(54) **BROWN FAT CELL COMPOSITIONS AND METHODS**

BRAUNFETTZELLENZUSAMENSETZUNGEN UND VERFAHREN

COMPOSITIONS DE CELLULES DE GRAISSE BRUNE ET MÉTHODES

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Description

FIELD OF THE INVENTION

[0001] This invention relates to the field of cell culture and more specifically to the culture and use of stem cells.

RELATED APPLICATIONS

[0002] This application claims priority to and benefit of U.S. Provisional application 61/502,508 filed on June 29 2011, U.S. Provisional application 61/632,122 filed on January 18, 2012, and U.S. Provisional application 61/632,516 filed on January 25, 2012 the entirety of each of these applications is incorporated by reference herein.

BACKGROUND

[0003] Brown fat is one of two types of adipose tissue found in the human body. During embryogenesis, brown fat is derived from the differentiation of the mesoderm. Brown fat is involved in development and homeostasis by providing metabolic tissue capable of providing heat. Brown fat helps regulate metabolism and nonshivering thermogenesis. In addition, brown fat plays a larger role than white adipose tissue in regulating metabolism. Brown fat makes up 5 percent of the body mass of a human neonate, and less than 1 percent of the body mass of an adult.

[0004] Metabolic activity of brown fat decreases with increased body mass index. Similarly, metabolic activity of brown fat decreases with increased body fat percent.

[0005] Stem cells have been identified and isolated in various tissues, and white adipose tissue stem cells have been isolated, expanded and shown to have functional therapeutic characteristics. To date, no stem cell population population has been identified in brown adipose tissue.

[0006] Cannon B. et al., "Cultures of adipose precursor cells from brown adipose tissue and of clonal brown-adipocyte cell lines.", Methods in Molecular Biology, vol. 155, 2001, pages 213-224, discloses a method of isolation of stem cells from brown adipose tissue comprising isolating cells from the stromal vascular fraction and plating the cells in a serum-containing medium or alternatively a defined medium still comprising however animal components.

[0007] Casteilla L. et al., "Differentiation of ovine brown adipocyte precursor cells in a chemically defined serum-free medium. Importance of glucocorticoids and age of animals", European Journal of Biochemistry, vol. 198, no. 1, 23 May 1991, pages 195-199, discloses the isolation of stem cells from brown adipose tissue (i.e. the stromal vascular fraction) and their culture in a serum-free medium. The serum-free medium comprises however insulin of bovine origin.

[0008] WO 2009/137613 A2-discloses the differentiation of muscle-derived progenitor cells into mature brown adipose tissue (BAT) cells with BMP7. The differentiated cells are transplanted for the treatment of diabetes. Different routes of administration are contemplated, the preferred route being subcutaneous. The medium used to obtain the BAT cells comprises bovine serum.

[0009] JP 2010 130968 A discloses the differentiation of different stem cells into brown adipocytes. The differentiated brown adipocytes are transplanted intraperitoneally into recipient mice to treat obesity. Every medium used comprises bovine serum.

[0010] Tran Thien T. et al., "Transplantation of adipose tissue and stem cells: role in metabolism and disease.", Nature Reviews, Endocrinology, vol. 6, no. 4, April 2010, pages 195-213, discloses the transplantation of brown adipose tissue to "lower white adipose tissue mass and improve overall metabolism".

SUMMARY OF THE INVENTION

[0011] Described are methods of developing cell lines, such as stem cell lines, for therapeutic or cosmetic use. For example, the cell lines can be used to treat a wide range of degenerative and metabolic disorders including, but not limited to, obesity, diabetes, hypertension, and cardiac deficiency. Also described are methods of using such cell lines to screen for compounds that play a role in regulating a variety of processes, such as, but not limited to, methylation, homeostasis, and genes involved in the regulation of metabolism, thermogenesis, activation, and/or maintenance of brown and white fat levels in the body.

[0012] In one aspect, the invention features a method of generating a stem cell. In one embodiment the steps include obtaining brown fat tissue; isolating a stem cell from the brown fat tissue; and culturing the stem cell in a medium not comprising an animal product, thereby generating a stem cell. In another embodiment the brown fat tissue is from a sample obtained from a subject. In another embodiment the stem cell is positive for one or more of the following cell surface markers: CD63, CD90, HLA ABC, CD105, CD73, CD166, CD 9, CD44. In another embodiment the stem cell is negative for one or more of the following cell surface markers: CD34, CD19, CD86, HLA DR, Lin, CD106, CD80, CD 117. In still yet another embodiment the stem cell is autogeneic. In another embodiment the stem cell is allogeneic. In another embodiment the medium includes Low Glucose DMEM without phenol red, 0.5-20% Human Platelet Lysate, 1X NEAA, 1X Glutamax, 1X Gentamycin, and 1000 units of Heparin.

[0013] In another aspect, the invention features a method of generating a cellular extract of stem cells, progenitor cells or differentiated cells such as activated brown fat adipocytes (e.g., brown fat adipocytes expressing UCP-1 and/or PRDM16) and administrating this extract into fat deposits within the subject, thereby treating obesity.

[0014] Another aspect of the invention is a method of

treating obesity in a subject. In one embodiment the method includes the steps of removing brown fat from a subject; and administering the brown fat to a fat deposit within the subject, thereby treating obesity in the subject. In one embodiment the fat deposit is a white fat deposit. In another embodiment the fat deposit is a brown fat deposit.

[0015] In another aspect, the invention features a method of identifying a compound involved in metabolism regulation, brown fat activation and/or maintenance, comprising contacting an autologous stem cell, progenitor cell or differentiated cell produced by a method described herein with a test compound; determining the level of metabolic activity, thermogenesis, and/or activation of certain genes such as PRDM-16 myf-5 UCP-1, CYC1, NDUFA11, NDUFA13, CMT1A, ELOVL3, DIO2, LHX8, COX8A, CYFIP2, CIDEA, Cox8b, Glut4, of the stem cell, progenitor cell, or differentiated cell in the presence of the test compound; and comparing the level of metabolic activity, thermogenesis and/or gene activity of the stem cell, progenitor cell or differentiated cell in the presence of the test compound to a level of metabolic activity, thermogenesis and/or gene activity of the stem cell, progenitor cell or differentiated cell in the absence of the test compound, wherein a level of metabolic activity, thermogenesis and/or gene activity in the presence of the test compound that is different from a level of metabolic activity, thermogenesis and/or gene activity in the absence of the test compound identifies the test compound as a compound involved in metabolism regulation or brown fat activation, thermogenesis and/or maintenance.

[0016] In yet another aspect the invention relates to a method of treating a metabolic disorder in a subject. In one embodiment the method includes the steps of removing a stem cell from a donor; differentiating the stem cell into a brown adipocyte in a medium not comprising an animal product; and administering the brown adipocyte to a fat deposit within the subject, thereby treating the metabolic disorder in the subject. In another embodiment the metabolic disorder is selected from the group consisting of obesity, central obesity, diabetes, hypertension, cardiac deficiency, ischemic cardiac disease, high blood pressure, triglyceride dyslipidemia, HDL dyslipidemia, cholesterol dyslipidemia, elevated fasting plasma glucose, leptin dysregulation, and adipon dysregulation. In still another embodiment the brown adipocyte is administered by subcutaneous injection. In still yet another embodiment the brown adipocyte is administered by systemic injection. In another embodiment the medium contains a differentiation agent selected from the group consisting of insulin, dexamethasone, isobutylmethylxanthine, and indomethacin.

[0017] Still yet another aspect of the invention is a method of identifying a compound involved in metabolism regulation. In one embodiment the method includes the steps of isolating a stem cell from the brown fat tissue; culturing the stem cell in a medium not comprising an animal product; contacting the stem cell with a test compound; determining the level of metabolic activity of the stem cell in the presence of the test compound; and comparing the level of metabolic activity of the stem cell in the presence of the test compound to a level of metabolic activity of the stem cell in the absence of the test compound, wherein a level of metabolic activity in the presence of the test compound that is different from a level of metabolic activity in the absence of the test compound identifies the test compound as a compound involved in metabolism regulation. In one embodiment the determining step includes measuring adipogenesis. In another embodiment the method further comprises detecting the levels of one or more of PRDM16, BMP7, UCP1, UCP2, or MYF5. In still yet another embodiment the test compound is selected from the group consisting of protein, antibody, peptide, mutein, polynucleotide, nucleic acid aptamer, and small molecule.

[0018] An aspect of the invention relates to differentiating a stem cell into a brown fat cell. In one embodiment the method includes contacting a stem cell with a medium not comprising an animal product, thereby generating a brown fat stem cell. In another embodiment the medium includes an agent selected from the group consisting of: insulin, dexamethasone, isobutylmethylxanthine, indomethacin, T3, rosiglitazone, FNDC5 and combinations thereof. In still yet another embodiment the medium includes insulin, dexamethasone, isobutylmethylxanthine, and indomethacin. In yet another embodiment the brown fat stem cell expresses PRDM-16, PGC-1, or a combination thereof. In still yet another embodiment the brown fat tissue is from a sample obtained from a subject.

BRIEF DESCRIPTION OF DRAWINGS

[0019] The present teachings described herein will be more fully understood from the following description of various illustrative embodiments, when read together with the accompanying drawings. It should be understood that the drawings described below are for illustration purposes only and are not intended to limit the scope of the present teachings in any way.

FIG. 1 is a schematic diagram depicting exemplary methods of injecting brown fat cells or brown fat precursor cells into a subject.

FIG. 2 is a micrograph of a cell culture ready for passaging.

FIG. 3 are images showing gene expression by RT-PCR in white adipose depots and brown adipose depots.

FIGS. 4A and B are fluorescent micrographs of cells stained with stem cell markers.

FIGS. 5A-F is a series of graphs showing detection by flow cytometry of various cell type markers.

FIG. 6 is a micrograph showing presence of fat droplets in cell culture.

FIGS. 7A and B are micrographs of cells stained with oil red O. FIG. 7A shows large fat droplets (200 μm) and FIG. 7B shows small fat droplets (50 μm).

FIGS. 8A and B are micrographs of cells stained with markers of differentiation. FIG. 8A shows staining with alizarin red, a marker of osteogenesis. FIG. 8B shows staining with osteocalcin antibody and alcian blue, to show chondrogenesis.

FIG. 9 is a scanning electron micrograph of exosomes isolated from brown fat cells.

FIG. 10 shows a timecourse of glucose levels and body weight in mice treated with brown fat progenitor cells.

FIG. 11 shows a timecourse of triglyceride levels and cholesterol levels in mice treated with brown fat progenitor cells.

FIG. 12 shows a timecourse of leptin levels and adipon levels in mice treated with brown fat progenitor cells.

DETAILED DESCRIPTION

[0020] The invention is based, at least in part, on the discovery that cells, such as autologous or nonautologous brown fat cells or stem cells, can be isolated and used to modify metabolism and/or thermogenesis in a subject, and/or to treat a wide range of disorders in a subject, such as metabolic disorders. (Fig. 1)

[0021] As described herein, brown fat cells and brown fat stem cells can be used therapeutically to treat a number of metabolic disorders. In some embodiments, the metabolic disorder is selected from central obesity (waist circumference of 40 inches or greater in males or 36 inches or greater in females); triglyceride dyslipidemia (1.7 mmol/L (150 mg/dl) or greater); HDL dyslipidemia (less than 40 mg/dl in males, less than 50 mg/dl in females); blood pressure (130/85 mmHg or greater); and elevated fasting plasma glucose (6.1 mmol/L (110 mg/dl) or greater).

[0022] The cells isolated can be from, for example, an autologous source or an allogeneic source. However, other sources also can be used.

[0023] Cellular products can be made from these cultured stem cells derived from mediastinal fat depots, such as cellular extracts. These cellular extracts then can be used therapeutically.

[0024] Cells or stem cells described in this application isolated from brown fat depots also can be used to screen compounds (including naturally occurring compounds) that increase the levels of brown fat, metabolism, and or gene expression profiles that favor higher metabolic activity. Similarly, cells or stem cells described herein can be used to identify compounds (including naturally occurring compounds) that decrease the levels of brown fat, metabolism, and or gene expression profiles that disfavor higher metabolic activity. In some embodiments, the screening is small molecule screening.

[0025] As used herein, "treatment" means any manner in which one or more of the symptoms of a disease or disorder are ameliorated or otherwise beneficially altered. As used herein, amelioration of the symptoms of a particular disorder refers to any lessening, whether permanent or temporary, lasting or transient, of the symptoms, which can be attributed to or associated with treatment by the compositions and methods of the present invention.

[0026] The terms "effective amount" and "effective to treat," as used herein, refer to an amount or a concentration of one or more of the compositions described herein utilized for a period of time (including acute or chronic administration and periodic or continuous administration) that is effective within the context of its administration for causing an intended effect or physiological outcome.

[0027] As used herein, the term "subject" means an animal, human or non-human, to whom treatment according to the methods of the present disclosure is provided. Veterinary and non-veterinary applications are contemplated. The term includes, but is not limited to, mammals. Typical subjects include humans, farm animals, and domestic pets such as cats and dogs. In some embodiments, the subject is a mammal. In some embodiments, the subject is a human subject, e.g., an obese human subject. In some embodiments, the subject is a non-human mammal, e.g., an experimental animal, a companion animal, or an animal that is raised for food.

[0028] As used herein, an "isolated" or "purified" cell is a cell substantially free of contaminating components from a cell culture or tissue source from which the cell is derived. "Substantially free" means that a preparation of a selected cell has less than about 50%, (e.g., less than about 40%, 30%, 20%, or 10%) of non-selected components. Such a non-selected component is also referred to herein as "contaminating component." When the isolated cells are recombinantly produced, they can be substantially free of culture medium, i.e., culture medium represents less than about 20%, (e.g., less than about 10% or 5%) of the volume of the cell preparation.

[0029] As used herein, "stem cells" are cells capable of both self-renewal and differentiation into many different cell lineages (is pluripotent). "Progenitor cells" refers to a subset of stem cells with phenotypes similar to that of a stem cell. A progenitor cell is capable of self-renewal and is typically multipotent. As used herein, "differentiated cells" refers to a subset of cells with phenotypes of a mature cell type specific to a particular tissue or organ system.

Methods of Obtaining Cells

[0030] In general, cells useful in the methods described herein can be obtained from a subject, such as by isolating the cells from a subject, or clinical grade embryonic stem cells can be obtained from a commercial source. In certain embodiments, the cells are part of a tissue sample, such as a brown fat sample, from a subject. The tissue sample can be administered directly into a subject or the cells can be isolated from the tissue and processed as described herein. In other embodiments, the cells are stem cells, such as from a bone marrow biopsy, and can be processed as described herein.

[0031] Certain cells that can be used in the methods described herein are unipotent, multipotent, or pluripotent cells and can be of mesodermal origin. In some embodiments, the cells are brown adipose progenitor cells, mature brown fat cells or stem cells. Brown adipose progenitor cells, mature brown fat cells or stem cells can be identified by determining the presence or absence of one or more cell surface expression markers. Exemplary cell surface markers that can be used to identify a brown adipose progenitor cell, mature brown fat cell or stem cell include, but are not limited to, MYF5, UCP-1, PRDM-16, SSEA-4, Sca-1, CD45, Mac-1, CD29 (integrin β1), CD105 (Endoglin), CD166 (ALCAM), desmin, vimentin, and c-kit. In yet other methods, the cells are brown adipose cells.

[0032] In any of the methods described herein, the cells can be autologous, syngeneic, allogeneic, or xenogeneic.

[0033] Cells suitable for use in the methods described herein can be found in a variety of tissues and organs including, but not limited to, for example, skeletal muscle, cardiac muscle, smooth muscle, prostate, dermis, the cardiovascular system, mammary gland, liver, neonatal skin, calvaria, bone marrow, the intestine, adipose tissue (*e.g.*, white adipose tissue, brown adipose tissue), peripheral blood, mobilized peripheral blood, and umbilical cord. Cells can be isolated from such tissues and organs in a number of known ways, including, *e.g.,* biopsy, apheresis, or liposuction.

[0034] In some embodiments, cells are obtained from adipose tissue, *e.g.,* white or brown adipose deposits. For example, brown adipose tissue can be harvested using known means (*e.g.*, by biopsy) from specific anatomical regions of a subject, such as cervical-supraclavicular regions, superior mediastinal regions, and regions superficial or lateral to sternocleidomastoid muscles. Brown adipose tissue can be identified in a number of ways, such as by imaging (*e.g.*, by PET scan). The brown adipose tissue can be subjected to minimal processing (such as by washing with buffer, *e.g.,* DPBS, and mechanically separated into portions) and administered into a subject as described herein.

[0035] In other methods, cells, such as brown fat cells, mononuclear cells, progenitor cells, or stem cells, can be isolated from contaminating components of the tissue sample. For example, the tissue sample can be treated with an enzyme such as collagenase (*e.g.*, Type I, II, or III), dispase, hyaluronidase, or elastase and cells can be isolated by filtration or centrifugation. Alternatively, the tissue sample can be treated chemically, such as with EDTA, and cells can be isolated using, *e.g.,* mechanical disruption (*e.g.*, vortexing). The isolated cells can be washed with a suitable buffer, such as DPBS.

[0036] In some methods, cells are isolated from a bone marrow sample of a subject. For example, a bone marrow sample can be obtained using needle aspiration or other known technique. In certain instances, cells can be isolated from a bone marrow sample using a Ficoll - Hypaq density gradient.

[0037] In yet other methods, cells are isolated from skin of a subject. For example, a punch biopsy can be used to obtain a skin sample. In one exemplary method, a punch biopsy is used to obtain a 0.5 cm piece of skin, which is washed three times with DPBS, and the dermis is removed from the biopsy. The skin is then cut into small (about 3 mm) sections and plated onto the wells of a 6-well tissue culture plate with a sterile cover slip over the tissue and the cells cultured.

Methods of Culturing Cells

[0038] In certain methods, cells obtained as described herein are maintained in a suitable culture medium, e.g., a culture medium not comprising an animal-based product (such as bovine serum or calf serum) in order to obtain a larger population of cells. The culture methods can include allowing the cells to undergo sufficient rounds of doubling, *e.g.,* to produce either a clonal cell strain or a heterogeneous cell strain of desired size, *e.g.,* a sufficient number to provide a therapeutic effect to a subject, or a sufficient number to establish a stable cell line. For example, cells can be cultured at 37°C for a period of 3-6 weeks after an initial biopsy.

[0039] The culture media used in the methods described herein do not include animal-based products. An exemplary culture medium suitable for the methods described herein can include human platelet lysate and an anticoagulant, such as heparin or acid-citrate-dextrose. Another exemplary culture medium includes human pooled AB type serum and an anticoagulant. Other examples of culture medium can include Modified Eagle Medium (MEM) (such as Dulbecco's MEM or alpha MEM) supplemented with glycine, L-alanine, L-asparagine, L-aspartic acid, L-glutamic acid, L-proline, L-serine, L-glutamine, and an antibiotic. One exemplary culture medium includes: DMEM (low glucose, without phenol red); 0.5-20% human platelet lysate; 1X non-essential amino acids (NEAA); 1X Glutamax; 1X gentamycin, and 1000 units of heparin.

[0040] In some embodiments, the cells can be maintained in the culture medium under standard conditions, such as described in, *e.g.,* Freshney (1994) Culture of Animal Cells, a Manual of Basic Technique, third edition,

Wiley-Liss, New York.

[0041] In certain embodiments, the cells are cultured in human platelet lysate or human pooled AB type serum derived from subjects who have been exposed to cold temperatures, e.g., a temperature suitable to generate a sympathetic nervous system response in the subject.

[0042] In other embodiments, the cells can be maintained in a hypoxic environment (e.g., 0 to about 5% O_2 at 37°C).

[0043] In certain embodiments, cultured cells can express MYF-5, BMP7, and/or PRDM16, SSEA4. In other embodiments, cells cultured from muscle tissue can express SCA1 and not express c-Kit or CD45.

Methods of Differentiating Cells

[0044] In certain embodiments, cells obtained as described herein are maintained in a suitable differentiation medium, e.g., a medium not comprising an animal-based product. For example, stem cells can be maintained in a differentiation medium for a time sufficient to result in the differentiation of the stem cells into brown adipocyte progenitor cells or brown adipocytes. In particular embodiments, cells are maintained in a differentiation medium for a time sufficient to result in the differentiation of the stem cells into MYF5 expressing cells. In certain instances, cells are maintained in the differentiation medium for, e.g., 1-5 days, 5-10 days, 10-14 days, 14-21 days, 21-28 days, or longer.

[0045] In some embodiments, the differentiation medium includes one or more chemical or hormone differentiation inducers and/or thiazolidinediones (as described in, e.g., Klien et al., J. Biol. Chem. 274:34795-34802 (1999); Hauner et al., J. Clin. Invest. 84:1663-1670 (1989)). In some instances, the differentiation medium includes insulin, dexamethasone, isobutylmethylxanthine, and indomethacin and rosiglitazone. One exemplary differentiation medium includes DMEM (low glucose without phenol red); 0.5% - 20% Human Platelet Lysate; 1X NEAA; 1X Glutamax; 1X gentamycin; 1000 units of heparin; 10 ug/mL of insulin; 1 uM dexamethasone; 200 uM indomethacin; and 0.5 mM isobutylmethylxanthine. In another embodiment, the differentiation medium includes BMP7. In another embodiment, the differentiation medium includes DMEM (low glucose without phenol red); 0.5% - 20% Human Platelet Lysate; 1X NEAA; 1X Glutamax; 1X gentamycin; 1000 units of-heparin;0.5 mM isobutylmethylxanthine, 125 nM indomethacin, 5 uM dexamethosone, 850 nM insulin, 1 nM T3, and 1 uM rosiglitazone. In another embodiment, the differentiation medium includes DMEM (low glucose without phenol red); 0.5% - 20% Human Platelet Lysate; 1X NEAA; 1X Glutamax; 1X gentamycin; 1000 units of-heparin;0.5 mM isobutylmethylxanthine, 125 nM indomethacin, 5 uM dexamethosone, 850 nM insulin, 1 nM T3, and 1 uM rosiglitazone and 20 nM FNDC5.

[0046] In some instances, the cells are maintained in differentiation medium until one or more markers of brown adipocyte differentiation are detected. Non-limiting examples of markers of differentiation include the expression of cell death-inducing DFF45-like effector A (CIDEA), Type II deiodinaie, PPAR gamma coactivator (PGC)-1 alpha, PGC-1 beta, uncoupling protein (e.g., UCP1), PRDM16, or CIG30, PRDM-16, CYC1, NDUFA11, NDUFA13, CMT1A, ELOVL3, DIO2, LHX8, COX8A, CYFIP2, Cox8b, Glut4.

[0047] In some embodiments, the methods include exposing the cells to cold shock by culturing or differentiating the cells during daily cycles of 4 °C - 25C for one hour followed by culture at standard conditions (37 °C, 5% CO_2 or hypoxic conditions).

[0048] In some embodiments, the methods include evaluating the level of adipogenesis following maintenance in a differentiation medium. Adipogenesis can be evaluated by measuring, e.g., lipid accumulation (e.g., using oil red-o (ORO) staining), cell morphology (e.g., using visual, e.g., microscopic, inspection of the cells), or cell thermodynamics (e.g., cytochrome oxidase activity, Na+-K+-ATPase enzyme units, or other enzymes involved in brown adipocyte thermogenesis). In addition, in some embodiments, functional brown fat adipogenesis following differentiation can be determined by fatty acid uptake assays or oxygen consumption rate.

Further Processing Methods

[0049] In some embodiments, cells obtained as described herein can be subjected to additional processing before administration into a subject. In certain embodiments, a cell obtained as described herein can be recombinantly modified to express one or more genes, e.g., a gene involved in brown adipogenesis. For example, a cell can be transfected with one or more nucleic acids encoding DFF45-like effector A (CIDEA), Type II deiodinaie, PPAR gamma coactivator (PGC)-1 alpha, PGC-1 beta, uncoupling protein (e.g., UCP1), PRDM16, or CIG30, PRDM-16, CYC1, NDUFA11, NDUFA13, CMT1A, ELOVL3, DIO2, LHX8, COX8A, CYFIP2, Cox8b, Glut4 or combinations of the genes, prior to administration into a subject.

[0050] In other embodiments, the cells can be pluripotent stem cells artificially derived (e.g., differentiated or partially differentiated) from a non-pluripotent cell. For example, skin cells, such as dermal fibroblasts, can be reprogrammed to a pluripotent state by transfection with OCT4, Nanog, or SSEA4. Such cells are known in the art as induced pluripotent stem cells. In other embodiments the cells can be further manipulated after becoming pluripotent by transfection with with one or more nucleic acids encoding DFF45-like effector A (CIDEA), Type II deiodinaie, PPAR gamma coactivator (PGC)-1 alpha, PGC-1 beta, uncoupling protein (e.g., UCP1), PRDM16, or CIG30, PRDM-16, CYC1, NDUFA11, NDUFA13, CMT1A, ELOVL3, DIO2, LHX8, COX8A, CYFIP2, Cox8b, Glut4 or combinations of the genes, (ie. PPARG2, CEBPB, PRDM16)

[0051] In further embodiments, a cell can be mitotically inactivated prior to administration into a subject. Without wishing to be bound by theory, it is believed that upon administration to a subject, cells obtained and/or treated as described herein express certain factors that may have a paracrine effect on surrounding cells and tissues. Accordingly, mitotic inactivation prevents further cell division while allowing the inactivated cells to maintain the paracrine effect. In certain embodiments, cells can be treated with chemical or gamma irradiation sufficient to mitotically inactivate the cells.

[0052] In some embodiments, a cell extract or lysate can be prepared from a cell described herein and administered to a subject. For example, cells can be obtained and cultured, and about 10^6 to about 10^{10} cells can be collected and used to prepare a cell-free extract using known methods. In one exemplary method, an extract is prepared by subjecting the collected cells to freeze-thaw cycles (*e.g.*, 1, 2, 3, 4, 5, or more freeze-thaw cycles) using an ethanol/dry ice bath. The extract is then centrifuged (*e.g.*, at about 14,000 rpm) to remove insoluble material. The extract can then be administered to a subject.

Expression Methods

[0053] In certain embodiments, a cell described herein can be recombinantly modified to express one or more genes. Such nucleic acids can be incorporated into an expression vector. Expression vectors comprising a nucleic acid sequence described herein can be administered in any effective carrier, *e.g.,* any formulation or composition capable of effectively delivering the component gene to cells *in vivo.* Approaches include insertion of the gene in viral vectors, including recombinant retroviruses, adenovirus, adeno-associated virus, lentivirus, poxvirus, alphavirus, and herpes simplex virus-1, or recombinant bacterial or eukaryotic plasmids. Viral vectors transfect cells directly; plasmid DNA can be delivered naked or with the help of, for example, cationic liposomes (lipofectamine) or derivatized (*e.g.*, antibody conjugated), polylysine conjugates, gramicidin S, artificial viral envelopes or other such intracellular carriers, as well as direct injection of the gene construct or $CaPO_4$ precipitation carried out *in vivo.*

[0054] In some embodiments, the expression vector is a viral vector containing one or more nucleic acid sequences (*e.g.*, cDNA). Retrovirus vectors can be used as a recombinant gene delivery system for the transfer of exogenous genes *in vivo,* particularly into humans as is known to one skilled in the art. (Protocols for producing recombinant retroviruses and for infecting cells *in vitro* or *in vivo* with such viruses can be found in Ausubel, et al., eds., Current Protocols in Molecular Biology, Greene Publishing Associates (1989), Sections 9.10-9.14, and other standard laboratory manuals. Another viral gene delivery system useful in the present methods utilizes adenovirus-derived. Yet another viral vector system useful for delivery of nucleic acids is the adeno-associated virus (AAV) as is known to one skilled in the art..

[0055] In addition to viral transfer methods, such as those illustrated above, non-viral methods can also be employed to express a nucleic acid into a cell described herein. Typically non-viral methods of gene transfer rely on the normal mechanisms used by mammalian cells for the uptake and intracellular transport of macromolecules. In some embodiments, non-viral gene delivery systems can rely on endocytic pathways for the uptake of the subject gene by the targeted cell. Exemplary gene delivery systems of this type include liposomal derived systems, poly-cationic conjugates such as polyamine and polylysine, and artificial viral envelopes. Other embodiments include plasmid injection systems as known to one skilled in the art. Other non-viral vectors include a scaffold/matrix attached region (S/MAR)-based vector. In particular embodiments, a cell described herein is transfected with an S/MAR-PRDM16 construct, an S/MAR-BMP-7/PRDM16 construct, or an S/MAR-BMP-7 construct. In another embodiment the cell is transfected with an S/MAR construct containing one or more nucleic acids encoding DFF45-like effector A (CIDEA), Type II deiodinaie, PPAR gamma coactivator (PGC)-1 alpha, PGC-1 beta, uncoupling protein (*e.g.*, UCP1), PRDM16, or CIG30, PRDM-16, CYC1, NDUFA11, NDUFA13, CMT1A, ELOVL3, DIO2, LHX8, COX8A, CYFIP2, Cox8b, Glut4 or combinations of the genes or a combination of them (i.e. PPARG2, CEBPB, PRDM16).

[0056] In some embodiments, a nucleic acid can be expressed using naked DNA constructs and/or DNA vector based constructs as is known to one skilled in the art. In some embodiments, DNA vectors can be introduced into target cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a target cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, electroporation, gene gun, sonoporation, or magnetofection.

[0057] All the molecular biological techniques required to generate an expression construct described herein are standard techniques that will be appreciated by one of skill in the art.

Methods of Administration

[0058] Methods described herein can include implanting tissue or cells, *e.g.,* cells obtained or isolated as described herein, into a subject to be treated. The cells can be differentiated brown adipocytes (*e.g.*, isolated brown adipocytes or differentiated adipocytes produced as described herein), or can be stem cells or undifferentiated cells, which cells, or their progeny (*i.e.,* daughter cells), will differentiate into brown adipocytes after implantation. These methods are useful, *e.g.,* for modifying metabolism in a subject as described herein.

[0059] Prior to administration into a subject, the cells can be washed (*e.g.*, in isotonic PBS) to remove any contaminants, including contaminating components of tissue sample, culture media or differentiation media, before implantation. The number of required cells is variable and depends on a variety of factors, including but not limited to, the cell type used, the site of implantation of the cells (for example, the number of cells that can be used can be limited by the anatomical site of implantation), and the age, surface area, and clinical condition of the subject. In some embodiments, at least about 10^5, 10^6, 10^7, 10^8, 10^9, or about 10^{10} cells are implanted into the subject.

[0060] Methods for implanting cells within a subject are known in the art, *e.g.*, using a delivery system configured to allow the introduction of cells into a subject. In general, the delivery system can include a reservoir containing cells and a needle in fluid communication with the reservoir. Such delivery systems are also within the scope of the invention. Generally, such delivery systems are maintained in a sterile manner. Various routes of administration and various sites (*e.g.*, renal sub capsular, subcutaneous, central nervous system (including intrathecal), intravascular, intrahepatic, intrasplanchnic, intraperitoneal (including intraomental), intramuscularly implantation) can be used.

[0061] The cells can be in a pharmaceutically acceptable carrier, with or without a scaffold, matrix, or other implantable device to which the cells can attach (examples include carriers made of, *e.g.*, collagen, fibronectin, elastin, cellulose acetate, cellulose nitrate, polysaccharide, fibrin, gelatin, and combinations thereof). Initially 1,000,000 cells where seeded onto porous extracellular scaffolds and cultured for 5 days. Differentiation into brown adipose was initiated by adding DMEM (low glucose without phenol red) containing

10% Human Platelet Lysate;

1X NEAA; 1X Glutamax;

1X gentamycin;

1000 units of heparin;

0.5 mM isobutylmethylxanthine,

125 nM indomethacin,

5 uM dexamethosone,

850 nM insulin,

1 nM T3,

1 uM rosiglitazone.

and the cells were grown in this medium for 2 days fol-

lowed by further differentiation for an additional 18 days in media composed of:
DMEM (low glucose without phenol red);

10% Human Platelet Lysate;

1X NEAA; 1X Glutamax;

1X gentamycin;

1000 units of heparin;0.5 mM isobutylmethylxanthine,

125 nM indomethacin,

5 uM dexamethosone,

850 nM insulin,

1 nM T3,

1 uM rosiglitazone

20 nM FNDC5.

[0062] Cells implanted using scaffolding were shown under scanning electron microscopy to attach to the scaffold. Further measurements of fatty acid uptake by the cells showed that the cells were metabolically active.

[0063] In particular instances, the cells are implanted into white fat or brown fat in the subject, and/or into or near an anatomical region containing white fat or brown fat.

[0064] Where non-immunologically compatible cells are used (*e.g.*, where non-autologous cells are administered to a subject), an immunosuppressive compound, *e.g.*, a drug or antibody, can be administered to the subject at a dosage sufficient to achieve inhibition of rejection of the cells. Dosage ranges for immunosuppressive drugs are known in the art. Dosage values may vary according to factors such as the disease state, age, sex, and weight of the individual.

Subjects

[0065] The methods and compositions described herein are useful for the treatment of metabolic disorders. Generally, the methods include administering an effective amount of cells described herein to a subject in need thereof, including a subject that has been diagnosed to be in need of such treatment. Subjects can include mammals.

[0066] In some embodiments, the methods include identifying a subject in need of treatment (*e.g.*, a subject having or at risk of developing a metabolic disorder), and administering to the subject an effective amount of tissue or cells described herein. In certain instances, the subject is diagnosed as being an overweight or obese subject,

e.g., with a body mass index (BMI) of 25-29 or 30 or above or a subject with a weight related disorder. A subject in need of treatment with the methods described herein can be selected based on the subject's body weight or body mass index. In some embodiments, the methods include evaluating the subject for one or more of: weight, adipose tissue stores, adipose tissue morphology, insulin levels, insulin metabolism, glucose levels, thermogenic capacity, and cold sensitivity. In some embodiments, subject selection can include assessing the amount or activity of brown adipose tissue in the subject and recording these observations.

[0067] The evaluation can be performed before, during, and/or after the administration of the cells described herein. For example, the evaluation can be performed at least 1 day, 2 days, 4, 7, 14, 21, 30 or more days before and/or after the administration of cells described herein.

Metabolism and Metabolic Disorders

[0068] Metabolism is a cascade of chemical reactions that regulate the mechanisms by which living organisms regulate, maintain and respond to intrinsic and extrinsic factors that affect their ability to maintain, grow, and reproduce. An imbalance of this metabolic activity can lead to downstream cellular events that can give rise to a number of degenerative disorders. Such disorders can bring about not only symptoms of a wide range of diseases, but can interfere with proper methylation of the imprinting pattern of male and female germ cells, thus establishing a genetic predisposition in the new generation to degenerative disorders.

[0069] In certain embodiments, the tissue or cells described herein are administered to a subject to modify, *e.g.,* increase, metabolic activity in the subject. In certain embodiments, the subject has a disorder such as, but not limited to, obesity, osteoarthritis, hypertension, diabetes, an auto-immune disorder, stroke, kidney failure, neoplasia, or a cardiac deficiency such as ischemic heart disease. In particular instances, the administration of cells described herein treat the disorder.

[0070] In other embodiments, subjects can be screened for levels of adipogenesis markers, such as DFF45-like effector A (CIDEA), Type II deiodinaie, PPAR gamma coactivator (PGC)-1 alpha, PGC-1 beta, uncoupling protein (*e.g.,* UCP1), PRDM16, or CIG30, PRDM-16, CYC1, NDUFA11, NDUFA13, CMT1A, ELOVL3, DIO2, LHX8, COX8A, CYFIP2, Cox8b, Glut4, and/or BMP7, and the information used as a diagnostic tool for obesity, hypertension, diabetes or ischemic cardiac disease. For example, an adipose tissue sample or a blood sample can be obtained from a subject and the level of DFF45-like effector A (CIDEA), Type II deiodinaie, PPAR gamma coactivator (PGC)-1 alpha, PGC-1 beta, uncoupling protein (*e.g.,* UCP1), PRDM16, or CIG30, PRDM-16, CYC1, NDUFA11, NDUFA13, CMT1A, ELOVL3, DIO2, LHX8, COX8A, CYFIP2, Cox8b, Glut4, and/or BMP7 can be measured in the sample. A level of DFF45-

like effector A (CIDEA), Type II deiodinaie, PPAR gamma coactivator (PGC)-1 alpha, PGC-1 beta, uncoupling protein (*e.g.,* UCP1), PRDM16, or CIG30, PRDM-16, CYC1, NDUFA11, NDUFA13, CMT1A, ELOVL3, DIO2, LHX8, COX8A, CYFIP2, Cox8b, Glut4, and/or BMP7 below a predetermined level indicates the subject has, or is at risk of developing, a metabolic disorder, such as obesity, hypertension, diabetes or ischemic cardiac disease. The predetermined level can be the level of a marker in a corresponding sample from a subject not having the metabolic disorder. In yet other embodiments, the levels of brown fat and white fat in a subject can be measured, *e.g.,* using PET scan, and a ratio of brown fat to white fat can be determined. A ratio of brown fat to white fat less than a predetermined level can indicate the subject has, or is at risk of developing, a metabolic disorder, such as obesity, hypertension, diabetes or ischemic cardiac disease.

Methods of Screening for Adipogenesis Compounds

[0071] Cells isolated or cultured as described herein can be used to screen for compounds that modify, e.g., increase or reduce, metabolic activity. In some embodiments, a cell, e.g., a brown adipocyte progenitor cell, differentiated cell or stem cell, can be contacted with a test compound and the level of a marker for adipogenesis, e.g., a marker described herein (e.g., PRDM16, BMP7, UCP1 or MYF5) can be measured. A test compound that increases the level of a marker for adipogenesis relative to a cell not contacted with the test compound is identified as a compound that increases metabolic activity, whereas a test compound that reduces a marker for adipogenesis is identified as a compound that decreases metabolic activity. In another embodiment the cell is a white adipocyte and it is transfected with a construct expressing a reporter gene (i.e. GFP, luciferase) under the control of DFF45-like effector A (CIDEA), Type II deiodinaie, PPAR gamma coactivator (PGC)-1 alpha, PGC-1 beta, uncoupling protein (*e.g.,* UCP1), PRDM16, or CIG30, PRDM-16, CYC1, NDUFA11, NDUFA13, CMT1A, ELOVL3, DIO2, LHX8, COX8A, CYFIP2, Cox8b, Glut4 or a combination of them. A test compound that increases the level of a marker for adipogenesis relative to a cell not contacted with the test compound is identified as a compound that increases metabolic activity, whereas a test compound that reduces a marker for adipogenesis is identified as a compound that decreases metabolic activity

[0072] In one exemplary method, a cell described herein is transfected with an expression vector that includes a nucleic acid encoding DFF45-like effector A (CIDEA), Type II deiodinaie, PPAR gamma coactivator (PGC)-1 alpha, PGC-1 beta, uncoupling protein (*e.g.,* UCP1), PRDM16, or CIG30, PRDM-16, CYC1, NDUFA11, NDUFA13, CMT1A, ELOVL3, DIO2, LHX8, COX8A, CYFIP2, Cox8b, Glut4, BMP7, MYF5 or a combination of them, under the control of an inducible promoter. Non-

limiting examples of such promoters include chemically-regulated promoters (i.e tetracycline, steroids and metals), or temperature specific promoters that will activate and promote expression at a permissive temperature, such as below normal body temperature of a subject.

[0073] In one example, a temperature specific promoter is placed in a viral or non viral vector upstream of a nucleic acid encoding DFF45-like effector A (CIDEA), Type II deiodinaie, PPAR gamma coactivator (PGC)-1 alpha, PGC-1 beta, uncoupling protein (*e.g.*, UCP1), PRDM16, or CIG30, PRDM-16, CYC1, NDUFA11, NDUFA13, CMT1A, ELOVL3, DIO2, LHX8, COX8A, CYFIP2, Cox8b, Glut4 or a combination of them, MYF5 and/or BMP7. A cell isolated from adipose tissue (white or brown), bone marrow, skin, muscle, or umbilical cord is transfected with the vector. The cell is then cultured under the permissive temperature (which allows gene expression from the promoter) in the presence or absence of a test compound, and the effect of the test compound on adipogenesis is determined.

[0074] Test compounds include, e.g., proteins (including antibodies), muteins, polynucleotides, nucleic acid aptamers, hormones (i.e Irisin) and peptide and nonpeptide small organic molecules. Test compounds can be isolated from natural sources, prepared synthetically or recombinantly, or any combination of the same. Particular, nonlimiting examples of test compounds include 2,4-dinitrophenol, Ephedrine, Sibutramine, FGF21, Bile acids, and Beta-3 adrenergic receptor agonists.

[0075] All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described herein.

[0076] The disclosure is further illustrated by the following examples. The examples are provided for illustrative purposes only. They are not to be construed as limiting the scope or content of the disclosure in any way.

EXAMPLES

Example 1 - Brown fat isolation processing

[0077] In one example, a patient first underwent a ^{18}F-fluorodeoxyglucose (^{18}F-FDG) PET-CT scan to identify brown fat deposits. Prior to undergoing the PET-CT scan, the patient was exposed to temperatures ranging from 1°C -25°C (to increase the uptake of ^{18}F-FDG) for 1-2 hours per day for a period of one month. Deposits identified within the cervical-supraclavicular and mediastinal area were biopsied using a needle. Brown fat biopsies

were washed three times in DPBS (-) and either treated with 0.075%-0.2% Collagenase Type 1A with vigorous shaking at 37°C for 30-60 minutes or treated with EDTA for 30 minutes and subjected mechanical tissue disruption for 5-30 minutes. The tissue/cells were washed three times with DPBS (-), and $1 \times 10^6 - 1 \times 10^{10}$ cells were injected into white fat deposits in the patient. Optionally, following enzymatic or mechanical tissue/cell isolation, the tissue/cells were exposed to cold temperature (0°C - 4°C) for 1-48 hours prior to injection.

Example 2 - Brown fat culture

[0078] In this example, cells were cultured prior to injection into a patient. The cells obtained from Example 1 were plated into hyper flasks at a concentration of 1000 cells/cm^2 in a culture medium comprising in this embodiment:

DMEM Low Glucose without phenol red

0.5-20% Human Platelet Lysate

1X NEAA

1X Glutamax

1X Gentamycin

1000 units of Heparin

[0079] The cells were cultured for 3-6 weeks. The cells were then characterized by flow-cytometry and analyzed for the expression of one or more of the following markers: SCA-1, CD34, SSEA1, SSEA4, OCT4, CD31, Wnt5a, Telomerase activity, alpha-SMA, STRO-1, MYF5, PRDM16, or UCP1. The cells were then expanded to concentrations of $1 \times 10^6 - 1 \times 10^{10}$ for implantation into white fat deposits of the patient.

Example 3 - Differentiation of cells into brown fat cells

[0080] In this example, cells (such as stem cells or precursor brown adipocytes) obtained from a brown fat deposit of a patient were differentiated and subsequently injected into a patient. The cells obtained from Example 1 were plated out at 1000 cells/cm^2 in hyper flasks containing a differentiation medium comprising, in this embodiment:

DMEM Low Glucose with out phenol red

0.5 - 20% Human Platelet Lysate

1X NEAA

1X Glutamax

1X Gentamycin

1000 units of Heparin

10 ug/mL of insulin

1 uM Dexamethasone

200 uM Indomethacin

0.5mM Isobutylmethylxanthine

[0081] In an alternative embodiment the differentiation medium included:

DMEM (low glucose without phenol red)
0.5% - 20% Human Platelet Lysate;
1X NEAA; 1X Glutamax;
1X gentamycin;
1000 units of heparin;
0.5 mM isobutylmethylxanthine,
125 nM indomethacin,
5 uM dexamethosone,
850 nM insulin,
1 nM T3,
1 uM rosiglitazone.

and the cells were grown in this medium for 2-6 days followed by further differentiation for an additional 6-21 days in media composed of:

DMEM (low glucose without phenol red);
0.5% - 20% Human Platelet Lysate;
1X NEAA; 1X Glutamax;
1X gentamycin;
1000 units of heparin;0.5 mM isobutylmethylxanthine,
125 nM indomethacin,
5 uM dexamethosone,
850 nM insulin,
1 nM T3,
1 uM rosiglitazone
20 nM FNDC5.

[0082] After being maintained in the differentiation medium for 10-30 days, cells were characterized by expression of DFF45-like effector A (CIDEA), Type II deiodinaie, PPAR gamma coactivator (PGC)-1 alpha, PGC-1 beta, uncoupling protein (*e.g.*, UCP1), PRDM16, or CIG30, PRDM-16, CYC1, NDUFA11, NDUFA13, CMT1A, ELOVL3, DIO2, LHX8, COX8A, CYFIP2, Cox8b, Glut4. $1 \times 10^6 - 1 \times 10^{10}$ of the cells are then prepared for injection into the patient.

Example 4 - Culturing and differentiating embryonic stem cells

[0083] In this example clinical grade embryonic stem cells were first grown and expanded. The cells were then differentiated into brown fat pre-adipocytes or fully differentiated brown fat adipocytes by exposing the cells, in one embodiment, to the following media:

DMEM Low Glucose with out phenol red

0.5 - 20% Human Platelet Lysate

1X NEAA

1X Glutamax

1X Gentamycin

1000 units of Heparin

10 ug/mL of insulin

1 uM Dexamethasone

200 uM Indomethacin

0.5 mM Isobutylmethylxanthine

[0084] In another embodiment, the cells were grown for 2-6 days in a medium comprising:

DMEM (low glucose without phenol red);

0.5% - 20% Human Platelet Lysate;

1X NEAA;

1X Glutamax;

1X gentamycin;

1000 units of heparin;

0.5 mM isobutylmethylxanthine,

125 nM indomethacin,

5 uM dexamethosone,

850 nM insulin,

1 nM T3,

1 uM rosiglitazone.

[0085] This is followed by further differentiation for an additional 6-21 days in media composed of:

DMEM (low glucose without phenol red);

0.5% - 20% Human Platelet Lysate;

1X NEAA;

1X Glutamax;

1X gentamycin;

1000 units of heparin;

0.5 mM isobutylmethylxanthine,

125 nM indomethacin,

5 uM dexamethosone, 850 nM insulin,

1 nM T3,

1 uM rosiglitazone,

20 nM FNDC5.

[0086]   Optionally, the cells were transfected, prior to differentiation or after differentiation, with a non-viral vector (scaffold/matrix attached region (S/MAR)) with a PRDM-16 gene by itself or in tandem with BMP-7 or MYF-5 or DFF45-like effector A (CIDEA), Type II deiodinaie, PPAR gamma coactivator (PGC)-1 alpha, PGC-1 beta, uncoupling protein (*e.g.*, UCP1), PRDM16, or CIG30, PRDM-16, CYC1, NDUFA11, NDUFA13, CMT1A, ELOVL3, DIO2, LHX8, COX8A, CYFIP2, Cox8b, Glut4 or a combination of them driven by a strong promoter such as CAGGS or PGK. The cells were then inactivated with a chemical based method such as mitomycin C or gamma irradiation and implanted into a patient at concentrations of 1×10^6 - 1×10^{10} cells.

Example 5 - Brown fat human depot biopsy

[0087]   In this example a patient underwent ^{18}F-PET-CT scan to identify tissue that has metabolic activity. An area with high metabolic activity was identified within the human mediastinum region of the patient. In addition, this patient was to undergo routine cardiothoracic surgery. At the time of surgery the region was exposed, and within this region 5 x 5 cm brown fat depot was dissected out and placed into sterile saline.

[0088]   A brown fat depot was discovered in the mediastinum that contains a population of stem cells that express unique markers different than those found in other stem cells, including stem cells isolated from white adipose depots. These newly identified stem cells were separated from the mediastinum brown fat depot and cultured for greater than 20 passages and still retained a normal karyotype. These cells also are capable of undergoing adipogenesis (white and brown), osteogenesis, and chondrogenesis.

Example 6 - Isolation of cells from human brown fat depot biopsy

[0089]   The brown fat human depot isolated in Example 5 was washed 5-10 times in sterile saline. The biopsy was cut using scissors until pieces are approximately 0.1 - 0.5 cm in size. The material was washed 3 times by centrifuging at 1200 RPM for 5 minutes. The material was then digested using either collagenase or dispase for no longer than an hour in a shaking water bath set at 37C. After digestion was complete the enzymatic reaction was terminated by adding equal volume of complete media. The material was then filtered using a 100 uM filter and the single cell suspension was washed 3 times with complete medium.

Example 7 - Culture of cells isolated from brown fat human depot biopsy

[0090]   The single cell suspension described in Example 6 was plated onto cell culture flasks at a concentration of 5,000 - 10,000 cells/cm2. After approximately 3-6 days the cells were ready for passaging (Fig. 2). The cells after undergoing more than 10 passages showed a normal karyotype. RT-PCR analysis of the cultured brown fat cells demonstrated that they were positive for CEBPB, UCP-1, UCP-2, PPARG, PGC-1, PRDM-16. It is important to note that cells isolated from white adipose depots were negative for PRDM-16, and PGC-1. (Fig. 3). Accordingly, PRDM-16 and/or PGC-1 expression can be used to differentiate between white adipose cells and brown adipose cells. Moreover, CEBPB, UCP-1, UCP-2, PPARG, PGC-1, PRDM-16 can be used as markers to identify brown adipose cells.

[0091]   Staining of the cells showed that they are positive for the stem cell markers HCAM and CD90 (Figs. 4A and B). The expanded cells also expressed exosomes (Fig. 9). Flow cytometry of the cell population demonstrated that the cells were negative for the following cell surface markers: CD34, CD19, CD86, HLA DR, Lin, CD106, CD80, CD 117. The cell population was also low for SSEA4, and Stro-1. The cells were positive for CD63, CD90, HLA ABC, CD105, CD73, CD166, CD 9, CD44 (Figs. 5A-F) which are markers for undifferentiated brown adipose stem cells.

Example 8 - Differentiation of cells into Adipose (white and brown)

[0092]   Expanded cells were plated onto cell culture plates and supplemented with adipogenic differentiation medium (Life Technologies). The media was changed every 3 days and differentiation was allowed to continue for 14 days. After 7 days in induction media, fat droplets were visible within the culture (Fig. 6), indicating that the cells had begun differentiating into adipose cells.

[0093]   Staining with oil red O demonstrated positive differentiation into adipose cells (Fig. 7).

[0094] Identification of positive brown fat droplets in the differentiation was observed by the accumulation of smaller fat droplets (Fig. 7B) compared to those found in cells differentiated from cells derived from white fat depots, which produce larger fat droplets (Fig. 7A).

[0095] In another embodiment stem cells were plated in 6-well dishes at a density of 50,000 cells/well. White or brown adipogenesis differentiation medium was added. For brown adipogenesis, FNDC5 was added 6 days post induction as previously described. 0.3% Oil Red O (Sigma Aldrich) was used for staining to detect intracellular lipid accumulation.

Example 9 - Chondrogenic and Osteogenic Induction of cells

[0096] Expanded cells were plated onto cell culture plates and supplemented with osteogenic and chondrogenic medium (Life Technologies). The media was changed every 3 days and differentiation was allowed to continue for 21 days. Confirmation of differentiation into chondroblasts and osteoblasts was observed by staining of the cells with alizarian red or osteocalcin antibody and alcian blue (Figs. 8A and B). These data indicate that progenitor cells isolated from brown fat depots maintain the ability to differentiate in to a variety of cell types other than white adipose or brown adipose.

Example 10 - Therapeutic use of Brown Fat Progenitor Cells

[0097] NOD-SCID mice are severely immune deficient and are used as a model system to test human cell-based therapies. Mice were raised on high fat/high carbohydrate chow throughout the test period. Mice were split into two groups: 10 untreated mice served as controls and 10 mice received a single subcutaneous injection underneath the dorsal skin of about 1 million human brown fat progenitor cells cultured as described in examples 5-9. The following analytes were measured for four months following injection: plasma glucose, triglycerides, cholesterol, leptin, and adipon. Body weight also was monitored. Baseline is 23 +/-5 g in the control group and 25+/-7 g in the treated group. The control group received a sham injection of carrier solution (DPBS) with no cells.

[0098] Compared to the control group that received a sham injection of carrier solution only (DPBS) with no cells, the experimental cohort which received a dose of 1,000,000 cells demonstrated a decrease in plasma glucose, triglycerides, cholesterol, and weight gain over a four month monitoring period. Adiponectin and leptin levels also correlated to improved levels due to the having received the cell dose. This is in sharp contrast compared to the untreated control mice that received the sham carrier solution (Figs. 10-12).

EQUIVALENTS

[0099] It is to be understood that while the disclosure has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

Claims

1. A method of generating a brown fat cell, the method comprising:

    isolating a stem cell from an obtained brown fat tissue; and
    culturing the stem cell in a differentiation medium not comprising an animal-based product, wherein the differentiation medium comprises insulin, dexamethasone, isobutylmethylxanthine, indomethacin, triiodothyronine (T3), rosiglitazone, fibronectin type III domain-containing protein 5 (FNDC5), and 0.5 to 20% human platelet lysate:

    thereby generating the brown fat cell,
    wherein the stem cell is positive for one or more of the following cell surface markers: CD63, CD90, HLA ABC, CD105, CD73, CD166, CD9, CD44, and
    wherein the stem cell is negative for one or more of the following cell surface markers: CD34, CD19, CD86, HLA DR, Lin, CD106, CD80, CD117.

2. The method of claim 1, wherein the differentiation medium further comprises an anticoagulant selected from a group consisting of heparin and acid-citrate dextrose.

3. The method of claim 1, further comprising exposing the brown fat cell in the differentiation medium to hypoxic conditions.

4. The method of claim 1, wherein the differentiation medium comprises 10 to 20% human platelet lysate.

5. A method of identifying a compound that modifies metabolic activity of a brown fat cell, the method comprising:

    isolating a stem cell from an obtained brown fat tissue;
    culturing the stem cell in a differentiation medium not comprising an animal-based product, wherein the differentiation medium comprises

insulin, dexamethasone, isobutylmethylxanthine, indomethacin, triiodothyronine (T3), rosiglitazone, fibronectin type III domain-containing protein 5 (FNDC5) and 0.5 to 20% human platelet lysate, thereby generating the human brown fat cell;

contacting the brown fat cell with the compound; determining the metabolic activity of the brown fat cell in the presence of the compound; and comparing the metabolic activity of the human brown fat cell in the presence of the compound to metabolic activity of the brown fat cell in the absence of the compound,

wherein the difference between the metabolic activity of the brown fat cell in the presence of the compound and the metabolic activity of the brown fat cell in the absence of the compound identifies the compounds that modify the metabolic activity of the brown fat cell,

wherein the stem cell is positive for one or more of the following cell surface markers: CD63, CD90, HLA ABC, CD105, CD73, CD166, CD9, CD44, and

wherein the stem cell is negative for one or more of the following cell surface markers: CD34, CD19, CD86, HLA DR, Lin, CD106, CD80, CD117.

6. The method of claim 5, wherein the determining the metabolic activity of the brown fat cell in the presence of the compound comprises measuring adipogenesis.

7. The method of claim 5, wherein the determining the metabolic activity of the brown fat cell in the presence of the compound comprises measuring gene expression of at least one marker of adipogenesis selected from a group consisting of: PR domain containing 16 (PRDM16), bone morphogenetic protein 7 (BMP7), uncoupling protein-1 (UCP1), uncoupling protein-2 (UCP2), or myogenic factor 5 (MYF5).

8. The method of claim 5, wherein the compound is selected from the group consisting of protein, antibody, peptide, mutein, polynucleotide, nucleic acid aptamer, hormone, peptide small organic molecule, and nonpeptide small molecule.

9. A method of differentiating a stem cell into a brown fat cell, the method comprising:

contacting an isolated stem cell with a differentiation medium not comprising an animal-based product, thereby generating the brown fat cell, wherein the differentiation medium comprises insulin, dexamethasone, isobutylmethylxanthine, indomethacin, triiodothyronine (T3), rosiglitazone, fibronectin type III domain-containing

protein 5 (FNDC5) and 0.5 to 20% human platelet lysate;

wherein the stem cell is positive for one or more of the following cell surface markers: CD63, CD90, HLA, ABC, CD105, CD73, CD166, CD9, CD44, and

wherein the stem cell is negative for one or more of the following cell surface markers: CD34, CD19, CD86, HLA DR, Lin, CD106, CD80, CD117.

10. The method of claim 9, wherein the brown fat cell expresses PR domain containing 16 (PRDM-16), peroxisome proliferator-activated receptor-gamma coactivator (PGC-1), or a combination thereof.

11. A method of differentiating a stem cell into a brown fat cell, the method comprising:

contacting the stem cell with a first differentiation medium not comprising an animal-based product, wherein the first differentiation medium comprises insulin, dexamethasone, isobutylmethylxanthine, indomethacin, triiodothyronine (T3), rosiglitazone, and 0.5 to 20% human platelet lysate;

contacting the stem cell with a second differentiation medium not comprising an animal-based product, wherein the second differentiation medium comprises insulin, dexamethasone, isobutylmethylxanthine, indomethacin, triiodothyronine (T3), rosiglitazone, fibronectin type III domain-containing protein 5 (FNDC5), and 0.5 to 20% human platelet lysate;

thereby generating a brown fat cell.

Patentansprüche

1. Verfahren zum Erzeugen einer braunen Fettzelle, das Verfahren umfassend:

Isolieren einer Stammzelle von einem erhaltenen braunen Fettgewebe; und
Kultivieren der Stammzelle in einem Differenzierungsmedium, welches kein tierisches Produkt umfasst, wobei das Differenzierungsmedium Insulin, Dexamethason, Isobutylmethylxanthin, Indomethacin, Triiodthyronin (T3), Rosiglitazon, Fibronectin Type III Domain-Containing Protein 5 (FNDC5) und 0,5 bis 20 % menschliches Blutplättchenlysat umfasst:

dadurch Erzeugen der braunen Fettzelle, wobei die Stammzelle für einen oder mehrere der folgenden Zelloberflächenmarker positiv ist: CD63, CD90, HLA ABC, CD105, CD73, CD166, CD9, CD44 und

wobei die Stammzelle für einen oder mehrere der folgenden Zelloberflächenmarker negativ ist: CD34, CD19, CD86, HLA DR, Lin, CD106, CD80, CD117.

2. Verfahren nach Anspruch 1, wobei das Differenzierungsmedium ferner ein Antikoagulans umfasst, das ausgewählt ist aus einer Gruppe, bestehend aus Heparin und Acid-Citrate-Dextrose.

3. Verfahren nach Anspruch 1, ferner umfassend das Aussetzen der braunen Fettzelle in dem Differenzierungsmedium unter hypoxische Bedingungen.

4. Verfahren nach Anspruch 1, wobei das Differenzierungsmedium 10 bis 20 % menschliches Blutplättchenlysat umfasst.

5. Verfahren zum Identifizieren einer Verbindung, welche die metabolische Aktivität einer braunen Fettzelle modifiziert, das Verfahren umfassend:

Isolieren einer Stammzelle von einem erhaltenen braunen Fettgewebe;
Kultivieren der Stammzelle in einem Differenzierungsmedium, welches kein tierisches Produkt umfasst, wobei das Differenzierungsmedium Insulin, Dexamethason, Isobutylmethylxanthin, Indomethacin, Triiodthyronin (T3), Rosiglitazon, Fibronectin Type III Domain-Containing Protein 5 (FNDC5) und 0,5 bis 20 % menschliches Blutplättchenlysat umfasst, wodurch die menschliche braune Fettzelle erzeugt wird;
Kontaktieren der braunen Fettzelle mit der Verbindung;
Bestimmen der metabolischen Aktivität der braunen Fettzelle in der Gegenwart der Verbindung; und
Vergleichen der metabolischen Aktivität der menschlichen braunen Fettzelle in der Gegenwart der Verbindung mit der metabolischen Aktivität der braunen Fettzelle in der Abwesenheit der Verbindung,
wobei die Differenz zwischen der metabolischen Aktivität der braunen Fettzelle in der Gegenwart der Verbindung und der metabolischen Aktivität der braunen Fettzelle in der Abwesenheit der Verbindung die Verbindungen identifiziert, welche die metabolische Aktivität der braunen Fettzelle modifizieren,
wobei die Stammzelle für einen oder mehrere der folgenden Zelloberflächenmarker positiv ist: CD63, CD90, HLA ABC, CD105, CD73, CD166, CD9, CD44, und
wobei die Stammzelle für einen oder mehrere der folgenden Zelloberflächenmarker negativ ist: CD34, CD19, CD86, HLA DR, Lin, CD106, CD80, CD117.

6. Verfahren nach Anspruch 5, wobei das Bestimmen der metabolischen Aktivität der braunen Fettzelle in der Gegenwart der Verbindung das Messen der Adipogenese umfasst.

7. Verfahren nach Anspruch 5, wobei das Bestimmen der metabolischen Aktivität der braunen Fettzelle in der Gegenwart der Verbindung das Messen der Genexpression von mindestens einem Marker der Adipogenese umfasst, der ausgewählt ist aus der Gruppe, bestehend aus: PR Domain Containing 16 (PRDM16), Bone Morphogenetic Protein 7 (BMP7), Uncoupling Protein-1 (UCP1), Uncoupling Protein-2 (UCP2) oder Myogenic Factor 5 (MYF5).

8. Verfahren nach Anspruch 5, wobei die Verbindung ausgewählt ist aus der Gruppe, bestehend aus Protein, Antikörper, Peptid, Mutein, Polynukleotid, Nukleinsäure-Aptamer, Hormon, kleinem organischen Peptid-Molekül und kleinem Nichtpeptid-Molekül.

9. Verfahren zum Differenzieren einer Stammzelle in eine braune Fettzelle, das Verfahren umfassend:

Kontaktieren einer isolierten Stammzelle mit einem Differenzierungsmedium, welches kein tierisches Produkt umfasst, wodurch die braune Fettzelle erzeugt wird,
wobei das Differenzierungsmedium Insulin, Dexamethason, Isobutylmethylxanthin, Indomethacin, Triiodthyronin (T3), Rosiglitazon, Fibronectin Type III Domain-Containing Protein 5 (FNDC5) und 0,5 bis 20 % menschliches Blutplättchenlysat umfasst;
wobei die Stammzelle für einen oder mehrere der folgenden Zelloberflächenmarker positiv ist: CD63, CD90, HLA, ABC, CD105, CD73, CD166, CD9, CD44, und
wobei die Stammzelle für einen oder mehrere der folgenden Zelloberflächenmarker negativ ist: CD34, CD19, CD86, HLA DR, Lin, CD106, CD80, CD117.

10. Verfahren nach Anspruch 9, wobei die braune Fettzelle PR Domain Containing 16 (PRDM-16), Peroxisome Proliferator-Activated Receptor-Gamma Coactivator (PGC-1) oder eine Kombination daraus exprimiert.

11. Verfahren zum Differenzieren einer Stammzelle in eine braune Fettzelle, das Verfahren umfassend:

Kontaktieren der Stammzelle mit einem ersten Differenzierungsmedium, welches kein tierisches Produkt umfasst, wobei das erste Differenzierungsmedium Insulin, Dexamethason, Isobutylmethylxanthin, Indomethacin, Triiodthyronin (T3), Rosiglitazon und 0,5 bis 20 %

menschliches Blutplättchenlysat umfasst;
Kontaktieren der Stammzelle mit einem zweiten Differenzierungsmedium, welches kein tierisches Produkt umfasst, wobei das zweite Differenzierungsmedium Insulin, Dexamethason, Isobutylmethylxanthin, Indomethacin, Triiodthyronin (T3), Rosiglitazon, Fibronectin Type III Domain-Containing Protein 5 (FNDC5) und 0,5 bis 20 % menschliches Blutplättchenlysat umfasst; dadurch Erzeugen einer braunen Fettzelle.

Revendications

1. Procédé de génération d'une cellule adipeuse brune, le procédé comprenant :

l'isolement d'une cellule souche à partir d'un tissu adipeux brun obtenu ; et
la mise en culture de la cellule souche dans un milieu de différentiation ne comprenant pas de produit d'origine animale, dans lequel le milieu de différentiation comprend de l'insuline, de la dexaméthasone, de l'isobutylméthylxanthine, de l'indométhacine, de la triiodothyronine (T3), de la rosiglitazone, de la protéine 5 contenant le domaine de type III de la fibronectine (FNDC5), et du lysat de plaquettes humaines à 0,5 à 20 % :

générant ainsi la cellule adipeuse brune, dans lequel la cellule souche est positive à un ou plusieurs des marqueurs de surface cellulaire suivants : CD63, CD90, HLA ABC, CD105, CD73, CD166, CD9, CD44, et dans lequel la cellule souche est négative à un ou plusieurs des marqueurs de surface cellulaire suivants : CD34, CD19, CD86, HLA DR, Lin, CD106, CD80, CD117.

2. Procédé selon la revendication 1, dans lequel le milieu de différentiation comprend en outre un anticoagulant choisi dans un groupe consistant en l'héparine et l'acide-citrate-dextrose.

3. Procédé selon la revendication 1, comprenant en outre l'exposition de la cellule adipeuse brune dans le milieu de différentiation à des conditions hypoxiques.

4. Procédé selon la revendication 1, dans lequel le milieu de différentiation comprend du lysat de plaquettes humaines à 10 à 20 %.

5. Procédé d'identification d'un composé qui modifie l'activité métabolique d'une cellule adipeuse brune, le procédé comprenant :

l'isolement d'une cellule souche à partir d'un tissu adipeux brun obtenu ; et
la mise en culture de la cellule souche dans un milieu de différentiation ne comprenant pas de produit d'origine animale, dans lequel le milieu de différentiation comprend de l'insuline, de la dexaméthasone, de l'isobutylméthylxanthine, de l'indométhacine, de la triiodothyronine (T3), de la rosiglitazone, de la protéine 5 contenant le domaine de type III de la fibronectine (FNDC5), et du lysat de plaquettes humaines à 0,5 à 20 %, générant ainsi la cellule adipeuse brune humaine ;
la mise en contact de la cellule adipeuse brune avec le composé ;
la détermination de l'activité métabolique de la cellule adipeuse brune en présence du composé ; et
la comparaison de l'activité métabolique de la cellule adipeuse brune humaine en présence du composé à une activité métabolique de la cellule adipeuse brune en l'absence du composé, dans lequel la différence entre l'activité métabolique de la cellule adipeuse brune en présence du composé et l'activité métabolique de la cellule adipeuse brune en l'absence du composé identifie les composés qui modifient l'activité métabolique de la cellule adipeuse brune, dans lequel la cellule souche est positive à un ou plusieurs des marqueurs de surface cellulaire suivants : CD63, CD90, HLA ABC, CD105, CD73, CD166, CD9, CD44, et dans lequel la cellule souche est négative à un ou plusieurs des marqueurs de surface cellulaire suivants : CD34, CD19, CD86, HLA DR, Lin, CD106, CD80, CD117.

6. Procédé selon la revendication 5, dans lequel la détermination de l'activité métabolique de la cellule adipeuse brune en présence du composé comprend la mesure de l'adipogenèse.

7. Procédé selon la revendication 5, dans lequel la détermination de l'activité métabolique de la cellule adipeuse brune en présence du composé comprend la mesure de l'expression génique d'au moins un marqueur d'adipogenèse choisi dans un groupe consistant en : domaine de PR contenant 16 (PRDM16), protéine 7 morphogénétique osseuse (BMP7), protéine découplante 1 (UCP1), protéine découplante 2 (UCP2), ou facteur myogénique 5 (MYF5).

8. Procédé selon la revendication 5, dans lequel le composé est choisi dans le groupe consistant en une protéine, un anticorps, un peptide, une mutéine, un polynucléotide, un aptamère d'acide nucléique, une hormone, une petite molécule organique peptidique, et une petite molécule non peptidique.

9. Procédé de différentiation d'une cellule souche en une cellule adipeuse brune, le procédé comprenant :

la mise en contact d'une cellule souche isolée avec un milieu de différentiation ne comprenant pas de produit d'origine animale, générant ainsi la cellule adipeuse brune,
dans lequel le milieu de différentiation comprend l'insuline, la dexaméthasone, l'isobutylméthylxanthine, l'indométhacine, la triiodothyronine (T3), la rosiglitazone, la protéine 5 contenant le domaine de type III de la fibronectine (FNDC5), et du lysat de plaquettes humaines à 0,5 à 20 % ;
dans lequel la cellule souche est positive à un ou plusieurs des marqueurs de surface cellulaire suivants : CD63, CD90, HLA ABC, CD105, CD73, CD166, CD9, CD44, et
dans lequel la cellule souche est négative à un ou plusieurs des marqueurs de surface cellulaire suivants : CD34, CD19, CD86, HLA DR, Lin, CD106, CD80, CD117.

10. Procédé selon la revendication 9, dans lequel la cellule adipeuse brune exprime le domaine de PR contenant 16 (PRDM16), le coactivateur du récepteur-gamma activé par le proliférateur de péroxisome (PGC-1), ou l'une de leur combinaison.

11. Procédé de différentiation d'une cellule souche en une cellule adipeuse brune, le procédé comprenant :

la mise en contact de la cellule souche avec un premier milieu de différentiation ne comprenant pas un produit d'origine animale, dans lequel le premier milieu de différentiation comprend l'insuline, la dexaméthasone, l'isobutylméthylxanthine, l'indométhacine, la triiodothyronine (T3), la rosiglitazone, et du lysat de plaquettes humaines à 0,5 à 20 % ;
la mise en contact de la cellule souche avec un second milieu de différentiation ne comprenant pas un produit d'origine animale, dans lequel le second milieu de différentiation comprend l'insuline, la dexaméthasone, l'isobutylméthylxanthine, l'indométhacine, la triiodothyronine (T3), la rosiglitazone, la protéine 5 contenant le domaine de type III de la fibronectine (FNDC5), et du lysat de plaquettes humaines à 0,5 à 20 % ;
générant ainsi une cellule adipeuse brune.

FIG. 1
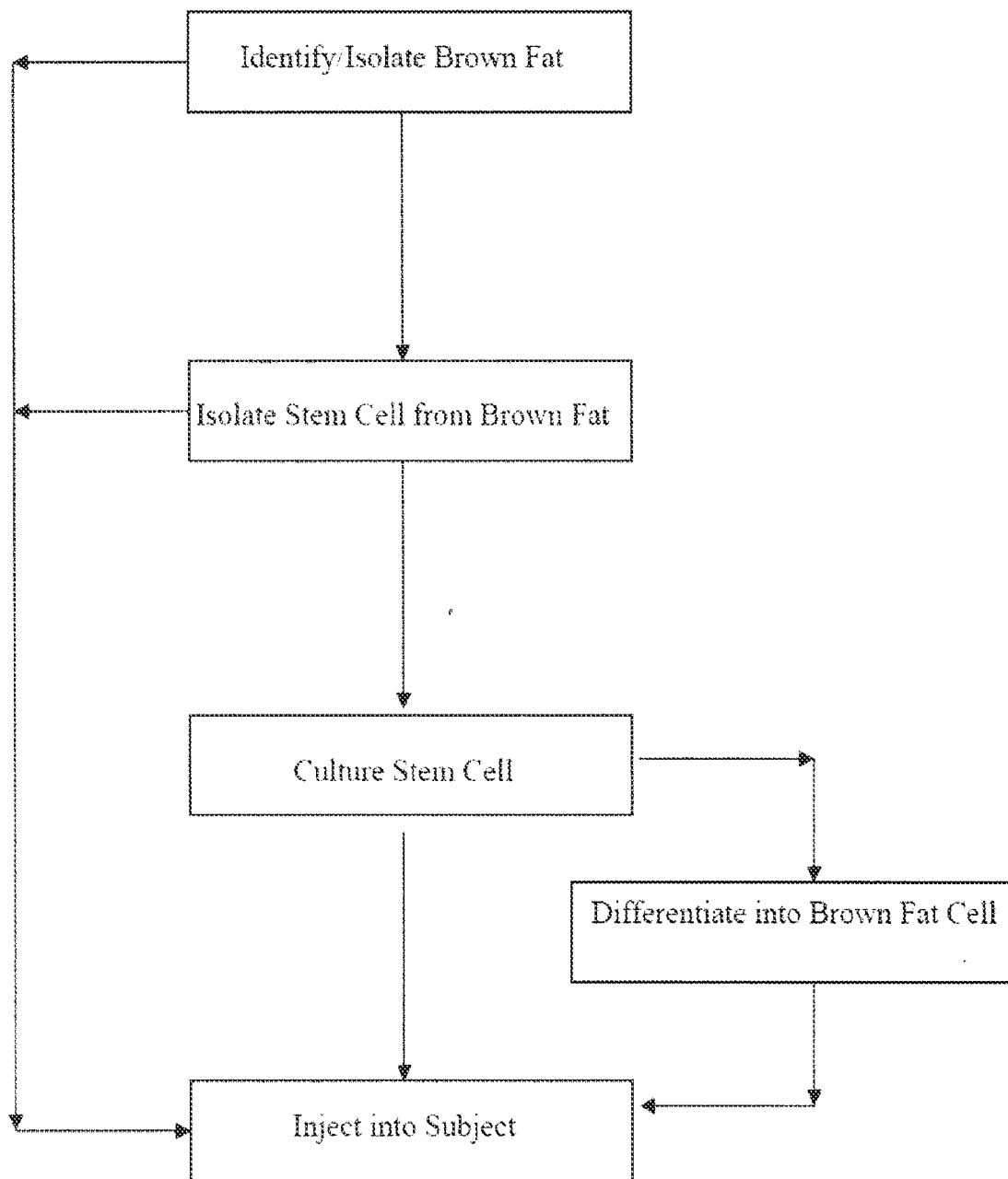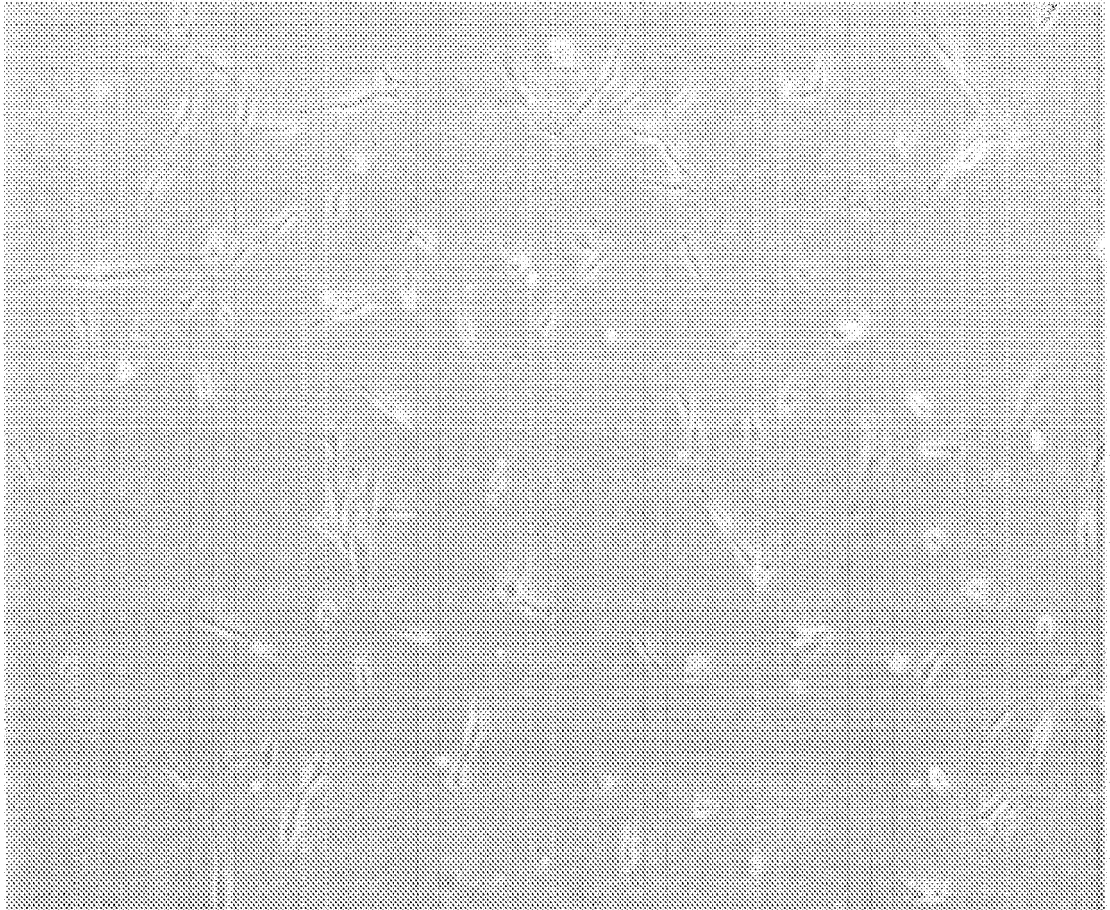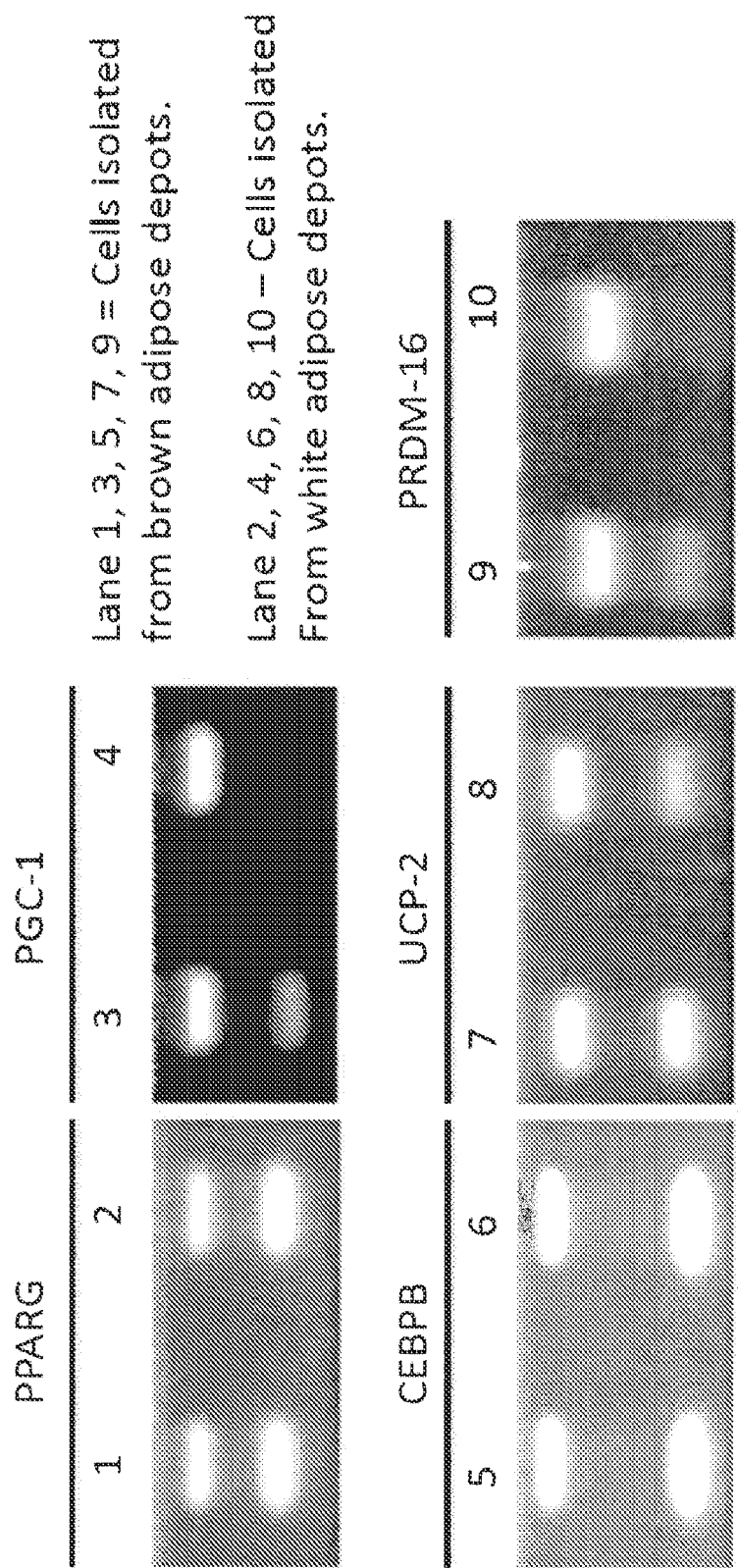
FIG. 2

FIG. 3

Lane 1, 3, 5, 7, 9 = Cells isolated from brown adipose depots.
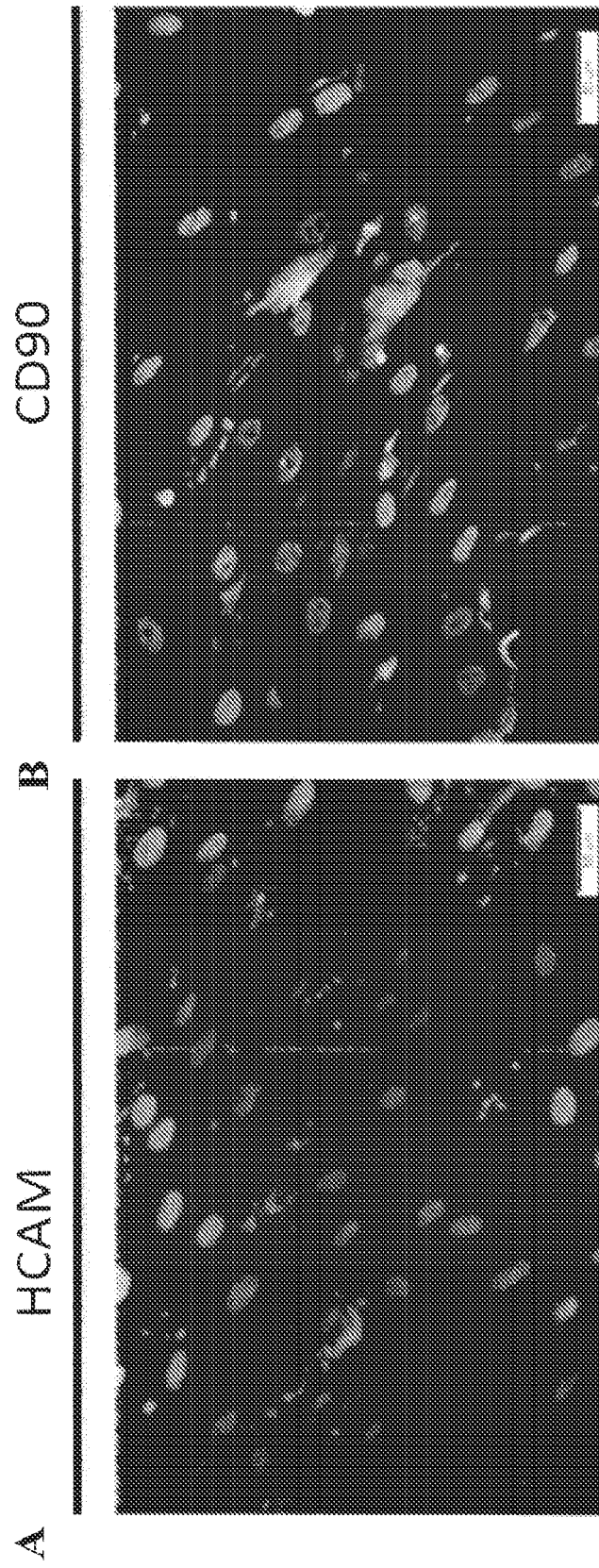
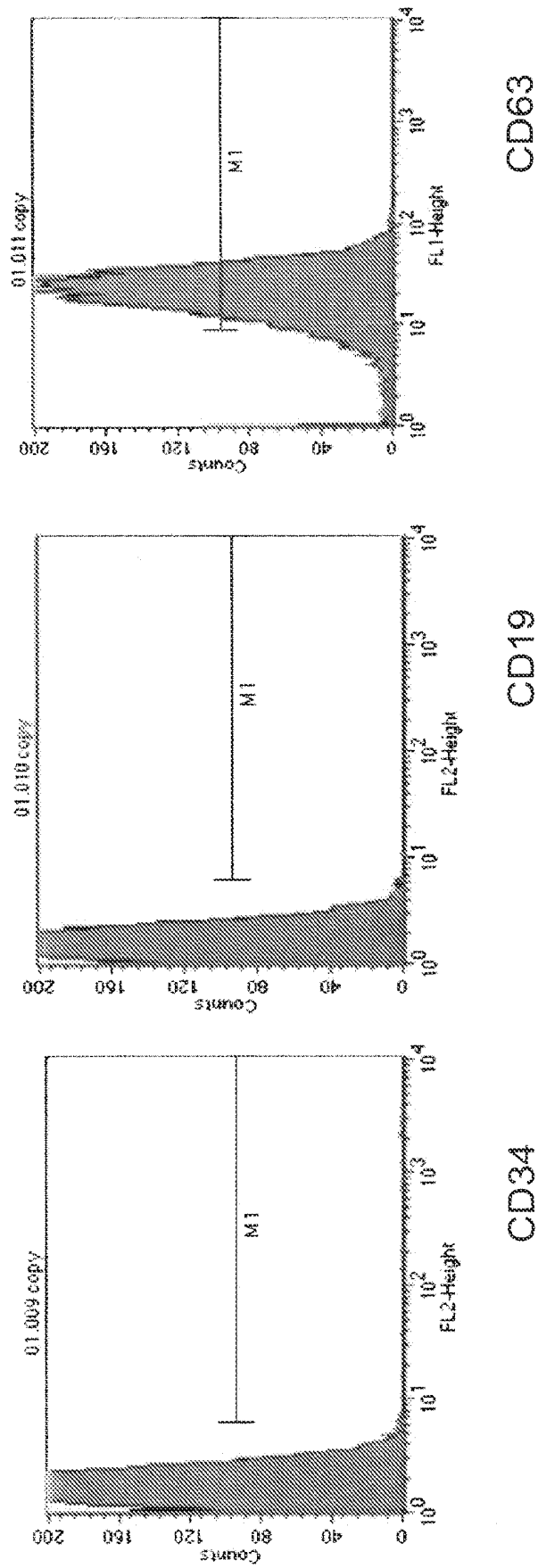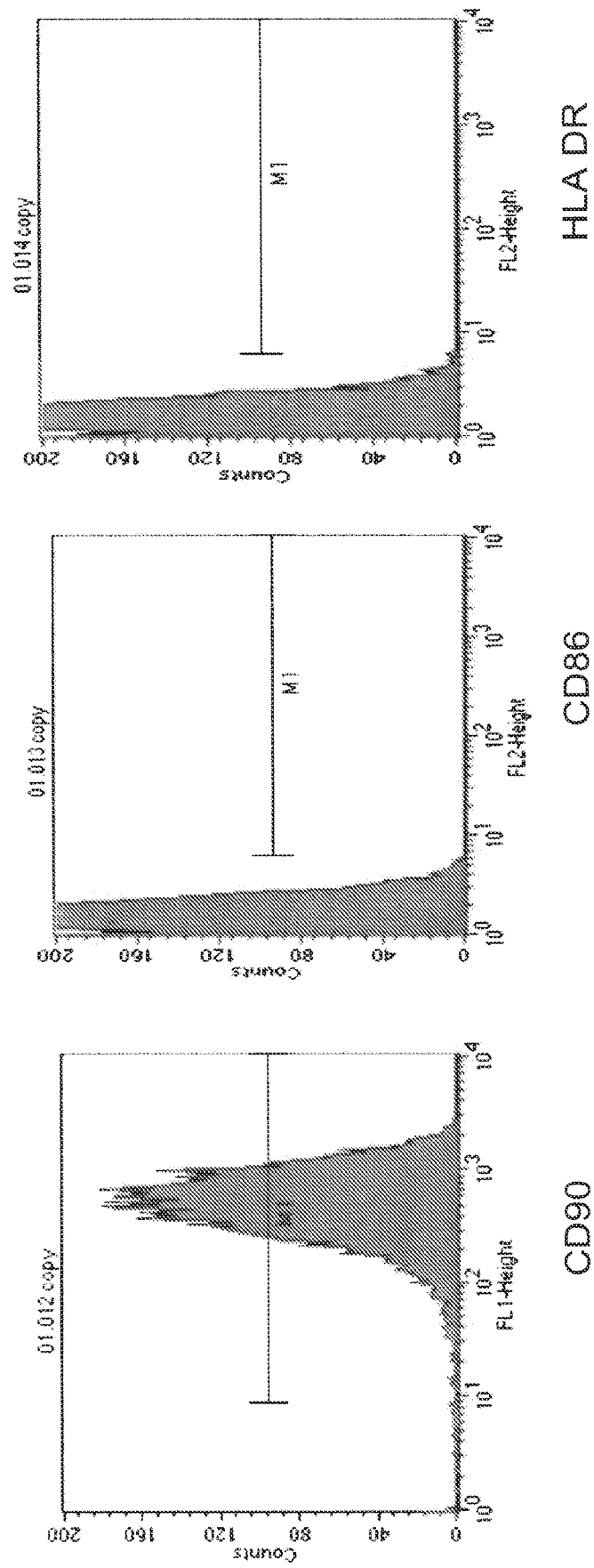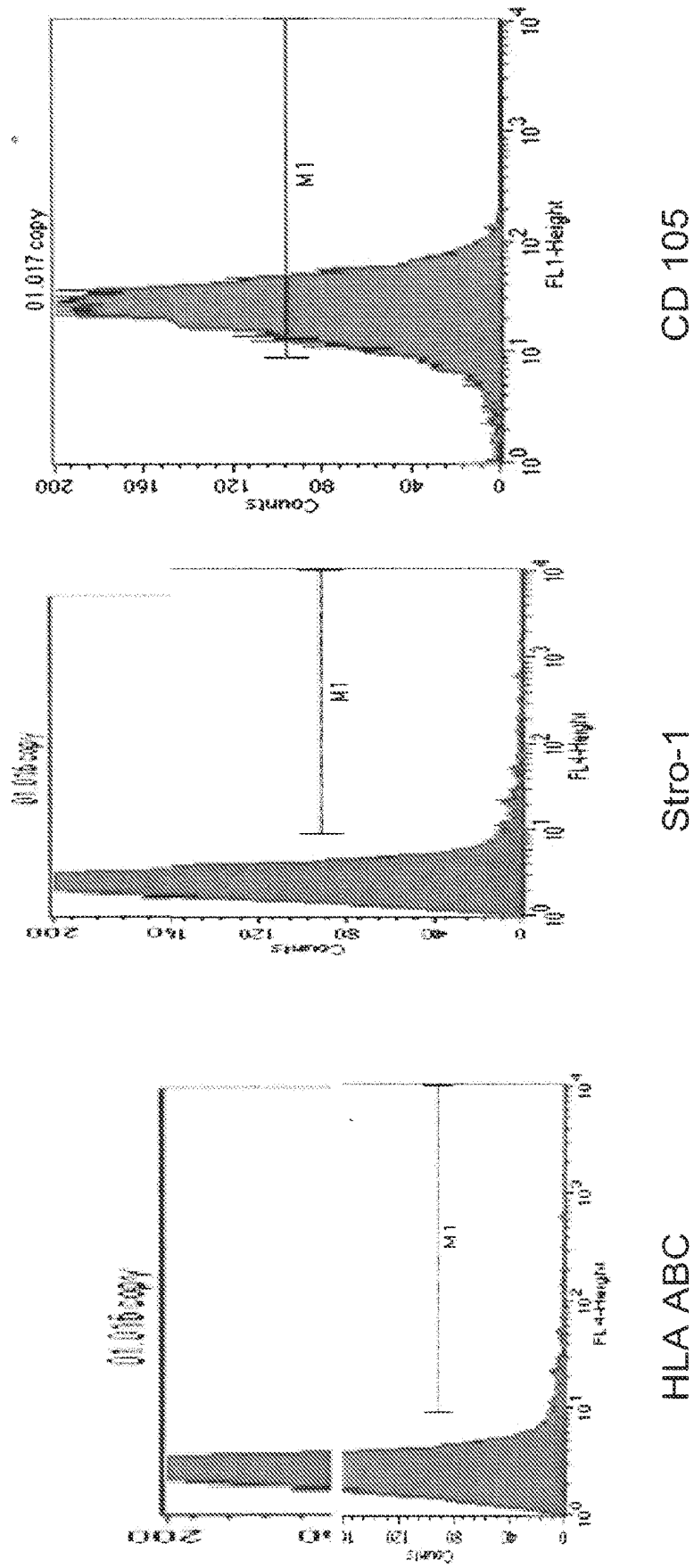Lane 2, 4, 6, 8, 10 – Cells isolated From white adipose depots.
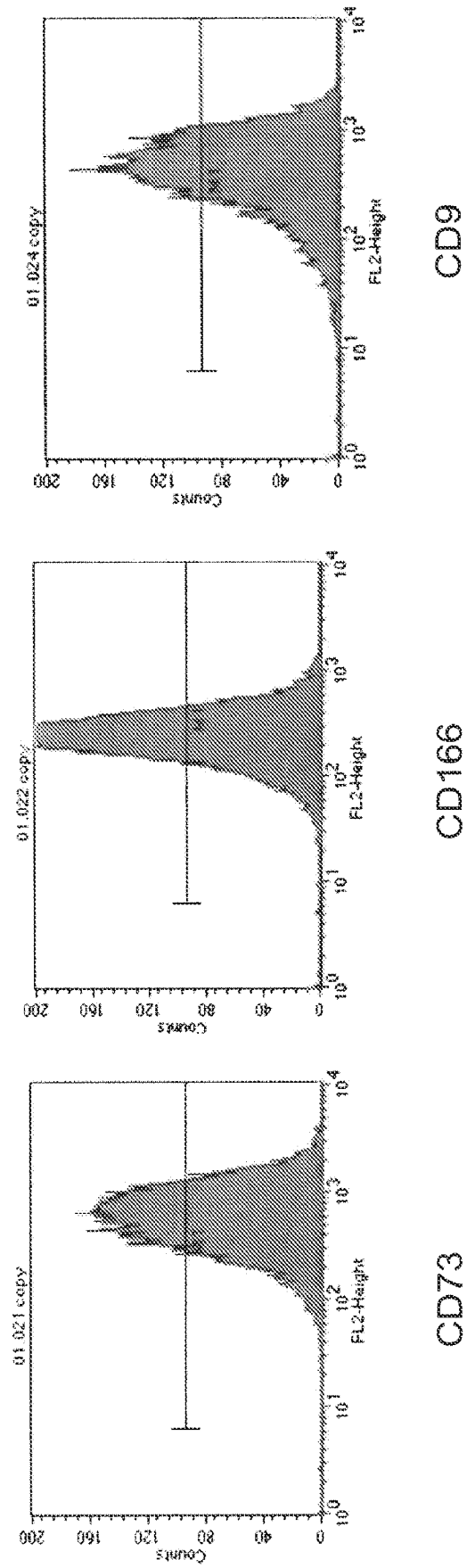
HCAM

CD90

A

B

FIG. 4

FIG. 5A

FIG. 5B

FIG. 5C
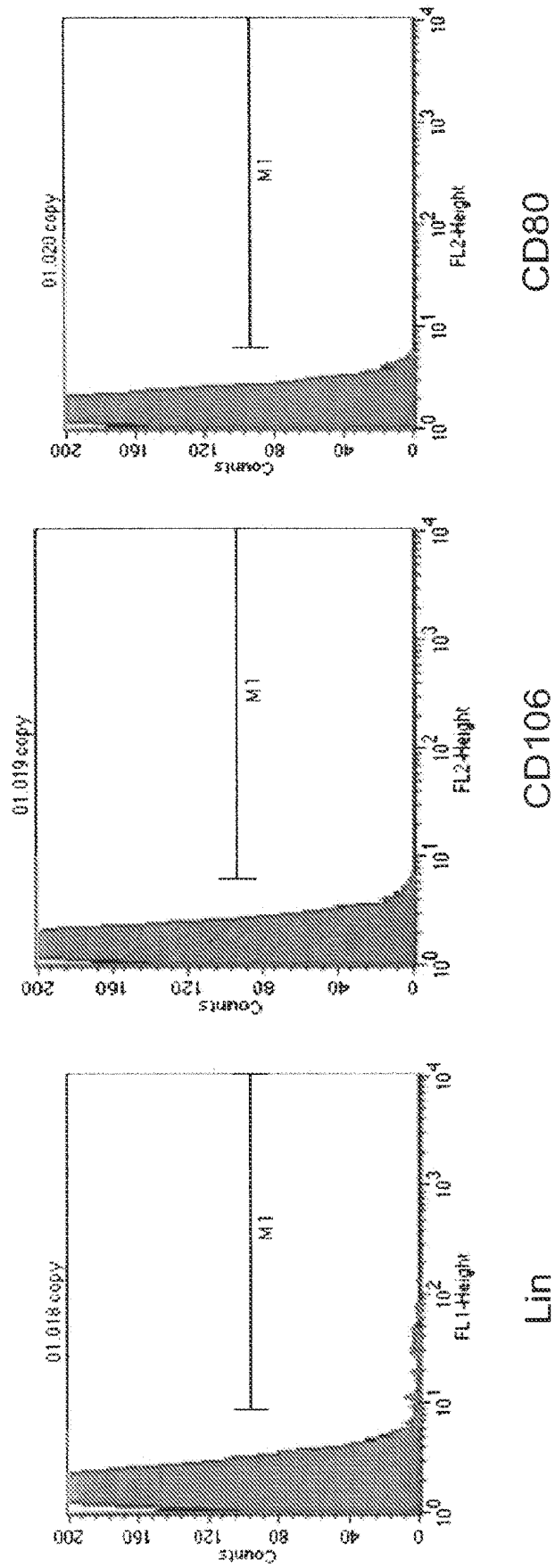
FIG. 5D

FIG. 5E
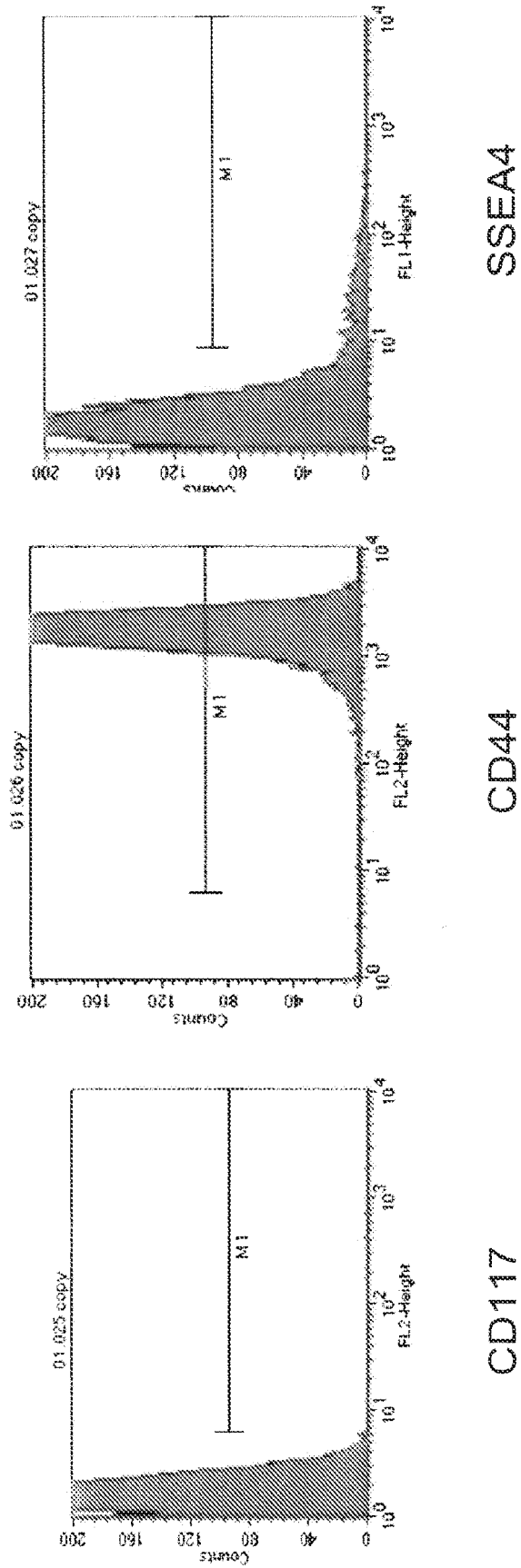
FIG. 5F
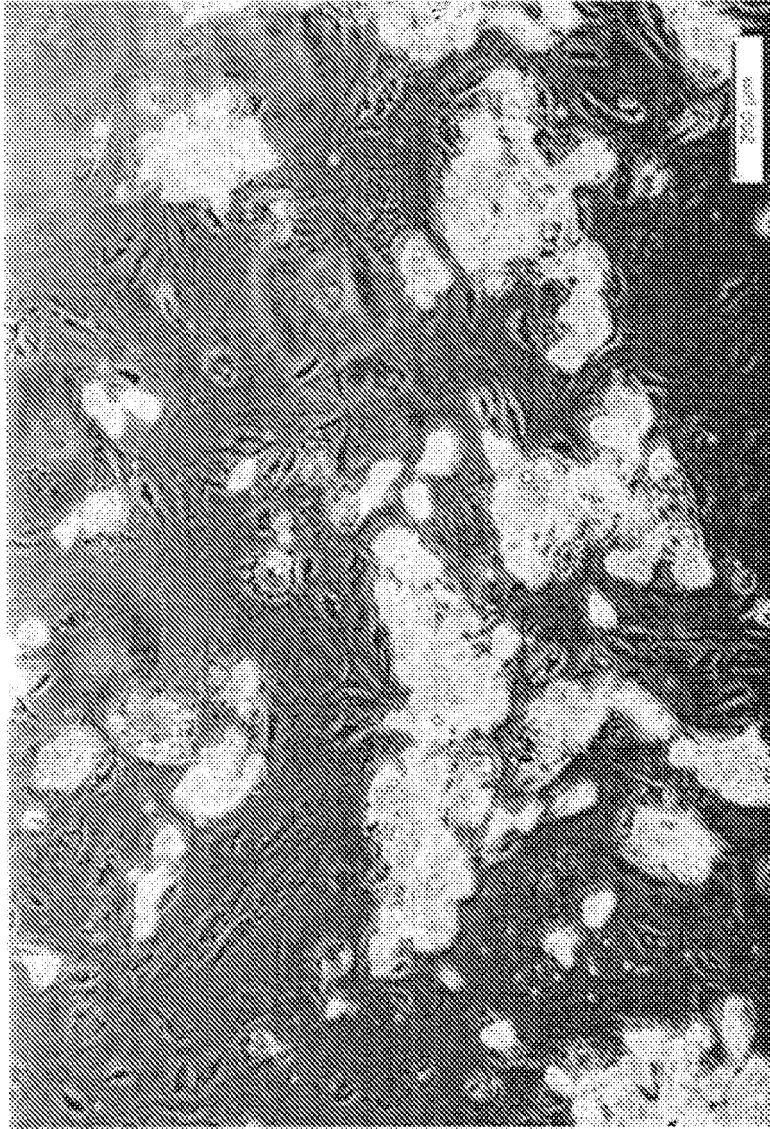
FIG. 6

A   Large fat droplets
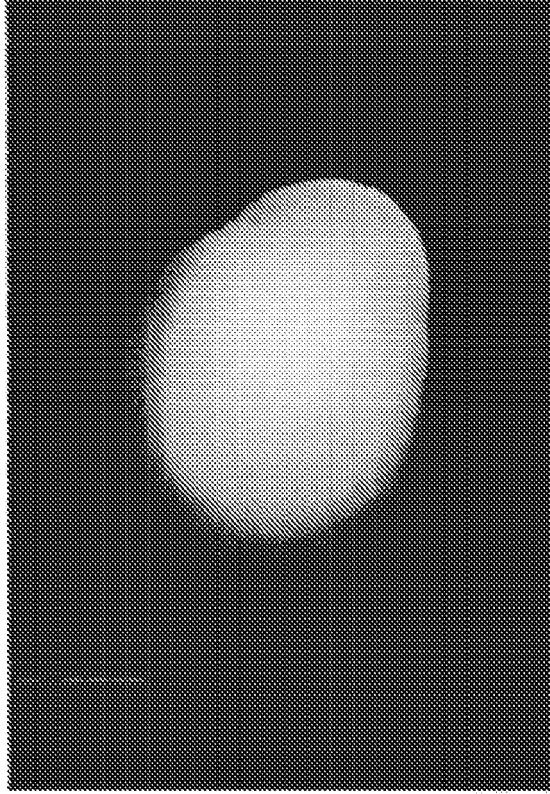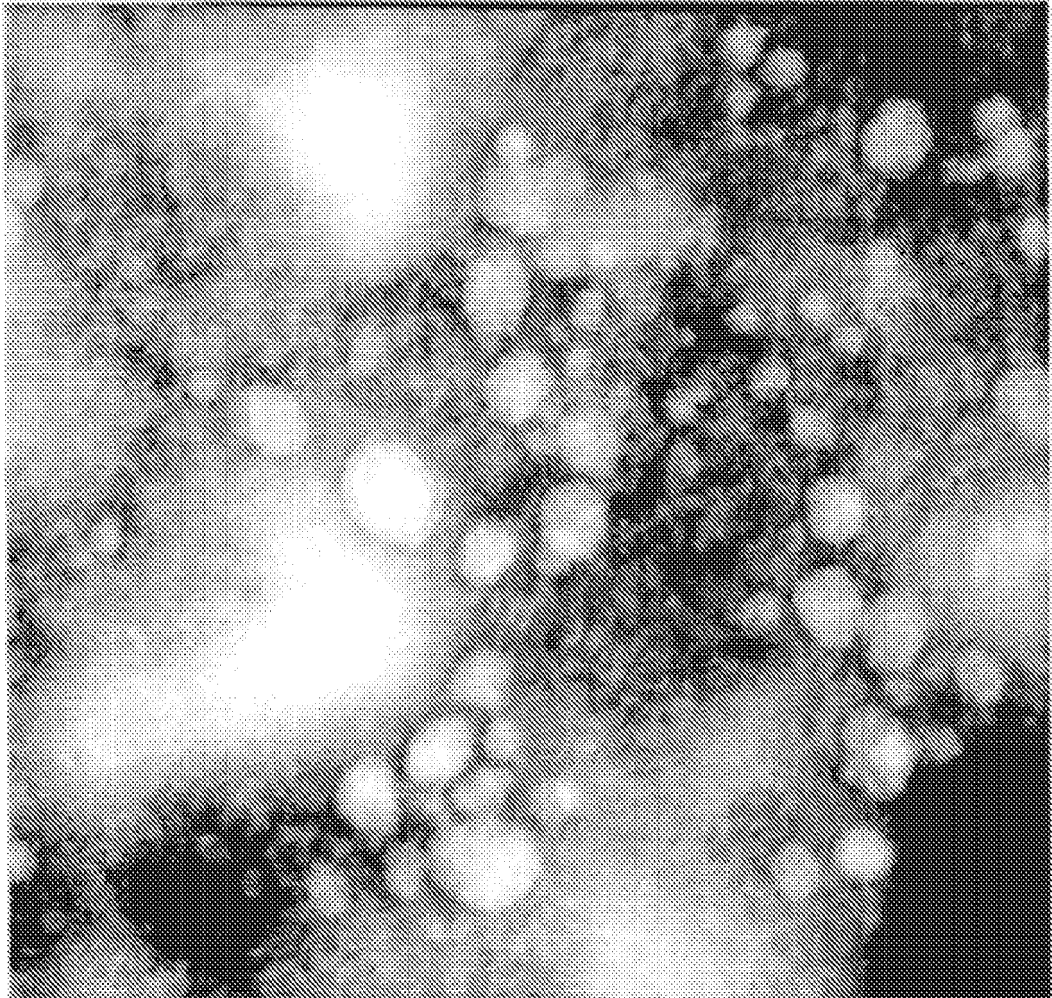
B   Small fat droplets

FIG. 7

A Osteogenesis

B Chondrogenesis

FIG. 8

FIG. 9
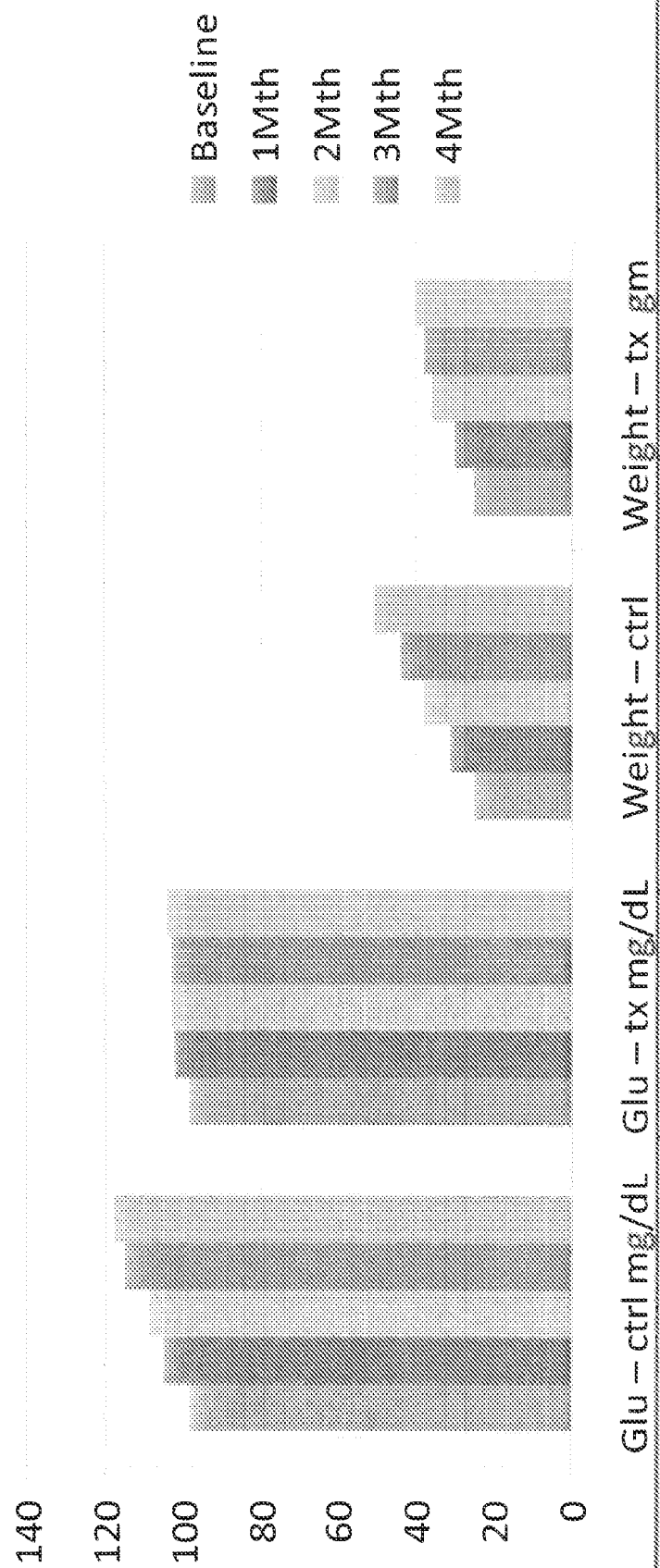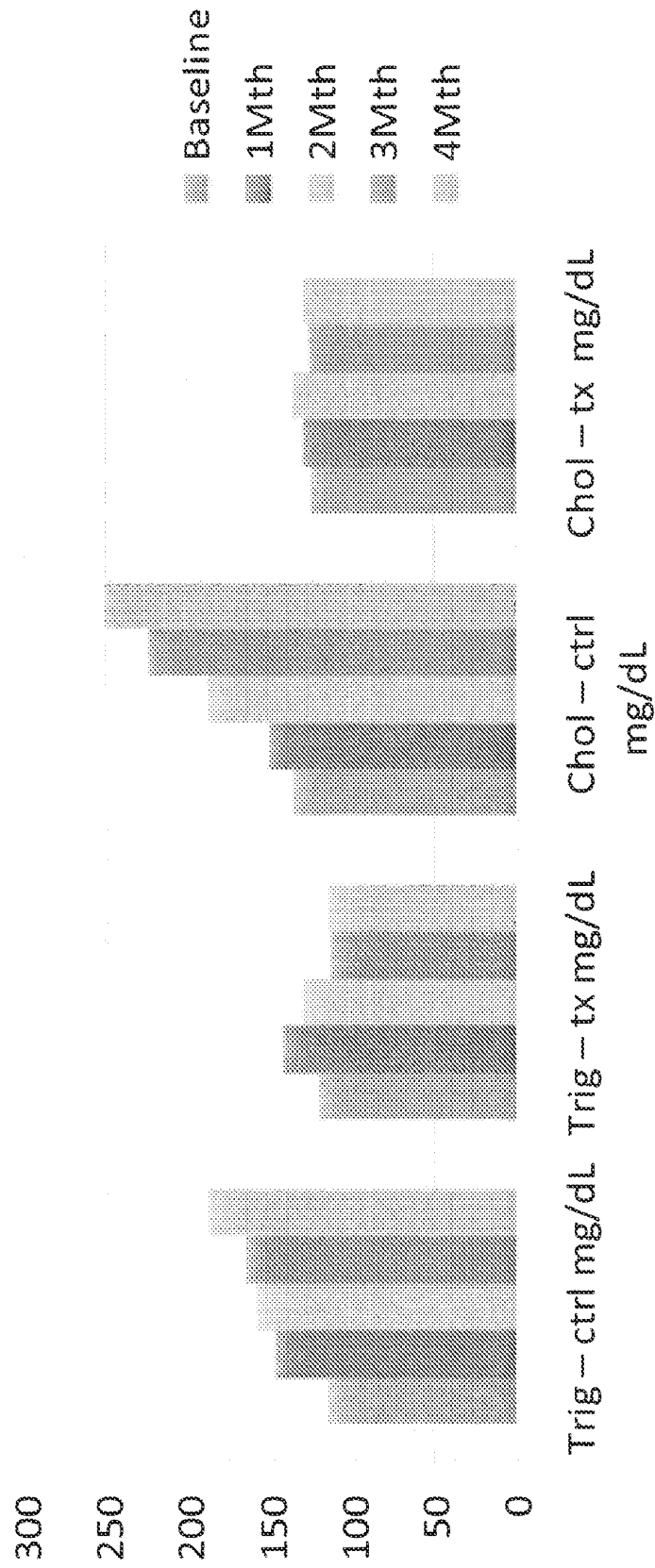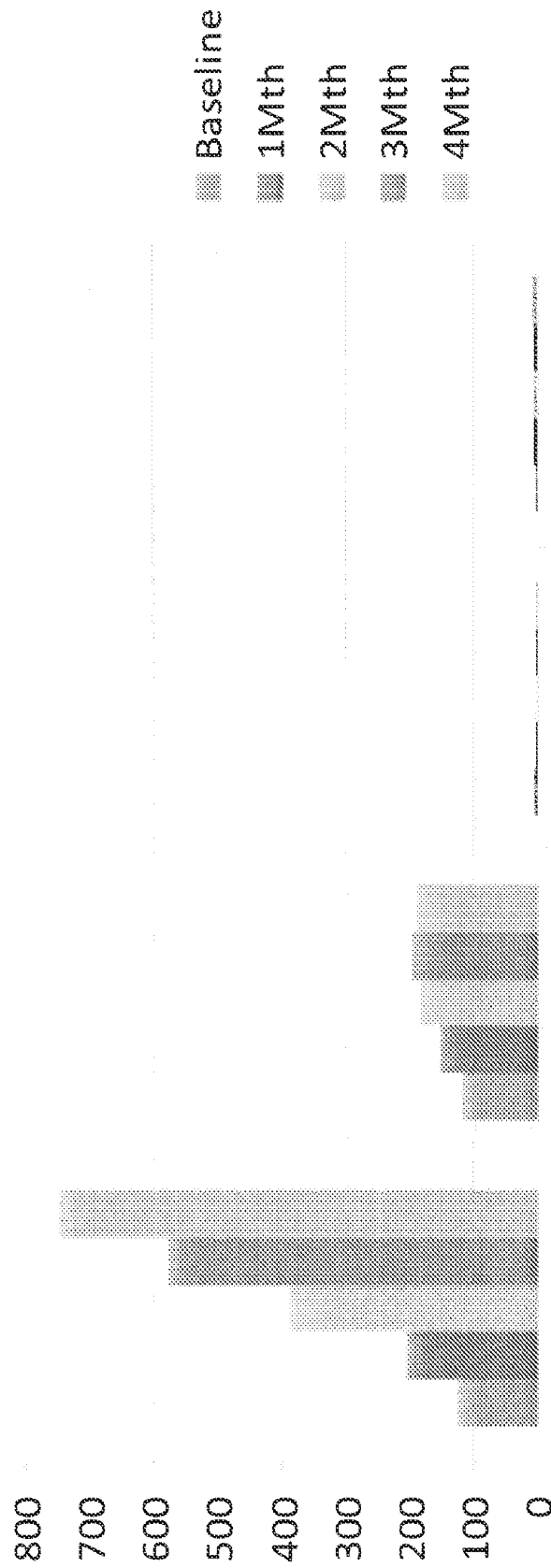
	Baseline	1Mth	2Mth	3Mth	4Mth
Glu – ctrl mg/dL	98±7	105±9	109±6	115±7	118±6
Glu – tx mg/dL	96±8	102±7	103±5	103±4	104±5
Weight – ctrl gm	23±5	31±6	38±6	44±4	51±6
Weight – tx gm	26±7	30±5	36±4	38±5	40±5

FIG. 10

	Baseline	1Mth	2Mth	3Mth	4Mth
Trig – ctrl mg/dL	115±10	147±13	159±21	165±14	188±16
Trig – tx mg/dL	120±14	142±15	131±19	113±11	114±10
Chol – ctrl mg/dL	130±16	151±15	188±26	224±34	251±22
Chol – tx mg/dL	125±12	130±14	136±24	126±19	130±15

FIG. 11

	Baseline	1Mth	2Mth	3Mth	4Mth
Leptin – ctrl pg/mL	125±15	205±23	389±29	577±46	748±69
Leptin – tx pg/mL	117±9	152±16	183±14	198±17	190±20
Adipon – ctrl ng/mL	4.2±1.1	3.1±1.2	3.8±1.1	2.9±0.8	2.8±0.6
Adipon – tx ng/mL	3.9±0.9	4.5±0.8	4.9±1.1	5.7±1.1	6.6±1.4

FIG. 12

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