general health, identification of orally
active agents that mimic the effects of exercise is a long
standing, albeit elusive medical goal.

[0066]   Disclosed herein are methods for enhancing an exercise
effect or promote/maintain muscle tone or mass in a subject
comprising administering an AMPK agonist sufficient to produce an
exercise effect, enhance mitochondrial expression or activity. In
one embodiment, the subject is a an exercising subject. In another
embodiment, the subject is a sedentary subject. In another
embodiment, the subject is immobilized or has an immobilized limb.
The disclosure further includes administering to the subject an
effective amount of a PPARδ agonist (e.g., GW1516). The exercise
effect that is enhanced can be, for example, improved running
endurance (such as, improved running distance or improved running
time or a combination thereof, increased fatty acid oxidation in at
least one skeletal muscle of the subject, and/or body fat (e.g.,
white adipose tissue) reduction). In some method embodiments, a
subject is a mammal (such as a racing mammal, like a horse, a dog,
or a human), and/or an adult, and/or an exercise-trained subject.
In other exemplary methods, a PPARδ agonist is administered on the
same day(s) on which the AMPK agonist is administered or on the
same day upon which physical exertion will be performed. In
another embodiment, the combination of an AMPK agonist and PPAR
agonist are administered on the same day as a physical exertion
will be performed. In some methods, administration of the AMPK
agonist is by oral administration, intravenous injection,
intramuscular injection, and/or subcutaneous injection. In other
method embodiments, the effective amount of the AMPK agonist is
from about 1 mg per day to about 20 mg per day in a single dose or
in divided doses. In some methods, administration of the PPARδ
agonist is by oral administration, intravenous injection,
intramuscular injection, and/or subcutaneous injection. In other
method embodiments, the effective amount of the PPARδ agonist is

from about 1 mg per day to about 20 mg per day in a single dose or in divided doses.

[0067]   The disclosed methods envision the use of any PPARδ agonist. Preferably such agonist would be non-toxic in the subject to which it is administered. Exemplary PPARδ agonists include GW1516, L- 165041 (as described by, *e.g.*, Leibowitz *et al.*, FEBS Lett., 473(3):333-336, 2000), any one or more compounds described in PCT Publication Nos. WO/2006/018174, WO/2005/113506, WO/2005/105754, WO/2006/041197, WO/2006/032023, WO/01/00603, WO/02/092590, WO/97/28115, WO/97/28149, WO/97/27857, WO/97/28137, WO/97/27847, and/or WO/98/27974, and/or a published U.S. national phase application or issued U.S. patent corresponding to any of the foregoing (each of which is expressly incorporated herein by reference). Moreover, other PPARδ agonists can be identified using the methods described, for example, in PCT Publication No. WO/1998/049555 or any corresponding published U.S. national phase application or issued U.S. patent (each of which is expressly incorporated herein by reference).

[0068]   In a specific example, the PPARδ agonist is GW1516 (also referred to in the art as GW501516). GW1516 is (2-methyl-4(((4-methyl-2-(4- trifluoromethylphenyl)-1,3-thiazol-5-yl)methyl)sulfanyl)phenoxy)acetic acid as has been shown to be is bioactive in humans (Sprecher *et al.*, Arterioscler. Thromb. Vase. Biol. 27(2): 359-65, 2007). In specific examples, GW1516 is administered orally, for example 1 mg - 20 mg/day, such as 2.5 mg or 10 mg per day.

[0069]   Disclosed herein are methods for enhancing or stimulating one or more exercise effects by combining, at least, an AMPK agonist with physical activity and/or administration of one or more PPARδ agonists.

[0070]   The disclosed methods envision the use of any method of administration, dosage, and/or formulation of an AMPK agonist alone or in combination with a PPARδ agonist that has the desired outcome of enhancing an exercise effect in a subject receiving the formulation, including, without limitation, methods of

administration, dosages, and formulations well known to those of
ordinary skill in the pharmaceutical arts.

[0071]   AMPK agonist of the disclosure may be administered in
the form of a drug to a human or an animal. Alternatively, the AMPK
agonist may be incorporated into a variety of foods and beverages
or pet foods so as to be consumed by humans or animals. The AMPK
agonist may be applied to a common food or beverage; or may be
applied to a functional food or beverage, a food for a subject
suffering a disease, or a food for specified health use, the food
(or beverage) bearing a label thereon indicating that it has a
physiological function; for example, energy supplement, exercise
enhancer or the like.

[0072]   The AMPK agonist alone or in combination with a PPARδ
agonist may be formulated into a drug product; for example, a
peroral solid product such as a tablet or a granule, or a peroral
liquid product such as a solution or a syrup.

[0073]   Modes of administering an AMPK agonist (or a formulation
including a PPARδ agonist) in a disclosed method include, but are
not limited to, intrathecal, intradermal, intramuscular,
intraperitoneal (ip), intravenous (iv), subcutaneous, intranasal,
epidural, intradural, intracranial, intraventricular, and oral
routes. In a specific example, the AMPK agonist or AMPK agonist and
PPARδ agonist is administered orally. Other convenient routes for
administration of an AMPK agonist (or a formulation including a
PPARδ agonist) include for example, infusion or bolus injection,
topical, absorption through epithelial or mucocutaneous linings
(for example, oral mucosa, rectal and intestinal mucosa, and the
like) ophthalmic, nasal, and transdermal. Administration can be
systemic or local. Pulmonary administration also can be employed
(for example, by an inhaler or nebulizer), for instance using a
formulation containing an aerosolizing agent.

[0074]   In specific method embodiments, it may be desirable to
administer an AMPK agonist or an AMPK agonist and PPARδ agonist
locally. This may be achieved by, for example, local or regional
infusion or perfusion, topical application (for example, wound
dressing), injection, catheter, suppository, or implant (for

23

example, implants formed from porous, non-porous, or gelatinous
materials, including membranes, such as sialastic membranes or
fibers), and the like.

[0075]   In other embodiments, a pump (such as a transplanted
minipump) may be used to deliver an AMPK agonist or a combination
of an AMPK agonist and a PPARδ agonist (or a formulation including
a PPARδ agonist) (see, e.g., Langer Science 249, 1527, 1990; Sefton
Crit. Rev. Biomed. Eng. 14, 201, 1987; Buchwald et al., Surgery 88,
507, 1980; Saudek et al., N. Engl. J. Med. 321, 574, 1989). In
another embodiment, an AMPK agonist (or a formulation including a
PPARδ agonist) is delivered in a vesicle, in particular liposomes
(see, e.g., Langer, Science 249, 1527, 1990; Treat et al., in
Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-
Berestein and Fidler (eds.), Liss, N. Y., pp. 353-365, 1989).

[0076]   In yet another method embodiment, an AMPK agonist alone
or in combination with a PPARδ agonist can be delivered in a
controlled-release formulation. Controlled-release systems, such as
those discussed in the review by Langer (Science 249, 1527 1990),
are known. Similarly, polymeric materials useful in controlled-
released formulations are known (see, e.g., Ranger et al.,
Macromol. ScL Rev. Macromol. Chem. 23, 61, 1983; Levy et al.,
Science 228, 190, 1985; During et al., Ann. Neurol. 25, 351, 1989;
Howard et al., J. Neurosurg. 71, 105, 1989). For example, an
agonists may be coupled to a class of biodegradable polymers useful
in achieving controlled release of a compound, including polylactic
acid, polyglycolic acid, copolymers of polylactic and polyglycolic
acid, polyepsilon caprolactone, polyhydroxy butyric acid,
polyorthoesters, polyacetals, polydihydropyrans, polycyanoacrylates
and cross- linked or amphipathic block copolymers of hydrogels.

[0077]   The disclosed methods contemplate the use of any dosage
form of an AMPK agonist alone or in combination with a PPARδ
agonist (or formulation containing the same) that delivers the
agonist(s) and achieves a desired result. Dosage forms are commonly
known and are taught in a variety of textbooks, including for
example, Allen et al., Ansel's Pharmaceutical Dosage Forms and Drug
Delivery Systems, Eighth Edition, Philadelphia, PA:Lippincott

Williams & Wilkins, 2005, 738 pages. Dosage forms for use in a
disclosed method include, without limitation, solid dosage forms
and solid modified-release drug delivery systems (e.g., powders and
granules, capsules, and/or tablets); semi-solid dosage forms and
transdermal systems (e.g., ointments, creams, and/or gels);
transdermal drug delivery systems; pharmaceutical inserts (e.g.,
suppositories and/or inserts); liquid dosage forms (e.g., solutions
and disperse systems); and/or sterile dosage forms and delivery
systems (e.g., parenterals, and/or biologies). Particular exemplary
dosage forms include aerosol (including metered dose, powder,
solution, and/or without propellants); beads; capsule (including
conventional, controlled delivery, controlled release, enteric
coated, and/or sustained release); caplet; concentrate; cream;
crystals; disc (including sustained release); drops; elixir;
emulsion; foam; gel (including jelly and/or controlled release);
globules; granules; gum; implant; inhalation; injection; insert
(including extended release); liposomal; liquid (including
controlled release); lotion; lozenge; metered dose (e.g., pump);
mist; mouthwash; nebulization solution; ocular system; oil;
ointment; ovules; powder (including packet, effervescent, powder
for suspension, powder for suspension sustained release, and/or
powder for solution); pellet; paste; solution (including long
acting and/or reconstituted); strip; suppository (including
sustained release); suspension (including lente, ultre lente,
reconstituted); syrup (including sustained release); tablet
(including chewable, sublingual, sustained release, controlled
release, delayed action, delayed release, enteric coated,
effervescent, film coated, rapid dissolving, slow release);
transdermal system; tincture; and/or wafer. Typically, a dosage
form is a formulation of an effective amount (such as a
therapeutically effective amount) of at least one active
pharmaceutical ingredient (such as an AMPK agonist or PPARδ
agonist) with pharmaceutically acceptable excipients and/or other
components (such as one or more other active ingredients). An aim
of a drug formulation is to provide proper administration of an
active ingredient (such as an AMPK agonist alone or in combination

with a PPARδ agonist) to a subject. A formulation should suit the
mode of administration. The term "pharmaceutically acceptable"
means approved by a regulatory agency of the federal or a state
government or listed in the U.S. Pharmacopoeia or other generally
recognized pharmacopoeia for use in animals, and, more
particularly, in humans. Excipients for use in exemplary
formulations include, for instance, one or more of the following:
binders, fillers, disintegrants, lubricants, coatings, sweeteners,
flavors, colorings, preservatives, diluents, adjuvants, and/or
vehicles. In some instances, excipients collectively may constitute
about 5%-95% of the total weight (and/or volume) of a particular
dosage form.

[0078]   Pharmaceutical excipients can be, for instance, sterile
liquids, such as water and/or oils, including those of petroleum,
animal, vegetable, or synthetic origin, such as peanut oil, soybean
oil, mineral oil, sesame oil, and the like. Water is an exemplary
carrier when a formulation is administered intravenously. Saline
solutions, blood plasma medium, aqueous dextrose, and glycerol
solutions can also be employed as liquid carriers, particularly for
injectable solutions. Oral formulations can include, without
limitation, pharmaceutical grades of mannitol, lactose, starch,
magnesium stearate, sodium saccharine, cellulose, magnesium
carbonate, and the like. A more complete explanation of parenteral
pharmaceutical excipients can be found in Remington, The Science
and Practice of Pharmacy, 19th Edition, Philadelphia, PA:Lippincott
Williams & Wilkins, 1995, Chapter 95. Excipients may also include,
for example, pharmaceutically acceptable salts to adjust the
osmotic pressure, lipid carriers such as cyclodextrins, proteins
such as serum albumin, hydrophilic agents such as methyl cellulose,
detergents, buffers, preservatives and the like. Other examples of
pharmaceutical excipients include starch, glucose, lactose,
sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium
stearate, glycerol monostearate, talc, sodium chloride, dried skim
milk, glycerol, propylene, glycol, water, ethanol, and the like. A
formulation, if desired, can also contain minor amounts of wetting
or emulsifying agents, or pH buffering agents.

[0079]   A dosage regimen utilizing a PPARδ agonist is selected in accordance with a variety of factors including type, species, age, weight, sex and physical condition of the subject; the route of administration; and/or the particular PPARδ agonist formulation employed. An ordinarily skilled physician or veterinarian can readily determine an effective amount of a PPARδ agonist (or formulation thereof) useful for enhancing an exercise effect in a subject.

[0080]   In some embodiments involving oral administration, oral dosages of an AMPK agonist alone or in combination with a PPARδ agonist will generally range between about 0.001 mg per kg of body weight per day (mg/kg/day) to about 100 mg/kg/day, and such as about 0.01-10 mg/kg/day (unless specified otherwise, amounts of active ingredients are on the basis of a neutral molecule, which may be a free acid or free base). For example, an 80 kg subject would receive between about 0.08 mg/day and 8 g/day, such as between about 0.8 mg/day and 800 mg/day. A suitably prepared medicament for once a day administration would thus contain between 0.08 mg and 8 g, such as between 0.8 mg and 800 mg. In some instance, formulation including an AMPK agonist alone or in combination with a PPARδ agonist may be administered in divided doses of two, three, or four times daily. For administration twice a day, a suitably prepared medicament as described above would contain between 0.04 mg and 4 g, such as between 0.4 mg and 400 mg. Dosages outside of the aforementioned ranges may be necessary in some cases. Examples of daily dosages that may be given in the range of 0.08 mg to 8 g per day include 0.1 mg, 0.5 mg, 1 mg, 2.5 mg, 5 mg, 10 mg, 25 mg, 50 mg, 100 mg, 200 mg, 300 mg, 400 mg, 500 mg, 600 mg, 800 mg, 1 g, 2 g, 4 g and 8 g. These amounts can be divided into smaller doses if administered more than once per day (e.g., one-half the amount in each administration if the drug is taken twice daily).

[0081]   For some method embodiments involving administration by injection (e.g., intravenously or subcutaneous injection), a subject would receive an injected amount that would deliver the active ingredient in approximately the quantities described above.

27

The quantities may be adjusted to account for differences in
delivery efficiency that result from injected drug forms bypassing
the digestive system. Such quantities may be administered in a
number of suitable ways, *e.g.* large volumes of low concentrations
of active ingredient during one extended period of time or several
times a day, low volumes of high concentrations of active
ingredient during a short period of time, *e.g.* once a day.
Typically, a conventional intravenous formulation may be prepared
which contains a concentration of active ingredient of between
about 0.01-1.0 mg/ml, such as for example 0.1 mg/ml, 0.3 mg/ml, or
0.6 mg/ml, and administered in amounts per day equivalent to the
amounts per day stated above. For example, an 80 kg subject,
receiving 8 ml twice a day of an intravenous formulation having a
concentration of active ingredient of 0.5 mg/ml, receives 8 mg of
active ingredient per day.

[0082]   In other method embodiments, an AMPK agonist (or a
formulation thereof) can be administered at about the same dose
throughout a treatment period, in an escalating dose regimen, or in
a loading-dose regime (for example, in which the loading dose is
about two to five times a maintenance dose). In some embodiments,
the dose is varied during the course of an AMPK agonist formulation
usage based on the condition of the subject receiving the
composition, the apparent response to the composition, and/or other
factors as judged by one of ordinary skill in the art. In some
embodiments long-term administration of an AMPK agonist or
combination therapy (or formulation thereof) is contemplated, for
instance in order to effect sustained enhancement of an exercise
effect (such as aerobic endurance, *e.g.*, running endurance).

[0083]   Also disclosed herein are methods for identifying the
use of performance-enhancing substances in an exercise-trained
subject, which include determining in a biological sample taken
from an exercise-trained subject (*e.g.*, a skeletal muscle biopsy)
the presence of ZMP or other non-naturally occurring AMP analog
and/or the expression of the molecules listed in Table 2 or listed
in Table 4, or a subset thereof, such as expression of at least 1,

28

at least 5, at least 10, at least 20, at least 40 of the molecules listed in Table 2 or in Table 4.

[0084]   In some methods for identifying the use of performance-enhancing substances in an exercise-trained subject the presence of a ZMP or AMP analog will be measured alone or in combination with whether (i) expression is upregulated in one or more of (such as at least 5, at least 10, at least 20, at least 35, or all of) adipose differentiation related protein; stearoyl-Coenzyme A desaturase 2; acetyl-Coenzyme A acetyltransferase 2; ATP citrate lyase; adiponectin, C1Q and collagen domain containing; diacylglycerol O-acyltransferase 2; lipase, hormone sensitive; monoglyceride lipase; resistin; CD36 antigen; fatty acid binding protein 4, adipocyte; lipoprotein lipase; microsomal glutathione S-transferase 1; GPI-anchored membrane protein 1; dual specificity phosphatase 7; homeodomain interacting protein kinase 3; insulin-like growth factor binding protein 5; protein phosphatase 2 (formerly 2A), regulatory subunit A (PR 65), beta isoform; protein tyrosine phosphatase-like (proline instead of catalytic arginine); member b; CCAAT/enhancer binding protein (C/EBP), alpha; nuclear receptor subfamily 1, group D, member 2(Reverb-b); transferring; archain 1; solute carrier family 1 (neutral amino acid transporter), member 5; RIKEN cDNA 1810073N04 gene; haptoglobin; retinol binding protein 4, plasma; phosphoenolpyruvate carboxykinase 1, cytosolic; cell death-inducing DFFA-like effector c; interferon, alpha-inducible protein 27; carbonic anhydrase 3; cysteine dioxygenase 1, cytosolic; DNA segment, Chr 4, Wayne State University 53, expressed; dynein cytoplasmic 1 intermediate chain 2; Kruppel-like factor 3 (basic); thyroid hormone responsive SPOT14 homolog (Rattus); cytochrome P450, family 2, subfamily e, polypeptide 1; complement factor D (adipsin); and/or transketolase; and/or (ii) expression is downregulated in one or more of gamma-glutamyl carboxylase; 3-oxoacid CoA transferase 1; solute carrier family 38, member 4; annexin A7; CD55 antigen; RIKEN cDNA 1190002H23 gene; fusion, derived from t(12;16) malignant liposarcoma (human); lysosomal membrane glycoprotein 2; and/or neighbor of Punc El 1, such as 1, 2, 3, 4, 5, 6, 7, 8 or 9 of these molecules.

[0085]   Exemplary methods for identifying the use of
performance-enhancing substances in an exercise-trained subject
involve determining protein expression and/or determining
expression of a gene encoding the protein. Such methods are routine
in the art. In some examples, the level of protein or nucleic acid
expression is quantified. Methods of identifying an agent having
potential to enhance exercise performance in a subject also are
disclosed herein. Such methods can include (i) providing a first
component comprising a PPARδ receptor or an AMPK-binding fragment
thereof; (ii) providing a second component comprising an AMP-
activated protein kinase (AMPK), AMPKα1, AMPKα2, or a PPARδ-binding
fragment of any thereof; (iii) contacting the first component and
the second component with at least one test agent under conditions
that would permit the first component and the second component to
specifically bind to each other in the absence of the at least one
test agent; and (iv) determining whether the at least one test
agent affects the specific binding of the first component and the
second component to each other. An effect on specific binding of
the first component and the second component to each other
identifies the at least one test agent as an agent having potential
to enhance exercise performance in a subject.

[0086]   The use of performance-enhancing substances (PES),
particularly by children and professional athletes, has been in the
news because of potential adverse health consequences and the
arguable effects that such practices have on moral development of
the individual and on fair athletic competition for all (Committee
on Sports Medicine and Fitness, Reginald L. Washington, MD,
Chairperson, Pediatrics, 115(4): 1103- 1106, 2005). One of the
discoveries provided herein is that certain genes (and/or the
proteins encoded thereby) are uniquely regulated by a combination
of exercise and a pharmaceutical agent (a PPARδ agonist) that
results in enhanced physical performance (see Table 2). In some
cases, the particular genes (and/or proteins encoded thereby) were
up- or down-regulated by the combined treatment but were not
affected by either intervention alone. In other cases, the
particular genes (and/or proteins encoded thereby) were not

affected by the combined treatment but were up- or down-regulated by one or both intervention when practiced alone. The unique regulation of these genes (and/or the encoded proteins) makes them useful markers (either alone or in any combination) for identifying exercising subjects who are taking (or receiving) PES.

[0087]   A PES is any substance taken in nonpharmacologic doses specifically for the purpose of improving sports performance (e.g., by increasing strength, power, speed, or endurance (ergogenic) or by altering body weight or body composition). Exemplary PES include the following: (i) pharmacologic agents (prescription or nonprescription) taken in doses that exceed the recommended therapeutic dose or taken when the therapeutic indication(s) are not present (e.g., using decongestants for stimulant effect, using bronchodilators when exercise-induced bronchospasm is not present, increasing baseline methylphenidate hydrochloride dose for athletic competition); (ii) agents used for weight control, including stimulants, diet pills, diuretics, and laxatives, when the user is in a sport that has weight classifications or that rewards leanness; (iii) agents used for weight gain, including over-the-counter products advertised as promoting increased muscle mass; (iv) physiologic agents or other strategies used to enhance oxygen-carrying capacity, including erythropoietin and red blood cell transfusions (blood doping); (v) any substance that is used for reasons other than to treat a documented disease state or deficiency; (vi) any substance that is known to mask adverse effects or detectability of another performance-enhancing substance, and/or (vii) nutritional supplements taken at super physiologic doses or at levels greater than required to replace deficits created by a disease state, training, and/or participation in sports. In one example the PES is as AMPK agonist (e.g., one that provides an MP analog) or GW1516.

[0088]   For example, to quantify the levels of an AMPK agonist such as AICAR in a sample of body fluid such as serum, as sensitive LC-MS/MS assay can be used. The assay utilizes a structurally related analog to the AMPK activator as an internal standard. For example, the adenosine analog tubercidin (4-amino-7-beta-D-

ribufuranosyl-7H-pyrrolo[2,3-d]pyrimindine; 7-beta-D-riburuanosyl-
7H-pyrrolo[2,3-d]pyrimindin-4-amine; 7-deazaadenosine) is
structurally related to AICAR and can be used as the internal
standard when assaying for AICAR. Samples containing a known
concentration of the internal standard can be directly analyzed by
LC-MS/MS. The substance of interest and the internal standard can
be resolved on a LC-MS/MS (tandem mass spec) system, for example,
suing a hydrophobic column with a solvent or acidified solvent
gradient, and detected as positive ions using selective reaction
monitoring. Other analyzers and detection methods known in the art
can be used. A standard curve over one two , three or four orders
of magnitude constructed using agonist-spiked serum can be
constructed to facilitate quantification of AICAR levels.

[0089]   The biomarkers of substance-induced performance
enhancement identified herein and useful in a disclosed method
include one or more (or any combination of) of an AMP analog or the
genes (and/or proteins encoded thereby) listed in Table 2, and in
some examples listed in Table 4. In particular method embodiments,
at least 2, at least 3, at least 5, at least 7, at least 10, at
least 15, at least 20, at least 30, or at least 40 of the genes
(and/or proteins encoded thereby) listed in Table 2 (or Table 4)
are detected in a disclosed method. In one example at least one
gene (and/or protein encoded thereby) from each class listed in
Table 2 (e.g., cytokines, fat metabolism) is analyzed.

[0090]   In one embodiment, upregulated expression is detected
for one or more of the following genes (or proteins encoded
thereby): adipose differentiation related protein; stearoyl-
Coenzyme A desaturase 2; acetyl-Coenzyme A acetyltransferase 2; ATP
citrate lyase; adiponectin, C1Q and collagen domain containing;
diacylglycerol O-acyltransferase 2; lipase, hormone sensitive;
monoglyceride lipase; resistin; CD36 antigen; fatty acid binding
protein 4, adipocyte; lipoprotein lipase; microsomal glutathione S-
transferase 1; GPI- anchored membrane protein 1; dual specificity
phosphatase 7; homeodomain interacting protein kinase 3; insulin-
like growth factor binding protein 5; protein phosphatase 2
(formerly 2A), regulatory subunit A (PR 65), beta isoform; protein

32

tyrosine phosphatase-like (proline instead of catalytic arginine);
member b; CCAAT/enhancer binding protein (C/EBP), alpha; nuclear
receptor subfamily 1, group D, member 2(Reverb-b); transferring;
archain 1; solute carrier family 1 (neutral amino acid
transporter), member 5; RIKEN cDNA 1810073N04 gene; haptoglobin;
retinol binding protein 4, plasma; phosphoenolpyruvate
carboxykinase 1, cytosolic; cell death-inducing DFFA-like effector
c; interferon, alpha-inducible protein 27; carbonic anhydrase 3;
cysteine dioxygenase 1, cytosolic; DNA segment, Chr 4, Wayne State
University 53, expressed; dynein cytoplasmic 1 intermediate chain
2; Kruppel-like factor 3 (basic); thyroid hormone responsive SPOT14
homolog (Rattus); cytochrome P450, family 2, subfamily e,
polypeptide 1; complement factor D (adipsin); and/or transketolase.
In particular method embodiments, upregulation of at least 2, at
least 3, at least 5, at least 7, at least 10, at least 15, at least
20, at least 30, or at least 38 of the foregoing genes (and/or
proteins encoded thereby) are detected in a disclosed method. In
other method embodiments, downregulated expression is detected in
one or more of the following genes (and/or proteins encoded
thereby): gamma- glutamyl carboxylase; 3-oxoacid CoA transferase 1;
solute carrier family 38, member 4; annexin A7; CD55 antigen; RIKEN
cDNA 1190002H23 gene; fusion, derived from t(12;16) malignant
liposarcoma (human); lysosomal membrane glycoprotein 2; and/or
neighbor of Punc El 1. In particular method embodiments,
downregulation of at least 2, at least 3, at least 5, or at least 7
of the foregoing genes (and/or proteins encoded thereby) are
detected in a disclosed method.

[0091]   In still other method embodiments, a combination of
upregulated genes (and/or proteins encoded thereby) and
downregulated genes (and/or proteins encoded thereby) as described
above is detected in a sample from a subject (such as, an exercised
or exercise-trained subject).

[0092]   Yet other method embodiments involve the detection in a
sample of a combination of an AMP analog (e.g., ZMP) and one or
more of the above-described upregulated genes (and/or proteins
encoded thereby) and/or the above-described downregulated genes

(and/or proteins encoded thereby), and/or the above-described exercise-regulated genes that are not affected by exercise combined with PPARδ administration.

[0093]   Disclosed methods may be used for detecting PES use in any subject capable of taking or receiving one or more such PES. In some method embodiments, a subject is a living multi-cellular vertebrate organism (e.g., human and/or non-human animals). In other exemplary methods, a subject is a mammal (including humans and/or non-human mammals) or, in more particular examples, a racing mammal (such as a horse, a dog, or a human). In still other methods, a subject is an exercise-trained subject. Some representative exercised-trained subjects have performed physical activity (such described in detail above) for at least 4 weeks, for at least 6 weeks, for at least 3 months, or for at least 6 months. Other exercise-trained subjects may be student athletes and/or professional athletes (including, in some examples, non-human professional athletes, such as race horses and/or racing dogs).

[0094]   Any sample from a subject (e.g., a biological sample) in which can be detected an AMP analog and/or one or more genes and/or proteins uniquely regulated by exercise in combination with PPARδ agonist intake (as described in detail throughout this specification) is contemplated for use in a disclosed method. Exemplary samples for use in a disclosed method include blood, saliva, urine, muscle biopsy (e.g., skeletal muscle biopsy), cheek swab, fecal sample, sweat, and/or sperm.

[0095]   Methods of detecting the expression of genes and/or proteins in a sample (e.g., biological sample) are very well known (see, e.g., U.S. Patent Nos. 6,911,307; 6,893,824; 5,972,692; 5,972,602; 5,776,672; 7,031,847; 6,816,790; 6,811,977; 6,806,049; 6,203,988; and/or 6,090,556). In particular embodiments, expression of one or more genes identified herein can be detected by any method of nucleic acid amplification (such as, polymerase chain reaction (PCR) or any adaptation thereof, ligase chain reaction, transcription-based amplification systems, cycling probe reaction, Qβ replicase amplification, strand displacement amplification, and/or rolling circle amplification), solid-surface

hybridization assays (such as Northern blot, dot blot, gene chips, and/or reversible target capture), solution hybridization assays (such as MAP technology (which uses a liquid suspension array of 100 sets of 5.5 micron probe-conjugated beads, each internally dyed with different ratios of two spectrally distinct fluorophores to assign it a unique spectral address)), and/or in situ hybridization. Various of the foregoing nucleic acid detection methods are described in detail in the review by Wolcott (Clin. Microbiol. Rev., 5(4):370-386, 1992). Other detailed and long-established protocols for practicing some such nucleic acid detection methods are found in Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd edition, Cold Spring Harbor Laboratory Press, 1989; Sambrook et al., Molecular Cloning: A Laboratory Manual, 3rd edition, Cold Spring Harbor Press, 2001; Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates, 1992 (and Supplements to 2000); and/or Ausubel et al., Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology, 4th edition, Wiley & Sons, 1999.

[0096]   In other embodiments, expression of one or more proteins encoded by corresponding genes identified herein can be detected by Western blot, immunohistochemistry, immunoprecipitation, antibody microarrays, ELISA, and/or by functional assay (e.g., kinase assay, ATPase assay, substrate (or ligand) binding assay, protein-protein binding assay, or other assay suitable for measuring a particular protein function).

[0097]   If the pattern of expression identified in the test subject is similar to that shown in Table 2 (e.g., the genes shown as upregulated and downregulated in Table 2 are observed in the subject to be upregulated and downregulated, respectively), this indicates that the subject is taking a PES, such as a PPARδ agonist (e.g., GW1516). In contrast, if the pattern of expression identified in the test subject is different to that shown in Table 2 (e.g., the genes shown as upregulated and downregulated in Table 2 are observed in the subject to be not differentially expressed or show a different pattern of regulation), this indicates that the

subject is not taking a PES, such as a PPARδ agonist (*e.g.*, GW 1516).

[0098]   This disclosure identifies a previously unknown protein-protein interaction between PPARδ and particular exercise-induced kinases (*e.g.*, AMPK, such as the AMPKα1 and/or AMPKα2 subunit(s) of AMPK). The interaction between PPARδ and AMPK may have important functional outcomes, such as enhancing exercise performance (*e.g.*, aerobic exercise performance, such as running endurance) in a subject.

[0099]   The foregoing discoveries enable methods for identify agents, *e.g.*, having potential to enhance exercise performance (*e.g.*, aerobic exercise performance, such as running endurance) in a subject. In some such methods, agents that affect (*e.g.*, enhance, weaken, or substantially disrupt) the protein-protein interaction are identified. In other such methods, agents that affect (*e.g.*, increase, decrease, or substantially eliminate) AMPK-dependent phosphorylation of a PPARδ complex are identified.

[00100]   An "agent" is any substance or any combination of substances that is useful for achieving an end or result; for example, a substance or combination of substances useful for modulating a protein activity associated with AMPK activation cascade (*e.g.*, AMPK-dependent phosphorylation of a PPARδ complex), or useful for modifying or affecting a protein-protein interaction (*e.g.*, PPARδ- AMPK interaction) or ATP metabolism. Any agent that has potential (whether or not ultimately realized) to modulate any aspect of the PPARδ- AMPK interaction disclosed herein is contemplated for use in the screening methods of this disclosure.

[00101]   Exemplary agents include, but are not limited to, peptides such as, for example, soluble peptides, including but not limited to members of random peptide libraries (see, *e.g.*, Lam *et al.*, Nature, 354:82-84, 1991; Houghten *et al.*, Nature, 354:84-86, 1991), and combinatorial chemistry-derived molecular library made of D- and/or L-configuration amino acids, phosphopeptides (including, but not limited to, members of random or partially degenerate, directed phosphopeptide libraries; see, *e.g.*, Songyang *et al.*, Cell, 72:767-778, 1993), antibodies (including, but not

limited to, polyclonal, monoclonal, humanized, anti-idiotypic,
chimeric or single chain antibodies, and Fab, F(ab')2 and Fab
expression library fragments, and epitope-binding fragments
thereof), small organic or inorganic molecules (such as, so-called
natural products or members of chemical combinatorial libraries),
molecular complexes (such as protein complexes), or nucleic acids.

[00102]   Libraries (such as combinatorial chemical libraries)
useful in the disclosed methods include, but are not limited to,
peptide libraries (see, *e.g.*, U.S. Pat. No. 5,010,175; Furka, Int.
J. Pept. Prot. Res., 37:487-493, 1991; Houghton *et al.*, Nature,
354:84-88, 1991; PCT Publication No. WO 91/19735), encoded peptides
(*e.g.*, PCT Publication WO 93/20242), random bio-oligomers (*e.g.*,
PCT Publication No. WO 92/00091), benzodiazepines (*e.g.*, U.S. Pat.
No. 5,288,514), diversomers such as hydantoins, benzodiazepines and
dipeptides (Hobbs *et al.*, Proc. Natl. Acad. Sci. USA, 90:6909-6913,
1993), vinylogous polypeptides (Hagihara *et al.*, J. Am. Chem. Soc,
114:6568, 1992), nonpeptidal peptidomimetics with glucose
scaffolding (Hirschmann *et al.*, J. Am. Chem. Soc, 114:9217-9218,
1992), analogous organic syntheses of small compound libraries
(Chen *et al.*, J. Am. Chem. Soc, 116:2661, 1994), oligocarbamates
(Cho *et al.*, Science, 261: 1303, 1003), and/or peptidyl
phosphonates (Campbell *et al.*, J. Org. Chem., 59:658, 1994),
nucleic acid libraries (see Sambrook *et al.* Molecular Cloning, A
Laboratory Manual, Cold Springs Harbor Press, N. Y., 1989; Ausubel
et al., Current Protocols in Molecular Biology, Green Publishing
Associates and Wiley Interscience, N.Y., 1989), peptide nucleic
acid libraries (see, *e.g.*, U.S. Pat. No. 5,539,083), antibody
libraries (see, *e.g.*, Vaughn *et al.*, Nat. Biotechnol, 14:309-314,
1996; PCT App. No. PCT/US96/10287), carbohydrate libraries (see,
e.g., Liang *et al.*, Science, 274:1520-1522, 1996; U.S. Pat. No.
5,593,853), small organic molecule libraries (see, *e.g.*,
benzodiazepines, Baum, C&EN, Jan 18, page 33, 1993; isoprenoids,
U.S. Pat. No. 5,569,588; thiazolidionones and methathiazones, U.S.
Pat. No. 5,549,974; pyrrolidines, U.S. Pat. Nos. 5,525,735 and
5,519,134; morpholino compounds, U.S. Pat. No. 5,506,337;
benzodiazepines, 5,288,514) and the like.

[00103]   Libraries useful for the disclosed screening methods can
be produce in a variety of manners including, but not limited to,
spatially arrayed multipin peptide synthesis (Geysen, *et al.*, Proc
Natl. Acad. Sci., 81(13):3998-4002, 1984), "tea bag" peptide
synthesis (Houghten, Proc Natl. Acad. Sci., 82(15):5131-5135,
1985), phage display (Scott and Smith, Science, 249:386-390, 1990),
spot or disc synthesis (Dittrich *et al.*, Bioorg. Med. Chem. Lett.,
8(17):2351-2356, 1998), or split and mix solid phase synthesis on
beads (Furka *et al.*, Int. J. Pept. Protein Res., 37(6):487-493,
1991; Lam *et al.*, Chem. Rev., 97 (2):411-448, 1997). Libraries may
include a varying number of compositions (members), such as up to
about 100 members, such as up to about 1000 members, such as up to
about 5000 members, such as up to about 10,000 members, such as up
to about 100,000 members, such as up to about 500,000 members, or
even more than 500,000 members.

[00104]   In one embodiment, high throughput screening methods
involve providing a combinatorial chemical or peptide library
containing a large number of potential therapeutic compounds (*e.g.*,
affectors of AMPK-PPARδ protein-protein interactions). Such
combinatorial libraries are then screened in one or more assays as
described herein to identify those library members (particularly
chemical species or subclasses) that display a desired
characteristic activity (such as increasing or decreasing an AMPK-
PPARδ protein-protein interaction). The compounds thus identified
can serve as conventional "lead compounds" or can themselves be
used as potential or actual therapeutics. In some instances, pools
of candidate agents may be identify and further screened to
determine which individual or subpools of agents in the collective
have a desired activity. PPARδ forms a protein-protein interaction
with AMPK or one or more of its subunits (such as AMPKα1 and/or
AMPKcc2). Agents that affect (*e.g.*, increase or decrease) an AMPK-
PPARδ interaction or AMP-dependent phosphorylation of a PPARδ
complex may have the effect of enhancing exercise performance
(*e.g.*, aerobic exercise performance, such as running endurance) in
a subject and, therefore, are desirable to identify.

38

[00105]   In screening methods described here, tissue samples,
isolated cells, isolated polypeptides, and/or test agents can be
presented in a manner suitable for high-throughput screening; for
example, one or a plurality of isolated tissue samples, isolated
cells, or isolated polypeptides can be inserted into wells of a
microtitre plate, and one or a plurality of test agents can be
added to the wells of the microtitre plate. Alternatively, one or a
plurality of test agents can be presented in a high-throughput
format, such as in wells of microtitre plate (either in solution or
adhered to the surface of the plate), and contacted with one or a
plurality of isolated tissue samples, isolated cells, and/or
isolated polypeptides under conditions that, at least, sustain the
tissue sample or isolated cells or a desired polypeptide function
and/or structure. Test agents can be added to tissue samples,
isolated cells, or isolated polypeptides at any concentration that
is not lethal to tissues or cells, or does not have an adverse
effect on polypeptide structure and/or function. It is expected
that different test agents will have different effective
concentrations. Thus, in some methods, it is advantageous to test a
range of test agent concentrations.

[00106]   Disclosed methods envision, as appropriate, the use of
PPARδ or AMPK (such as AMPKα1 or AMPKcc2) or functional fragments
of any thereof as contained, independently, in a subject, one or a
plurality of cells or cellular extracts, one or a plurality of
tissue or tissue extracts, or as an isolated polypeptide. PPARδ
ligand optionally is included (or is omitted) in disclosed methods.

[00107]   A "direct association" between two or more polypeptides
(such as, PPARδ and AMPK (such as AMPKα1 or AMPKα2) is
characterized by physical contact between at least a portion of the
interacting polypeptides that is of sufficient affinity and
specificity that, for example, immunoprecipitation of one of the
polypeptides also will specifically precipitate the other
polypeptide; provided that the immunoprecipitating antibody does
not also affect the site(s) involved in the interaction. A direct
association between polypeptides also may be referred to as a
"protein-protein interaction." The binding of one polypeptide to

another in a protein-protein interaction (e.g., PPARδ to AMPK (or
AMPKα1 and/or AMPKα2) and vice versa) is considered "specific
binding". Agents that affect an AMPK-PPARδ interaction can be
identified by a variety of assays, including solid-phase or
solution-based assays. In an exemplary solid-phase assay, PPARδ or
an AMPK-binding fragment thereof and AMPK or a subunit thereof
(such as AMPKα1 and/or AMPKα2) or a PPARδ-binding fragment thereof
are mixed under conditions in which PPARδ and AMPK (or its
subunit(s) or functional fragments) normally interact (e.g., co-
immunoprecipitate). One of the binding partners is labeled with a
marker such as biotin, fluorescein, EGFP, or enzymes to allow easy
detection of the labeled component. The unlabeled binding partner
is adsorbed to a support, such as a microtiter well or beads. Then,
the labeled binding partner is added to the environment where the
unlabeled binding partner is immobilized under conditions suitable
for interaction between the two binding partners. One or more test
compounds, such as compounds in one or more of the above-described
libraries, are separately added to individual microenvironments
containing the interacting binding partners. Agents capable of
affecting the interaction between the binding partners are
identified, for instance, as those that increase or decrease (e.g.,
increase) retention or binding of the signal (i.e., labeled binding
partner) in the reaction microenvironment, for example, in a
microtiter well or on a bead for example. As discussed previously,
combinations of agents can be evaluated in an initial screen to
identify pools of agents to be tested individually, and this
process is easily automated with currently available technology.

[00108]   In other embodiments, solution phase selection can be
used to screen large complex libraries for agents that specifically
affect protein-protein interactions (see, e.g., Boger et al.,
Bioorg. Med. Chem. Lett., 8(17):2339-2344, 1998); Berg et al.,
Proc. Natl. Acad. Sci., 99(6):3830-3835, 2002). In one such
example, each of two proteins that are capable of physical
interaction (for example, PPARδ (or AMPK-binding fragments thereof)
and AMPK or AMPKα1 or AMPKα2 (or PPARδ-binding fragments of any
thereof) are labeled with fluorescent dye molecule tags with

different emission spectra and overlapping adsorption spectra. When
these protein components are separate, the emission spectrum for
each component is distinct and can be measured. When the protein
components interact, fluorescence resonance energy transfer (FRET)
occurs resulting in the transfer of energy from a donor dye
molecule to an acceptor dye molecule without emission of a photon.
The acceptor dye molecule alone emits photons (light) of a
characteristic wavelength. Therefore, FRET allows one to determine
the kinetics of two interacting molecules based on the emission
spectra of the sample. Using this system, two labeled protein
components are added under conditions where their interaction
resulting in FRET emission spectra. Then, one or more test
compounds, such as compounds in one or more of the above-described
libraries, are added to the environment of the two labeled protein
component mixture and emission spectra are measured. An increase in
the FRET emission, with a concurrent decrease in the emission
spectra of the separated components indicates that an agent (or
pool of candidate agents) has affected (*e.g.*, enhanced) the
interaction between the protein components.

[00109]   Interactions between PPARδ (or AMPK-binding fragments
thereof) and AMPK or AMPKα1 or AMPKα2 (or PPARδ-binding fragments
of any thereof) also can be determined (*e.g.*, quantified) by co-
immunoprecipitation of the relevant component polypeptides (*e.g.*,
from cellular extracts), by GST-pull down assay (*e.g.*, using
purified GST-tagged bacterial proteins), and/or by yeast two-hybrid
assay, each of which methods is standard in the art. Conducting any
one or more such assays in the presence and, optionally, absence of
a test compound can be used to identify agents that improve or
enhance (or, in other embodiments, decrease or inhibit) the
interaction between PPARδ (or AMPK-binding fragments thereof) and
AMPK or AMPKα1 or AMPKα2 (or PPARδ-binding fragments of any
thereof) in the presence of a test compound as compared to in the
absence of the test compound or as compared to some other standard
or control.

[00110]   In certain embodiments, one or more AMPK (such as AMPKα1
and/or AMPKcc2)-binding fragments of PPARδ and/or one or more

PPARδ-binding fragments of AMPK (such as AMPKα1 and/or AMPKα2) are used. Polypeptide fragments having the desired binding activities can be identified by making a series of defined PPARδ fragments and/or AMPK (such as AMPKα1 or AMPKα2) fragments using methods standard in the art. For example, cDNA encoding the protein(s) of interest (e.g., PPARδ or AMPK) can be serially truncated from the 3' or 5' end (provided that a start codon is engineered into 5' truncations) using conveniently located restriction enzyme sites (or other methods) and leaving intact (or otherwise correcting) the proper reading frame. Conveniently, a nucleic acid sequence encoding an epitope tag (such as a FLAG tag) is placed in frame with (and substantially adjacent to) the truncated protein-encoding sequence to produce a nucleic acid sequence encoding an epitope-tagged protein fragment. The epitope-tagged protein fragment can be expressed in any convenient expression system (such as a bacterial expression system), isolated or not, and mixed with a sample containing a protein or other protein fragment to which the epitope-tagged protein fragment may bind. An antibody specific for the tag (or other region of the protein fragment) can be used to immunoprecipitate the fragment of interest together with any protein(s) or protein fragment(s) that bind to it. Protein(s) or protein fragment(s) that bind to the epitope-tagged protein fragment of interest can be particular identified, e.g., by Western blot.

[00111]   In particular methods, the formation of a PPARδ-AMPK (such as AMPKα1 and/or AMPKcc2) complex (including complexes including one or both of PPARδ-binding AMPK fragments and/or AMPK-binding PPARδ fragments) or the affinity of PPARδ (or AMPK-binding fragments thereof) and AMPK (or PPARδ-binding fragments thereof) for each other is increased when the amount of such complex or the binding affinity is at least 5%, at least 10%, at least 20%, at least 30%, at least 50%, at least 100% or at least 250% higher than a control measurement (e.g., in the same test system prior to addition of a test agent, or in a comparable test system in the absence of a test agent). In other particular methods, the formation of a PPARδ-AMPK (such as AMPKα1 and/or AMPKcc2) complex

42

(including complexes including one or both of PPARδ-binding AMPK fragments and/or AMPK-binding PPARδ fragments) or the affinity of PPARδ (or AMPK-binding fragments thereof) and AMPK (or PPARδ-binding fragments thereof) for each other is decreased when the amount of such complex or the binding affinity is at least 5%, at least 10%, at least 20%, at least 30%, at least 50%, at least 100% or at least 250% lower than a control measurement (e.g., in the same test system prior to addition of a test agent, or in a comparable test system in the absence of a test agent).

[00112]   Disclosed are methods of screening test agents for those that affect (e.g., increase or decrease) AMPK (e.g., AMPKα1 and/or AMPKα2)-dependent phosphorylation of the PPARδ complex. Agents that affect AMPK-dependent phosphorylation of the PPARδ complex can be identified by a variety of assays, such adaptations of solid-phase- or solution-based assays described above, where the end point to be detected is phosphorylation of one or more components of the PPARδ complex.

[00113]   Methods for detecting protein phosphorylation are conventional (see, e.g., Gloffke, The Scientist, 16(19):52, 2002; Screaton et al., Cell, 119:61-74, 2004) and detection kits are available from a variety of commercial sources (see, e.g., Upstate (Charlottesville, VA, USA), Bio-Rad (Hercules, CA, USA), Marligen Biosciences, Inc. (Ijamsville, MD, USA), Calbiochem (San Diego, CA, USA). Briefly, phosphorylated protein (e.g., phosphorylation of one or more components of the PPARδ complex) can be detected using stains specific for phosphorylated proteins in gels. Alternatively, antibodies specific phosphorylated proteins can be made or commercially obtained. Antibodies specific for phosphorylated proteins can be, among other things, tethered to the beads (including beads having a particular color signature) or used in ELISA or Western blot assays.

[00114]   In one example, a PPARδ complex (or a fragment thereof containing an AMPK phosphorylation site) and AMPK or one or more of it subunits (such as AMPKα1 and/or AMPKcc2) or functional fragments thereof that are capable of phosphorylation are mixed under conditions whereby a PPARδ complex is phosphorylated by AMPK. A

PPARδ complex is adsorbed to a support, such as a microtiter well or beads. Then, AMPK (or its one or more subunits (such as AMPKα1 and/or AMPKcc2) or phosphorylation-capable fragments thereof) is added to the environment where the complex is immobilized. A phosphate donor typically is also included in the environment. The phosphate to be donated, optionally, can be labeled. One or more test compounds, such as compounds in one or more of the above-described libraries, are separately added to the individual microenvironments. Agents capable of affecting AMPK-dependent phosphorylation are identified, for instance, as those that enhance (or inhibit) phosphorylation of immobilized PPARδ complex. In embodiments involving a labeled phosphate donor, phosphorylation of immobilized PPARδ complex can be determined by retention or binding of a labeled phosphate in the reaction microenvironment, for example, in a microtiter well or on a bead for example. In other embodiments, such reactions can take place in solution (*i.e.*, with no immobilized components), PPARδ complex can be isolated from the solution (*e.g.*, by immunoprecipitation with PPARδ- specific or phosphate-specific antibodies), and its level of phosphorylation in the presence (and, optionally, absence) of one of more test agents determined as previously discussed. In yet another embodiment, the phosphorylation of an AMPK is measured, wherein an agent that modulates AMPK activity is thus identified as an AMPK agonist.

[00115]   In particular methods, the phosphorylation of a PPARδ complex is increased when such posttranslational modification is detectably measured or when such posttranslational modification is at least 20%, at least 30%, at least 50%, at least 100% or at least 250% higher than control measurements (*e.g.*, in the same test system prior to addition of a test agent, or in a comparable test system in the absence of a test agent, or in a comparable test system in the absence of AMPK).

[00116]   In particular methods, the phosphorylation of PPARδ complex is decreased when such posttranslational modification is detectably reduced or when such posttranslational modification is at least 20%, at least 30%, at least 50%, at least 100% or at least 250% lower than control measurements (*e.g.*, in the same test system

prior to addition of a test agent, or in a comparable test system
in the absence of a test agent, or in a comparable test system in
the absence of AMPK).

[00117]   A PPARδ polypeptide useful in a disclosed screening
method is any known PPARδ receptor. Also useful in the disclosed
screening methods are homologs, functional fragments, or functional
variants of a PPARδ that retains at least AMPK-binding activity as
described herein for a prototypical PPARδ polypeptide (see Example
6).

The amino acid sequences of prototypical PPARδ polypeptides (and
PPARδ-encoding nucleic acid sequences) are well known. Exemplary
PPARδ amino acid sequences and PPARδ-encoding nucleic acid
sequences are described, for instance, in U.S. Patent No.
5,861,274, and U.S. Pat. Appl. Pub. No. 20060154335 (each of which
is expressly incorporated herein by reference), and in GenBank
Accession Nos. NP_035275 (GI:33859590)(Mus musculus amino acid
sequence); NM_011145.3 (GI:89001112)(Mus musculus nucleic acid
sequence); NP_006229 (GI:5453940)(Homo sapiens amino acid
sequence); NM_006238.3 (GI:89886454)(Homo sapiens nucleic acid
sequence); NP_037273 (GI:69S13S4)(Rattus norvegicus amino acid
sequence); NM_013141.1 (GL6981383) (Rattus norvegicus nucleic acid
sequence); NP_990059 (gi45382025) (Gallus gallus amino acid
sequence) or NM_204728.1 (GI:45382024)(Gallus gallus nucleic acid
sequence). In some method embodiments, a PPARδ homolog or
functional variant shares at least 60% amino acid sequence identity
with a prototypical PPARδ polypeptide; for example, at least 75%,
at least 80%, at least 85%, at least 90%, at least 95%, or at least
98% amino acid sequence identity with an amino acid sequence as set
forth in U.S. Patent No. 5,861,274, U.S. Pat. Appl. Pub. No.
20060154335, or GenBank Accession No. NP_035275 (GI:33859590)(Mus
musculus amino acid sequence); NP_006229 (GI:5453940)(Homo sapiens
amino acid sequence); NP_037273 (GI.69$13$4)(Rattus norvegicus
amino acid sequence); or NP_990059 (gi45382025) {Gallus gallus
amino acid sequence). In other method embodiments, a PPARδ homolog
or functional variant has one or more conservative amino acid
substitutions as compared to with a prototypical PPARδ polypeptide;

for example, no more than 3, 5, 10, 15, 20, 25, 30, 40, or 50
conservative amino acid changes compared to an amino acid sequence
as set forth in U.S. Patent No. 5,861,274, U.S. Pat. Appl. Pub. No.
20060154335, or GenBank Accession No. NP_035275
(GI:33859590)(M***Unknown: ***s musculus amino acid sequence);
NP_006229 (GI:5453940)(Homo sapiens amino acid sequence); NP_037273
(GI.69$13$4)(Rattus norvegicus amino acid sequence); or NP_990059
(gi45382025) (Gallus gallus amino acid sequence).

[00118]   Some method embodiments involve a PPARδ functional
fragment (such as an AMPK-binding fragment), which can be any
portion of a full-length known PPARδ polypeptide, including, e.g.,
about 20, about 30, about 40, about 50, about 75, about 100, about
150 or about 200 contiguous amino acid residues of same; provided
that the fragment retains a PPARδ function of interest (e.g., AMPK
binding). PPARδ encompasses known functional motifs (such as
ligand-binding domain, a DNA-binding domain, and a transactivation
domain).

[00119]   Mammalian AMP-activated kinase (AMPK) is a
heterotrimeric protein composed of 1 alpha subunit, 1 beta subunit,
and 1 gamma subunit. There are, at least, two known isoforms of the
alpha subunit (α1 and α2). AMPKα1 and AMPKα2 have 90% amino acid
sequence identity within their catalytic cores but only 61% in
their C-terminal tails (see Online Mendelian Inheritance in Man
(OMIM) Database Accession No. 602739; publicly available at the
following website: ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=602739).

[00120]   An AMPK (such as AMPKα1 and/or AMPKα2) polypeptide
useful in a disclosed screening method is any known AMPK protein or
subunit thereof (such as AMPKα1 and/or AMPKα2). Also useful in the
disclosed screening methods are homologs, functional fragments, or
functional variants of an AMPK protein or subunit thereof (such as
AMPKα1 and/or AMPKα2) that retains at least PPARδ-binding activity
as described herein (see Example 6). The amino acid sequences of
prototypical AMPK subunits (such as AMPKα1 and/or AMPKα2) (and
nucleic acids sequences encoding prototypical AMPK subunits (such
as AMPKα1 and/or AMPKα2)) are well known. Exemplary AMPKα1 amino
acid sequences and the corresponding nucleic acid sequences are

described, for instance, in GenBank Accession Nos. NM_206907.3
(GI:94557298)(Homo sapiens transcript variant 2 REFSEQ including
amino acid and nucleic acid sequences); NM_006251.5
(GI:94557300)(Homo sapiens transcript variant 1 REFSEQ including
amino acid and nucleic acid sequences); NM_001013367.3
(GI:94681060)(Mus musculus REFSEQ including amino acid and nucleic
acid sequences); NMJ)01039603.1 (GI:88853844)(Gallus gallus REFSEQ
including amino acid and nucleic acid sequences); and NM_019142.1
(GI: 11862979XRaJfWS norvegicus REFSEQ including amino acid and
nucleic acid sequences). Exemplary AMPKα2 amino acid sequences and
the corresponding nucleic acid sequences are described, for
instance, in GenBank Accession Nos. NM_006252.2 (GI:46877067)(Homo
sapiens REFSEQ including amino acid and nucleic acid sequences);
NM_178143.1 (GI:54792085)(Mus musculus REFSEQ including amino acid
and nucleic acid sequences); NM_001039605.1 (GI:88853850)(Gallus
gallus REFSEQ including amino acid and nucleic acid sequences); and
NM_214266.1 (GI:47523597)(Mus musculus REFSEQ including amino acid
and nucleic acid sequences).

[00121]   In some method embodiments, a homolog or functional
variant of an AMPK subunit shares at least 60% amino acid sequence
identity with a prototypical AMPKα1 and/or AMPKα2 polypeptide; for
example, at least 75%, at least 80%, at least 85%, at least 90%, at
least 95%, or at least 98% amino acid sequence identity with an
amino acid sequence as set forth in the GenBank Accession Nos.
NM_206907.3; NM_006251.5; NMJ)01013367.3; NM_001039603.1;
NM_019142.1; NM_006252.2; NM_178143.1; NM_001039605.1; or
NM_214266.1. In other method embodiments, a homolog or functional
variant of an AMPK subunit has one or more conservative amino acid
substitutions as compared to a prototypical AMPKα1 and/or AMPKα2
polypeptide; for example, no more than 3, 5, 10, 15, 20, 25, 30,
40, or 50 conservative amino acid changes compared to an amino acid
sequence as set forth in as set forth in GenBank Accession Nos.
NM_206907.3; NM_006251.5; NM_001013367.3; NM_001039603.1;
NM_019142.1; NM_006252.2; NM_178143.1; NM_001039605.1; or
NM_214266.1. Exemplary conservative amino acid substitutions have
been previously described herein.

[00122]   Some method embodiments involve a functional fragment of
AMPK or a subunit thereof (such as AMPKα1 and/or AMPKα2), including
a PPARδ-binding fragment or a fragment with PPARδ phosphorylation
activity. Functional fragments of AMPK or a subunit thereof (such
as AMPKα1 and/or AMPKα2) can be any portion of a full-length or
intact AMPK polypeptide complex or subunit thereof (such as AMPKα1
and/or AMPKα2), including, e.g., about 20, about 30, about 40,
about 50, about 75, about 100, about 150 or about 200 contiguous
amino acid residues of same; provided that the fragment retains at
least one AMPK (or AMPKα1 and/or AMPKα2) function of interest
(e.g., PPARδ binding and/or PPARδ phosphorylation activity).
Protein-protein interactions between PPARδ and AMPK are believed to
involve, at least, an AMPKα subunit (such as AMPKα1 and/or AMPKα2).
Moreover, because PPARδ specifically binds both AMPKα1 and AMPKα2
(see Example 6), such interaction likely is mediated by the
portions of these AMPKα isoforms that share the most sequence
homology (as discussed above). Accordingly, in some method
embodiments, an AMPK PPARδ-binding fragment includes a functional
fragment encompassing (or consisting of) the catalytic core domain
of an alpha subunit of AMPK (such as AMPKα1 and/or AMPKα2).

[00123]   An "isolated" biological component (such as a
polynucleotide, polypeptide, or cell) has been purified away from
other biological components in a mixed sample (such as a cell or
tissue extract). For example, an "isolated" polypeptide or
polynucleotide is a polypeptide or polynucleotide that has been
separated from the other components of a cell in which the
polypeptide or polynucleotide was present (such as an expression
host cell for a recombinant polypeptide or polynucleotide).

[00124]   The term "purified" refers to the removal of one or more
extraneous components from a sample. For example, where recombinant
polypeptides are expressed in host cells, the polypeptides are
purified by, for example, the removal of host cell proteins thereby
increasing the percent of recombinant polypeptides in the sample.
Similarly, where a recombinant polynucleotide is present in host
cells, the polynucleotide is purified by, for example, the removal

of host cell polynucleotides thereby increasing the percent of recombinant polynucleotide in the sample.

[00125] Isolated polypeptides or nucleic acid molecules, typically, comprise at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95% or even over 99% (w/w or w/v) of a sample.

[00126] Polypeptides and nucleic acid molecules are isolated by methods commonly known in the art and as described herein. Purity of polypeptides or nucleic acid molecules may be determined by a number of well-known methods, such as polyacrylamide gel electrophoresis for polypeptides, or agarose gel electrophoresis for nucleic acid molecules.

[00127] The similarity between two nucleic acid sequences or between two amino acid sequences is expressed in terms of the level of sequence identity shared between the sequences. Sequence identity is typically expressed in terms of percentage identity; the higher the percentage, the more similar the two sequences.

[00128] Methods for aligning sequences for comparison are well known in the art. Various programs and alignment algorithms are described in: Smith and Waterman, Adv. Appl. Math. 2:482, 1981; Needleman and Wunsch, J. Mol. Biol. 48:443, 1970; Pearson and Lipman, Proc. Natl. Acad. ScL USA 85:2444, 1988; Higgins and Sharp, Gene 73:237-244, 1988; Higgins and Sharp, CABIOS 5:151-153, 1989; Corpet *et al.*, Nucleic Acids Research 16:10881-10890, 1988; Huang, *et al.*, Computer Applications in the Biosciences 8:155-165, 1992; Pearson *et al.*, Methods in Molecular Biology 24:307-331, 1994; Tatiana *et al.*, (1999), FEMS Microbiol. Lett., 174:247-250, 1999. Altschul *et al.* present a detailed consideration of sequence alignment methods and homology calculations (J. Mol. Biol. 215:403-410, 1990). The National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST™, Altschul *et al.*, J. Mol. Biol. 215:403-410, 1990) is available from several sources, including the National Center for Biotechnology Information (NCBI, Bethesda, MD) and on the Internet, for use in connection with the sequence-analysis programs blastp, blastn, blastx, tblastn and tblastx. A description of how to determine sequence identity using

this program is available on the internet under the help section
for BLAST™.

[00129]   For comparisons of amino acid sequences of greater than
about 30 amino acids, the "Blast 2 sequences" function of the
BLAST™ (Blastp) program is employed using the default BLOSUM62
matrix set to default parameters (cost to open a gap [default = 5];
cost to extend a gap [default = 2]; penalty for a mismatch [default
= -3]; reward for a match [default = 1]; expectation value (E)
[default = 10.0]; word size [default = 3]; number of one-line
descriptions (V) [default = 100]; number of alignments to show (B)
[default = 100]). When aligning short peptides (fewer than around
30 amino acids), the alignment should be performed using the Blast
2 sequences function, employing the PAM30 matrix set to default
parameters (open gap 9, extension gap 1 penalties). Proteins with
even greater similarity to the reference sequences will show
increasing percentage identities when assessed by this method.

[00130]   For comparisons of nucleic acid sequences, the "Blast 2
sequences" function of the BLAST™ (Blastn) program is employed
using the default BLOSUM62 matrix set to default parameters (cost
to open a gap [default = 11]; cost to extend a gap [default = 1];
expectation value (E) [default = 10.0]; word size [default = 11];
number of one-line descriptions (V) [default = 100]; number of
alignments to show (B) [default = 100]). Nucleic acid sequences
with even greater similarity to the reference sequences will show
increasing percentage identities when assessed by this method.

[00131]   Specific binding refers to the particular interaction
between one binding partner (such as a binding agent) and another
binding partner (such as a target). Such interaction is mediated by
one or, typically, more noncovalent bonds between the binding
partners (or, often, between a specific region or portion of each
binding partner). In contrast to non-specific binding sites,
specific binding sites are saturable. Accordingly, one exemplary
way to characterize specific binding is by a specific binding
curve. A specific binding curve shows, for example, the amount of
one binding partner (the first binding partner) bound to a fixed
amount of the other binding partner as a function of the first

binding partner concentration. As the first binding partner concentration increases under these conditions, the amount of the first binding partner bound will saturate. In another contrast to non-specific binding sites, specific binding partners involved in a direct association with each other (e.g., a protein-protein interaction) can be competitively removed (or displaced) from such association (e.g., protein complex) by excess amounts of either specific binding partner. Such competition assays (or displacement assays) are very well known in the art.

[00132]   The disclosure also provides methods for identifying agents useful for effecting muscle tone or mass. The disclosure provides endurance gene signatures (see, e.g., Table 2 and Table 4) comprising genes that are modulated in the presence of AICAR or GW1516, or a combination of AICAR and GW1516. Such gene signature are useful for identifying agents that provides an increase in muscle tone or mass and thereby can modulate physical endurance. A GW-AI endurance gene signature refers to a set of genes described in Table 4 or a subset thereof. A GW-TR endurance gene signature refers to a set of genes described in Table 2 or a subset thereof.

[00133]   As depicted in Figures 6 and 8, an overlap of gene expression profile for each of the agonists is provided. An agent to be tested can be administered to a subject and the gene expression profile measure in a muscle sample (e.g., a biopsy) or other biological sample. Where the gene expression profile comprises a set of the endurance gene signatures (e.g., the overlapping 52 genes associated with GW1516 and AICAR administration, see Table 4) or a subset thereof such an agent can be identified as an agent or drug useful for treating or modifying muscle activity.

EXAMPLES

[00134]   The following examples are provided to illustrate certain particular features and/or embodiments. These examples should not be construed to limit the invention to the particular features or embodiments described.

Example 1

[00135]   Administration of PPARδ agonist does not enhance
physical performance in non-exercised subjects. Wang et a.
previously demonstrated that skeletal muscle-specific expression of
a constitutively active form of PPARδ receptor resulted in
transgenic mice with skeletal muscles that had an increased number
of slow, oxidative (type I) muscle fibers and markedly increased
running endurance (Wang et al., PLoC Biol., 2:e294, 2004). This
Example demonstrates that administration of a PPARδ agonist
(GW1516) to non-transgenic mice also results in the expression in
skeletal muscle of some biomarkers of oxidative metabolism.
However, in unexpected contrast to the results obtained by genetic
activation of the PPARδ pathway, PPARδ activation by
pharmacological treatment did not modify fiber-type composition of
skeletal muscle, nor improve running endurance in non-transgenic,
sedentary (also referred to as "non-exercised" or "untrained")
mice. Male C57B/6J mice (8 wks old) were obtained from Jackson
Laboratory and housed in the Salk Institute animal care facility.
The animals were acclimated to their surroundings for one week
prior to experimentation, and had access at all times to standard
mouse chow and water ad libitum.

[00136]   Mice were acclimated to moderate treadmill running (10
m/min for 15 min) every other day for 1 week. After acclimation,
basal running endurance was determined by placing each mouse on a
treadmill, gradually increasing the speed from 0 to 15 m/min, and
maintaining 15 m/min until the mouse was exhausted. The time and
distance run until exhaustion were recorded as the basal endurance
values (Week 0).

[00137]   Mice then were treated once per day for 4 weeks with
vehicle or the PPARδ agonist, GW 1516 (5 mg/kg). Treatments were
administered orally. During the treatment period, mice were housed
in standard laboratory cages and received only the amount of
physical activity that could be had by normal movements about such
cage.

[00138]   Animals were euthanized by carbon dioxide asphyxiation
72 hours after the final treatment. Gastrocnemius and quadriceps
muscles were isolated, frozen and stored at -80°C for future

analysis. Total RNA was prepared from quadriceps muscle using TRIzoL™ reagent (Invitrogen, Carlsbad, CA, USA) in conformance with manufacturer's instructions. Real time quantitative PCR (QPCR) was used to determine expression levels of uncoupling protein 3 (UCP3), muscle carnitine palmitoyl transferase I (mCPT I) and pyruvate dehydrogenase kinase 4 (PDK4) using primers known to those of ordinary skill in the art.

[00139]   As shown in FIG. 1A, four weeks of GW1516 treatment induced the expression of UCP3, mCPT I, and PDK4, in quadriceps muscle of treated mice (compare V to GW). These changes in gene expression were detected as early as 4 days after treatment and with drug concentrations ranging from 2-5 mg/kg/day.

[00140]   Moreover, in the gene expression studies, maximal effects of PPARδ activation were detected in pre-dominantly fast-twitch (quadricep and gastrocnemius) but not slow- twitch (soleus) muscles.

[00141]   Using primary muscle cells cultured from wild type and PPARδ null mice (Chawla et al., Proc. Natl. Acad. ScL U SA. 100(3): 1268-73, 2003; Man et al., J. Invest. Dermatol. 2007; Rando and Blau, J. Cell. Biol. 125(6): 1275-87, 1994), it was confirmed that the induction of oxidative genes by GW1516 is mediated via activation of PPARδ in skeletal muscles (FIGS. 1B-D). Moreover, this is similar to the expression changes found in the same gene set in muscles from mice expressing the constitutively active VP1β-PPARδ transgene (Wang et al., PLoC Biol, 2:e294, 2004) (FIG. IA, see TG). Collectively, these results indicate that pharmacological activation of PPARδ can initiate an oxidative response in adult skeletal muscle.

[00142]   Expression of biomarkers characteristic of an oxidative phenotype in skeletal muscle, typically, has been correlated with increased oxidative performance (e.g., increased running endurance) of such skeletal muscle. This correlation was observed, for instance, in the VP1β-PPARδ transgenic mouse (Wang et al., PLoC Biol., 2:e294, 2004). For this and other reasons, it was expected that GW1516 treatment similarly would increase running performance. Accordingly, to determine the functional effects of ligand, age and

weight matched cohorts of treated and control mice were subjected
to an endurance treadmill performance test before (week 0) and
after (week 5) treatment. Following four weeks of treatment and
housing in standard laboratory cages without additional exercise,
the running endurance of GW1516-treated and control mice again was
determined as described above. Remarkably, and despite expectations
for improvement, GW1516-treated mice did not significantly differ
from controls in either the time spent or distance run on the
treadmill prior to exhaustion (FIG. 1E). Furthermore, long-term
drug treatment of up to 5 months also did not change running
endurance.

[00143]   These results indicate that although in non-trained
adult muscle pharmacological activation of PPARδ induces some
transcriptional changes, it fails to alter either fiber type
composition or endurance. In summary, pharmacologic activation of
the PPARδ genetic program in adult C57B1/6J mice is insufficient to
promote a measurable enhancement of treadmill endurance.

Example 2

[00144]   Administration of PPARδ agonist remodels skeletal muscle
in exercised-trained subjects. Fiber type proportions in skeletal
muscle are believed to be determined by heredity and environmental
factors, such as physical activity level (Simoneau and Bouchard,
FASEB J., 9(11): 1091-1095, 1995; Larsson and Ansved, Muscle Nerve,
8(8):714-722, 1985). Endurance exercise training is known to
remodel the skeletal muscle by increasing type I slow-twitch
fibers, oxidative enzymes, and mitochondrial density, which
progressively alter performance (Holloszy *et al.*, J. Appl. Physiol.
56:831-8, 1984; Booth *et al.*, Physiol Rev. 71:541-85, 1991; Schmitt
et al., . Physiol. Genomics. 15:148-57, 2003; Yoshioka *et al.*,
FASEB J. 17:1812-9, 2003; Mahoney *et al.*, Phys. Med. Rehabil Clin.
N. Am. 16:859-73, 2005; Mahoney *et al.*, FASEB J. 19:1498-500, 2005;
Siu *et al.*, J. Appl Physiol. 97:277-85, 2004; Gamier *et al.*, FASEB
J. 19:43-52, 2005; Short *et al.*, J Appl Physiol. 99:95-102, 2005;
Timmons *et al.*, FASEB J. 19: 750-60, 2005). This example
demonstrates that PPARδ agonist treatment influences skeletal
muscle on a molecular level.

[00145]   To determine whether co-administration of GW1516 in the context of endurance exercise can enhance changes in fiber type composition and mitochondrial biogenesis, the effect of GW1516 treatment on muscle fiber-type composition was determined by meta-chromatic staining of cryo-sections of gastrocnemius as described by Wang et al (Phys Biol, 2:e294, 2004). Meta-chromatic staining was used, following a routine myofibrillar ATPase reaction, to demonstrate quantitative differences in phosphate deposition among different skeletal muscle fiber types and, thereby, differentiate skeletal muscle fiber types (Doriguzzi et al., Histochem., 79(3):289-294, 1983; Ogilvie and Feeback, Stain Technol, 65(5):231-241, 1990). In this assay, muscle fibers with high ATPase activity {e.g., type I (slow oxidative) muscle fibers) are darkly stained.

[00146]   As shown in FIG. 2A, there was no significant difference in the proportion of type I (slow, oxidative) muscle fibers in the gastrocnemius muscles of vehicle- and GW1516-treated sedentary mice. In contrast, hind limb muscles of VP16-PPARS transgenic mice exhibited an increased number of type I muscle fibers when assayed by monochromatic staining. In trained mice, GW1516 increased the proportion of type I fibers (by ~38%) compared to the vehicle-treated sedentary mice (FIGS. 2A and 2B). Therefore, administration of a PPARδ agonist (e.g., GW1516) alone to sedentary subjects does not significantly affect the number of type I (slow-twitch, oxidative) muscle fibers in hind limb muscles, but can increase the number of type I muscle fibers in hind limb muscles of trained subjects.

[00147]   In addition to its effects on the fiber type, exercise training increased skeletal muscle mitochondrial biogenesis, which can be measured as a function of mitochondrial DNA expression levels using quantitative real time PCR (QPCR). Mitochondrial DNA expression levels were determined in muscles of V, GW, Tr, and GW+Tr subjects using quantitative real time PCR. As shown in FIG. 2C, similar to type I fiber changes, mitochondrial DNA expression was not changed by drug alone but was increased by approximately 50% with the combination of exercise and GW1516 treatment (FIG. 2C). Such an increase is known to contribute to enhanced endurance

capacity (*e.g.*, Holloszy, Med. Sci. Sports 7:155-64, 1975). Slow-twitch and fast- twitch muscle fiber types also can be distinguished by myosin isoform expression (Gauthier and Lowey, J. Cell Biol. 81:10-25, 1979; Fitzsimons and Hoh, Biochem. J. 193:229-33, 1981). Myosin isoform expression in skeletal muscle adapts to various conditions, such as changes in muscle mechanics, muscle innervation, or exercise paradigm (for review, see, *e.g.*, Baldwin and Haddad, J. Appl. Physiol., 90(1):345-57, 2001; Baldwin and Haddad, Am. J. Phys. Med. Rehabil., 81(11 Suppl):S40-51, 2002; Parry, Exerc. Sport Sci Rev., 29(4): 175-179, 2001). The effect of GW1516 administration on myosin heavy chain (MHC) expression (MHC I, MHC Ha, MHC lib) was determined by methods known to those of ordinary skill in the art. GW1516 treatment in sedentary mice increased the expression of MHC I (a marker of slow-twitch, oxidative muscle fibers) and decreased the expression of MHC lib (a marker of fast-twitch, glycolytic muscle fibers) as compared to vehicle-treated, control mice. In comparison, GW1516 treatment did not alter the expression of MHC Ha (a marker of fast-twitch oxidative/glycolytic muscle fibers) in sedentary mice. Therefore, at least at the transcriptional level, the PPARδ agonist was capable of inducing some proteins characteristic of a slow-twitch muscle fiber phenotype.

[00148]   Expression of constitutively active PPARδ in the skeletal muscles of VP1β-PPARδ transgenic mice resulted in a "long-distance running phenotype" with "profound and coordinated increases in oxidative enzymes, mitochondrial biogenesis and production of specialized type I fiber contractile proteins-the three hallmarks of muscle fiber type switching" (Wang *et al.*, PLoC Biol., 2:e294, 2004). In contrast, pharmacological activation of PPARδ in normal subjects only partially recapitulated VP1β-PPARδ transgenesis by regulating some metabolic genes. Markedly, administration of a PPARδ agonist to sedentary subjects did not lead to a change in fiber type specification (as measured by monochromatic staining) or enhance exercise endurance. Transgenic over- expression of activated PPARδ at birth pre-programs the nascent myofibers to trans- differentiate into slow-twitch fibers,

thus imparting a high basal endurance capacity to adult transgenic mice. In contrast, since fiber type specification is completed prior to exposure of adults to PPARδ agonist, the potential plasticity of muscle to drug treatment alone is virtually non-existent.

[00149]   This example illustrates that the genetic or pharmacologic activation of the PPARδ regulatory program in skeletal muscles of adult, sedentary subjects does not have the same outcome. The ability to genetically manipulate skeletal muscle specification by activation of the PPARδ receptor in a transgenic mouse from early development in the absence of exercise is not necessarily predictive of the result of pharmacologically activating the PPARδ program in the sedentary, normal adult. The cellular "template" for PPARδ effects on skeletal muscle is very different in a normal subject as compared to a genetically engineered transgenic subject. For example, in a normal adult, muscle fiber specification of individual muscle groups is already determined and the connections between muscle fibers and spinal motor neurons are established prior to pharmacological activation of the PPARδ-regulated program. In the transgenic mouse, the constitutively active PPARδ transgene is active all the while muscle fiber specification is being determined and connections between muscle fibers and motor neurons are being made. In addition, the effects of activation of endogenous PPARδ receptor by a single daily dose of a PPARδ agonist, which is expect to have a transient peak exposure followed by clearance, likely are much different from the effects of the constitutive activation of a VP1β-PPARδ transgene.

Example 3

[00150]   The combination of PPARδ agonist treatment and exercise training significantly affected fatty acid metabolism and markers of fatty acid oxidation. In addition to affecting the contractile apparatus of skeletal muscle, exercise training also increases skeletal muscle mitochondrial density (*e.g.*, Freyssenet *et al.*, Arch. Physiol. Biochem., 104(2): 129-141, 1996). This Example illustrates that PPARδ agonist treatment (*e.g.*, GW1516) in

exercise-trained subjects affected fatty acid metabolism in exercised muscle. The effects of GW1516 treatment and exercise, alone or in combination, on components of the oxidative metabolism of fatty acids were determined by measuring gene expression levels of selective biomarkers for fatty acid β-oxidation (FAO). Male C57B/6J mice (8-10 wks old) were randomly divided into four groups (nine per group): (i) vehicle-treated and sedentary (V), (ii) GW1516-treated and sedentary (GW), (iii) vehicle-treated and exercise trained (Tr) and (iv) GW1516-treated and exercise trained (GW+Tr). Mice in all groups were acclimated to moderate treadmill running and basal running endurance was determined as described in Example 1. Thereafter, mice in the exercise-trained groups received four weeks (5 days/week) of exercise training on a treadmill inclined at 5 degrees. Intensity and time of training were gradually increased. At the end of four weeks, all exercise-trained mice were running for 50 min/day at 18 m/min. Vehicle or GW1516 was administered to the respective exercise-treated or sedentary groups as described in Example 1. Unless otherwise noted, V, GW, Tr and GW+Tr subjects described in this and the examples below were similarly treated. At the end of the drug treatment and/or training protocol (Week 5) 6 mice per group were subjected to the running test. These interventions do not affect body weight and food intake in mice. RNA was prepared real time quantitative PCR performed as described in Example 1.

[00151]   Confirming the results obtained in Example 1, UCP3, mCPT I, and PDK4 were upregulated by GW1516 but showed no further induction with exercise (see FIGS. 1A and 3A). Unexpectedly, a second set of genes were identified that showed no response to exercise or GW1516 alone but were robustly induced by the combination. This intriguing response profile includes a series of genes involved in the regulation of fatty acid storage (such as steroyl-CoA-desaturase (SCD1), fatty acyl coenzyme A synthase (FAS) and serum response element binding protein Ic (SREBPIc)) and fatty acid uptake [such as the fatty acid transporter (FAT/CD36) and lipoprotein lipase (LPL)] adding a new set of target genes to exercise and drug treated mice (FIGS. 3B, 3C and 6A-C).

[00152]   In addition to gene expression, protein expression was
determined for selective oxidative biomarkers including myoglobin,
UCP3, cytochrome c (CYCS) and SCD1, using Western blotting. Protein
homogenates were prepared from quadriceps muscle, separated by SDS
polyacrylamide gel electrophoresis, transferred to blotting
membrane and probed with antibodies specific for myoglobin (Dako),
UCP3 (Affinity Bioreagents), cytochrome c (Santacruz) SCD1
(Santacruz), and, as a loading control, tubulin (Sigma). A robust
up regulation of myoglobin, UCP3, cytochrome c, and SCD1 protein
expression was observed with combined exercise and GW1516 treatment
in comparison to treatment with the PPARδ agonist or exercise alone
(FIG. 3D).

[00153]   Altered triglycerides can be used to access changes in
muscle oxidative capacity. Muscle triglyceride (mTG) content was
measured as previously described (Wang *et al.*, PLoC Biol, 2:e294,
2004) using a kit from Thermo Electron Corporation. As shown in
FIG. 4, mTG content was comparable between vehicle and GW1516-
treated sedentary mice and was substantially increased in muscle of
mice receiving only exercise training. In contrast, dramatic
increase in triglycerides in exercised muscle was completely
reversed in GW1516-treated exercise trained mice, indicating
increased fat utilization (FIG. 4). Gene and/or protein expression
that is induced by a combination of exercise and drug treatment
(e.*g.*, PPARδ agonist administration) but not by either input alone
is believed to be a new discovery. This type of response can be
used to further characterize the intersection of pharmacologic and
physiologic genetic networks. For example, one or more genes and/or
proteins uniquely regulated by one or more drugs {e.*g.*, PPARδ
agonists) and exercise can be used as markers, for instance, of
illicitly boosting performance in professional and/or amateur
athletes.

Example 4

[00154]   Administration of PPARδ agonist enhances the physical
performance of exercise-trained subjects. As described in Example
1, although GW1516 treatment induces wide- spread genomic changes
associated with oxidative metabolism, nonetheless alone it failed

to increase running endurance. This finding was unexpected because it was known that constitutive activation of the PPARδ gene network (in the VP1β-PPARδ transgenic mouse) lead to a distance-running phenotype (familiarly, a "marathon mouse"). On the other hand, as surprisingly shown in Example 3, PPARδ agonist (e.g., GW1516) treatment in conjunction with exercise produced an enriched remodeling program that included a series of transcriptional and post-translational adaptations in the skeletal muscle. This indicates that exercise training serves as a trigger to unmask a set of PPARδ target genes. This Example provides methods used to demonstrate that administration of a PPARδ agonist (e.g., GW1516) surprisingly improves physical performance in exercised (trained) subjects.

[00155]   Male C57B/6J mice (8-10 wks old) were randomly divided into four groups (nine per group): (i) vehicle-treated and sedentary (V), (ii) GW1516-treated and sedentary (GW), (iii) vehicle-treated and exercise trained (Tr) and (iv) GW1516-treated and exercise trained (GW+Tr), acclimated to moderate treadmill running as described in Example 1, and exercise-trained as described in Example 3. At the end of the drug treatment and/or training protocol (Week 5) 6 mice per group were subjected to the running test.

[00156]   At the end of the drug treatment and/or training protocol (Week 5), running endurance of six mice per group was determined in the same manner as was basal running endurance. No follow-up endurance tests were performed on three mice in each group to confirm that changes observed in the skeletal muscle were not due to the acute run, but were related to the exercise training.

[00157]   As shown in FIGS. 5A and 5B, the same dose and duration of GW1516 treatment that failed to alter running endurance in sedentary mice, when paired with 4 weeks of exercise training, increases running time by 68% and running distance by 70% over vehicle-treated trained mice (FIGS. 5A and 5B, compare Week 5). Comparison of running time and distance before (week 0) and after (week 5) exercise and drug treatment revealed a 100% increment in

endurance capacity for individual mice, underscoring the robustness
of the combination paradigm (FIGS. 5 A and 5B). In contrast, the
same exercise protocol without concurrent GW1516 treatment did not
significantly increase running endurance in C57B1/6J mice.
Hematoxylin and eosin (H&E) staining of white adipose tissue
paraffin sections was performed as previously described (Wang *et
al.*, PLoS Biol., 2:e294, 2004; Wang *et al.*, Cell, 113:159-70,
2003). As shown in FIG. 5C, GW1516 treatment in combination with
exercise produced a significant (32%) reduction in the epididymal
fat to body weight ratio, which was further evident in the
decreased cross-sectional area of the adipocytes in the same group
(FIG. 5D). Therefore, the combined effects of GW1516 and exercise
are not restricted to muscle.

[00158]   Using the methods described in Example 2, it was also
demonstrated that the combination of GW1516 treatment and exercise
training significantly increased the number of type I muscle fibers
in exercised muscle. However, combining GW1516 treatment with
exercise did not induce additional changes in MHC I and MHC lib
expression. Therefore, although orally administered PPARδ agonist
(GW1516) alone is capable of inducing the expression of at least
some of the contractile proteins in the PPARδ-regulated gene
network (see Example 5) the transcriptional effect observed was not
sufficient to induce a post-transcriptional change in fiber-type
composition as was observed by meta-chromatic staining in GW1516-
treated, exercised mice.

[00159]   This Example illustrates that PPARδ agonist (*e.g.*,
GW1516) treatment unexpectedly augments the performance of aerobic
exercise (*e.g.*, running distance and endurance) in an exercised
subject. Endurance exercise is known to channel extra-muscular fat
to muscle triglyceride stores by inducing adipose tissue lipolysis
to meet increased oxidative demands (Despres *et al.*, Metabolism,
33:235-9, 1984; Mauriege *et al.*, Am. J. Physiol, 273:E497-506,
1997; Mader *et al.*, Int. J. Sports Med., 22:344-9, 2001; Schmitt *et
al.*, Physiol. Genomics, 15:148-57, 2003; Schrauwen-Hinderling *et
al.*, J. Clin. Endocrinol. Metab., 88:1610-6, 2003). In addition,
the induction of FAO components and selective up-regulation of

fatty acid storage and up-take components in GW1516-treated, exercised mice described in Example 3 indicate enhanced mobilization of fat as fuel in skeletal muscle.

[00160]   Therefore, combined exercise and GW1516 treatment dramatically increases muscle oxidative capacity in subjects, for example by increasing local fatty acid synthesis and/or mobilizing fatty acid stores from adipose tissue.

[00161]   This is the first demonstration of how an orally active PPARδ agonist and exercise can co-operatively re -program the muscle genome and raise endurance limits.

Example 5

[00162]   The combination of PPARδ agonist treatment and exercise training produced a unique gene expression signature. A comprehensive study of the skeletal muscle transcriptional program in V, GW, Tr and Tr+GW mice was conducted using microarray analysis. Affymetrix™ high-density oligonucleotide array mouse genome 430A 2.0 chips were used.

[00163]   Preparation of *in vitro* transcription products, oligonucleotide array hybridization, and scanning were performed in conformance with Affymetrix™-provided protocols. To minimize discrepancies due to variables, the raw expression data were scaled by using Affymetrix™ MICROARRAY SUITE™ 5.0 software, and pairwise comparisons were performed. The trimmed mean signal of all probe sets was adjusted to a user-specified target signal value (200) for each array for global scaling. No specific exclusion criteria were applied. Additional analyses were performed using the freeware program BULLFROG 7 (available on the internet Barlow-LockhartBrainMapNIMHGrant.org) and the Java-based statistical tool VAMPIRE (Hsiao *et al.*, Bioinformatics, 20:3108-3127, 2004).

[00164]   Genome-wide analysis of the quadriceps muscle revealed that GW1516 treatment, exercise, and the combination regulated 96, 113 and 130 genes, respectively (FIG. 6). Approximately 50% of the target genes regulated by GW1516 or exercise alone were the same, demonstrating that PPARδ activation of the gene network partially mimics exercise effects on the same network.

[00165]   The 130 genes regulated by the combination of GW1516
treatment and exercise training and a classification of each such
gene are shown in Table 1. The 130 regulated genes included 30 fat
metabolism genes, 5 oxygen carriers, 5 mitochondrial genes, 3
carbohydrate metabolism genes, 15 signal transduction genes, 16
transcription genes, 10 transport genes, 3 steroid biogenesis
genes, 5 heat shock genes, 2 angiogenesis genes, 5 proliferation
and apoptosis genes, 2 cytokines, and 29 others. The majority of
the genes in the exercise-trained/GW1516-treated (GW+Tr) gene
signature shown in Table 1 were induced (109/130). The 109
upregulated genes are shown in non-bold font in Table 1 (final
column >1). Down-regulated genes are shown in bold italics in Table
1 (final column <1).

TABLE 1

Genes regulated by GW1516 treatment and exercise training

FEATURE	LOCUS	DESCRIPTION	GW + Tr
		ANGIOGENESIS	
1417130_s_at	Angptl4	angiopoietin-like 4	5.495
1418762_at	*Cd55*	*CD55 antigen*	*0.56*
		CARBOHYDRATE METABOLISM	
1449088_at	Fbp2	fructose bisphosphatase 2	2.808
1423439_at	Pck1	phosphoenolpyruvate carboxykinase 1, cytosolic	3.518
1434499_a_at	Ldhb	lactate dehydrogenase B	2.541
		PROLIFERATION & APOPTOSIS	
1425621_at	Trim35	tripartite motif-containing 35	1.856
1418003_at	*1190002H23Rik*	*RIKEN cDNA 1190002H23 gene*	*0.543*
1448272_at	Btg2	B-cell translocation gene 2, anti-proliferative	1.601
1452260_at	Cidec	cell death-inducing DFFA-like effector c	4.771
1417956_at	Cidea	cell death-inducing DNA fragmentation factor, alpha subunit-like effector A	49.625
		CYTOKINES	
1426278_at	Ifi27	interferon, alpha-inducible protein 27	1.714
1421239_at	Il6st	interleukin 6 signal transducer	1.972
		FAT METABOLISM	
1448318_at	Adfp	adipose differentiation related protein	2.009
1424729_at	BC054059	cDNA sequence BC054059	5.08
1424937_at	2310076L09Rik	RIKEN cDNA 2310076L09 gene	1.868
1450010_at	Hsd17b12	hydroxysteroid (17-beta) dehydrogenase 12	2.376
1415965_at	Scd1	stearoyl-Coenzyme A desaturase 1	6.494
1415822_at	Scd2	stearoyl-Coenzyme A desaturase 2	1.849
1423828_at	Fasn	fatty acid synthase	6.323
1455061_a_at	Acaa2	acetyl-Coenzyme A acyltransferase 2 (mitochondrial 3-oxoacyl-Coenzyme A thiolase)	1.926
1448987_at	Acadl	acetyl-Coenzyme A dehydrogenase, long-chain	2.549
1422651_at	Adipoq	adiponectin, C1Q and collagen domain containing	3.082
1422820_at	Lipe	lipase, hormone sensitive	3.032
1449964_a_at	Mlycd	malonyl-CoA decarboxylase	1.781
1426785_s_at	Mgll	monoglyceride lipase	1.907
1420658_at	Ucp3	uncoupling protein 3 (mitochondrial, proton carrier)	2.943
1425326_at	Acly	ATP citrate lyase	2.606
1460409_at	Cpt1a	carnitine palmitoyltransferase 1a, liver	2.753
1422677_at	Dgat2	diacylglycerol O-acyltransferase 2	2.784
1456702_x_at	*Ggcx*	*gamma-glutamyl carboxylase*	*0.575*
1425834_a_at	Gpam	glycerol-3-phosphate acyltransferase, mitochondrial	2.207
1417273_at	Pdk4	pyruvate dehydrogenase kinase, isoenzyme 4	2.27
1449182_at	Retn	resistin	4.114
1435630_s_at	Acat2	acetyl-Coenzyme A acetyltransferase 2	1.625
1425829_a_at	Abcb1a	ATP-binding cassette, sub-family B (MDR/TAP), member 1A	10.322
1423166_at	Cd36	CD36 antigen	1.584
1422811_at	Slc27a1	solute carrier family 27 (fatty acid transporter), member 1	3.58
1416023_at	Fabp3	fatty acid binding protein 3, muscle and heart	1.833
1424155_at	Fabp4	fatty acid binding protein 4, adipocyte	2.189
1431056_a_at	Lpl	lipoprotein lipase	1.659
1422432_at	Dbi	diazepam binding inhibitor	1.936
1422811_at	Slc27a1	solute carrier family 27 (fatty acid transporter), 1	3.58
		HEAT SHOCK RESPONSE	
1448881_at	Hp	haptoglobin	1.679
1427126_at	Hspa1b	heat shock protein 1B	8.845
1438902_a_at	Hsp90aa1	heat shock protein 90 kDa alpha (cytosolic), class A member 1	1.513

TABLE 1-continued

Genes regulated by GW1516 treatment and exercise training

FEATURE	LOCUS	DESCRIPTION	GW + Tr
1431274_a_at	Hspa9a	heat shock protein 9A	1.61
1416755_at	Dnajb1	DnaJ (Hsp40) homolog, subfamily B, member 1	3.59
		MISCELLANEOUS	
1460256_at	Car3	carbonic anhydrase 3	2.339
1415841_at	Dyncli2	dynein cytoplasmic 1 intermediate chain 2	1.705
1432344_a_at	Aplp2	amyloid beta (A4) precursor-like protein 2	1.937
1416429_a_at	Cat	catalase	1.82
1418306_at	Crybb1	crystallin, beta B1	2.457
1448842_at	Cdo1	cysteine dioxygenase 1, cytosolic	3.266
1434503_s_at	*Lamp2*	*lysosomal membrane glycoprotein 2*	*0.608*
1416473_a_at	*Nope*	*neighbor of Punc E11*	*0.452*
1453527_a_at	Neurl	neuralized-like homolog (*Drosophila*)	1.941
1451603_at	Rtbdn	retbindin	2.32
1453724_a_at	Serpinf1	serine (or cysteine) peptidase inhibitor, clade F, member 1	7.765
1448680_at	*Serpina1a*	*serine (or cysteine) proteinase inhibitor, clade A, member 1a*	*0.396*
1427285_s_at	Surf4	surfeit gene 4	2.091
1424737_at	Thrsp	thyroid hormone responsive SPOT14 homolog (*Rattus*)	2.685
1431609_a_at	Acp5	acid phosphatase 5, tartrate resistant	3.91
1448538_a_at	D4Wsu53e	DNA segment, Chr 4, Wayne State University 53, expressed	1.586
1452406_x_at		*erythroid differentiation regulator 1*	*0.619*
1451286_s_at	*Fus*	*fusion, derived from t(12;16) malignant liposarcoma (human)*	*0.605*
1425552_at	Hip1r	huntingtin interacting protein 1 related	1.75
1428091_at	*Klhl7*	*kelch-like 7 (Drosophila)*	
1429360_at	Klf3	Kruppel-like factor 3 (basic)	1.901
1449413_at	Mpv17l	Mpv17 transgene, kidney disease mutant-like	1.988
1451667_at	C530043G21Rik	RIKEN cDNA C530043G21 gene	1.5
1425865_a_at	Lig3	ligase III, DNA, ATP-dependent	2.693
1415994_at	Cyp2e1	cytochrome P450, family 2, subfamily e, polypeptide 1	2.941
1417867_at	Cfd	complement factor D (adipsin)	2.828
1451015_at	Tkt	transketolase	2.256
1432344_a_at	Aplp2	amyloid beta (A4) precursor-like protein 2	1.937
1419487_at	Mybph	Myosin binding protein H	1.578
		MITOCHONDRIAL PROTEINS	
1436750_a_at	*Oxct1*	*3-oxoacid CoA transferase 1*	*0.574*
1415897_a_at	Mgst1	microsomal glutathione S-transferase 1	1.916
1434970_a_at	*Mrpl15*	*mitochondrial ribosomal protein L15*	*0.61*
1423109_s_at	Slc25a20	solute carrier family 25 (mitochondrial carnitine/acylcarnitine translocase), member 20	1.865
1416014_at	*Abce1*	*ATP-binding cassette, sub-family E (OABP), member 1*	*0.556*
		OXYGEN CARRIERS	
1448348_at	Gpiap1	GPI-anchored membrane protein 1	1.83
1451203_at	Mb	myoglobin	1.578
1428111_at	*Slc38a4*	*solute carrier family 38, member 4*	*0.579*
1428361_x_at	Hba-a1	hemoglobin alpha, adult chain 1	1.632
1417184_s_at	Hbb-b2|Hbb-y	hemoglobin, beta adult minor chain|hemoglobin Y, beta-like embryonic chain	1.626
		SIGNAL TRANSDUCTION	
1416137_at	*Anxa7*	*annexin A7*	*0.544*
1455918_at	Adrb3	adrenergic receptor, beta 3	3.83
1417163_at	*Dusp10*	*dual specificity phosphatase 10*	*0.579*
1452097_a_at	Dusp7	dual specificity phosphatase 7	1.661
1419191_at	Hipk3	homeodomain interacting protein kinase 3	1.694
1448152_at	Igf2	insulin-like growth factor 2	1.635
1422313_a_at	Igfbp5	insulin-like growth factor binding protein 5	1.772
1428265_at	Ppp2r1b	protein phosphatase 2 (formerly 2A), regulatory subunit A (PR 65), beta isoform	2.509
1438562_a_at	*Ptpn2*	*Protein tyrosine phosphatase, non-receptor type 2*	*0.432*
1449342_at	Ptplb	protein tyrosine phosphatase-like (proline instead of catalytic arginine), member b	2.38
1422119_at	Rab5b	RAB5B, member RAS oncogene family	1.603
1437016_x_at	*Rap2c*	*RAP2C, member of RAS oncogene family*	*0.601*
1425444_a_at	Tgfbr2	transforming growth factor, beta receptor II	2.13
1431164_at	Rragd	Ras-related GTP binding D	2.101
1420816_at	Ywhag	3-monooxygenase/tryptophan 5-monooxygenase activation protein, gamma polypeptide	1.87

TABLE 1-continued

Genes regulated by GW1516 treatment and exercise training

FEATURE	LOCUS	DESCRIPTION	GW + Tr
		STEROID BIOGENESIS	
1418601_at	Aldh1a7	aldehyde dehydrogenase family 1, subfamily A7	3.862
1426225_at	Rbp4	retinol binding protein 4, plasma	2.065
1455913_x_at	*Ttr*	*transthyretin*	*0.026*
		TRANSCRIPTION	
1417794_at	Zfp261	zinc finger protein 261	1.847
1424731_at	Nle1	notchless homolog 1 (*Drosophila*)	1.831
1454791_a_at	Rbbp4	retinoblastoma binding protein 4	2.865
1460281_at	Asb15	ankyrin repeat and SOCS box-containing protein 15	1.78
1449363_at	Atf3	activating transcription factor 3	1.802
1418982_at	Cebpa	CCAAT/enhancer binding protein (C/EBP), alpha	2.168
1417065_at	Egr1	early growth response 1	2.577
1434831_a_at	*Foxo3a*	*forkhead box O3a*	*0.634*
1415899_at	Junb	Jun-B oncogene	1.792
1421554_at	Lmx1a	LIM homeobox transcription factor 1 alpha	4.106
1416959_at	Nr1d2	nuclear receptor subfamily 1, group D, member 2(Reverb-b)	1.794
1450749_a_at	Nr4a2	nuclear receptor subfamily 4, group A, member 2 (NURR1)	1.776
1460215_at	Rpol-4	RNA polymerase 1-4	2.498
1417719_at	*Sap30*	*sin3 associated polypeptide*	*0.551*
1420892_at	Wnt7b	wingless-related MMTV integration site 7B	4.449
1423100_at	Fos	FBJ osteosarcoma oncogene	3.9
		TRANSPORT PROTEINS	
1427222_a_at	*Svp2*	*seminal vesicle protein 2*	*0.014*
1456124_x_at	*Svs5*	*seminal vesicle secretion 5*	*0.095*
1425546_a_at	Trf	transferrin	1.907
1423743_at	Arcn1	archain 1	1.617
1451771_at	Tpcn1	two pore channel 1	2.842
1416629_at	Slc1a5	solute carrier family 1 (neutral amino acid transporter), member 5	1.939
1420295_x_at	Clcn5	chloride channel 5	2.333
1417839_at	Cldn5	claudin 5	1.545
1425260_at	*Alb1*	*albumin 1*	*0.245*
1434617_x_at	1810073N04Rik	RIKEN cDNA 1810073N04 gene	2.326

Data is average of N=3 samples in each group (p<0.05).

[00166]   Surprisingly, the combination of GW1516 treatment and exercise established a unique gene expression pattern ("a GW+TR profile") that was neither an amalgamation nor a complete overlap of the two interventions (FIG. 6). This unique signature included 48 target genes (Table 2) not regulated by GW1516 and exercise alone and excluded 74 genes regulated by GW1516 or exercise alone (some of which are shown in Table 3). This signature for the combination of GW1516 treatment and exercise (Table 2) was highly enriched in genes encoding regulatory enzymes for energy homeostasis, angiogenesis, oxygen transport, signal transduction,

transcription and substrate transport, which are processes that are
involved in endurance adaptation. Particularly, a predominance of
genes involved in oxidative metabolism, is selectively up-regulated
by combined exercise and drug treatment (see unbolded genes in
Tables 1 and 2). In addition, several stress-related genes
activated by either intervention, including heat shock proteins,
metallothioneins and other stress biomarkers (Table 3) are not
changed by the combination possibly reflecting a potential
lessening of exercise-based damage.

TABLE 2

Gene targets unique to combined GW1516 treatment and exercise training.		
DESCRIPTION	LOCUS	GW + Tr
ANGIOGENESIS		
CD55 antigen	*Cd55*	*0.56*
CARBOHYDRATE METABOLISM		
phosphoenolpyruvate carboxykinase 1, cytosolic	Pck1	3.518
CYTOKINES		
interferon, alpha-inducible protein 27	Ifi27	1.714

TABLE 2-continued

Gene targets unique to combined GW1516 treatment and exercise training.

DESCRIPTION	LOCUS	GW + Tr
FAT METABOLISM		
adipose differentiation related protein	Adrp	2.009
stearoyl-Coenzyme A desaturase 2	Scd2	1.849
acetyl-Coenzyme A acetyltransferase 2	Acat2	1.625
ATP citrate lyase	Acly	2.606
adiponectin, C1Q and collagen domain containing	Adipoq	3.082
diacylglycerol O-acyltransferase 2	Dgat2	2.784
gamma-glutamyl carboxylase	*Ggcx*	*0.575*
lipase, hormone sensitive	Lipe	3.032
monoglyceride lipase	Mgll	1.907
resistin	Retn	4.114
CD36 antigen	Cd36	1.584
fatty acid binding protein 4, adipocyte	Fabp4	2.189
lipoprotein lipase	Lpl	1.659
HEAT SHOCK RESPONSE		
haptoglobin	Hp	1.679
MITOCHONDRIAL PROTEINS		
3-oxoacid CoA transferase 1	*Oxct1*	*0.574*
microsomal glutathione S-transferase 1	Mgst1	1.916
OTHERS		
carbonic anhydrase 3	Car3	2.339
cysteine dioxygenase 1, cytosolic	Cdo1	3.266
DNA segment, Chr 4, Wayne State University 53, expressed	D4Wsu53e	1.586
dynein cytoplasmic 1 intermediate chain 2	Dync1i2	1.705
	Fus	*0.605*
Kruppel-like factor 3 (basic)	Klf3	1.901
lysosomal membrane glycoprotein 2	*Lamp2*	*0.608*
neighbor of Punc E11	*Nope*	*0.452*
thyroid hormone responsive SPOT14 homolog (*Rattus*)	Thrsp	2.685
cytochrome P450, family 2, subfamily e, polypeptide 1	Cyp2e1	2.941
complement factor D (adipsin)	Cfd	2.828
transketolase	Tkt	2.256
OXYGEN CARRIERS		
GPI-anchored membrane protein 1	Gpiap1	1.83
solute carrier family 38, member 4	*Slc38a4*	*0.579*
PROLIFERATION & APOPTOSIS		
RIKEN cDNA 1190002H23 gene	*1190002H23Rik*	*0.543*
cell death-inducing DFFA-like effector c	Cidec	4.771
SIGNAL TRANSDUCTION		
annexin A7	*Anxa7*	*0.544*
dual specificity phosphatase 7	Dusp7	1.661
homeodomain interacting protein kinase 3	Hipk3	1.694
insulin-like growth factor binding protein 5	Igfbp5	1.772
protein phosphatase 2 (formerly 2A), regulatory subunit A (PR 65), beta isoform	Ppp2r1b	2.509
protein tyrosine phosphatase-like (proline instead of catalytic arginine), member b	Ptplb	2.38
STEROID BIOGENESIS		
retinol binding protein 4, plasma	Rbp4	2.065
TRANSCRIPTION		
CCAAT/enhancer binding protein (C/EBP), alpha	Cebpa	2.168
nuclear receptor subfamily 1, group D, member 2(Reverb-b)	Nr1d2	1.794
TRANSPORT		
transferrin	Trf	1.907
archain 1	Arcn1	1.617
solute carrier family 1 (neutral amino acid transporter), member 5	Slc1a5	1.939
RIKEN cDNA 1810073N04 gene	1810073N04Rik	2.326

TABLE 3

Gene targets regulated by GW1516 treatment or exercise training alone.

FEATURE	LOCUS	DESCRIPTION	GW	Tr	GW + Tr
	Hspb1	heat shock protein 1	1.815	1.965	—
1451284_at	Hspb7	heat shock protein family, 7 (cardiovascular)	3.414	1.753	—
1422943_a_at	Dnaja1	DnaJ (Hsp40) homolog, subfamily A, 1	—	1.545	—
1421290_at	Hsp110	heat shock protein 110	—	1.587	—
1416288_at	Serpinh1	serine (or cysteine) peptidase inhibitor, H, 1	—	2.198	—
1423566_a_at	Dnaja4	DnaJ (Hsp40) homolog, subfamily A, 4	1.756	1.545	—
1417872_at	Mt1	metallothionein 1	2.364	—	—
1424596_s_at	Mt2	metallothionein 2	2.151	—	—
1416157_at	Cryab	crystallin, alpha B	1.561	1.52	—
1423139_at	Crygf	crystallin, gamma F	1.801	3.56	—
1448830_at	Smad3	MAD homolog 3 (*Drosophila*)	1.841	1.886	—
1450637_a_at	Ankrd1	ankyrin repeat domain 1 (cardiac muscle)	4.235	—	—
1416029_at	Tnfrsf12a	TNF receptor superfamily, 12a	1.759	1.782	—
1426464_at	Jun	Jun oncogene	—	1.521	—

Data is average of N=3 samples in each group (p<0.05)

[00167]   Thirty-two percent of the GW+Tr-regulated genes encode enzymes of metabolic pathways such as fatty acid biosynthesis/storage (*e.g.*, FAS, SCD 1 & 2), uptake [*e.g.*, FAT/CD36, fatty acid binding proteins (FABP) and LPL] and oxidation [*e.g.*, adiponectin, hormone sensitive lipase (HSL), PDK4, UCP3]; and carbohydrate metabolism [*e.g.*, fructose bisphosphate 2 (FBP2), phosphoenolpyruvate carboxykinase 1 (PEPCK1), lactate dehydrogenase B], which along with oxygen transporters and mitochondrial proteins form the largest class of genes directly linked to muscle performance (Ikeda *et al.*, Biochem. Biophys. Res. Commun. 296:395-400, 2002; Achten and Jeukendrup, Nutrition. 20:716-27, 2004; Hittel *et al.*, J. Appl. Physiol. 98: 168-79, 2005; Civitarese *et al.*, Cell Metab. 4:75-87, 2006; Nadeau *et al.*, FASEB J. 17:1812-9, 2006; Kiens, Physiol. Rev. 86:205-43, 2006; Yamauchi *et al.*, Nat. Med. 8:1288-95, 2006). Unexpectedly, established PPARa target genes fatty acyl-CoA oxidase and medium chain acyl-CoA dehydrogenase (MCAD) were not represented in the signature. All but four of these metabolic genes were induced, which indicated a general increase in oxidative capacity of skeletal muscle in exercise-trained subjects that received GW1516 treatment.

[00168]   Other genes regulated in quadriceps muscle by the combination of exercise and GW1516 treatment encoded proteins

involved in pathways such as angiogenesis (*e.g.*, angiopoietin-like 4 protein/also a known regulator of lipid metabolism), (*e.g.*, adrenergic receptor β3, insulin-like growth factor, insulin-like growth factor binding protein 5), transcription (*e.g.*, C/EBP α, Reverb β, NURR1) and substrate transport (*e.g.*, transferrin, chloride channel 5) (Nagase *et al.*, J. Clin. Invest. 97:2898-904, 1996; Singleton and Feldman, Neurobiol. Dis. 8:541-54, 2001; Adams, J. Appl. Physiol. 93:1159-67, 2002; Centrella *et al.*, Gene. 342: 13-24, 2004; Lundby *et al.*, Eur. J. Appl Physiol. 96: 363-9, 2005; Mahoney *et al.*, FASEB J. 19: 1498-500, 2005; Mahoney *et al.*, Phys. Med. Rehabil Clin. N. Am. 16: 859-73, 2005; Ramakrishnan *et al.*, J. Biol. Chem. 280:8651-9, 2005). Without wishing to be bound to a particular theory, such other genes are likely involved, at least in part, in muscle remodeling and increased endurance observed in GW1516-treated, exercise-trained subjects.

[00169]　　　Interestingly, comparative expression analysis of the 48 gene subset of the endurance signature (Table 2), but not of either intervention alone, revealed a striking similarity to 'untrained' VP16-PPAR5 transgenic mice. This observation confirms the primary dependence of the 48 genes on PPARδ and indicates that exercise-generated signals may function to synergize PPARδ transcriptional activity to levels comparable to transgenic over-expression. Therefore, exercise cues along with PPARδ agonist may function to hyper-activate receptor transcriptional activity to re-program of adult muscle.

[00170]　　　Genes and/or proteins uniquely affected (*e.g.*, up-regulated or down-regulated or not substantially regulated) by exercise in the presence of one or more pharmaceutical agents (*e.g.*, PPARδ agonists) can be used as markers, for instance, of "drug doping" in exercise-trained subjects (*e.g.*, athletes). It is expected that the unique set of 48 genes regulated by GW+Tr, but not GW1516 treatment or exercise training alone, can be used to identify exercised subjects who have received a variety performance-enhancing drugs.

Example 6

[00171]   PPARδ directly interacts with exercise-activated kinases, p44/42 MAPK and AMPK. Exercise training is known to activate kinases, such as p44/42 MAPK and AMPK, which regulate gene expression in skeletal muscle (Chen *et al.*, Diabetes, 52:2205-12, 2003; Goodyear *et al.*, Am. J. Physiol, 271:E403-8, 1996). AMPK affects skeletal muscle gene expression and oxidative metabolism (Chen *et al.*, Diabetes. 52: 2205-12, 2003, Reznick *et al.*, J. Physiol. 574: 33-9, 2006). The interaction between exercise-regulated kinases and PPARδ signaling is described in this Example. The levels of phospho-p44/42 MAPK and phospho-AMPK α subunit and total AMPK were determined in protein homogenates of quadriceps muscle by Western blot. Antibodies specific for phospho-p44/42 MAPK, phospho- and total-AMPK α1 antibodies were obtained from Cell Signaling. The phospho- specific AMPK α1 antibody recognizes the key activating threonine in the activation loop.

[00172]   Active forms of both kinases (phospho-p44/42 MAPK and phospho-AMPK α subunit) were expressed at higher levels in the quadriceps muscles of exercised mice relative to the sedentary controls (FIG. 7A). Previous reports claim that PPARδ is not required for activation of AMPK by GW1516 in cultured cells (Kramer *et al.*, Diabetes. 54(4): 1157-63, 2005 and Kramer *et al.*, J. Biol .Chem. 282(27): 19313-2, 2007). In contrast, it was observed that GW1516 failed to activate p44/42 or AMPK in either sedentary or trained muscles, which indicated that PPARδ-regulated effects are downstream to the exercise-induced signals that activate these kinases. Furthermore, AMPK appears to be constitutively active in muscles of VP16-PPARδ transgenic mice in absence of exercise or drug (FIG. 7B). These results indicate that synergy is AMPK and PPARδ co-dependent.

[00173]   If synergy is AMPK and PPARδ co-dependent, selective co-activation of AMPK and PPARδ would induce gene expression changes that mimic those triggered by combined exercise and PPARδ as well as VP16-PPARδ over-expression. To demonstrate this, transcriptional changes induced in skeletal muscle by combined exercise and GW1516 treatment (as described in Example 5) were compared to that of

combined AMPK activator (the cell permeable AMP analog AICAR; 250 mg/kg/day, i.p.) and GW1516 (5 mg/kg/day, oral gavage) treatment for 6 days. Genome analysis was performed using the methods described in Example 5.

[00174]   Simultaneous GW1516 and AICAR treatment for 6 days created a unique gene expression signature in the quadriceps of untrained C57B1/6J mice (FIG. 8A, which includes target genes associated with translation, protein processing, amino acid metabolism, fat metabolism, oxygen carriers, carbohydrate metabolism, signal transduction, transcription, transport, steroid biogenesis, heat shock response, angiogenesis, proliferation and apoptosis, cytokines, contractile proteins, stress, and others) that shares 40% of the genes with that of combined GW1516 treatment and exercise (FIG. 8B). Classification of the 52 genes common to the two signatures (combined PPARδ activation and exercise or PPARδ and AMPK co-activation) (listed in Table 4) revealed that the majority of the targets were linked to oxidative metabolism.

TABLE 4

Targets common to exercise-PPARδ and AMPK-PPARδ gene signatures.

DESCRIPTION	LOCUS	Tr + GW	AI + GW
ANGIOGENESIS			
angiopoietin-like 4	Angptl4	5.495	2.917
APOPTOSIS			
cell death-inducing DFFA-like effector c	Cidec	4.771	1.838
cell death-inducing DNA fragmentation factor, alpha subunit-like effector A	Cidea	49.625	1.842
CARBOHYDRATE METABOLISM			
lactate dehydrogenase B	Ldhb	2.541	1.917
fructose bisphosphatase 2	Fbp2	2.808	2.478
FAT METABOLISM			
stearoyl-Coenzyme A desaturase 1	Scd1	6.494	1.78
fatty acid binding protein 3, muscle and heart	Fabp3	1.833	1.5
pyruvate dehydrogenase kinase, isoenzyme 4	Pdk4	2.27	2.486
uncoupling protein 3 (mitochondrial, proton carrier)	Ucp3	2.943	2.792
adiponectin, C1Q and collagen domain containin	Adipoq	3.082	1.56
diacylglycerol O-acyltransferase 2	Dgat2	2.784	2.14
solute carrier family 27 (fatty acid transporter), member 1	Slc27a1	3.58	2.195
lipase, hormone sensitive	Lipe	3.032	1.746
solute carrier family 25 (mitochondrial carnitine/acylcarnitine translocase), member 20	Slc25a20	1.704	1.697
CD36 antigen	Cd36	1.584	1.513
phosphoenolpyruvate carboxykinase 1, cytosolic	Pck1	3.518	1.781
fatty acid synthase	Fasn	6.323	2.24
fatty acid binding protein 4, adipocyte	Fabp4	2.189	1.81
monoglyceride lipase	Mgll	1.907	1.51
acetyl-Coenzyme A acetyltransferase 2	Acat2	1.625	1.563
acetyl-Coenzyme A dehydrogenase, long-chain	Acadl	2.549	1.992
resistin	Retn	4.114	1.756
malonyl-CoA decarboxylase	Mlycd	1.781	1.962
transketolase	Tkt	2.256	1.983
ATP citrate lyase	Acly	2.458	1.91
HEAT SHOCK			
heat shock protein 90 kDa alpha (cytosolic), class A member 1	Hsp90aa1	1.455	0.616
DnaJ (Hsp40) homolog, subfamily B, member 1	Dnajb1	3.59	0.604
CYTOKINES			
interferon, alpha-inducible protein 27	Ifi27	1.714	1.537
OTHER			
sarcolipin	Sln	0.363	4.576
thyroid hormone responsive SPOT14 homolog (*Rattus*)	Thrsp	2.685	1.766
RIKEN cDNA 2310076L09 gene	2310076L09Rik	1.868	2.117
myosin, heavy polypeptide 2, skeletal muscle, adult	Myh2	2.194	1.797
surfeit gene 4	Surf4	2.091	0.654

TABLE 4-continued

Targets common to exercise-PPARδ and AMPK-PPARδ gene signatures.

DESCRIPTION	LOCUS	Tr + GW	AI + GW
acid phosphatase 5, tartrate resistant	Acp5	3.91	1.477
serine (or cysteine) proteinase inhibitor, clade A, member 1a	Serpina1a	0.396	3.891
cysteine dioxygenase 1, cytosolic	Cdo1	3.266	1.678
erythroid differentiation regulator 1		0.619	1.805
RIKEN cDNA 1810073N04 gene	1810073N04Rik	2.326	1.628
superoxide dismutase 3, extracellular	Sod3	1.606	1.617
complement factor D (adipsin)	Cfd	2.828	1.5
cytochrome P450, family 2, subfamily e, polypeptide 1	Cyp2e1	2.941	1.743
catalase	Cat	1.728	1.902
early growth response 1	Egr1	2.577	0.65
OXYGEN CARRIER			
hemoglobin, beta adult minor chain|hemoglobin Y, beta-like embryonic chain	Hbb-b2|Hbb-y	1.626	1.503
STEROID BIOGENESIS			
retinol binding protein 4, plasma	Rbp4	2.065	2.225
SIGNAL TRANSDUCTION			
adreneyrgic receptor, beta 3	Adrb3	3.83	1.56
protein tyrosine phosphatase-like (proline instead of catalytic arginine), member b	Ptplb	2.38	1.569
dual specificity phosphatase 7	Dusp7	1.661	1.672
TRANSCRIPTION			
nuclear receptor subfamily 4, group A, member 2	Nr4a2	1.776	0.437
TRANSPORT			
solute carrier family 1 (neutral amino acid transporter), member 5	Slc1a5	1.939	1.511
two pore channel 1	Tpcn1	2.842	1.487
seminal vesicle secretion 5	Svs5	0.095	2.243

Data is average of N=3 samples in each group (p<0.05).

[00175] Quantitative expression analysis of selective oxidative genes (eight of those listed in Table 4) was determined in quadriceps of mice treated with vehicle (V), GW 1516 (GW, 5mg/kg/day), AICAR (AI, 250 mg/kg/day) and the combination of the two drugs (GW+AI) for 6 days using the methods described in Example 1. As shown in FIGS. 9A-H, several of these biomarkers including PDK4, SCD1, ATP citrate lyase, HSL, mFABP and LPL were induced in a synergistic fashion by GW1516 and AICAR in the quadriceps (FIGS. 9C-9H). Intriguingly, synergism was undetectable in UCP3 and mCPT I (FIGS. 9A and B). These genes were induced in quadriceps of

untrained VP16-PPAR5 mice, where AMPK is constitutively active
(Table 5).

TABLE 5

Selective oxidative genes induced in muscle by combined
PPARδ and AMPK activation as well as
VP16-PPARδ over-expression

Description	Locus	GW + AI	VP-PPARδ
ATP citrate lyase	Acly	1.648	3.095
carnitine palmitoyltransferase 1b, muscle	Cpt1b	1.371	1.678
fatty acid binding protein 3, muscle and heart	Fabp3	1.447	5.904
fatty acid synthase	Fasn	2.24	2.749
lipoprotein lipase	Lpl	1.113	1.72
lipase, hormone sensitive	Lipe	1.746	2.203
pyruvate dehydrogenase kinase, isoenzyme 4	Pdk4	2.486	5.06
stearoyl-Coenzyme A desaturase 1	Scd1	1.78	7.353
uncoupling protein 3	Ucp3	2.792	4.107

[00176]   Collectively, these results demonstrate that while
interaction between AMPK and PPARδ may substantially contribute to
re-programming of the skeletal muscle transcriptome during
exercise, additional changes may involve cross-talk between other
components of the exercise signaling network and PPARδ. In summary,
PPARδ and exercise synergistically regulate running endurance.
Although not bound by theory, kinase activation may influence PPARδ
signaling during exercise in establishing an "endurance gene
expression signature" that effectively enhances performance.

Example 7

[00177]   AMPK increases transcription activation by PPARδ. The
genetic synergism described in Example 6 indicates that AMPK
directly regulates the transcriptional activity of PPARδ in
skeletal muscles. To demonstrate this, an analysis of the effects
of GW1516 and AICAR on gene expression in primary muscle cells

isolated from wild type and PPARδ null mice was performed. Primary muscle cells were isolated from wild type and PPARδ null mice as previously described (Rando and Blau, J. Cell. Biol. 125(6): 1275-87, 1994). Skeletal muscle C2C12 cells were cultured in DMEM containing 20% serum and penicillin/streptomycin cocktail. For differentiation, cells at 80% confluence were switched to a differentiation medium (DMEM + 2% serum) for 4 days to obtain differentiated myotubules. Cells were treated with vehicle, GW1516, AICAR, or GW1516 + AICAR (GW: 0.1 μM; AICAR: 500 μM) for 24 hours. RNA expression of UCP3, PDK4, LPL, and HSL was determined using real time quantitative PCR as described in Example 1. As shown in FIGS. 10A-D, synergism is dependent on PPARδ and lost in the null cells. Similar synergistic regulation of gene expression by GW1516 and AICAR was also observed in differentiated C2C12 cells. These results show that AMPK activation may enhance ligand-dependent transcriptional effects of PPARδ in muscles. To more directly address this, reporter gene expression assays were utilized.

[00178]   AD 293 cells were cultured in DMEM containing 10% serum and an antibiotic cocktail. Cells were transfected with one or more of CMX-Flag, CMX-Flag PPARδ, CMX-Tk-PPRE, or CMX-βGAL, or an hAMPK (α1 and α2 subunits, Origene) expression vector using Lipofectamine™ 2000 in accordance with the manufacturer's instructions. Anti-Flag antibody-conjugated beads were incubated overnight at 40C with lysates from transfected cells. Flag-tagged protein or protein complexes were immunoprecipitated by separating the beads from non-bound materials. The beads were washed in ice-cold lysis buffer followed by extraction in Laemmli buffer. For co-immunoprecipitation experiments SDS was excluded from the lysis buffer. Western blotting was performed with antibodies specific for the Flag tag or AMPK α subunit(s).

[00179]   Co-transfection of either catalytic AMPK α1 or cc2 subunits, but not control vector, with PPARδ increased the basal (FIG. 10E) and GW1516-dependent transcriptional activity (FIG. 10F) of PPARδ in inducing a PPRE-driven reporter gene in AD293 cells. AMPK over-expression or GW1516 treatment did not change reporter activity in transfections excluding the PPARδ expression vector

negating the possibility of an effect via RXR. Additional results
indicate that AMPK may modulate PPARδ transcriptional activity by
directly interacting with the receptor. In AD293 cells co-
transfected with Flag-PPARδ and with either catalytic AMPK α1 or α2
subunits, both of the subunits co-immunoprecipitated as a complex
with Flag- PPARδ (FIG. 10G). Furthermore, Flag-PPARδ also co-
immunoprecipitated endogenous AMPKα subunits from AD293 cells
confirming a direct physical interaction between the nuclear
receptor and the kinase (FIG. 10H). Despite physical interaction,
AMPK failed to increase PPARδ phosphorylation.

[00180]   While potential AMPK phosphorylation sites were found in
PPARδ, none of these sites were phosphorylated by AMPK in *in vitro*
kinase assays. This was further confirmed by measuring the p32
labeling of PPARδ in AD 293 cells in the presence or absence of
AMPK. AD 293 cells were transfected with PPARδ and hAMPk (α1 or α2
subunit) expression vectors as described above. Forty-eight hours
after transfection, the cells were washed three times with
phosphate-free DMEM and incubated with 32P-orthophosphate in
phosphate-free DMEM for 20 hours (100 μCi/5 ml). Cells were washed
three times with ice-cold phosphate-free DMEM and lysed in ice-cold
lysis buffer.

[00181]   As shown in FIG. 10I, overall PPARδ phosphorylation is
not increased by AMPK *in vivo*. However, co-transfection of AMPKcc2
and co-activator PGClα (a known phosphorylation target of AMPK) co-
operatively interact to further induce both the basal and ligand-
dependent transcriptional activity of PPARδ (FIG. 10J). Strikingly,
no significant physical interaction between Flag-PGClα and AMPK
(FIG. 10K) was detected, both of which independently interacted
with PPARδ. Collectively, these observations indicate that AMPK may
be present in a transcriptional complex with PPARδ where it can
potentiate receptor activity via direct protein-protein interaction
and/or by phosphorylating co-activators such as PGClα.

[00182]   These results indicate that AMPK directly interacts with
PPARδ and dramatically increases basal and ligand-dependent
transcription via the receptor. Despite physical interaction, AMPK
does not phosphorylate PPARδ. AMPK and its substrate PGClα

synergistically increased PPARδ transcription, indicating indirect regulation of receptor by AMPK via co-regulator modification.

[00183]   The conclusion that exercise-activated AMPK interacts with PPARδ in regulating gene expression *in vivo* is strengthened by the observation that treatment of animals with AICAR (AMPK activator) and GW1516 creates a gene signature in skeletal muscle that replicates up to 40% of the genetic effects of combined exercise and GW1516 treatment (see Table 4). Moreover, several candidate genes from this signature are synergistically induced by GW1516 and AICAR in wild type but not in PPARδ null primary muscle cells, demonstrating that the interactive effects of the two drugs are mediated through PPARδ. While 45% of the commonly regulated genes are linked to oxidative metabolism, additional common targets relevant to muscle performance include angiogenic, signal transduction and glucose sparing genes (Table 4). It is possible that the portion of the PPARδ-exercise signature that is independent of PPARδ-AMPK interaction (FIG. 8B) may depend on cross-talk between the receptor and other exercise signal transducers such as MAPK, calcineurin/NFAT and SIRT 1. These possibilities are summarized in FIG. 10L, where AMPK and additional components of the signaling network are proposed to interact with liganded PPARδ to generate a muscle endurance gene signature and enhanced endurance adaptation.

[00184]   The data show that synthetic PPARδ activation alone induces a set of genomic changes that fail to alter the preset muscle architecture and endurance levels in adult mice. However, the combination of PPARδ activation with exercise brings about novel transcriptional changes, potentially via interaction with kinases such as AMPK (as depicted in FIG. 10L), re-setting the muscle transcriptome to a phenotype that dramatically enhances muscle performance.

Example 8

[00185]   The data demonstrate that pharmacologic activation of PPARδ in adult mice can increase running endurance in conjunction with exercise signals. The central role for AMPK in this process is especially underscored by the observations that it is both robustly

stimulated by exercise as well as constitutively active in muscles
of VP16-PPARd transgenic mice that exhibit endurance without
exercise. Further, AMPK can integrate multiple transcriptional
programs by interacting not only with PPARδ but also other
transcriptional regulators of metabolism (e.g., PGC1α, PPARα) (Hong
et al., 2003; Leff, 2003; Bronner et al., 2004; Jaager et al.,
2007). This raises the interesting question as to whether chemical
activation of AMPK is sufficient to increase running endurance
without exercise.

[00186]   To test this idea C57B/6J mice were treated with AICAR
(500 mg/kg/day) for 4 weeks. AICAR increased phosphorylation of
AMPK a subunit and acetyl CoA carboxylase (ACC) and increased
expression of UCP3 in quadriceps, confirming effective activation
of AMPK signaling (Figure 11A). Interestingly, 4 weeks of drug
treatment decreased the ratio of epididymal fat mass to body weight
and increased oxygen consumption without changing body weight
(Figures 11B-11E), supporting the speculation that AICAR may
positively regulate endurance. Indeed, in a treadmill endurance
test, ACIAR-treated mice ran longer (~23%) and further (~44%) than
did vehicle-treated mice, revealing that increase in endurance can
be achieved without exercise (Figure 11F). Furthermore, global gene
expression analysis of quadriceps revealed that AICAR treatment
alone upregulated a set of 32 genes linked to oxidative metabolism
(Figure 11G and Table 6). Notably, 30 of these 32 genes were also
upregulated in VP16- PPARδ transgenic mice, suggesting that
stimulation of oxidative genes by AMPK may depend on PPARδ (Table
6).

Table 6: Oxidative genes induced by AMPK agonist AICAR as well as
    by transgenic over-expression of VP16-PPARδ in quadriceps of
untrained mice. Data is average of N=3 mice in each group (p<0.5).

Locus	Description	AICAR	VP-PPARd
Aacs	acetoacetyl-CoA synthetase	1.875	2.771
Acly	ATP citrate lyase	1.687	2.429
Adipoq	adiponectin	2.043	2.122
Ak2	adenylate kinase 2	1.486	1.173
Apoe	apolipoprotein E	1.403	1.959
Cd36	CD36 antigen	1.386	3.66
Ces3	carboxylesterase 3	1.516	1.869
Cidea	cell death-inducing DNA fragmentation factor, alpha subunit-like effector A	5.021	2.356
Dgat2	diacylglycerol O-acyltransferase 2	2.034	2.147
Elovl6	ELOVL family member 6	2.134	4.43
Fabp4	fatty acid binding protein 4, adipocyte	1.709	1.23
Fasn	fatty acid synthase	2.098	2.749
Lep	leptin	2.047	3.579
Lipe	lipase, hormone sensitive	1.64	2.203
Lpl	lipoprotein lipase	1.386	1.285
Mgll	monoglyceride lipase	1.503	2.816
Mgst1	microsomal glutathione S-transferase 1	1.602	1.905
Nrip1	nuclear receptor interacting protein 1	1.474	1.887
Pck1	phosphoenolpyruvate carboxykinase 1	2.68	2.169
Pcx	pyruvate carboxylase	1.393	-
Pdk4	pyruvate dehydrogenase kinase, isoenzyme 4	1.321	5.06
Pparg	peroxisome proliferator activated receptor gamma	3.027	2.334
Retn	resistin	1.891	1.641
Scd1	stearoyl-Coenzyme A desaturase 1	1.931	7.353
Sdhd	succinate dehydrogenase complex, subunit D	1.529	1.349
Sfxn1	sideroflexin 1	1.713	1.494
Slc25a5		1.372	1.348
Sult1a1	sulfotransferase family 1A	2.021	1.996
Tspo	translocator protein	1.441	2.133
Ucp1	uncoupling protein 1	3.623	0.695
Ucp3	uncoupling protein 3	1.258	4.107

[00187]   To test this possibility, wild-type and PPARδ null primary muscle cells were used. Treatment of wild-type primary cells with AICAR (for 72 hr) increased expression of key oxidative biomarker genes (Scd1, fasn [FAS], Ppargc 1a, Pdk4) (Figure 11H). In contrast, AICAR failed to increase the expression of the above genes in PPARδ null cells, demonstrating the requirement of the receptor for transcriptional effects of AMPK on oxidative genes.

[00188]   The data show that the AMP-mimetic AICAR can increase endurance in sedentary mice by genetically reprogramming muscle

metabolism in a PPARδ-dependent manner. The data also demonstrate
that PPARδ agonist in combination with exercise synergistically
induces fatigue resistant type I fiber specification and
mitochondrial biogenesis, ultimately enhancing physical
performance. These changes correlate with an unexpected but
interesting establishment of a muscle endurance gene signature that
is unique to the drug-exercise paradigm. Such a signature is an
outcome of molecular crosstalk and perhaps a physical association
between exercise-activated AMPK and PPARδ. These findings identify
a novel pharmacologic strategy to reprogram muscle endurance by
targeting AMPK- PPARδ signaling axis with orally active ligands.

[00189]   The AMPK activator AICAR increased oxygen consumption
and endurance in untrained adult mice in part by stimulating PPARδ-
dependent oxidative genes. Despite a demonstrated role for PPARδ in
endurance, 4 week treatment with a potent and selective agonist
failed to alter either fiber type composition or endurance,
revealing that direct and pharmacologic activation of PPARδ is
insufficient to enhance running performance. In contrast,
transgenic overexpression of activated PPARδ at birth preprograms
the nascent myofibers to transdifferentiate into slow-twitch
fibers, thus imparting a high basal endurance capacity to adult
transgenic mice. Apparently, once fiber type specification is
complete in adults, the potential plasticity of muscle to synthetic
activation of a single transcriptional pathway is constrained.

[00190]   Along these lines, the unexpected yet successful
reprogramming of endurance in untrained adults with synthetic AMP-
mimetic might be linked to the ability of AMPK to simultaneously
target multiple transcriptional programs governed by its substrates
such as PGC1a, PPARα and PPARδ, triggering a genetic effect akin to
exercise (Hong *et al.*, 2003; Leff, 2003; Bronner *et al.*, 2004;
Jager *et al.*, 2007).

[00191]   Interestingly, the recalcitrance of adult skeletal
muscle endurance to manipulation by PPARδ agonist alone is relieved
by combining drug treatment with exercise. Indeed, this strategy
generates an endurance gene signature that is unique from either
paradigm alone, reflecting a crosstalk between exercise and PPARδ

signaling. Although exercise activates a cascade of signaling events, AMPK is likely central to this genetic adaptation for several reasons. First, AMPK is a metabolic sensor that detects low ATP levels (such as occur during exercise) and in turn increases oxidative metabolism (Mu *et al.*, 2001; Reznick and Shulman, 2006). Second, long-term effects of AMPK are in part mediated via regulation of gene expression (Reznick and Shulman, 2006). Third, exercise induces activation and nuclear import of AMPK, where it can potentially interact with transcription factors.

[00192]   And finally, transgenic mice defective for AMPK activation exhibit reduced voluntary exercise (Mu *et al.*, 2001; Thomson *et al.*, 2007), making it an attractive exercise cue that modulates receptor signaling.

[00193]   The notion that exercise-activated AMPK interacts with PPARδ in regulating gene expression is supported by the demonstration that AMPK associates with PPARδ and dramatically increases basal and ligand-dependent transcription via the receptor. Despite physical interaction, AMPK does not induce PPARδ phosphorylation in metabolic labeling studies.

[00194]   Interestingly, AMPK and its previously reported substrate PGC1a synergistically increased PPARδ transcription, suggesting indirect regulation of receptor function by AMPK via coregulator modification. Nevertheless, it could be a possible regulation of PPARδ by AMPK via direct protein-protein interaction.

[00195]   Indeed, regulation of other transcription factors by AMPK via similar mechanisms has been previously demonstrated (Hong *et al.*, 2003; Leff, 2003; Bronner *et al.*, 2004). A physiological validation of AMPK- PPARδ interaction comes from the observation that GW1516 and AICAR (AMPK activator) synergistically induce several endurance-related genes in wild-type but not in PPARδ null primary muscle cells. More importantly, treatment of animals with AICAR and GW1516 creates a gene signature in skeletal muscle that replicates up to 40% of the genetic effects of combined exercise and GW1516 treatment. Notably, the shared genes between the two profiles are linked to oxidative metabolism, angiogenesis, and

glucose sparing, pathways that are directly relevant to muscle performance.

[00196]   Although not all genes regulated by either exercise or exercise- PPARδ interaction are AMPK dependent, two key findings assign a critical role for the kinase in promoting endurance compared to other known exercise signals (Bassel-Duby and Olson, 2006; Goodyear et al., 1996; Lagouge et al., 2006). First, AMPK is constitutively active in VP16- PPARδ transgenic muscles that exhibit endurance without exercise. Second, AMPK activation by AICAR was sufficient to increase running endurance without additional exercise signals. Strikingly, the majority of the oxidative genes (30 out of 32) upregulated by AICAR are active in super-endurance VP16-PPARd mice and perhaps are the core set of genes required to improve muscle performance.

[00197]   Interestingly, AICAR failed to induce oxidative gene expression in PPARδ null muscle cells, indicting the requirement of PPARδ, at least for regulation of oxidative metabolism by AMPK. Collectively, these findings demonstrate a molecular partnership between AMPK and PPARδ in reprogramming skeletal muscle transcriptome and endurance (Figure 11I) that can be readily exploited by orally active AMPK drugs to replace exercise.

[00198]   In humans, endurance exercise leads to physiological adaptations in the cardiopulmonary, endocrine, and neuromuscular systems (Jones and Carter, 2000; Lucia et al., 2001). Although the investigation focused on skeletal muscle, extramuscular effects of PPARd, AMPK, and exercise may also contribute to increased endurance. Although potentiation of extramuscular adaptations by PPARd and AMPK agonists remains to be studied, drug treatment can reduce epididymal fat mass, possibly conferring additional systemic benefits. It is noteworthy that PPARδ is important for normal cardiac contractility, as well as for the endocrine function of adipose tissue (Wang et al., 2003; Cheng et al., 2004). Similarly, the activation of AMPK by metformin is thought to mediate its ability to lower blood glucose levels (Shaw et al., 2005). In addition to increasing performance in athletes, exercise has beneficial effects in a wide range of pathophysiological

conditions, such as respiratory disorders, cardiovascular abnormalities, type 2 diabetes, and cancer risk. The disclosure demonstrates that synthetic PPARδ activation and exercise—and, more importantly, AMPK activation alone—provide a robust transcriptional cue that reprograms the skeletal muscle genome and dramatically enhances endurance. The disclosure provides a strategy for reorganizing the preset genetic imprint of muscle (as well as other tissues) with exercise mimetic drugs has therapeutic potential in treating certain muscle diseases such as wasting and frailty as well as obesity where exercise is known to be beneficial.

Example 9

[00199] Enhancing exercise effect in a subject. This example describes methods that can be used to increase or enhance an exercise in a healthy mammalian subject. Although specific conditions are described, one skilled in the art will appreciate that minor changes can be made to such conditions.

[00200] Healthy adult human subjects perform aerobic exercise (e.g., running) for at least 30 minutes (e.g., 30-90 minutes) for at least 3-4 days per week (e.g., 3-7 days per week) for at least 2 weeks (e.g., at least 4-12 weeks). The exercise is performed at 40%-50% maximal heart rate, 50%-60% maximal heart rate, 60%-70% maximal heart rate, or 75%-80% maximal heart rate, where maximum heart rate for a human subject is calculated as: 220 bps - (age of the subject).

[00201] During or after performing aerobic exercise as described above, the subjects are orally administered GW1516 [(2-methyl-4(((4-methyl-2-(4- trifluoromethylphenyl)-1,3-thiazol-5-yl)methyl)sulfanyl)phenoxy)acetic acid] at a dose of 1 to 20 mg per day, such as 2.5 or 10 mg per day. Subjects can continue to perform aerobic exercise while receiving GW1516. The subject can receive GW1516 for a period of at least 2 weeks, such as at least 4 weeks.

[00202] The exercise effect achieved in the treated subjects (e.g., running endurance) can be compared to such an effect in untreated subjects. Exercise effect can be measured using methods known in the art, such as measuring aerobic or running endurance (for example measuring distance run until exhaustion or amount of

time to run a particular distance). In some instances, the exercise
effect of interest is increased in treated subjects by at least 5%,
such as at least 10% as compared to untreated subjects.

Example 10

[00203]   Identifying performance enhancing substances in an
exercise-trained subject. This example describes methods that can
be used to identify performance- enhancing substances in an
exercised-trained subject. A biological sample obtained from a
healthy adult human is analyzed to determine if the subject is
taking a PES (e.*g.*, GW1516) by analyzing expression of one or more
of the molecules (nucleic acids or proteins) listed in Table 2 or
Table 4. Suitable biological samples include samples containing
genomic DNA or RNA (including mRNA) or proteins obtained from cells
of a subject, such as those present in peripheral blood, urine,
saliva, tissue biopsy, or buccal swab. For example, a biological
sample of the subject can be assayed for a change in expression
(such as an increase or decrease) of any combination of at least
four molecules (nucleic acids or proteins) listed in Table 2 or 4,
such as any combination of at least 10, at least 20, at least 30,
or at least 40 of those listed in Table 2 or 4, for example all of
those listed in Table 2 or 4.

[00204]   Methods of isolating nucleic acid molecules from a
biological sample are routine, for example using PCR to amplify the
molecules from the sample, or by using a commercially available kit
to isolate mRNA or cDNA. However, nucleic acids need not be
isolated prior to analysis. Nucleic acids can be contacted with an
oligonucleotide probe that will hybridize under stringent
conditions with one or more nucleic acid molecule listed in Table 2
or 4. The nucleic acids which hybridize with the probe are then
detected and quantified. The sequence of the oligonucleotide probe
can bind specifically to a nucleic acid molecule represented by the
sequences listed in Table 2 or 4.

[00205]   Increased or decreased expression of the molecules
listed in Table 2 or 4 can be detected by measuring the cellular
levels of mRNA. mRNA can be measured using techniques well known in
the art, including for instance Northern analysis, RT-PCR and mRNA

in situ hybridization. Details of mRNA analysis procedures can be found, for instance, in provided examples and in Sambrook *et al.* (ed.), Molecular Cloning: A Laboratory Manual, 2nd ed., vol. 1-3, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

[00206]   Oligonucleotides specific to sequences listed in Table 2 or 4 can be chemically synthesized using commercially available machines. These oligonucleotides can then be labeled, for example with radioactive isotopes (such as 32P) or with non-radioactive labels such as biotin (Ward and Langer *et al.*, Proc. Natl. Acad. Sci. USA 78:6633-57, 1981) or a fluorophore, and hybridized to individual DNA samples immobilized on membranes or other solid supports by dot- blot or transfer from gels after electrophoresis. These specific sequences are visualized, for example by methods such as autoradiography or fluorometric (Landegren *et al.*, Science 242:229 '-37 ', 1989) or colorimetric reactions (Gebeyehu *et al.*, Nucleic Acids Res. 15:4513-34, 1987).

[00207]   Analyzing Proteins in the biological sample can also be analyzed. In some examples, proteins are isolated using routine methods prior to analysis.

[00208]   In one example, surface-enhanced laser desorption-ionization time-of-flight (SELDI-TOF) mass spectrometry is used to detect changes in differential protein expression, for example by using the ProteinChip™ (Ciphergen Biosystems, Palo Alto, CA). Such methods are well known in the art (for example see U.S. Pat. No. 5,719,060; U.S. Pat. No. 6,897,072; and U.S. Pat. No. 6,881,586). SELDI is a solid phase method for desorption in which the analyte is presented to the energy stream on a surface that enhances analyte capture or desorption. Therefore, in a particular example, the chromatographic surface includes antibodies that recognize proteins listed in Table 2 or 4. Antigens present in the sample can recognize the antibodies on the chromatographic surface. The unbound proteins and mass spectrometric interfering compounds are washed away and the proteins that are retained on the chromato graphic surface are analyzed and detected by SELDI-TOF. The MS profile from the sample can be then compared using differential protein expression mapping, whereby relative expression levels of

proteins at specific molecular weights are compared by a variety of statistical techniques and bioinformatic software systems.

[00209]   In another examples, the availability of antibodies specific to the molecules listed in Table 2 or 4 facilitates the detection and quantification of proteins by one of a number of immunoassay methods that are well known in the art, such as those presented in Harlow and Lane (Antibodies, A Laboratory Manual, CSHL, New York, 1988). Methods of constructing such antibodies are known in the art. Any standard immunoassay format (such as ELISA, Western blot, or RIA assay) can be used to measure protein levels. Immunohistochemical techniques can also be utilized for protein detection and quantification. For example, a tissue sample can be obtained from a subject, and a section stained for the presence of the desired protein using the appropriate specific binding agents and any standard detection system (such as one that includes a secondary antibody conjugated to horseradish peroxidase). General guidance regarding such techniques can be found in Bancroft and Stevens (Theory and Practice of Histological Techniques, Churchill Livingstone, 1982) and Ausubel *et al.* (Current Protocols in Molecular Biology, John Wiley & Sons, New York, 1998).

[00210]   For the purposes of detecting or even quantifying protein or nucleic acid expression, expression in the test sample can be compared to levels found in cells from a subject who has not taken a PES. Alternatively, the pattern of expression identified in the test subject can be compared to that shown in Table 2 or 4.

[00211]   For example, if the test sample shows a pattern of expression similar to that in Table 2 or 4 (*e.g.*, the genes shown as upregulated and downregulated in Table 2 or 4 are observed in the subject to be upregulated and downregulated, respectively), this indicates that the subject is taking a PES, such as a PPARδ agonist (*e.g.*, GW1516). In contrast, If the pattern of expression identified in the test subject is different to that shown in Table 2 or 4 (*e.g.*, the genes shown as upregulated and downregulated in Table 2 or 4 are observed in the subject to be not differentially expressed or show a different pattern of regulation), this

indicates that the subject is not taking a PES, such as a PPARδ agonist (e.g., GW1516).

[00212]   A significant increase in the non-bolded proteins listed in Table 2 in the cells of a test subject compared to the amount of the same protein found in normal human cells is usually at least 2-fold, at least 3-fold, at least 4-fold or greater difference. Substantial overexpression of the non-bolded proteins listed in Table 2 in the subject's sample can be indicative of the subject taking a PES. Similarly, a significant decrease in the bolded proteins listed in Table 2 in the cells of a test subject compared to the amount of the same protein found in normal human cells is usually at least 2-fold, at least 3-fold, at least 4-fold or greater difference.

[00213]   Substantial underexpression of the bolded proteins listed in Table 2 in the subject's sample can be indicative of the subject taking a PES.

[00214]   While this disclosure has been described with an emphasis upon particular embodiments, it will be obvious to those of ordinary skill in the art that variations of the particular embodiments may be used and it is intended that the disclosure may be practiced otherwise than as specifically described herein. Accordingly, this disclosure includes all modifications encompassed within the spirit and scope of the disclosure as defined by the following claims:

We Claim:

1.   Use of an AMP kinase agonist for the manufacture of a
medament to improve or maintain muscle tone or mass in a subject
having a muscle wasting disease or disorder.

2.   The use of claim 1, wherein the AMPK agonist is AICAR.

3.   The use of claim 1, further comprising an effective amount of
a PPARδ agonist, thereby enhancing an exercise effect in the
subject.

4.   The use of claim 1, wherein the subject is a mammal.

5.   The use of claim 4, wherein the subject is a racing mammal.

6.   The use of claim 5, wherein the racing mammal is a horse, a
dog, or a human.

7.   The use of claim 4, wherein the subject is an adult.

8.   The use of claim 1, wherein the subject is an exercise-
trained subject.

9.   The use of claim 3, wherein the PPARδ agonist is GW1516.

10.   The use of claim 3, wherein the PPARδ agonist is administered
on the same day(s) on which the AMPK agonist is administered.

11.   The use of claim 8, wherein the AMPK agonist is AICAR and the
PPARδ agonist is GW1516.

12.   The use of claim 3, further comprising having the subject
perform aerobic exercise.

13. The use of claim 3, wherein the exercise effect is improved endurance.

14. The use of claim 13, wherein improved endurance comprises improved running distance or improved running time or a combination thereof.

15. The use of claim 1, wherein an effective amount is from about 0.5 mg/kg per day to about 100 mg/kg per day in a single dose or in divided doses.

16. The use of claim 1, wherein the compound is formulated for oral administration, intravenous injection, intramuscular injection, or subcutaneous injection.

17. The use of claim 3, wherein an exercise effect is increased fatty acid oxidation in at least one skeletal muscle of the subject.

18. The use of claim 3, wherein an exercise effect is body fat reduction.

19. The use of claim 18, wherein the body fat is white adipose tissue.

20. A composition comprising an AMP kinase agonist for use in treating muscle tone, endurance or mass in a subject.

21. The composition of claim 20, wherein the AMPK agonist is AICAR.

22. The composition of claim 20, further comprising an effective amount of a PPARδ agonist, thereby enhancing an exercise effect in the subject.

23. The composition of claim 20, wherein the subject is a mammal.

24.   The composition of claim 23, wherein the subject is a racing mammal.

25.   The composition of claim 24, wherein the racing mammal is a horse, a dog, or a human.

26.   The composition of claim 23, wherein the subject is an adult.

27.   The composition of claim 20, wherein the subject is an exercise-trained subject.

28.   The composition of claim 23, wherein the PPARδ agonist is GW1516.

29.   The composition of claim 23, wherein the PPARδ agonist is administered on the same day(s) on which the AMPK agonist is administered.

30.   The composition of claim 28, wherein the AMPK agonist is AICAR and the PPARδ agonist is GW1516.

31.   The composition of claim 23, further comprising having the subject perform aerobic exercise.

32. The composition of claim 23, wherein the exercise effect is improved endurance.

33.   The composition of claim 32, wherein improved endurance comprises improved running distance or improved running time or a combination thereof.

34.   The composition of claim 20, wherein an effective amount is from about 0.5 mg/kg per day to about 100 mg/kg per day in a single dose or in divided doses.

35.   The composition of claim 20, wherein the compound is formulated for oral administration, intravenous injection, intramuscular injection, or subcutaneous injection.

36.   The composition of claim 23, wherein an exercise effect is increased fatty acid oxidation in at least one skeletal muscle of the subject.

37.   The composition of claim 23, wherein an exercise effect is body fat reduction.

38.   The composition of claim 37, wherein the body fat is white adipose tissue.

39.   A method for identifying the use of performance-enhancing substances in an exercise-trained subject comprising determining in a biological sample taken from an exercise-trained subject the expression of one or more molecules listed in Tables 2 or 4.

40.   The method of claim 39, wherein: (i) expression is upregulated in one or more of adipose differentiation related protein; stearoyl-Coenzyme A desaturase 2; acetyl-Coenzyme A acetyltransferase 2; ATP citrate lyase; adiponectin, ClQ and collagen domain containing; diacylglycerol O-acyltransferase 2; lipase, hormone sensitive; monoglyceride lipase; resistin; CD36 antigen; fatty acid binding protein 4, adipocyte; lipoprotein lipase; microsomal glutathione S-transferase 1; GPI- anchored membrane protein 1; dual specificity phosphatase 7; homeodomain interacting protein kinase 3; insulin-like growth factor binding protein 5; protein phosphatase 2 (foraierly 2A), regulatory subunit A (PR 65), beta isoform; protein tyrosine phosphatase-like (proline instead of catalytic arginine); member b; CCAAT/enhancer binding protein (C/EBP), alpha; nuclear receptor subfamily 1, group D, member 2(Reverb-b); transferring; archain 1; solute carrier family 1 (neutral amino acid transporter), member 5; RIKEN cDNA 1810073N04 gene; haptoglobin; retinol binding protein 4, plasma;

phosphoenolpyruvate carboxykinase 1, cytosolic; cell death-inducing
DFFA-like effector c; interferon, alpha-inducible protein 27;
carbonic anhydrase 3; cysteine dioxygenase 1, cytosolic; DNA
segment, Chr 4, Wayne State University 53, expressed; dynein
cytoplasmic 1 intermediate chain 2; Kruppel-like factor 3 (basic);
thyroid hormone responsive SPOT14 homolog (Rattus); cytochrome
P450, family 2, subfamily e, polypeptide 1; complement factor D
(adipsin); and/or transketolase; or (ii) expression is
downregulated in one or more of gamma-glutamyl carboxylase; 3-
oxoacid CoA transferase 1; solute carrier family 38, member 4;
annexin A7; CD55 antigen, RIKEN cDNA 1190002H23 gene; fusion,
derived from t(12;16) malignant liposarcoma (human); lysosomal
membrane glycoprotein 2; and/or neighbor of Punc E1 1; or (iii) a
combination of (i) and (ii).

41.   The method of claim 39 or 40, wherein determining expression
comprises determining protein expression, determining expression of
a gene encoding the protein, or a combination thereof.

42.   The method of claim 41, comprising determining expression of
a gene encoding the protein.

43.   The method of claim 39, wherein the biological sample is a
skeletal muscle biopsy.

44.   A composition comprising an AMPK agonist and a PPARδ agonist
in a pharmaceutically acceptable carrier.

45.   The composition of claim 44, wherein the AMPK agonist is an
AICAR, salt, ester, prodrug, precursors or derivative thereof.

46.   The composition of claim 44, wherein the PPARδ agonist is
GW1516, salt, ester, prodrug, precursor, or derivative thereof.

47.   A method of treating a muscle wasting disease, muscle atrophy
or aging comprising administering to a subject a composition

comprising an AMPK agonist, wherein the muscle tone or mass is promoted.

48.   The method of claim 47, wherein the AMPK agonist is AICAR.

49.   The method of claim 47 or 48, wherein the composition further comprises a PPARδ agonist.

50.   A method of identifying a muscle promoting agent comprising contacting a subject with an agents and measuring the expression of a set of endurance gene signatures comprising the genes in Table 2 or 4 or a subset thereof, wherein the expression of a set of such genes is indicative of a muscle promoting agent.

51.   A method for improving or maintaining muscle mass or tone in a subject, comprising administering to the subject an AMP kinase (AMPK) agonist wherein the muscle tone or mass is maintained in a sedentary or immobilized subject or increased in the subject.

52.   The method of claim 51, wherein the AMPK agonist is AICAR.

53.   The method of claim 51, further comprising administering to the subject an effective amount of a PPARδ agonist, thereby enhancing an exercise effect in the subject.

54.   The method of claim 51, wherein the subject is a mammal.

55.   The method of claim 54, wherein the subject is a racing mammal.

56.   The method of claim 55, wherein the racing mammal is a horse, a dog, or a human.

57.   The method of claim 54, wherein the subject is an adult.

58. The method of claim 51, wherein the subject is an exercise-trained subject.

59. The method of claim 53, wherein the PPARδ agonist is GW1516.

60. The method of claim 53, wherein the PPARδ agonist is administered on the same day(s) on which the AMPK agonist is administered.

61. The method of claim 58, wherein the AMPK agonist is AICAR and the PPARδ agonist is GW1516.

62. The method of claim 53, further comprising having the subject perform aerobic exercise.

63. The method of claim 53, wherein the exercise effect is improved endurance.

64. The method of claim 53, wherein improved endurance is improved running distance or improved running time or a combination thereof.

65. The method of claim 51, wherein an effective amount is from about 0.5 mg/kg per day to about 100 mg/kg per day in a single dose or in divided doses.

66. The method of claim 51, wherein administration comprises oral administration, intravenous injection, intramuscular injection, or subcutaneous injection.

67. The method of claim 53, wherein an exercise effect is increased fatty acid oxidation in at least one skeletal muscle of the subject.

68. The method of claim 53, wherein an exercise effect is body fat reduction.

69.   The method of claim 58, wherein the body fat is white adipose tissue.

FIG. 1A
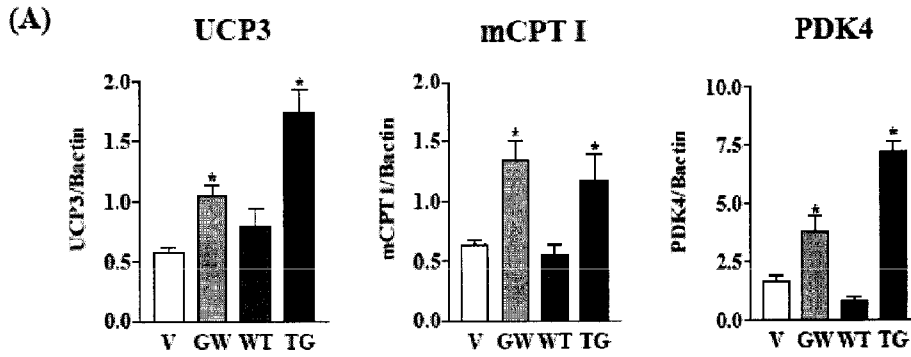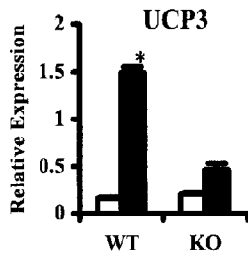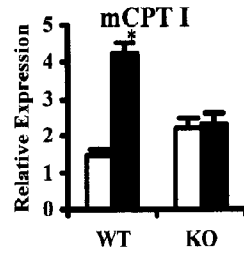
FIG. 1B   **FIG. 1C**   **FIG. 1D**
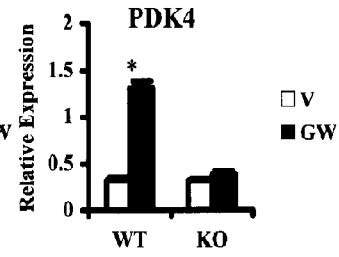
FIG. 1E
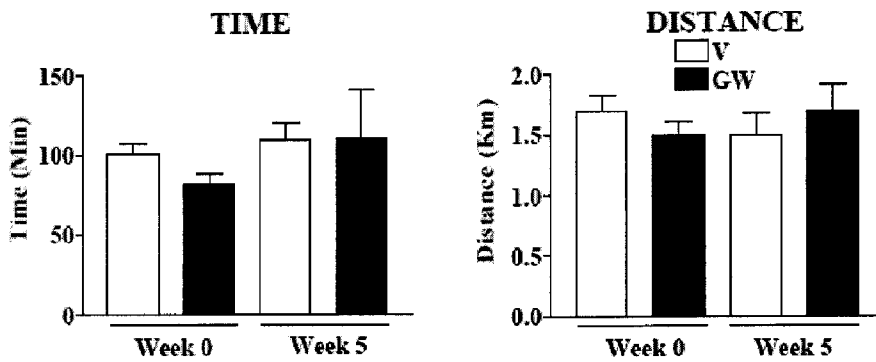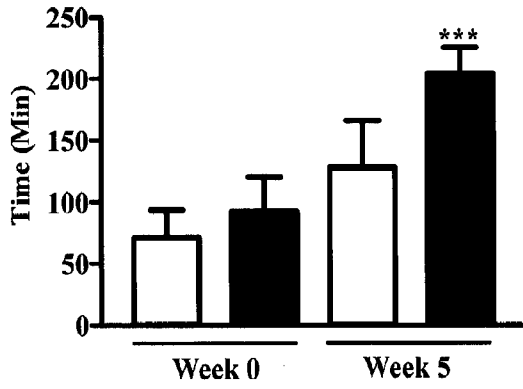

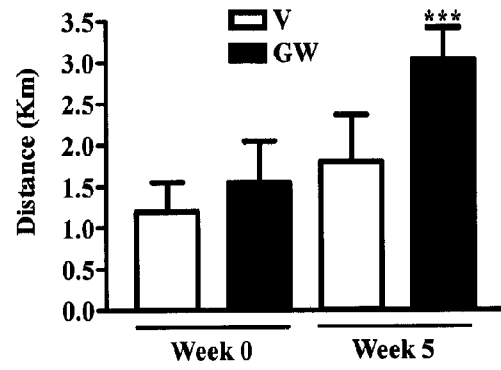FIG. 1A-E

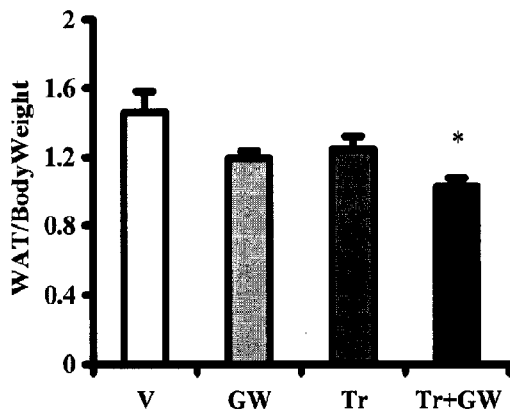## FIG. 2A
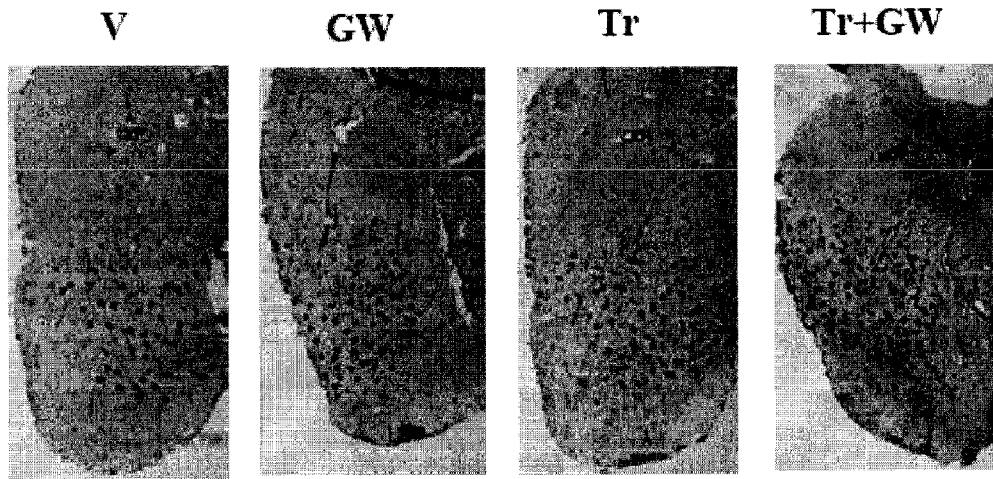
FIG. 2B
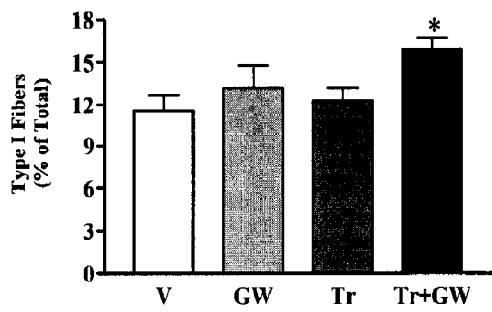
FIG. 2C
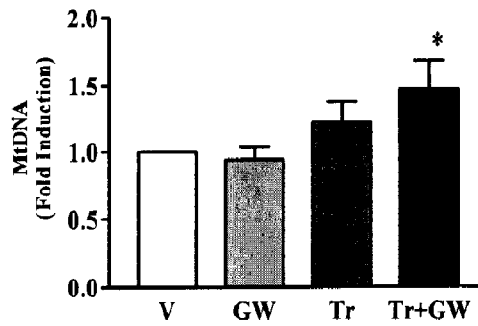
FIG. 2A-C
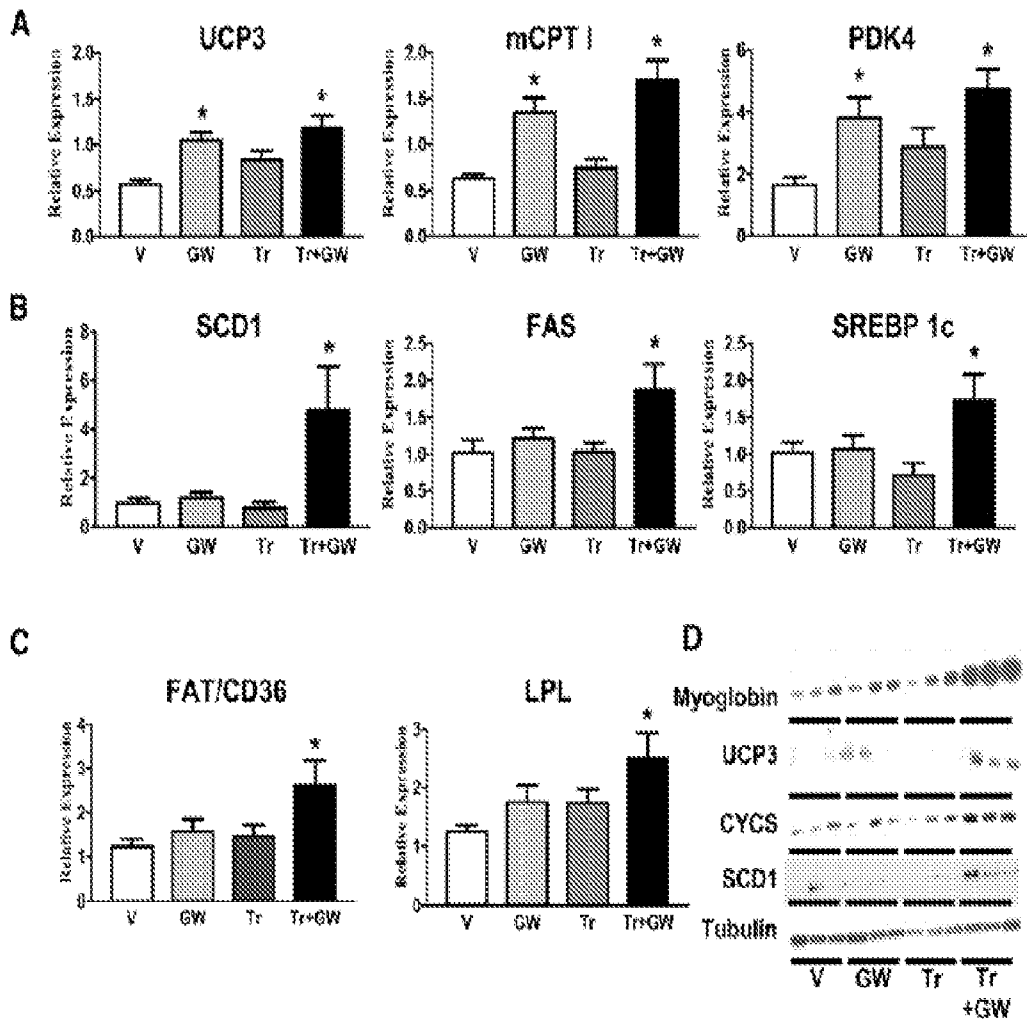
FIG. 3A-D
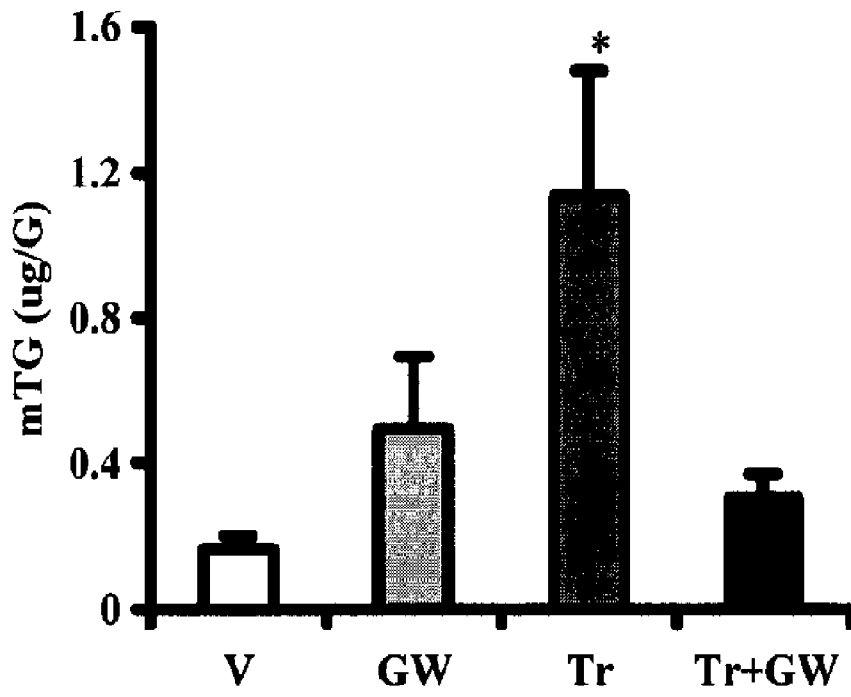
FIG. 4

FIG. 5A

FIG. 5B

FIG. 5C

FIG. 5D

FIG. 6

FIG. 7A-B

FIG. 8A-C

FIGS. 9A-H

(A) UCP3 (B) mCPT I (C) PDK4 (D) SCD1 (E) ATP Citrate Lyase (F) HSL (G) mFABP (H) LPL

FIG. 10A-L

FIGS. 11A-I

Locus	Description	Fold
Aacs	acetoacetyl-CoA synthetase	1.875
Acly	ATP citrate lyase	1.667
Dgat2	diacylglycerol O-acyltransferase 2	2.034
Fasn	fatty acid synthase	2.098
Lipe	lipase, hormone sensitive	1.64
Pdk4	pyruvate dehydrogenase kinase 4	1.321
Scd1	stearoyl-Coenzyme A desaturase 1	1.931
Ucp1	uncoupling protein 1	3.623