



(51) International Patent Classification:

G01N 33/53 (2006.01) *G01N 33/68* (2006.01)
G01N 33/48 (2006.01)

(21) International Application Number:

PCT/US2013/061265

(22) International Filing Date:

24 September 2013 (24.09.2013)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

61/704,926 24 September 2012 (24.09.2012)

US

(71) Applicant: TEXAS TECH UNIVERSITY SYSTEM
[US/US]; Office of Technology Transfer and Intellectual
Property, P.O. Box 42007, Lubbock, TX 79409-2007
(US).

(72) Inventor: JOHNSON, Bradley, J.; 6811 79th Street, Lub-
bock, TX 79424 (US).

(74) Agents: CHALKER, Daniel, J. et al.; Chalker Flores,
LLP, 14951 North Dallas Parkway, Suite 400, Dallaas, TX
75254 (US).

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

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

Published:

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

(54) Title: ENHANCEMENT OF INTRAMUSCULAR FAT IN BEEF CATTLE

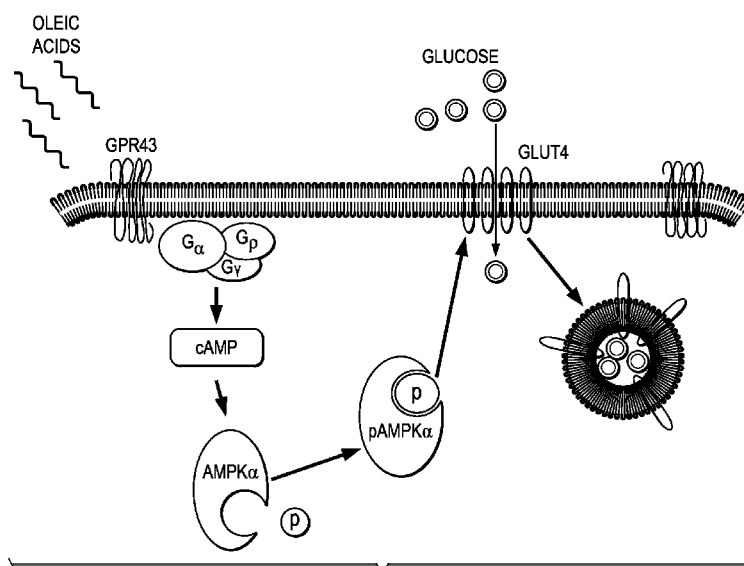


FIG. 4B

(57) Abstract: The present invention includes compositions and method of selectively modulating an intramuscular adipose tissue in beef cattle by providing a beef cattle and providing a pharmacologically effective amount of one or more agents that interact with a GPR43 receptor in the beef cattle, wherein the one or more agents modulate the GPR43 receptor to preferentially enhance the development of an intramuscular adipose tissue in the beef cattle but has no effect on subcutaneous adipose tissue accumulation in the beef cattle.

ENHANCEMENT OF INTRAMUSCULAR FAT IN BEEF CATTLE

Technical Field of the Invention

The present invention relates generally to compositions and methods for the enhancement of intramuscular fat and in particular, to compositions and methods for modulating the GPR43 receptor as a mechanism for enhancing intramuscular fat deposition in beef cattle.

5 Background Art

Without limiting the scope of the invention, its background is described in connection with mechanism for enhancing intramuscular fat in beef cattle. One important factor in the overall quality and economic value of animals including cattle is the adiposity. Specifically, the deposition location and quantity of the intramuscular fat or marbling contributes significantly to meat quality parameters, including juiciness, flavor and tenderness. This is specifically seen in beef cattle. Even though adipose tissue starts accumulation as early as the weaning period, marbling is the last adipose tissue to be deposited on a finishing animal. Many factors go into the accumulation of intramuscular fat, (e.g., genetics, age and nutrition) and to produce highly marbled beef requires a high-energy diet and long-term feeding which can result in subcutaneous fat accumulation instead of intramuscular fat because subcutaneous adipose tissue development precedes marbling development. Preferential elevation of intramuscular adipose tissue and reduction of inedible subcutaneous adipose tissue increases the economical benefits.

United States Patent Number 7,303,889 entitled "Ligand for G-Protein Coupled Receptor GPR43 and uses Thereof" discloses the unknown agonist and/or antagonist compounds identified and/or recovered by the method of the invention, as well as to a diagnostic kit comprising the unknown compounds or a pharmaceutical composition comprising an adequate pharmaceutical carrier and a sufficient amount of the unknown compound.

US Patent Application publication number 20080286806 entitled "Ligand for G-Protein Coupled Receptor GPR43 and uses Thereof" discloses the G-protein coupled orphan receptor GPR43 and the identification of short chain fatty acids as natural ligands of the receptor and provides agents that modulate GPR43 ligand binding and signaling activity, as well as compositions consisting essentially of an isolated GPR43 polypeptide and an isolated short chain fatty acid.

Disclosure of the Invention

The present invention provides compositions and methods of enhancing intramuscular fat or marbling in beef cattle through the GPR43 receptor. The present invention is directed to the discovery of the presence of the receptor GPR43 in only intramuscular adipose tissue and not in the subcutaneous adipose tissue which can be used in the modulation of the receptor GPR43 to enhancing intramuscular fat in beef cattle and to improve beef carcass quality and hence the value of the meat.

One embodiment of the present invention provides a method of selectively modulating an intramuscular adipose tissue in beef cattle by providing a beef cattle and providing a pharmacologically effective amount of one or more agents that interact with a GPR43 receptor in the beef cattle, wherein the one or more agents modulate the GPR43 receptor to preferentially enhance the development of an intramuscular adipose tissue in the beef cattle but has no effect on subcutaneous adipose tissue accumulation in the beef cattle. The one or more agents are selected from a peptide, a polypeptide, an antibody, an antigen-binding fragment, a lipid, a carbohydrate, a nucleic acid, and a small organic molecule. For example, the one or more agents is a recombinant GPR43 polypeptide, a virus coding for a GPR43 polypeptide or a ligand and the one or more agents may be disposed in a synthetic liposomes comprising a GPR43 polypeptide.

Another embodiment of the present invention provides a composition to selectively modulating an intramuscular adipose tissue in beef cattle including a carrier; and a pharmacologically effective amount of one or more agents that interact with a GPR43 receptor disposed in the carrier.

One embodiment of the present invention provides a method of enhance intramuscular adipose tissue development without affecting subcutaneous fat accumulation in an animal by providing an animal; and administering to the animal a pharmacologically effective amount of one or more agents that interact with a GPR43 receptor in the animal to preferentially enhance the development of an intramuscular adipose tissue but does not effect a subcutaneous adipose tissue accumulation in the animal. The animal may be cattle, swine, poultry, sheep, human, bison, lamb, horse, dog, cat or goat.

One embodiment of the present invention provides a method of modulating an adipose tissue by providing an intramuscular adipose tissue and providing a pharmacologically effective amount of one or more pharmacological agents that interact with a GPR43 receptor in the

intramuscular adipose tissue, wherein the GPR43 receptor preferentially enhances the development of an intramuscular adipose tissue but has no effect on a subcutaneous adipose tissue.

5 The present invention provides a composition to enhance intramuscular adipose tissue development without affecting subcutaneous fat accumulation including a pharmacologically effective amount of one or more compositions that interact with a GPR43 receptor disposed in a carrier to preferentially enhance the development of an intramuscular adipose tissue but does not effect a subcutaneous adipose tissue accumulation in the animal.

10 One embodiment of the present invention provides a method of enhancing marbling accumulation in beef cattle without excessive subcutaneous adipose tissue accumulation by providing a beef cattle; and providing an effective amount of one or more agents that interact with a GPR43 receptor in the beef cattle, wherein the one or more agents modulate the GPR43 receptor to preferentially enhance the marbling in the beef cattle but has no effect on subcutaneous adipose tissue accumulation in the beef cattle.

15 One embodiment of the present invention provides a method of modulating a GPR43 receptor activity to control intramuscular adipose tissue development without affecting subcutaneous fat accumulation by providing an intramuscular adipose tissue having a GPR43 receptor; and providing a pharmacologically effective amount of one or more agents that interact with a GPR43 receptor in the intramuscular adipose tissue to preferentially
20 enhance the development of an intramuscular adipose tissue in the intramuscular adipose tissue but has no effect on a subcutaneous adipose tissue accumulation.

One embodiment of the present invention provides a feed composition to selectively modulating an intramuscular adipose tissue including a feed composition; and an effective amount of one or more agents that interact with a GPR43 receptor disposed in the feed
25 composition, wherein the one or more agents interact with the GPR43 receptor in the intramuscular adipose tissue to preferentially enhance the development of an intramuscular adipose tissue in the intramuscular adipose tissue but has no effect on a subcutaneous adipose tissue accumulation.

Another embodiment of the present invention provides a feed supplement to selectively
30 modulate an intramuscular adipose tissue including a feed supplement; and an effective amount of one or more agents disposed in the feed supplement, wherein the one or more agents interact with a GPR43 receptor in the intramuscular adipose tissue to preferentially

enhance the development of an intramuscular adipose tissue in the intramuscular adipose tissue but has no effect on a subcutaneous adipose tissue accumulation.

Description of the Drawings

For a more complete understanding of the features and advantages of the present invention,
5 reference is now made to the detailed description of the invention along with the accompanying figures and in which:

FIGURE 1A is a graph of the relative GPR43 mRNA level was greater expressed in intramuscular adipose tissue than subcutaneous adipose tissue and muscle tissues.

FIGURE 1B is a graph of the GPR41 and similar isoforms of GPR43.

10 FIGURE 2A is an image of a gel of the Bovine GPR 43 protein level was greater in the intramuscular adipose tissue compared to subcutaneous adipose tissue.

FIGURE 2B is a graph of the level of relative quantification of GPR 43 per GAPDH was greater in intramuscular adipose tissue than subcutaneous adipose tissue.

FIGURE 2C is an image of a coomassie blue-staining data revealed that total protein
15 amounts from three tissues were loaded at same concentration.

FIGURES 3A and 3B are images of multi-locular lipid droplets accumulated in the cultured subcutaneous and intramuscular adipocytes and FIGURES 3C and 3D are images of the loaded samples.

FIGURE 3E is a graph of the oleic acid increased GPR43/GAPDH protein levels in the
20 intramuscular adipocyte compared to subcutaneous adipocytes.

FIGURE 3F is a graph of the oleic acid tended increase relative GPR 43 mRNA level in the intramuscular adipocyte compare to subcutaneous adipocyte.

FIGURE 4A is a schematic of the reaction of GPR 43 located in intramuscular and subcutaneous adipocytes.

25 FIGURE 4B is an image of the mechanism of action of fatty acid enhances glucose absorption through Glucose Transpoter 4(GLUT4).

FIGURES 5A-5D show IM and SC adipocyte treated with 100 μ M oleic acid differentiation cocktail; 10 μ M insulin, 100 μ M oleic acid, 4 μ M dexamethasone, and 10 μ M ciglitizone. FIGURES 5E-5G show the mRNA levels for PPAR γ and GPR 43.

FIGURES 6A-6F shows the effect of media fatty acids on gene transcription in IM and SC adipocytes.

FIGURES 7A-7C show GPR 43 mRNA levels in IM and SC adipocytes in response to media oleic acid.

- 5 FIGURES 8A shows the levels of GPR 43 protein in the total protein isolated from bovine peripheral tissues. FIGURES 8B shows the relative GPR 43 mRNA levels in IM adipose tissue, SC adipose tissue, and L.D. muscle (MUS). FIGURES 8C-8D show the protein level of GPR 43 per GAPDH was greater in IM adipose tissue and LD than in SC adipose tissue.

Description of the Invention

- 10 While the making and using of various embodiments of the present invention are discussed in detail below, it should be appreciated that the present invention provides many applicable inventive concepts that can be embodied in a wide variety of specific contexts. The specific embodiments discussed herein are merely illustrative of specific ways to make and use the invention and do not delimit the scope of the invention.
- 15 To facilitate the understanding of this invention, a number of terms are defined below. Terms defined herein have meanings as commonly understood by a person of ordinary skill in the areas relevant to the present invention. Terms such as "a", "an" and "the" are not intended to refer to only a singular entity, but include the general class of which a specific example may be used for illustration. The terminology herein is used to describe specific
- 20 embodiments of the invention, but their usage does not delimit the invention, except as outlined in the claims.

- As used herein, the term "GPR43 polypeptide" refers to a receptor having two essential properties: 1) a GPR43 polypeptide has at least 80% amino acid identity, preferably 85%, 90%, 95%, or higher, up to and including 100% identity; and 2) a GPR43 polypeptide has
- 25 GPR43 activity including either or both of GPR43 ligand binding activity or GPR43 signaling activity as defined herein.

- As used herein, "ligand" refers to a moiety that is capable of associating or binding to a receptor. According to the method of the invention, a ligand and a receptor have a binding constant that is sufficiently strong to allow detection of binding by an assay method that is
- 30 appropriate for detection of a ligand binding to a receptor. A ligand according to the invention includes the actual molecule that binds a receptor (e.g. propionate) or a ligand may

be any nucleotide, antibody, antigen, enzyme, peptide, polypeptide or nucleic acid capable of binding to the receptor. A ligand may be a short chain carboxylic acid but can also include a polypeptide, a peptide or a nucleic acid sequence.

As used herein, an "antagonist" is a ligand which competitively binds to a receptor at the same site as an agonist, but does not activate an intracellular response initiated by an active form of the receptor. An antagonist thereby inhibits the intracellular response induced by an agonist, by at least 10%, 15-25%, 25-50% and 50-100%, as compared to the intracellular response in the presence of an agonist and in the absence of an antagonist.

As used herein, an "agonist" refers to a ligand that activates an intracellular response when it binds to a receptor at concentrations equal to or lower than propionate concentrations which induce an intracellular response. An agonist according to the invention can increase the intracellular response mediated by a receptor by at least 2-fold, 5-fold, 10-fold and 100-fold or more (i.e., 150-fold, 200-fold, 250-fold, 500-fold, 1000-fold, 10,000-fold etc), as compared to the intracellular response in the absence of agonist.

As used herein, an "inverse agonist" refers to a ligand which decreases a constitutive activity of a cell surface receptor when it binds to a receptor. An inverse agonist according to the invention can decrease the constitutive intracellular response mediated by a receptor by at least 2-fold, 5-fold, 10-fold and 100-fold or more (i.e., 150-fold, 200-fold, 250-fold, 500-fold, 1000-fold, 10,000-fold etc.), as compared to the intracellular response in the absence of inverse agonist.

An "inhibitor" compound according to the invention is a molecule directed against the receptor or against the natural ligand for the receptor that decreases the binding of the ligand to the receptor by at least 10%, preferably 15-25%, more preferably 25-50% and most preferably, 50-100%, in the presence of acetate or propionate, as compared to the binding in the presence of acetate or propionate and in the absence of inhibitor. An "inhibitor" compound of the invention can decrease the intracellular response induced by an agonist, for example acetate or propionate, by at least 10%, 15-25%, 25-50% and 50-100%. An "inhibitor" also refers to a nucleotide sequence encoding an inhibitor compound of the invention. An inhibitor, useful according to the present invention, includes, but is not limited to an antibody which specifically binds to at least a portion of GPR43 which is required for signal transduction through GPR43 or chemical compounds which are capable of blocking or reducing (e.g., by at least 10%) the signal transduction pathway which is coupled to the

GPR43 receptor. Such inhibitors include, but are not limited to sub-lethal doses of pertussis toxin, N-ethylmaleimide, dibutyryl cAMP, and H-89 (N-[2-((p-bromocinnamyl)amino)ethyl]-5-isoquinolinesulfonamide-HCL).

As used herein, "natural ligand" refers to a naturally occurring ligand, found in nature, which
5 binds to a receptor. A "natural ligand" does not refer to an engineered ligand that is not found in nature and that is engineered to bind to a receptor, where it did not formerly do so in a manner different, either in degree or kind, from that which it was engineered to do. Such an engineered ligand is no longer naturally-occurring but is "non-natural" and is derived from a naturally occurring molecule.

10 As used herein, a "modulator" refers to a compound that increases or decreases the cell surface expression of a receptor of the invention, increases or decreases the binding of a ligand to a receptor of the invention, or any compound that increases or decreases the intracellular response initiated by an active form of the receptor of the invention, either in the presence or absence of an agonist, and in the presence of a ligand for the receptor. A
15 modulator includes an agonist, antagonist, inhibitor or inverse agonist, as defined herein. A modulator can be for example, a polypeptide, a peptide, an antibody or antigen-binding fragment thereof, a lipid, a carbohydrate, a nucleic acid, and a small organic molecule. Candidate modulators can be natural or synthetic compounds, including, for example, synthetic small molecules, compounds contained in extracts of animal, plant, bacterial or
20 fungal cells, as well as conditioned medium from such cells.

Pharmaceutical compositions for administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, foods, supplements, additives, and the
25 like, for ingestion.

Pharmaceutical preparations can be obtained through a combination of active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers such as sugars, including lactose,
30 sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethyl cellulose; and gums including arabic and tragacanth; and proteins such as gelatin and

collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.

Pharmaceutical formulations for parenteral administration include aqueous solutions of active compounds. For injection, the pharmaceutical compositions of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer' solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

One embodiment of the present invention provides a preferential enhancement of intramuscular adipose tissue without changing subcutaneous adipose tissue development in food animals, e.g., beef cattle, swine, poultry, sheep, etc. Therefore we could market cattle at lighter end points, thus improving the overall efficiency of growth in the beef cattle sector.

In one embodiment, an animal can be treated to modulate the signaling activity of a GPR43 receptor by administering a sub-lethal dose of an agent which inhibits or promotes the signaling activity of GPR43. A sub-lethal dose refers to a dose of an agent for inhibiting or stimulating a GPR43 signaling activity which is at or below the LD50 for the particular agent. In one embodiment, an agent useful for the modulation of GPR43 signaling may be an antibody which specifically binds to the ligand binding site of GPR43. An amount of anti-GPR43 antibody needed to achieve a dosage useful for the modulation of GPR43 signaling will depend upon the level of expression of GPR43, localization of receptor expression.

A modulator can be for example, a polypeptide, a peptide, an antibody or antigen-binding fragment thereof, a lipid, a carbohydrate, a nucleic acid, and a small organic molecule. Candidate modulators can be natural or synthetic compounds, including, for example, synthetic small molecules, compounds contained in extracts of animal, plant, bacterial or fungal cells, as well as conditioned medium from such cells.

Bovine adiposity is not only a factor affecting overall carcass composition but also an important determinant of beef carcass quality and economic value. Deposition of intramuscular fat or marbling in beef cattle contributes significantly to meat quality

parameters, including juiciness, flavor and tenderness. Marbling is often defined as the adipose tissue within muscle bundles or intramuscular adipose tissue. It is generally recognized that marbling is the last adipose tissue to be deposited on a finishing beef animal, although adipose tissue starts accumulating early in life. The accumulation of intramuscular fat is largely influenced by the genetic background of cattle, as well age and plane of nutrition. Highly-marbled beef requires not only long-term feeding but also cattle need to consume a high-energy diet for greater than 100 days. However, high energy diet and long term feeding causes subcutaneous fat accumulation instead of intramuscular fat because subcutaneous adipose tissue development precedes marbling development. Elevation of inedible subcutaneous adipose tissue reduces economical benefits. Because of this disparity in various adipose tissue development, animal pharmaceutical companies and feedlot nutritionists have been searching for compounds or diet programs that will preferentially enhance intramuscular adipose tissue development but have no effect on subcutaneous fat accumulation. An important key to exploiting this difference is to find novel receptors present in intramuscular adipose tissue that are not found in subcutaneous adipose tissue. The use of specific pharmacological agents to target these receptors and manipulate one adipose tissue type but not the other.

In contrast to mouse adipose tissue depot sites, both GPR (G-protein-coupled receptor) 41 and GPR43 mRNA were detected in a variety of bovine tissues with the exception of perirenal and subcutaneous adipose tissues. However, we found that GPR 43 protein is present in bovine intramuscular adipose tissue but not in bovine subcutaneous adipose tissue. This is a very novel finding. GPR 43 has been shown to be present in a variety of tissues, particularly abundant in adipose tissue stores, inflammatory cells and the gastrointestinal tract. GPR43 is also highly expressed in mouse perirenal, subcutaneous, mesenteric and parametrial adipose tissue as compared to other tissue tested. GPR43 once activated by specific ligands, plays a major role in adipogenic development and differentiation of mesenchymal precursor cells. Level of GPR43 in different adipose depot regulates different level of glucose absorption through cell membrane and glucose is more important for marbling development than for subcutaneous fat development in early age development. Because the proportion of carbon source from glucose for intramuscular adipose tissue development is higher than subcutaneous adipose tissue development. The present invention uses this novel finding to enhance marbling accumulation in beef without excessive subcutaneous adipose tissue accumulation.

Bovine adiposity is not only a factor affecting overall carcass composition but also an important determinant of beef carcass quality and economic value. Deposition of intramuscular fat or marbling in beef cattle contributes significantly to meat quality parameters, including juiciness, flavor and tenderness. Marbling is often defined as the adipose tissue within muscle bundles or intramuscular adipose tissue. High-energy diet and long-term feeding causes subcutaneous fat accumulation instead of intramuscular fat because subcutaneous adipose tissue development precedes marbling development. Elevation of inedible subcutaneous adipose tissue reduces economical benefits. Because of this disparity in various adipose tissue developments, animal pharmaceutical companies and feedlot nutritionists have been searching for compounds or diet programs that will preferentially enhance intramuscular adipose tissue development but have no effect on subcutaneous fat accumulation. An important key to exploiting this difference is to find novel receptors present in intramuscular adipose tissue that are not present in subcutaneous adipose tissue. The use of specific pharmacological agents to target these receptors will result in opportunities to manipulate one adipose tissue type but not the other. GPR 43 has been shown to be present in a variety of tissues, particularly abundant in certain adipose tissue stores, inflammatory cells and the gastrointestinal tract (Brown et al., 2003). Hong et al., 2003 reported that GPR 43 was highly expressed in mouse perirenal, subcutaneous, mesenteric and parametrial adipose tissue as compared to other tissue tested. This suggested that GPR 43, once activated by specific ligands, may play a major role in adipogenic development and differentiation of mesenchymal precursor cells. Level of GPR 43 in different adipose depot may regulate different level of glucose absorption through cell membranes. Wang et al., (2009) indicated that bovine GPR 43, regulated by volatile fatty acids was expressed in the rumen and pancreas but not expressed in the subcutaneous adipose tissue. Smith and Crouse (1984) presented that glucose is more important for marbling development than for subcutaneous fat development in early age development. Therefore, we hypothesized that the bovine intramuscular and subcutaneous adipose tissues have different expression patterns of GPR 43 levels and that GPR 43 may be related with glucose uptake in the intramuscular adipose tissue. We discovered that GPR 43 protein is present in bovine intramuscular adipose tissue but not in subcutaneous adipose tissue allowing to potentially differentially affect intramuscular adipose tissue but not subcutaneous adipose tissue.

Intramuscular and subcutaneous adipose tissues were collected from heifer and steers. Adipose tissue was transported to the laboratory in 0.2µm filter sterilized 38°C phosphate-buffered saline (PBS: 0.76M NaCl, 0.3M NaH₂PO₄, pH 7.2) containing 100U/ml penicillin, 100µg/ml streptomycin and 250ng/ml fungizone. The tissues were minced finely and

5 incubated in sterile digestion medium containing Dulbecco's modified Eagle's Medium (DMEM), 5% fetal bovine serum (FBS), collagenase, and penicillin-streptomycin (PS) for 40 minutes. The collagenase-digested adipose tissue was filtered through a 250 µm nylon membrane, and the cell suspension centrifuged at 2000 x g. After discarding the supernatant and lipid layer, the pellet was washed three times in DMEM. The pellet, containing stromal-

10 vascular (s.v.) cells, was resuspended and maintained in growth media composed of DMEM, 10% fetal bovine serum, and antibiotics at 37°C under a humidified atmosphere of 95% O₂ and 5% CO₂. Upon reaching confluence, the growth medium was replaced by a differentiation medium composed of DMEM and 10 µg/mL insulin, 10 µg/mL Dexamethsone, 10 µM oleic acid, 5 µM ciglitizone, and antibiotics.

15 Real time-PCR sample preparation and RNA isolation: Total RNA isolated using Sigma's Tri-reagent. Concentration of RNA was determined by absorbance at 260 nm. Integrity of RNA was determined by electrophoresis of totals RNA through a 1% agarose-formaldehyde gel followed by ethidium bromide staining to allow visualization of 28 and 18S ribosomal RNA (rRNA). After RNA integrity was assessed, 1ug of RNA was reverse-transcribed to

20 produce the first-strand complementary DNA (cDNA).

Real-time RT-PCR was used to measure the quantity of GPR43 mRNA relative to the quantity of ribosomal protein subunit 9 (RPS9) mRNA in total RNA isolated from cultured intramuscular and subcutaneous adipocytes and adipose tissues. Complementary DNA was produced from 1 ug RNA using Taq- Man Reverse Transcriptase Reagents (Applied

25 Biosystems, Foster City, CA) and the protocol recommended by the manufacturer. Measurement of the relative quantity of the cDNA of interest was carried out using TAMRA PCR Master Mix (Applied Biosystems, Foster City, CA), appropriate forward and reverse primers, and 1 uL of the cDNA mixture. Assays were performed in the 7900HT Real-Time PCR System (Applied Biosystems) using thermal cycling parameters recommended by the

30 manufacturer (40 cycles of 15 seconds at 95°C and 1 minute at 60°C). Titration of GPR 43 mRNA primers against increasing amounts of cDNA give linear responses with slopes between -2.8 and -3.0. In order to reduce the effect of assay-to-assay variation in the PCR

assay, all values were calculated relative to a calibration standard run on every real-time PCR assay. Bovine RPS9 primers and probes were used as an endogenous control.

Preparation of Protein Extracts: Total protein was isolated using the Mammalian Protein Extraction Reagent (M-PER; Pierce Biotechnology, Rockford, IL). Adipose tissues (0.5 g) were homogenized with 3mL of M-PER contained 2 mM Na_3VO_4 and Complete (Roche protease inhibitor tablets). Cells were rinsed with warm PBS and extraction reagent (200 μL /well) was added. The cultures were gently shaken for 5 minutes to ensure complete cell lysis then centrifuged for 10 minutes at 10,000 rpm. The supernatant was collected and protein concentration was determined using a ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE).

Western Blot Analysis: Protein samples were denatured using equal volume of sodium dodecyl sulfate (SDS)- β -mercaptoethanol and boiled for 2 minutes. Total protein (30 μg) was separated by gel electrophoresis using Novex 10-20% Tris-Glycine Gels (Invitrogen, Carlsbad, CA). Gels were run for 100 min at 125 V and 130 mA. The protein was transferred onto a PVDF membrane (Bio-Rad, Hercules, CA). The PVDF membranes were blocked using XCell SURELOCK™ Mini-Cell (Invitrogen, Carlsbad, CA) for 120 minutes at 4°C. The primary antibody GPR43 and GAPDH (ab12571 and ab9484; Abcam, Inc., Cambridge, MA) in blocking buffer was added to the membrane and incubated overnight at 4°C. Following overnight incubation, the membrane was washed three times with PBST. The secondary antibody for the GPR43 and GAPDH primary antibody was added to the membrane and incubated for 2 hours. Membranes were exposed to the X-ray film after treated chemiluminescent reagent. Detection and quantification of proteins were analyzed using a BioRad Imaging System (BioRad, Hercules, CA).

FIGURE 1A is a graph of the relative GPR43 mRNA level was greater expressed in intramuscular adipose tissue than subcutaneous adipose tissue and muscle tissues. Total RNA was isolated from intramuscular, subcutaneous, and muscle tissues. All analysis was triplicate. FIGURE 1B is a graph of the GPR41 and similar isoforms of GPR43. Relative GPR43 mRNA level was greater expressed in intramuscular adipose tissue than subcutaneous adipose tissue as seen in FIGURE 1A. We analyzed mRNA level isolated from intramuscular, subcutaneous, and muscle tissue. All analysis was triplicates from two animals. Relative levels of GPR 43 mRNA were highly expressed in intramuscular adipose tissue compared to subcutaneous adipose tissue from both cattle. From these data we

reported that GPR 43 mRNA is highly distributed in bovine intramuscular adipocyte but less distributed in bovine subcutaneous adipocyte.

FIGURE 2A is an image of a gel of the Bovine GPR 43 protein level was greater in the intramuscular adipose tissue compared to subcutaneous adipose tissue. FIGURE 2B is a graph of the level of relative quantification of GPR 43 per GAPDH was greater in intramuscular adipose tissue than subcutaneous adipose tissue. FIGURE 2C is an image of a coomassie blue-staining data revealed that total protein amounts from three tissues were loaded at same concentration. Western blotting data demonstrated that bovine GPR 43 protein is greater in intramuscular adipose tissue than in subcutaneous adipose tissue (FIGURE 2A). Interestingly, this data indicated that the protein level of intramuscular adipose tissue was similar as that level of LD muscle tissue. Level of relative quantification of GPR 43 per GAPDH is greater in intramuscular adipose tissue than SC adipose tissue (FIGURE 2B). FIGURE 2C demonstrated that the level of total protein in each lane was similar. Western gel stained with coomassie blue solution presented same amount of proteins loaded in each lane. Although the same concentration of protein was loaded in each lane, unique protein bands were shown in three different tissues.

Multilocular lipid droplets were accumulated in the cultured intramuscular adipocytes but unilocular lipid accumulated in the subcutaneous adipocytes (FIGURES 3A-3F). FIGURE 3A and 3B are images of multi-locular lipid droplets accumulated in the cultured subcutaneous and intramuscular adipocytes. FIGURES 3C and 3D are images of the loaded samples. FIGURE 3E is a graph of the oleic acid increased GPR43/GAPDH protein levels in the intramuscular adipocyte compared to subcutaneous adipocytes. FIGURE 3C is a graph of the oleic acid tended increase relative GPR 43 mRNA level in the intramuscular adipocyte compare to subcutaneous adipocyte. FIGURE 3A and 3B show multilocular lipid droplets were accumulated in the cultured subcutaneous and intramuscular adipocytes. These subcutaneous and intramuscular adipocytes showed different patterns of lipid droplet accumulation. Oleic acid increased, not only amount of GPR 43 protein but also increased relative level of GPR 43 mRNA in the intramuscular adipocyte cultures in a dose-dependent fashion. FIGURE 3E is an image of oleic acid increased GPR43/GAPDH protein levels in the intramuscular adipocyte compared to subcutaneous adipocytes. Bars are means $\pm$ SE relative to control. Bars abcde differ from control ($P < 0.05$). Values are the means of three experiments. FIGURE 3F shows that oleic acid tended to increase relative GPR 43 mRNA

level in the intramuscular adipocyte compare to subcutaneous adipocyte. Values are the means of 3 replicates. Both adipocytes were incubated with differentiation factors such as insulin, ciglitizone, and dexamethasone. GPR 43 protein levels were increased in dose-dependent treated oleic acid at intramuscular but not at subcutaneous adipocytes.

- 5 Intramuscular adipocytes significantly increased GPR 43 protein and mRNA levels depending on dose-titrated oleic acids (FIGURES 3E and 3F). Relative GPR 43 mRNA levels tended to be increased in intramuscular adipocytes. Oleic acid tended to increase relative GPR 43 mRNA level in the intramuscular adipocyte compare to subcutaneous adipocyte.
- 10 FIGURE 4A is a schematic of the reaction of GPR 43 located in intramuscular and subcutaneous adipocytes. FIGURE 4B is an image of the mechanism of action of fatty acid enhances glucose absorption through Glucose Transporter 4 (GLUT4). The reaction of GPR 43 located in subcutaneous and intramuscular adipocytes. FIGURE 4A shows GPR 43 distributed in plasma membrane was located greater in intramuscular adipocyte than
- 15 subcutaneous adipocytes. Level of GPR 43 in intramuscular adipocytes enhances absorption of glucose through plasma membrane. FIGURE 4B shows a mechanism of action of fatty acid enhances glucose absorption through Glucose Transporter 4 (GLUT4). Initiation of GPR 43 was activated G-protein complex and regulated cAMP signal pathway. Phosphorylation of AMPK was activated GLUT4 in the membrane protein. GLUT4 was activated absorption
- 20 of glucose molecule into the cytosol.

- This difference between IM and SC adipose tissue illustrated that metabolic regulation in these two fat depots was activated by tissue-specific mechanisms. Both G protein-coupled receptor (GPR) 41 and 43 are activated by short chain fatty acids (SCFA), such as acetate or propionate, and showed differential expression in adipose tissue during differentiation
- 25 (Brown et al., 2003; Hong et al. 2005). These reports indicated that GPR 43 is highly expressed in isolated adipocytes but has a lower expression in stromal-vascular cells. This suggests that the physiological effects of SCFA or long chain fatty acid (LCFA) in peripheral tissues may be regulated through the GPRs. Wang et al. (2009) indicated that the amino acid sequence of bovine GPR 41 and 43 were more than 75% identical to those of
- 30 human GPR 41 and 43. Additionally, Wang et al. (2009) showed that GPR 41 and 43 genes were not detected in bovine SC, perirenal, or intermuscular adipose tissues. We hypothesized that GPR 43 was differentially distributed between SC and IM adipose tissues and oleic acid may increase lipid synthesis via GPR 43 in IM adipose tissue, but not in SC adipose tissue.

Thus, the objective of this study was to determine the effect of fatty acids on bovine IM and SC adipocyte differentiation, GPR 43 mRNA expression and GPR 43 protein concentration.

The IM and SC preadipocytes were cultured in DMEM containing 10% FBS, and antibiotics. Plates were incubated for 96 h to reach approximately 80% confluency. Bovine IM and SC preadipocytes were differentiated with fatty acid-free DMEM containing 5% FBS with 10 μ M insulin, 4 μ M dexamethasone, and 10 μ M ciglitizone (FAF-Diff). Cultures were maintained with the FAF-Diff mixture as a control and additions of 1, 10, 100, or 500 μ M oleic acid (18:1 ; n-9), 100 μ M of stearic acid (18:0), 100 μ M of linoleic acid (18:2 ; n-6), or 100 μ M α -linolenic acid (18:3 ; n-3) for 96 h.

Oil-Red-O and hematoxylin staining were used to confirm accumulating lipid droplets in differentiated IM and SC adipocytes. The cells were fixed with 10% neutral buffer formalin. After washing, the cells were stained with 0.5% Oil-Red-O solution in the dark for 20 min and then washed with 60% propylene glycol. The cell nuclei were stained by Harris' hematoxylin in the dark for 3 min and then mounted with glycerin. Intramuscular and SC adipocytes were identified by the presence of lipid droplets in the cytosol, which were changed to a red color by Oil-red-O staining.

At 96 h, after fatty acid treatments, total RNA from IM and SC adipocytes was isolated with 500 μ L of TRI-Reagent (Sigma, St. Louis, MO). The concentration of RNA was determined by absorbance at 260 nm. Complementary DNA (cDNA) was produced from 1 μ g of RNA using Taq- Man Reverse Transcriptase Reagents (Applied Biosystems, Foster City, CA) according to the protocol recommended by the manufacturer. Random hexamers were used as primers for cDNA synthesis. Measurement of the relative quantity of the cDNA of interest was carried out using TAMRA PCR Master Mix (Applied Biosystems, Foster City, CA) with the appropriate forward and reverse primers and 1 μ L of the cDNA mixture. Real-time PCR was used to measure the quantity of CCAAT/enhancer-binding protein β (C/EBP β), peroxisome proliferator-activated receptor γ (PPAR γ), stearoyl-CoA desaturase (SCD), and GPR 43 mRNA relative to the quantity of ribosomal subunit 9 (RPS9) mRNA. Assays were performed in the GeneAmp 7900H Sequence Detection System (Applied Biosystems) using thermal cycling parameters recommended by the manufacturer (40 cycles of 15 s at 95°C and 1 min at 60°C).

Cultured IM and SC adipocytes were isolated with 200 μ L of ice-cold buffer containing M-PER (Fisher Scientific, Fair Lawn, NJ), protein inhibitor (Roche, Indianapolis, IN), and 2

mM sodium vanadate (Na_3VO_4 ; Fisher Scientific). Cell homogenate was mixed with an equal volume of 2x standard SDS sample loading buffer (Invitrogen). Ten to 20% gradient gels were used for SDS-PAGE separation of proteins. Proteins were transferred to membranes, blocked with non-fat dry milk, and then incubated overnight at 4°C in primary antibodies (GPR 43, PPAR γ , SCD; Abcam, Cambridge, MA) at a dilution of 1:1,000 in TBS/Tween. Membranes were incubated with horseradish peroxidase-conjugated secondary antibodies at dilution of 1:2000 for 2 hours. After three, 10 minutes washes in TBS/Tween, membranes were visualized using Enhanced Chemiluminescent Substrate (ECL) Western blotting reagents (GE Healthcare, Piscataway, NJ) and exposure to film (MR, Kodak, Rochester, NY). Density of bands was quantified by using Imager Scanner II and ImageQuant TL software. To reduce the variation between blots, tissue lysates of both groups were run on a single gel. Band density was normalized according to the GAPDH content.

FIGURES 5A-5D are images of IM and SC adipocyte treated with 100 μM oleic acid differentiation cocktail; 10 μM insulin, 100 μM oleic acid, 4 μM dexamethasone, and 10 μM ciglitizone. FIGURES 5A and 5C are microscopic images of Oil-red- O staining and FIGURES 5B and 5D are images of Oil-red-O staining indicated a differentiation of IM and SC adipocytes. Multilocular lipid droplets were detected in IM adipocytes, whereas, unilocular lipid droplets were detected in SC adipocytes.

FIGURES 5E-5G are images of the mRNA levels for PPAR γ and GPR 43 were compared to control and IM and SC adipocytes differentiated in 100 μM oleic acid. Oleic acid increased PPAR γ mRNA, decreased SCD mRNA, and did not affect GPR 43 mRNA in both IM and SC adipocytes.

Intramuscular and SC adipocytes displayed morphological differences (FIGURE 5). IM adipocytes had multilocular lipid droplets, whereas the SC adipocytes had unilocular lipid droplets (FIGURE 5A). Treatment with 100 μM oleic acid increased ($P < 0.05$) the mRNA level of PPAR γ but decreased ($P < 0.05$) the mRNA level of SCD in IM and SC adipocytes (FIGURES 5E-5G). Additionally, PPAR γ gene expression was lower in IM adipocytes than in SC adipocytes. No effect on GPR 43 mRNA was detected with oleic acid treatment.

FIGURES 6A-6F are graphs of the effect of media fatty acids on gene transcription in IM and SC adipocytes. Cont = control, without differentiation cocktail; FAF = Fatty acid free differentiation cocktail; Ste = FAF + Stearic Acid; Ole = FAF + Oleic acid; Li = FAF +

Linoleic acid; and Ln = FAF + Linolenic acid. Bars are means $\pm$ SEM (n=3). Means with common superscripts do not differ ($P > 0.05$). Fatty acids treatments had differential effects on bovine IM and SC adipocytes. Relative C/EBP β mRNA levels in SC adipocyte were lower in adipocytes treated with was stearic acid and linolenic acid than in adipocytes treated in FAF-Diff media. PPAR γ mRNA levels were increased ($P < 0.05$) in SC adipocytes treated with linoleic acid and α -linolenic acid, compared to other treatments. In IM adipocytes, the level of SCD mRNA compared to the FAF-Diff treatment was decreased ($P < 0.05$) when treated with any of the fatty acids. In both IM and SC adipocytes, linoleic and linolenic acid decreased ($P < 0.05$) SCD mRNA levels compared to the FAF-Diff treatment. The level of AMPK mRNA was greater ($P < 0.05$) in SC adipocytes treated with linoleic acid than adipocytes treated with stearic acid. GPR 43 mRNA levels were greater ($P < 0.05$) in linoleic acid than stearic acid in IM and SC adipocytes. There were no differences detected in GLUT4 levels between the fatty acid treatments in either IM or SC adipocytes.

FIGURES 7A-7C show GPR 43 mRNA levels in IM and SC adipocytes in response to media oleic acid. FIGURE 7A is a western blot image of oleic acid treatment on GPR 43 in bovine IM and SC adipocyte cultures. FIGURE 7B shows dose response of GPR 43 protein to oleic acid increased relative GPR 43 protein in IM adipocytes but not SC adipocytes. FIGURE 7B shows oleic acid tended increase relative GPR 43 mRNA levels in the IM adipocytes but not SC adipocytes ($P = 0.098$). Relative GPR 43 protein levels showed a dose-dependent increase in response to greater concentrations of oleic acid ($P < 0.05$) in IM adipocyte cultures. Oleic acid had no effect on GPR 43 mRNA ($P > 0.10$) in SC adipocytes (FIGURE 7B). Intramuscular adipocytes tended ($P = 0.098$) have elevated relative GPR 43 mRNA levels in response to treatment with oleic acid (FIGURE 7C). Again, SC adipocytes were not affected by oleic acid for relative GPR 43 mRNA levels ($P > 0.10$).

FIGURE 8A shows the levels of GPR 43 protein in the total protein isolated from bovine peripheral tissues. FIGURE 8B shows the relative GPR 43 mRNA levels in IM adipose tissue, SC adipose tissue, and L.D. muscle (MUS). FIGURES 8C-8D show the protein level of GPR 43 per GAPDH was greater in IM adipose tissue and LD than in SC adipose tissue. SI, small intestine; LI, large intestine; SM MUS, semimembranosus muscle; P AT, perirenal adipose tissue; and SC AT, subcutaneous adipose tissue. Means with common superscripts do not differ ($P > 0.05$). Data are means $\pm$ SEM (n=3). We also analyzed relative GPR 43 mRNA and protein level in IM adipose tissue, SC adipose tissue, and *longissimus* muscle

tissue (FIGURE 8). Relative GPR 43 protein levels were greater ($P < 0.05$) in IM adipose tissue than in muscle or SC adipose tissue (FIGURE 8B). IM adipose tissue had a greater expression ($P < 0.05$) of GPR 43 mRNA than SC adipose tissue.

The morphological difference between IM and SC adipocytes illustrated that these two cells types are fundamentally different and as a result may react differently to treatment with long chain fatty acids. The mRNA levels of C/EBP β and PPAR γ in adipocytes indicated that the adipogenic transcription factors expressed were more active in the SC adipocytes than in IM adipocytes. This suggests that IM and SC preadipocytes from stromal-vascular cells may represent different cell lines. Compared to IM adipocytes in pigs, SC adipocytes have been shown to have a greater expression of fatty acid binding protein (FABP) 4 and 5, proteins which are responsible for transporting fatty acids across the extracellular membrane into adipocytes (Zhou et al., 2010). This would support the theory that SC adipocytes have the ability to deposit a greater amount of lipid than IM adipocytes.

In SC adipocytes, stearic acid and oleic acid increased PPAR γ gene expression relative to FAF-Diff treatment. However, linoleic (18:2; n-6) and α -linolenic acid (18:3; n-3) both elicited greater PPAR γ expression than all other fatty acid treatments. This indicates that long-chain polyunsaturated fatty acids may be more effective in upregulating PPAR γ gene expression than mono- and saturated fatty acids in SC adipose tissue.

Unlike PPAR γ , linoleic and α -linolenic acid downregulated SCD gene expression in both IM and SC adipocytes. Stearoyl-CoA desaturase is responsible for converting stearic acid (18:0) to oleic acid (18:1;n-9) and is an indicator of adipocyte development. Pasture feeding can contain high levels of linoleic and α -linolenic acid. This suggests that pasture fed animals may experience a decreased SCD gene expression and could have slower adipocyte development as a result.

Hong et al. (2005) demonstrated that GPR 43 is highly expressed in isolated mouse adipocytes but has lower expression in stromal-vascular cells, indicating that the GPRs are expressed only in differentiated cells. Wang et al. (2009) reported that relative GPR 41 and GPR 43 mRNA levels were not detected in bovine adipose tissue. This was partially confirmed in the current study where no GPR protein was detected in bovine perirenal or SC adipose tissues. However, both GPR 43 protein and mRNA levels were greater in IM adipose tissue than in SC adipose tissue. Additionally, GPR 43 mRNA in IM adipose tissue was greater than that found in muscle tissue. The differences in GPR 43 mRNA and protein

levels between the IM and muscle tissues may be caused by different rates of GPR mRNA translation or protein turnover in those two tissues.

In both IM and SC adipocytes, linoleic acid increased GPR 43 gene expression. This was similar to the effect of linoleic acid on PPAR γ gene expression. These results indicate that
5 long-chain unsaturated fatty acids may have greater effects on GPR 43 expression than long-chain saturated fatty acids. Because GPR 43 activation can result in the inhibition of lipolysis and reduction in plasma free fatty acids (Ge et al., 2008), the increased GPR 43 expression seen with linoleic acid is likely linked to increased lipogenesis.

Wagyu cattle, which have the capacity for high levels of intramuscular fat, have a greater
10 genetic tendency to produce oleic acid and this production may be linked to marbling (Smith et al., 2006). In the current study, oleic acid treatment did not affect the early adipogenic transcriptional factors, C/EBP β and PPAR γ , in either IM or SC adipocytes. However, GPR 43 mRNA and protein levels in IM adipocytes showed a dose-dependent increase response to oleic acid. No difference was detected in SC adipocytes for either GPR 43 mRNA or
15 protein levels. Oleic acid may have autocrine or paracrine effects in stimulating marbling development in IM adipose tissue, whereas SC adipose development was less responsive to oleic acid. Therefore, oleic acid may be a critical factor in the GPR 43 pathway in bovine IM adipose tissue.

The current study illustrated that IM and SC adipocytes were morphologically different and
20 had differential responses to long chain fatty acids. It also showed that GPR 43 expression was enhanced by the treatment with oleic acid in IM adipocytes. Thus, oleic acid may be a critical factor in IM adipocyte differentiation, but not SC early differentiation.

It is contemplated that any embodiment discussed in this specification can be implemented with respect to any method, kit, reagent, or composition of the invention, and vice versa.
25 Furthermore, compositions of the invention can be used to achieve methods of the invention.

It will be understood that particular embodiments described herein are shown by way of illustration and not as limitations of the invention. The principal features of this invention can be employed in various embodiments without departing from the scope of the invention. Those skilled in the art will recognize, or be able to ascertain using no more than routine
30 experimentation, numerous equivalents to the specific procedures described herein. Such equivalents are considered to be within the scope of this invention and are covered by the claims.

The use of the word “a” or “an” when used in conjunction with the term “comprising” in the claims and/or the specification may mean “one,” but it is also consistent with the meaning of “one or more,” “at least one,” and “one or more than one.” The use of the term “or” in the claims is used to mean “and/or” unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and “and/or.” Throughout this application, the term “about” is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, or the variation that exists among the study subjects.

As used in this specification and claim(s), the words “comprising” (and any form of comprising, such as “comprise” and “comprises”), “having” (and any form of having, such as “have” and “has”), “including” (and any form of including, such as “includes” and “include”) or “containing” (and any form of containing, such as “contains” and “contain”) are inclusive or open-ended and do not exclude additional, unrecited elements or method steps.

The term “or combinations thereof” as used herein refers to all permutations and combinations of the listed items preceding the term. For example, “A, B, C, or combinations thereof” is intended to include at least one of: A, B, C, AB, AC, BC, or ABC, and if order is important in a particular context, also BA, CA, CB, CBA, BCA, ACB, BAC, or CAB. Continuing with this example, expressly included are combinations that contain repeats of one or more item or term, such as BB, AAA, AB, BBC, AAABCCCC, CBBAAA, CABABB, and so forth. The skilled artisan will understand that typically there is no limit on the number of items or terms in any combination, unless otherwise apparent from the context.

All of the compositions and/or methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

What is claimed is:

1. A method of selectively modulating an intramuscular adipose tissue in beef cattle comprising the steps of:
 - providing a beef cattle; and
 - providing a pharmacologically effective amount of one or more agents that interact
5 with a GPR43 receptor in the beef cattle, wherein the one or more agents modulate the GPR43 receptor to preferentially enhance the development of an intramuscular adipose tissue in the beef cattle but has no effect on subcutaneous adipose tissue accumulation in the beef cattle.
2. The method of claim 1, wherein the one or more agents are selected from a peptide, a
10 polypeptide, an antibody, an antigen-binding fragment, a lipid, a carbohydrate, a nucleic acid, and a small organic molecule.
3. The method of claim 1, wherein the one or more agents is a recombinant GPR43 polypeptide.
4. The method of claim 1, wherein the one or more agents is a ligand.
- 15 5. The method of claim 1, wherein the one or more agents comprise a virus coding for a GPR43 polypeptide.
6. The method of claim 1, wherein the one or more agents is disposed in a synthetic liposomes comprising a GPR43 polypeptide.
7. A composition to selectively modulating an intramuscular adipose tissue in beef
20 cattle comprising:
 - a carrier; and
 - a pharmacologically effective amount of one or more agents that interact with a GPR43 receptor disposed in the carrier.

8. The composition of claim 7, wherein the one or more agents are selected from a peptide, a polypeptide, an antibody or antigen-binding fragment thereof, a lipid, a carbohydrate, a nucleic acid, and a small organic molecule.
9. The composition of claim 7, wherein the one or more agents is a recombinant GPR43 polypeptide.
10. The composition of claim 7, wherein the one or more agents is a ligand.
11. The composition of claim 7, wherein the one or more agents comprise a virus coding for a GPR43 polypeptide.
12. The composition of claim 7, wherein the one or more agents comprise a synthetic liposomes comprising a GPR43 polypeptide.
13. A method of enhance intramuscular adipose tissue development without affecting subcutaneous fat accumulation in an animal comprising the steps of:
- providing an animal; and
- administering to the animal a pharmacologically effective amount of one or more agents that interact with a GPR43 receptor in the animal to preferentially enhance the development of an intramuscular adipose tissue but does not effect a subcutaneous adipose tissue accumulation in the animal.
14. The method of claim 13, wherein the animal is beef cattle, swine, poultry, sheep, human, bison, lamb, horse, dog, cat or goat.
15. The method of claim 13, wherein the one or more agents are selected from a ligand, a peptide, a polypeptide, a recombinant GPR43 polypeptide, an antibody or antigen-binding fragment thereof, a lipid, a carbohydrate, a nucleic acid, and a small organic molecule.
16. The method of claim 13, wherein the one or more agents comprise a virus coding for a GPR43 polypeptide.
17. The method of claim 13, wherein the one or more agents contacting are disposed in a synthetic liposomes comprising a GPR43 polypeptide.

18. A method of modulating an adipose tissue comprising the steps of:
- providing an intramuscular adipose tissue; and
- providing a pharmacologically effective amount of one or more pharmacological agents that interact with a GPR43 receptor in the intramuscular adipose tissue, wherein the GPR43 receptor preferentially enhances the development of an intramuscular adipose tissue but has no effect on a subcutaneous adipose tissue.
19. The method of claim 18, wherein the one or more agents are selected from a ligand, a peptide, a polypeptide, a recombinant GPR43 polypeptide, an antibody or antigen-binding fragment thereof, a lipid, a carbohydrate, a nucleic acid, and a small organic molecule.
20. A composition to enhance intramuscular adipose tissue development without affecting subcutaneous fat accumulation comprising:
- a pharmacologically effective amount of one or more compositions that interact with a GPR43 receptor disposed in a carrier to preferentially enhance the development of an intramuscular adipose tissue but does not effect a subcutaneous adipose tissue accumulation in the animal.
21. The composition of claim 20, wherein the one or more agents are selected from a ligand, a peptide, a polypeptide, a recombinant GPR43 polypeptide, an antibody or antigen-binding fragment thereof, a lipid, a carbohydrate, a nucleic acid, and a small organic molecule.
22. A method of enhancing marbling accumulation in beef cattle without excessive subcutaneous adipose tissue accumulation comprising the steps of:
- providing a beef cattle; and
- providing an effective amount of one or more agents that interact with a GPR43 receptor in the beef cattle, wherein the one or more agents modulate the GPR43 receptor to preferentially enhance the marbling in the beef cattle but has no effect on subcutaneous adipose tissue accumulation in the beef cattle.

23. The method of claim 22, wherein the one or more agents are selected from a ligand, a peptide, a polypeptide, a recombinant GPR43 polypeptide, an antibody or antigen-binding fragment thereof, a lipid, a carbohydrate, a nucleic acid, and a small organic molecule.

24. A method of modulating a GPR43 receptor activity to control intramuscular adipose tissue development without affecting subcutaneous fat accumulation comprising the steps of:

providing an intramuscular adipose tissue having a GPR43 receptor; and

providing a pharmacologically effective amount of one or more agents that interact with a GPR43 receptor in the intramuscular adipose tissue to preferentially enhance the development of an intramuscular adipose tissue in the intramuscular adipose tissue but has no effect on a subcutaneous adipose tissue accumulation.

25. The method of claim 24, wherein the one or more agents are selected from a ligand, a peptide, a polypeptide, a recombinant GPR43 polypeptide, an antibody or antigen-binding fragment thereof, a lipid, a carbohydrate, a nucleic acid, and a small organic molecule.

26. A feed composition to selectively modulating an intramuscular adipose tissue comprising:

a feed composition; and

an effective amount of one or more agents that interact with a GPR43 receptor disposed in the feed composition, wherein the one or more agents interact with the GPR43 receptor in the intramuscular adipose tissue to preferentially enhance the development of an intramuscular adipose tissue in the intramuscular adipose tissue but has no effect on a subcutaneous adipose tissue accumulation.

27. The method of claim 26, wherein the feed composition is an animal feed wherein the animal is selected from cattle, swine, poultry, sheep, human, bison, lamb, horse, dog, cat or goat.

28. The feed composition of claim 26, wherein the one or more agents is selected from a ligand, a peptide, a polypeptide, a recombinant GPR43 polypeptide, an antibody or antigen-binding fragment thereof, a lipid, a carbohydrate, a nucleic acid, and a small organic molecule.

29. A feed supplement to selectively modulate an intramuscular adipose tissue comprising:

a feed supplement; and

an effective amount of one or more agents disposed in the feed supplement, wherein
5 the one or more agents interact with a GPR43 receptor in the intramuscular adipose tissue to preferentially enhance the development of an intramuscular adipose tissue in the intramuscular adipose tissue but has no effect on a subcutaneous adipose tissue accumulation.

30. The feed supplement of claim 29, wherein the feed supplement is an animal feed
10 supplement wherein the animal is selected from beef cattle, swine, poultry, sheep, human, bison, lamb, horse, dog, cat or goat.

31. The feed supplement of claim 29, wherein the one or more agents are selected from a
ligand, a peptide, a polypeptide, a recombinant GPR43 polypeptide, an antibody or antigen-
binding fragment thereof, a lipid, a carbohydrate, a nucleic acid, and a small organic
15 molecule.

1/8

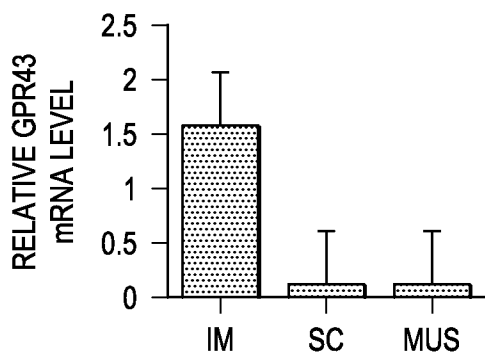


FIG. 1A

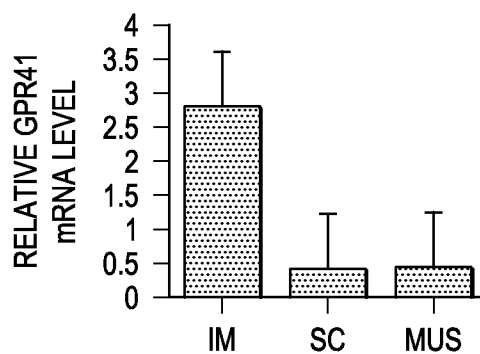


FIG. 1B

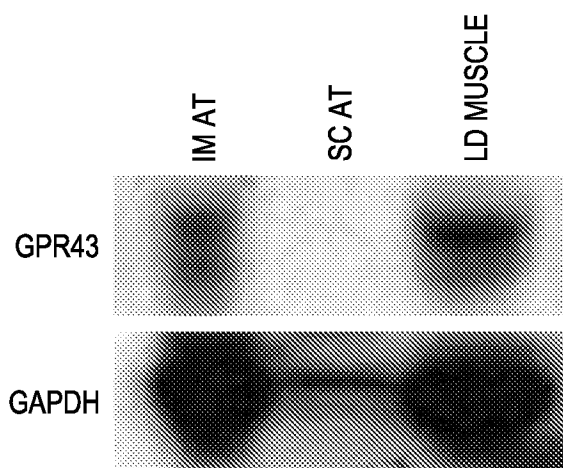


FIG. 2A

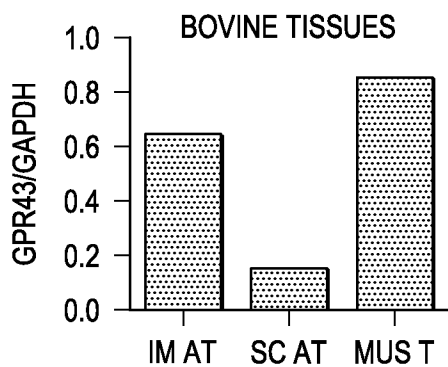


FIG. 2B

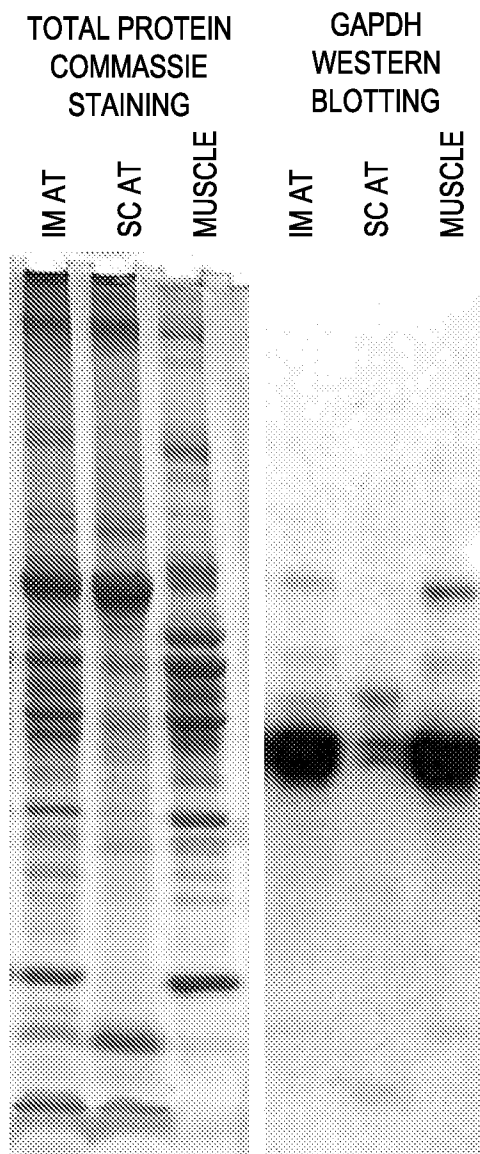


FIG. 2C

2/8

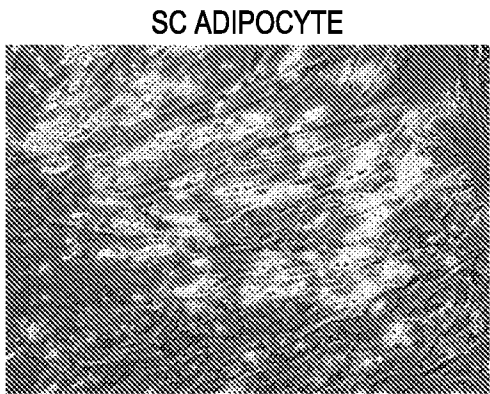


FIG. 3A

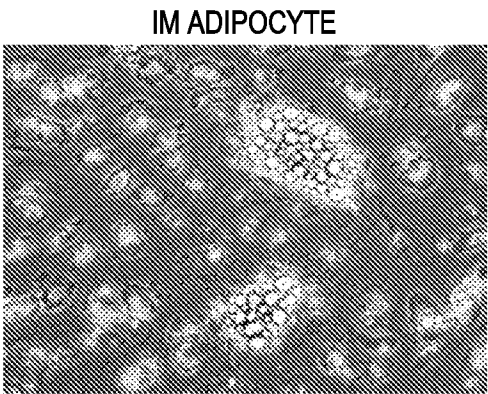


FIG. 3B

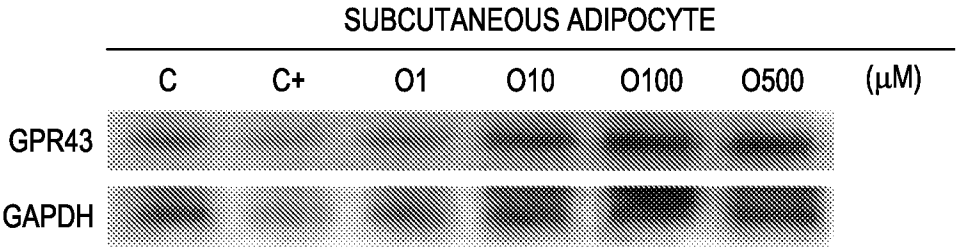


FIG. 3C

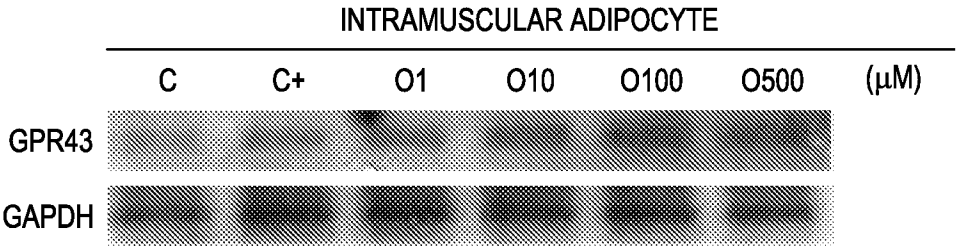


FIG. 3D

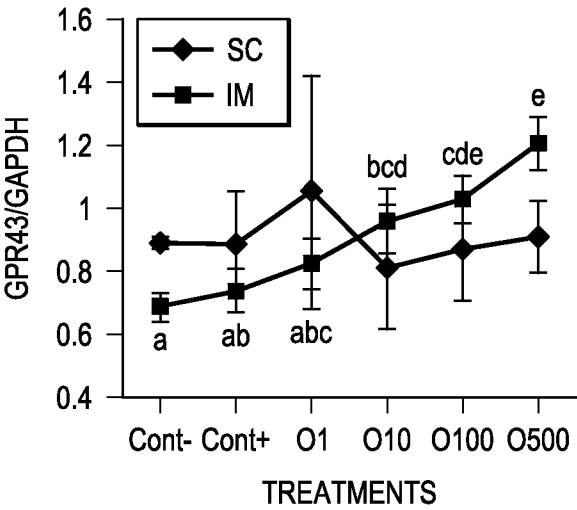


FIG. 3E

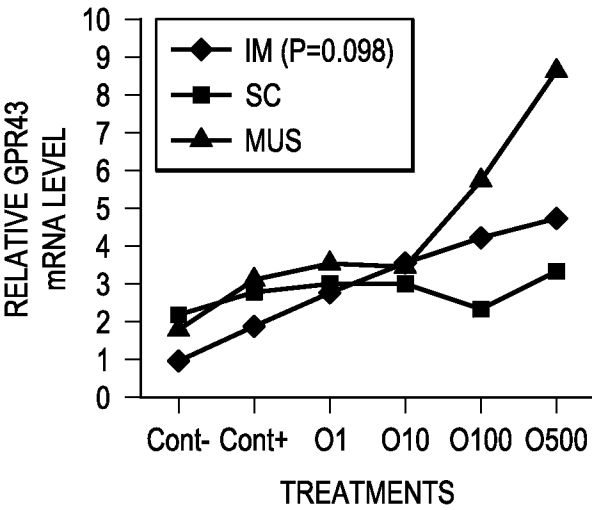


FIG. 3F

3/8

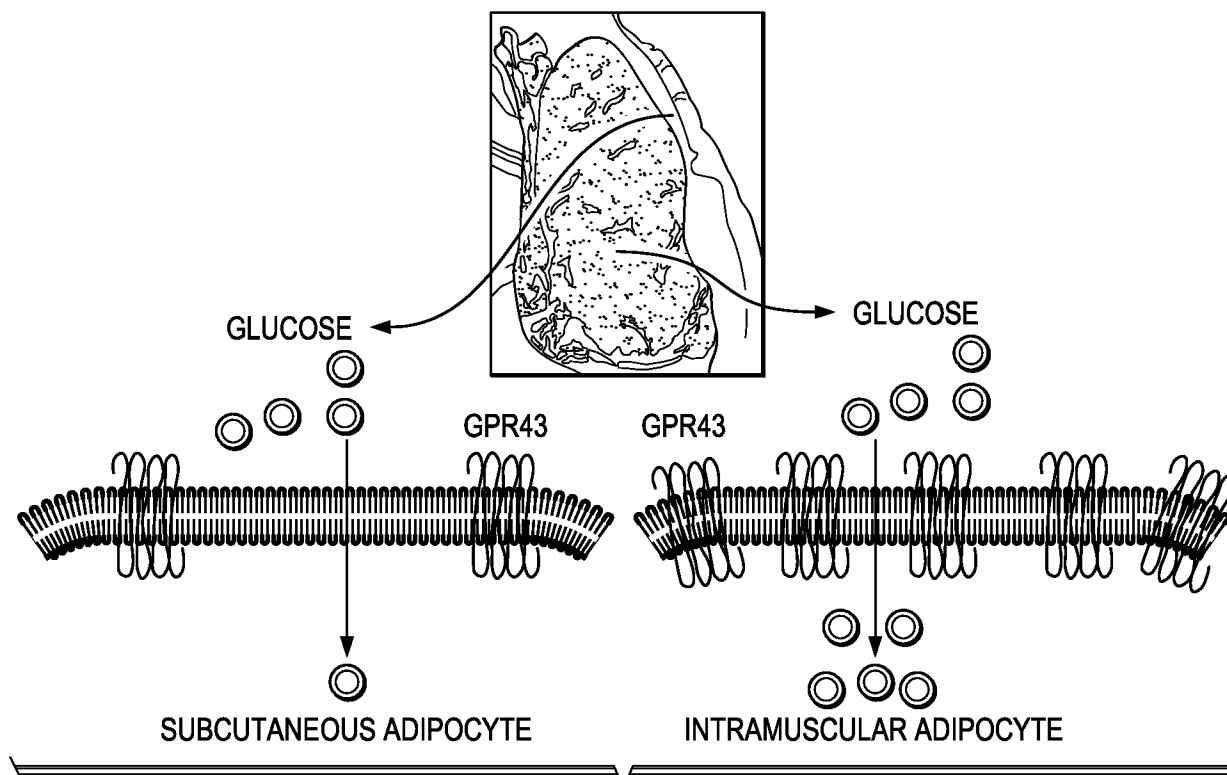


FIG. 4A

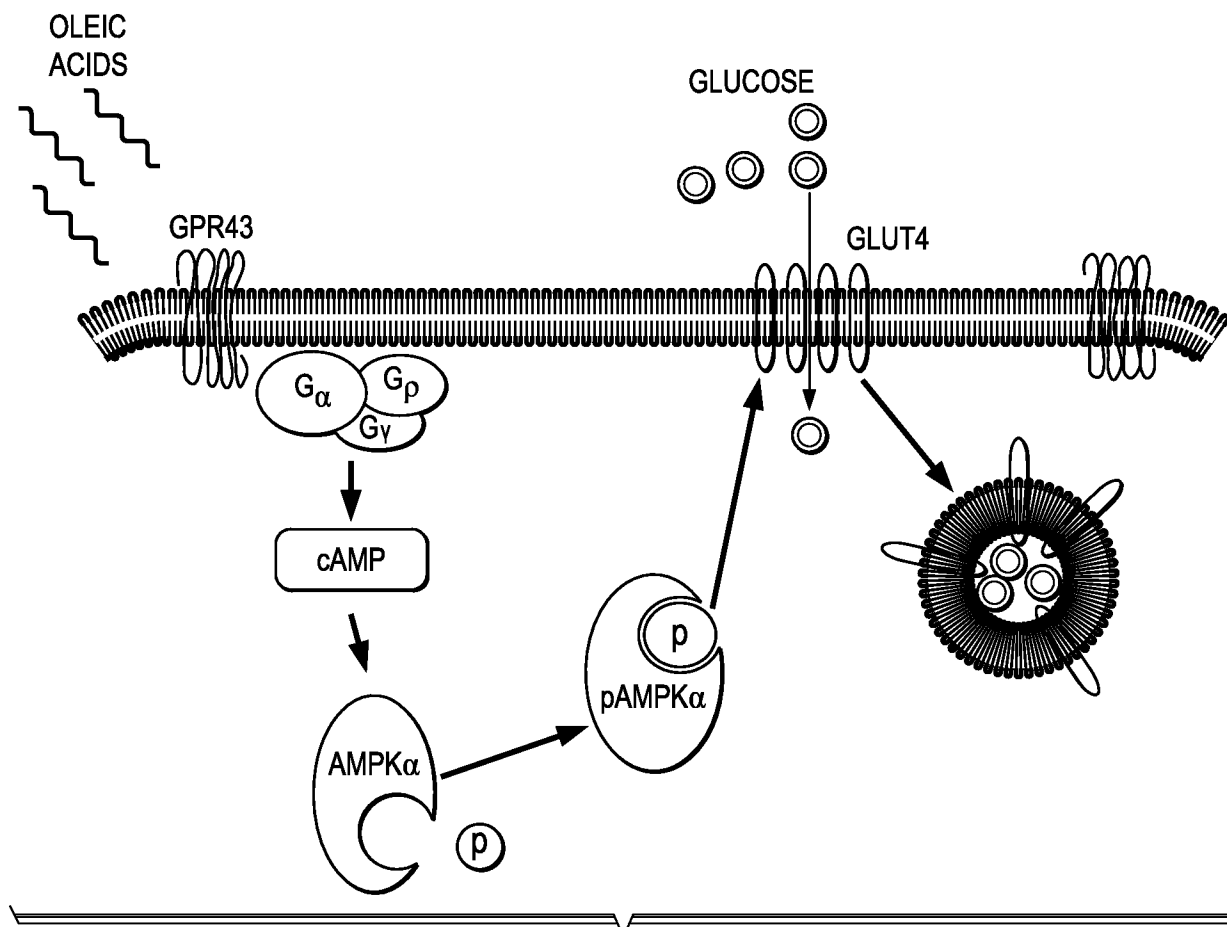


FIG. 4B

4/8

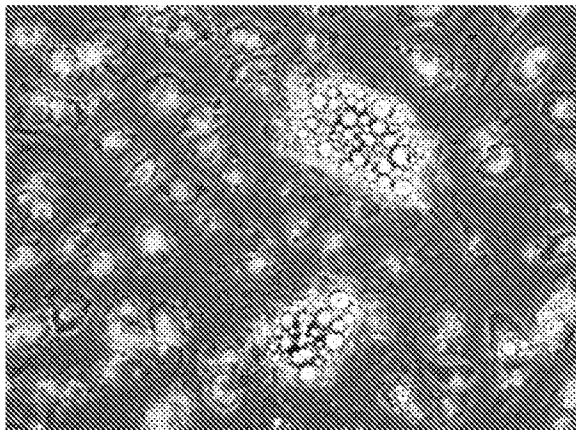


FIG. 5A

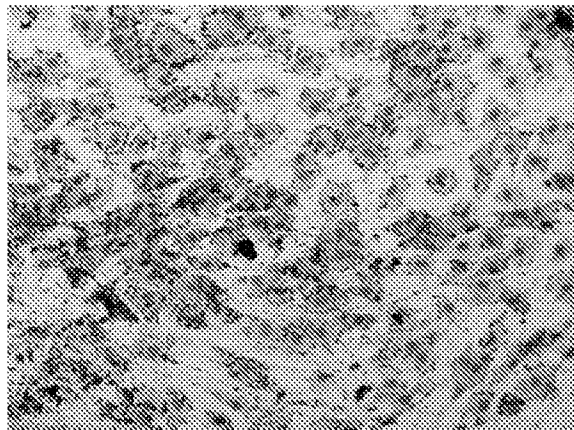


FIG. 5B

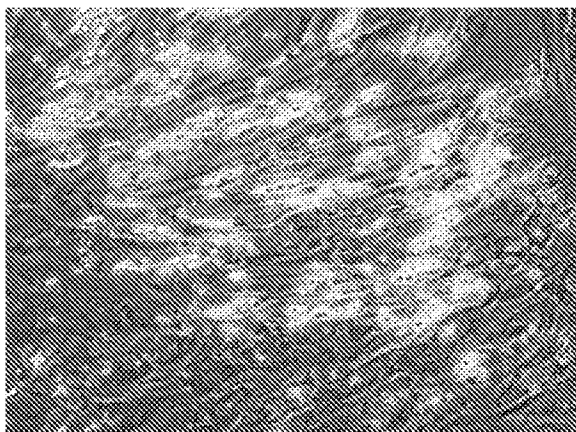


FIG. 5C

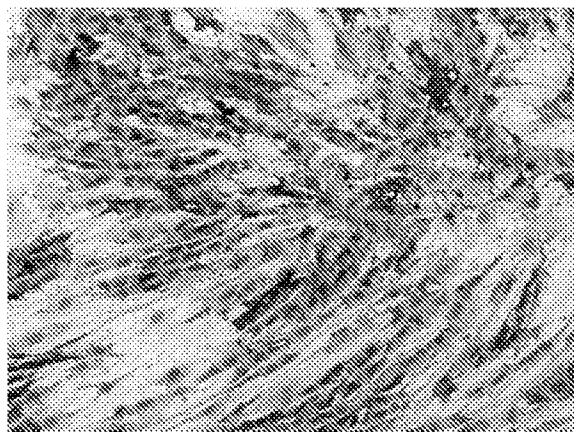


FIG. 5D

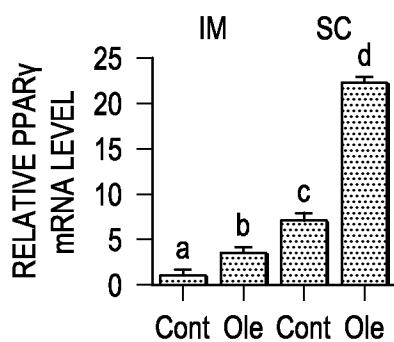


FIG. 5E

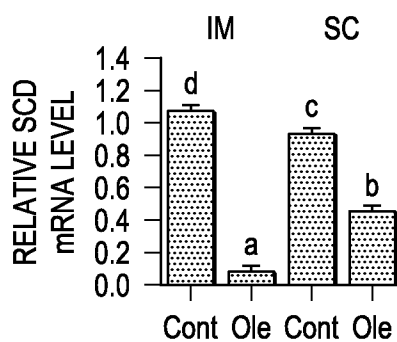


FIG. 5F

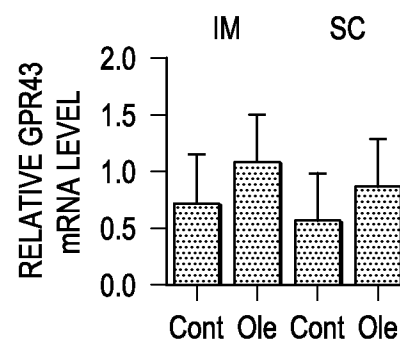


FIG. 5G

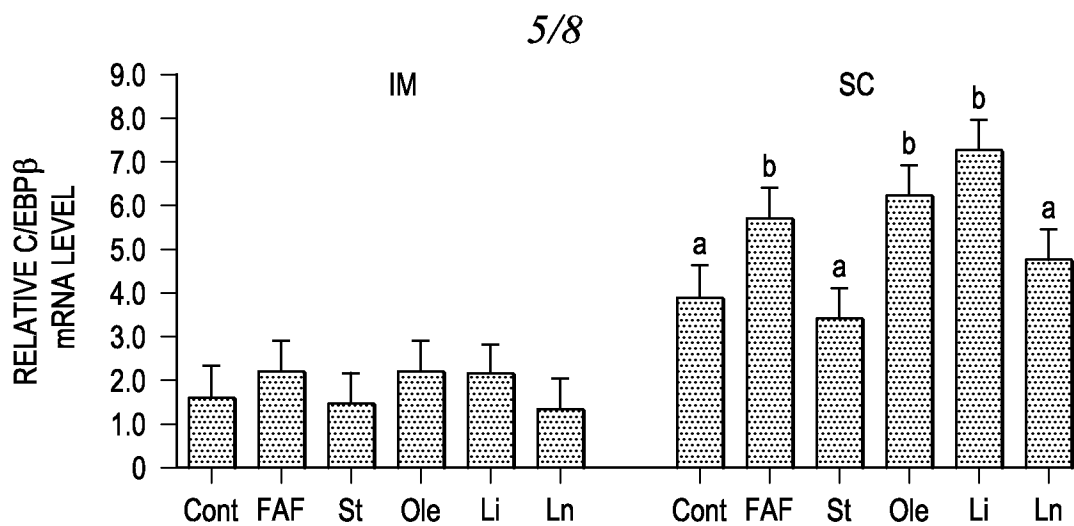


FIG. 6A

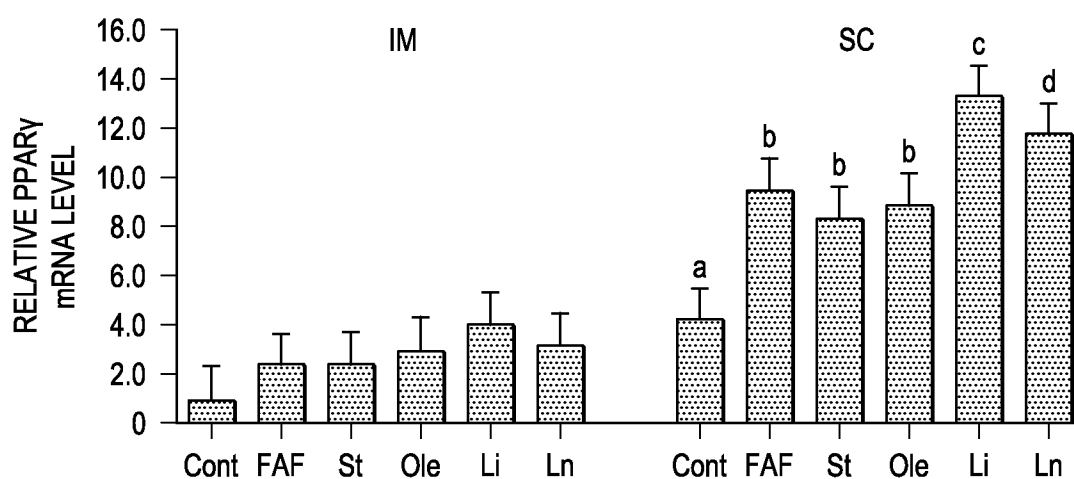


FIG. 6B

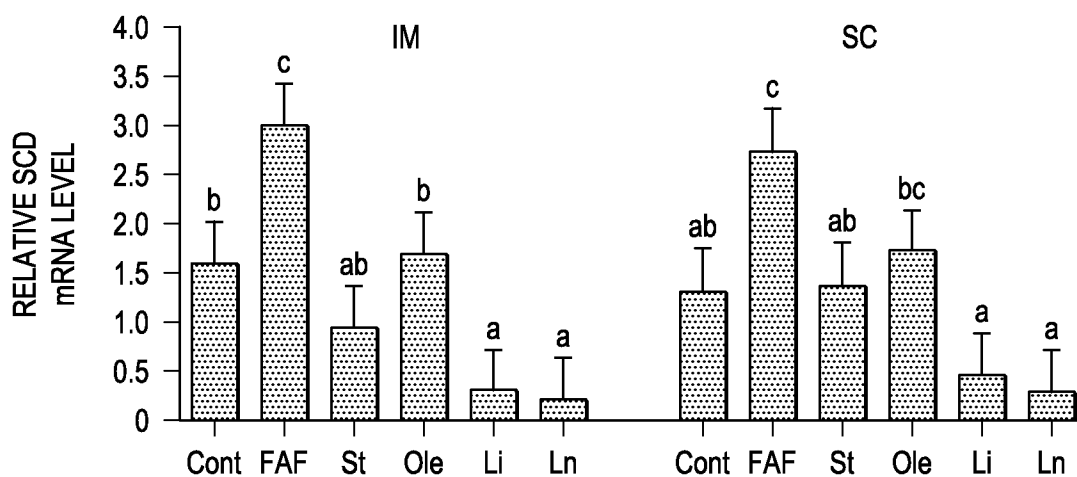


FIG. 6C

6/8

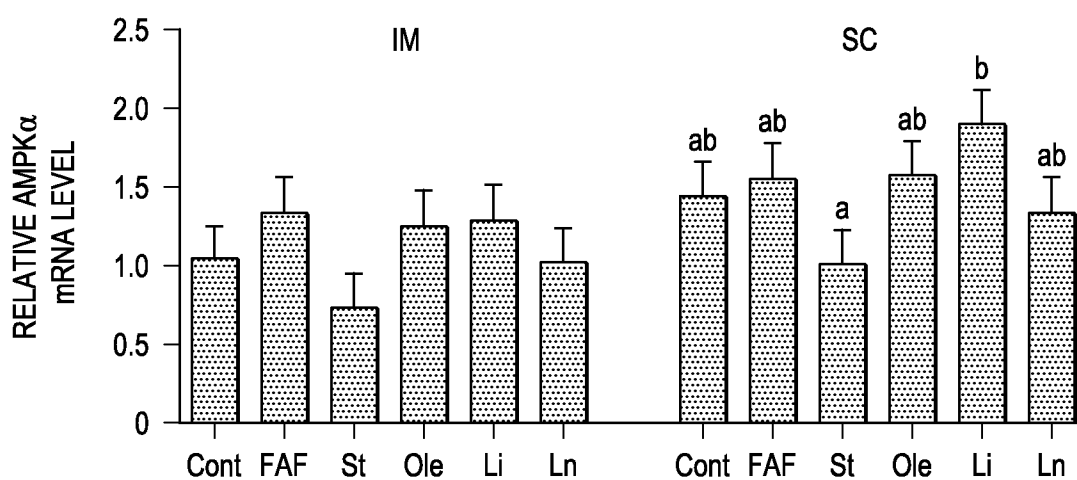


FIG. 6D

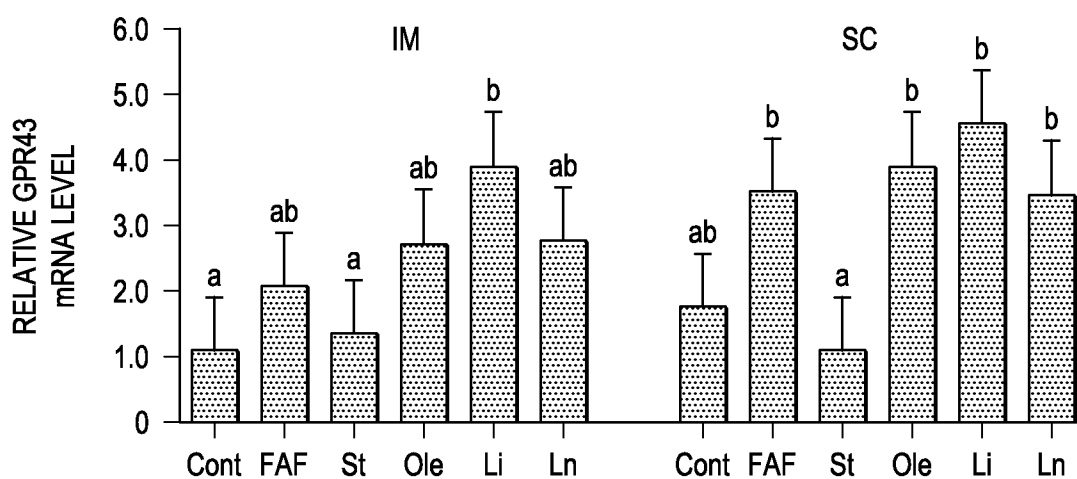


FIG. 6E

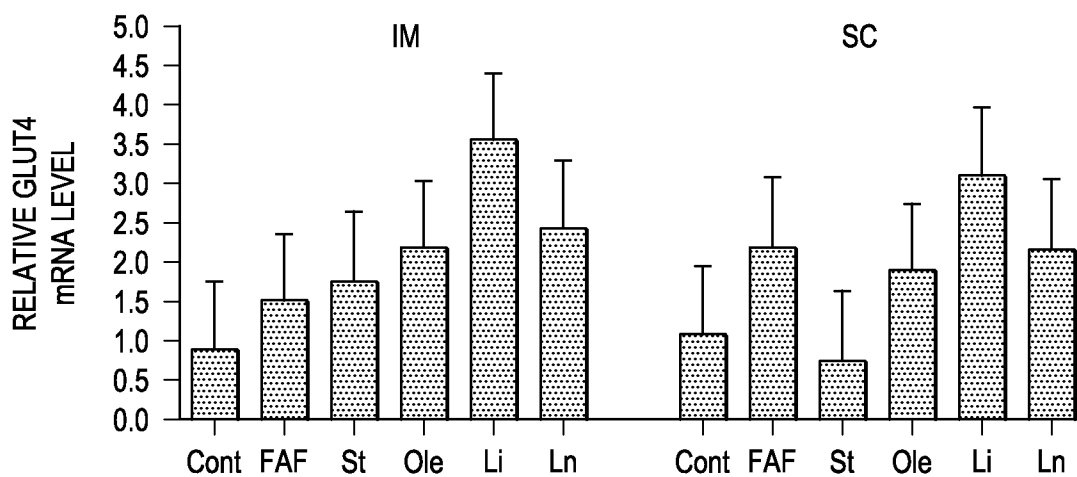


FIG. 6F

7/8

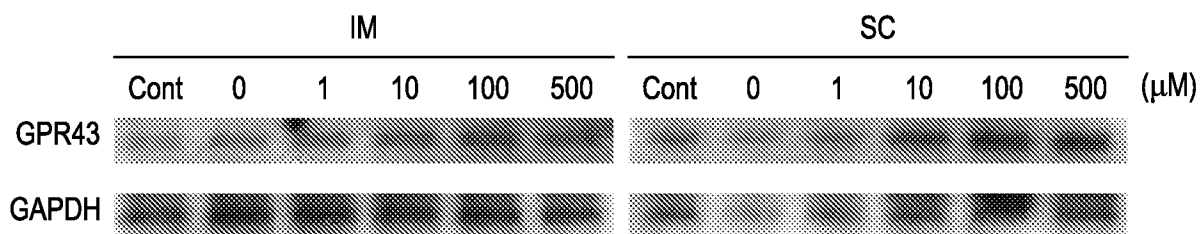


FIG. 7A

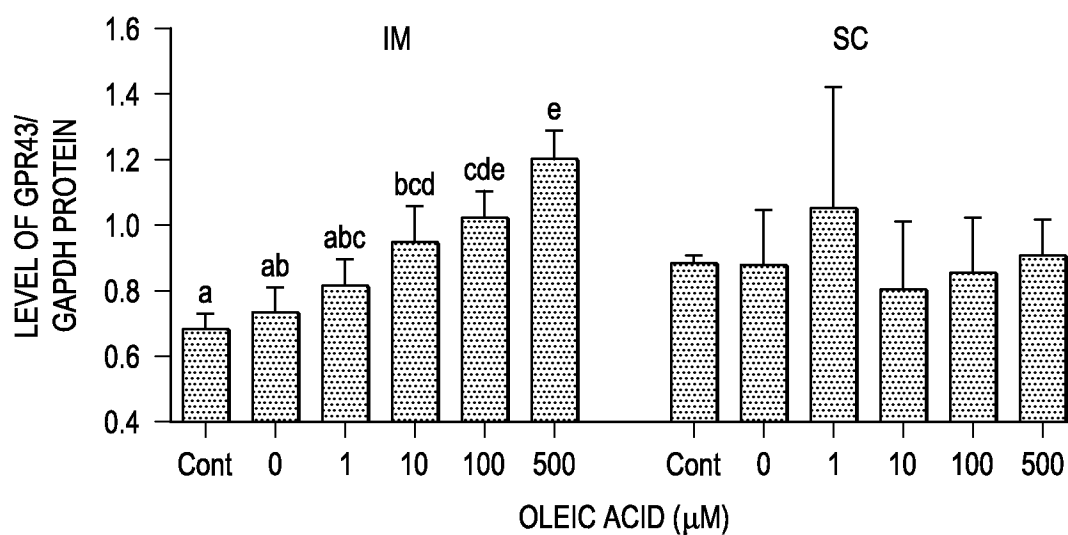


FIG. 7B

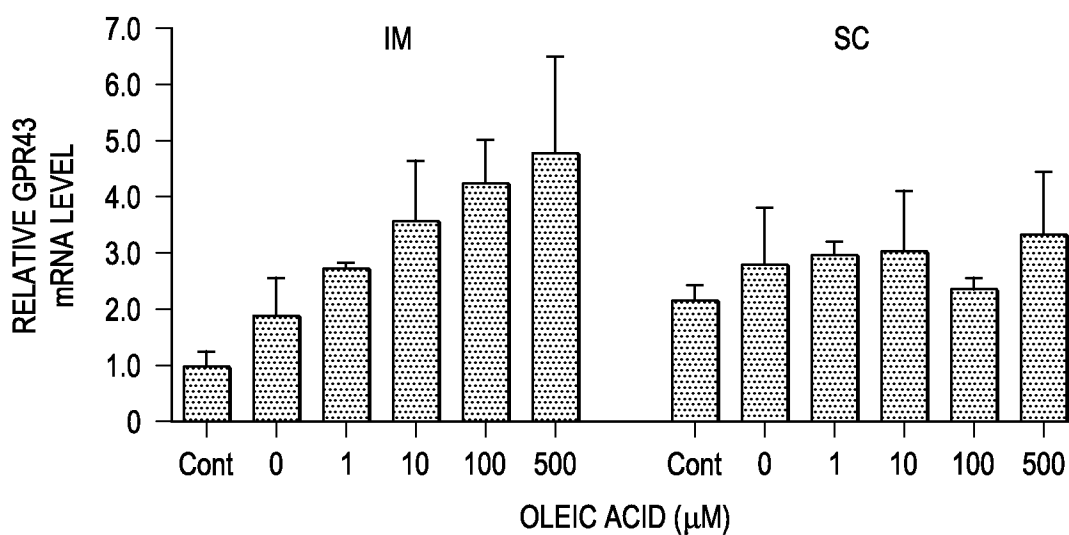


FIG. 7C

8/8

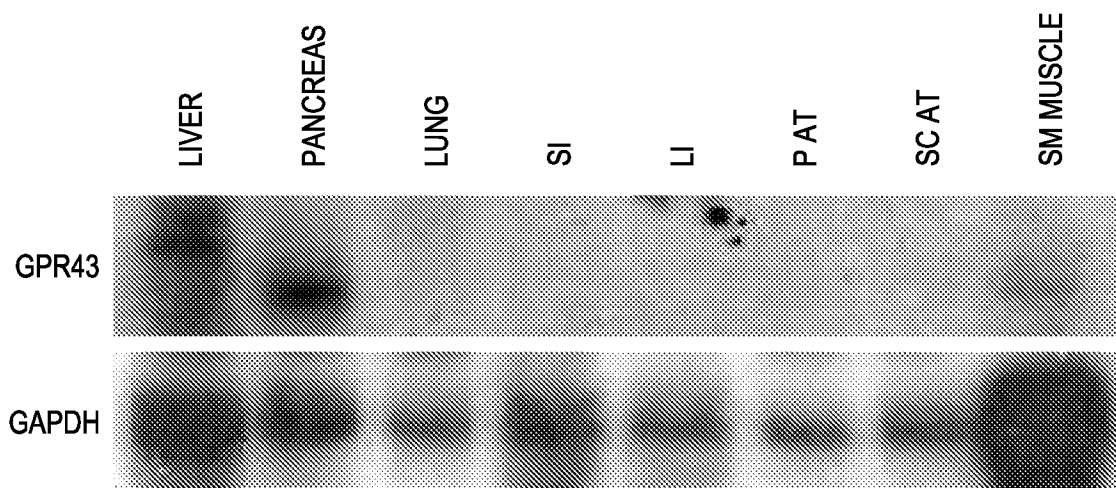


FIG. 8A

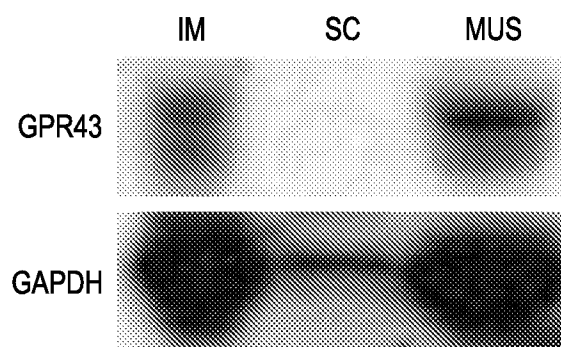


FIG. 8B

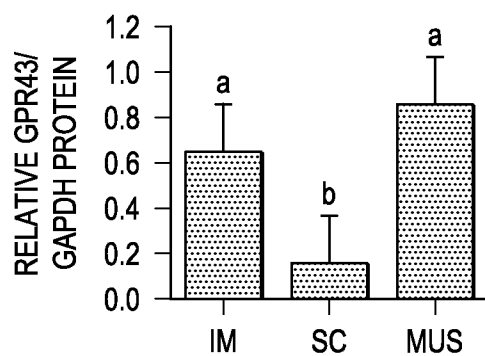


FIG. 8C

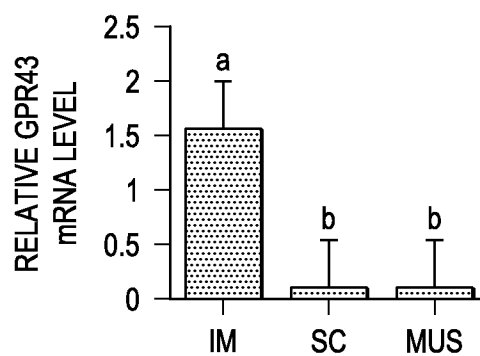


FIG. 8D

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/061265**A. CLASSIFICATION OF SUBJECT MATTER****G01N 33/53(2006.01)i, G01N 33/48(2006.01)i, G01N 33/68(2006.01)i**

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

G01N 33/53; G01N 33/566; G01N 33/48; G01N 33/68

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKOMPASS(KIPO internal) & Keywords: intramuscular adipose tissue, subcutaneous adipose tissue, GPR43

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHUNG et al., `The role of fatty acids on enhancing marbling development through bovine G coupled-protein receptors (GPR)` In: The Plains Nutrition Council, 2011 Spring Conference, pp.77-78 (2011) See the whole document.	1-12, 20-23, 26-31
A	US 7303889 B2 (LE POUL et al.) 04 December 2007 See the whole document.	1-12, 20-23, 26-31
A	SILVEY, `Effects of fatty acids on gene expression and lipid metabolism in bovine intramuscular and subcutaneous adipose tissues` Doctoral Dissertation, Texas A&M University (2011) See the whole document.	1-12, 20-23, 26-31
A	SMITH et al., `Cellular regulation of bovine intramuscular adipose tissue development and composition` Journal of Animal Science, Vol.87, No.14, pp.E72-E82 (2009) See the whole document.	1-12, 20-23, 26-31
A	HUTCHINGS et al., `Therapeutic antibodies directed at G protein-coupled receptors` mAbs, Vol.2, Issue 6, pp.594-606 (2010) See the whole document.	1-12, 20-23, 26-31



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

05 December 2013 (05.12.2013)

Date of mailing of the international search report

05 December 2013 (05.12.2013)

Name and mailing address of the ISA/KR

Korean Intellectual Property Office
189 Cheongsa-ro, Seo-gu, Daejeon Metropolitan City,
302-701, Republic of Korea

Facsimile No. +82-42-472-7140

Authorized officer

KIM, Seung Beom

Telephone No. +82-42-481-3371



INTERNATIONAL SEARCH REPORTInternational application No.
PCT/US2013/061265**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 13-19,24-25
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 13-19 and 24-25 pertain to methods for treatment of the human body by therapy, and thus relate to a subject matter which this International Searching Authority is not required, under Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of any additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2013/061265Patent document
cited in search reportPublication
datePatent family
member(s)Publication
date

US 7303889 B2

04/12/2007

AU 2003-202542 A1

24/07/2003

CA 2471504 A1

17/07/2003

EP 1463759 A1

06/10/2004

EP 1463759 B1

29/05/2013

JP 2005-533483 A

10/11/2005

JP 4360915 B2

11/11/2009

US 2003-175775 A1

18/09/2003

US 2008-286806 A1

20/11/2008

WO 2003-057730 A1

17/07/2003