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(54) **TREATMENT OF INFLAMMATORY  
CONDITIONS**

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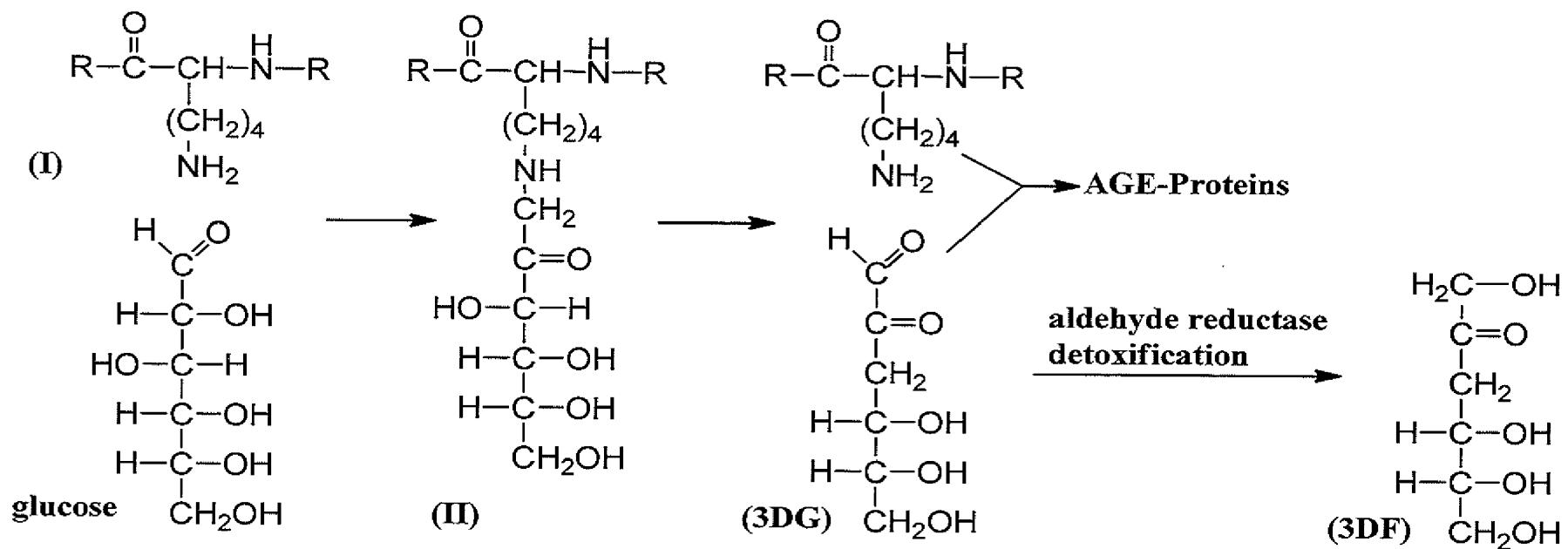
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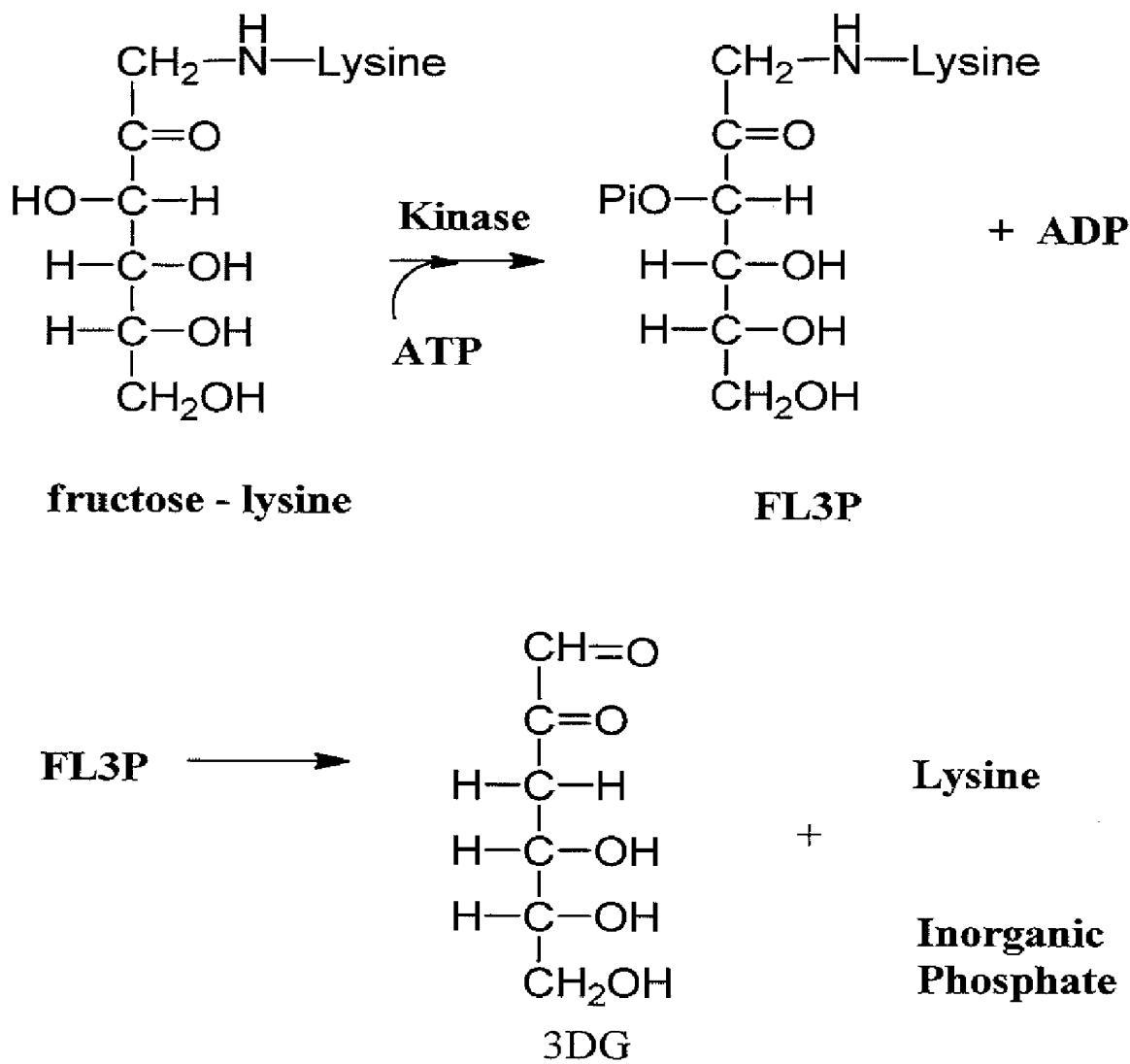
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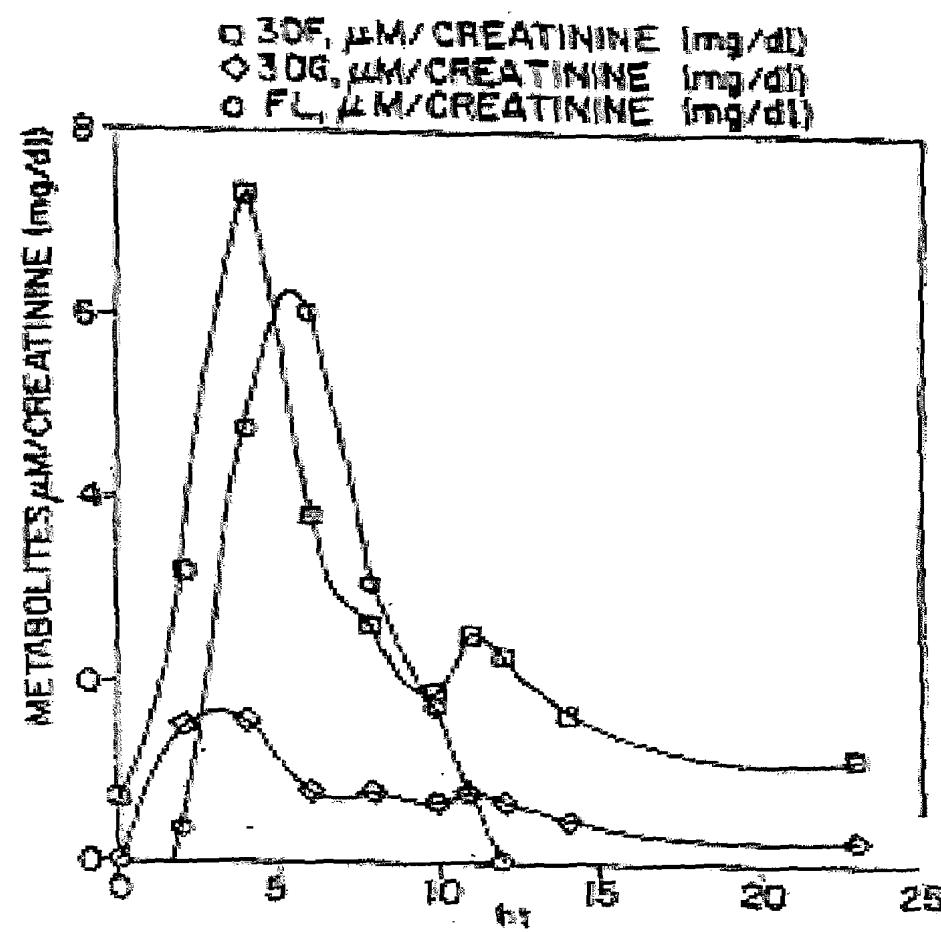
(57) **ABSTRACT**

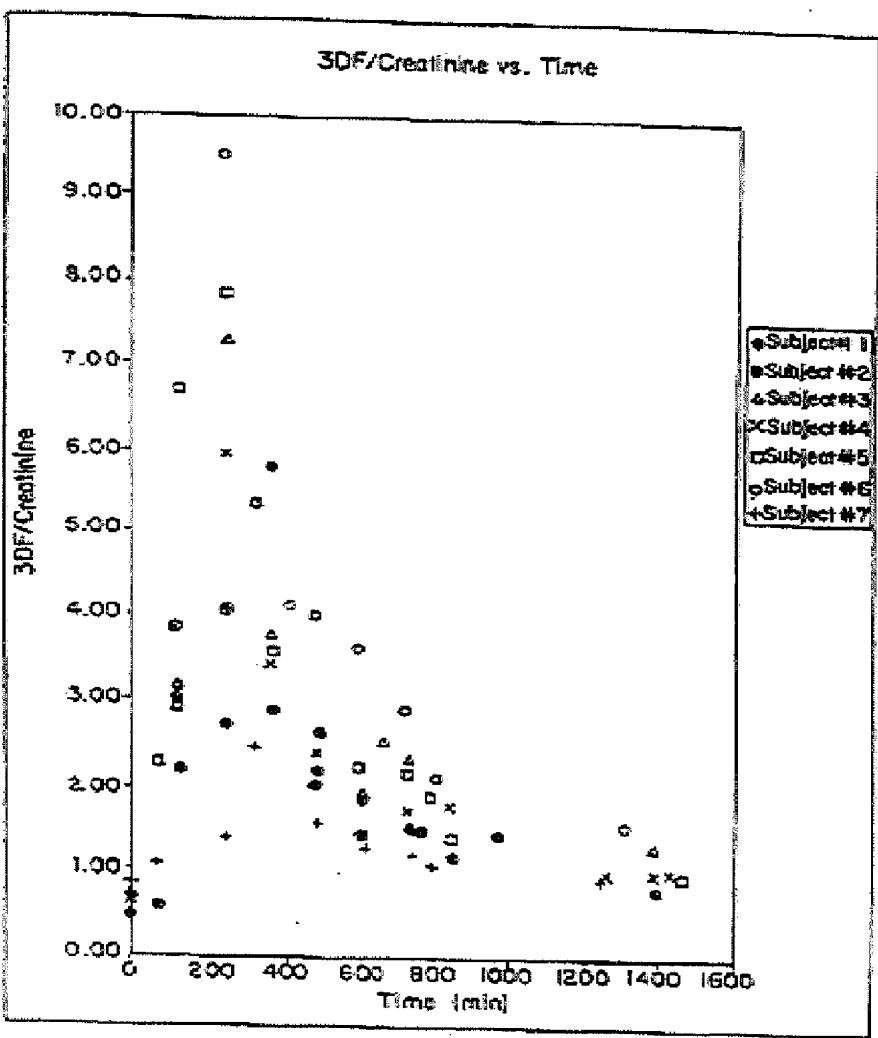
The invention relates to methods of inhibiting production and function of 3-deoxyglucosone and other alpha-dicarbonyl sugars in skin thereby treating or prevention various diseases, disorders or conditions. Additionally, the invention relates to treatment of various diseases, disorders or conditions associated with or mediated by oxidative stress since 3DG induces ROS and AGEs, which are associated with the inflammatory response caused by oxidative stress.

**Fig. 1**

**Fig. 2**



**Fig. 3**

**Fig. 4**

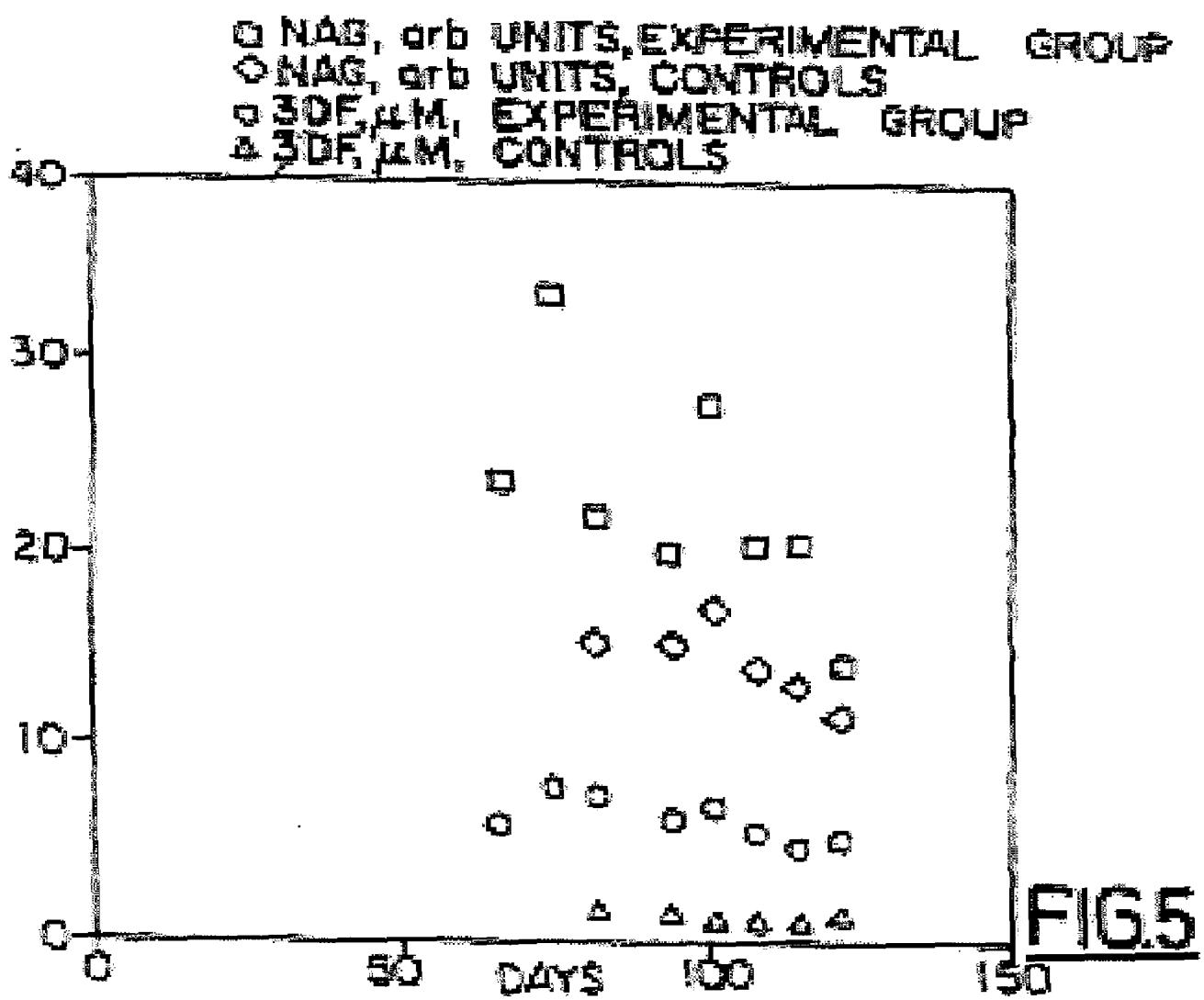
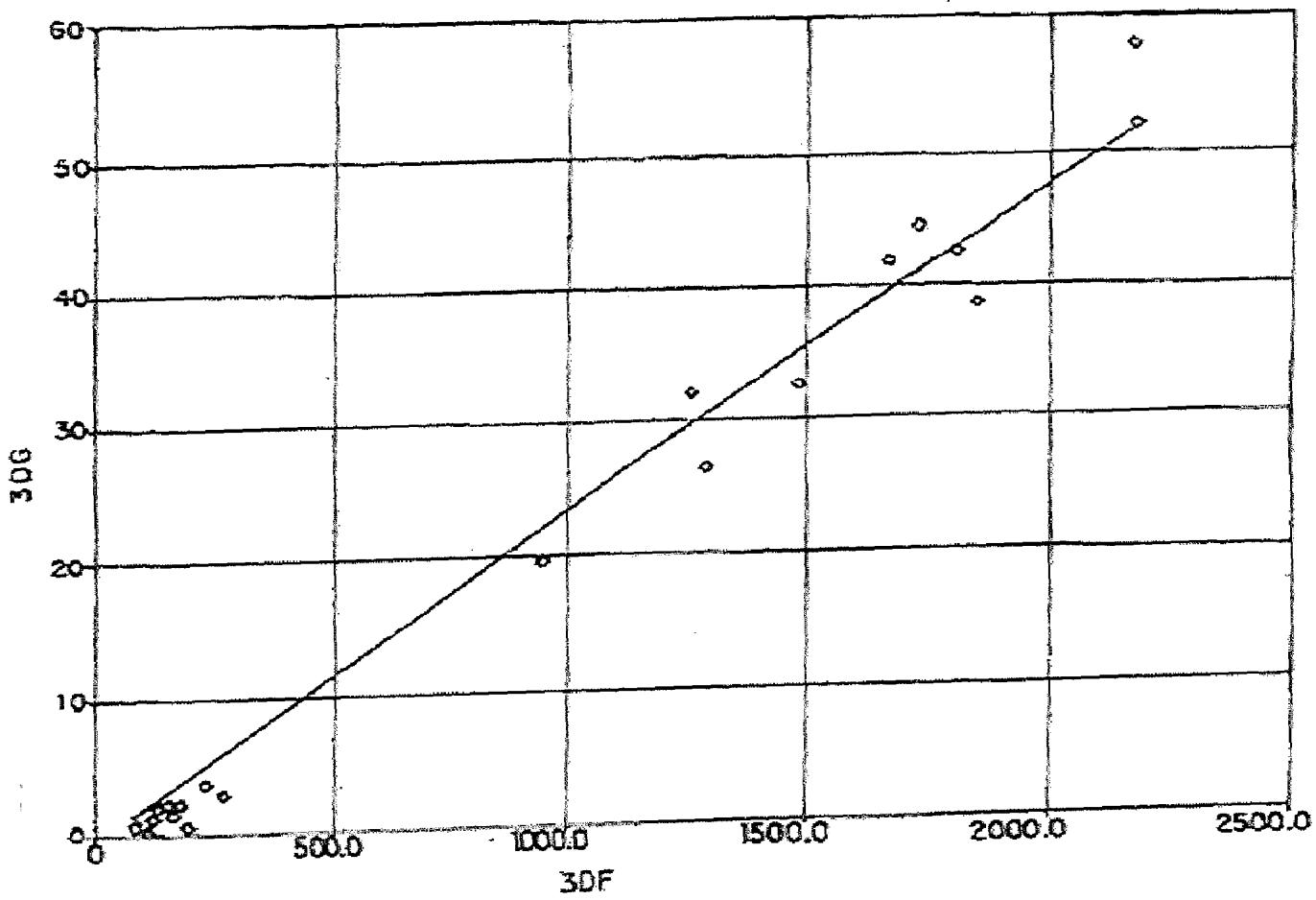


Fig. 6



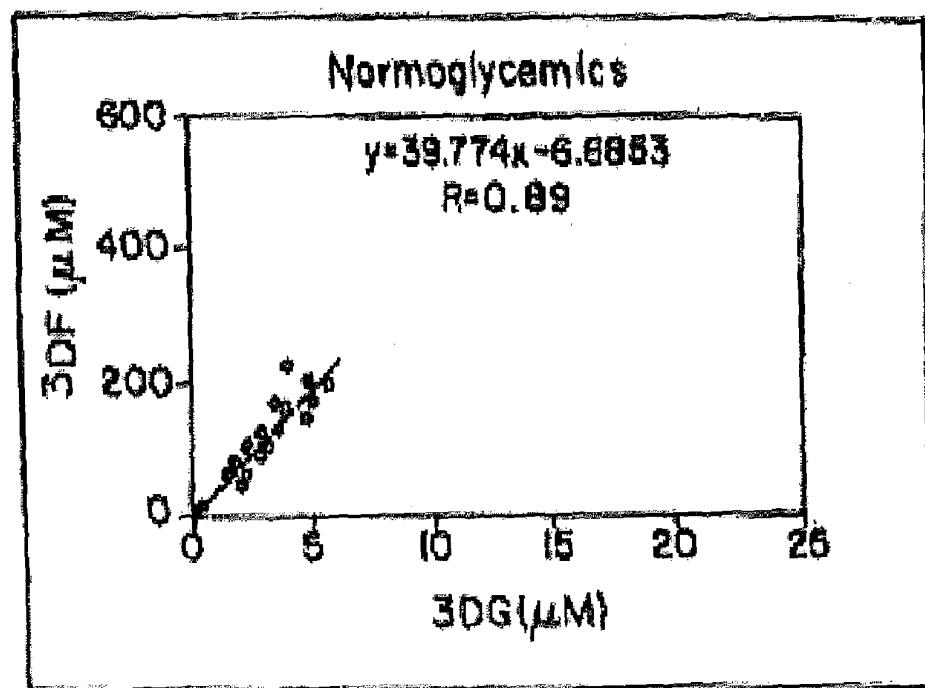


FIG.7A

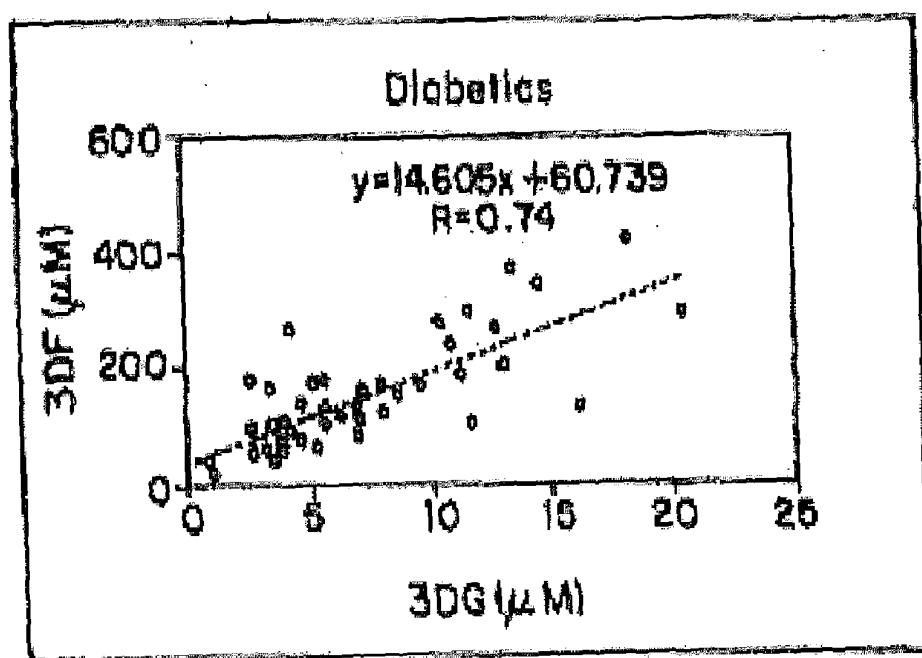
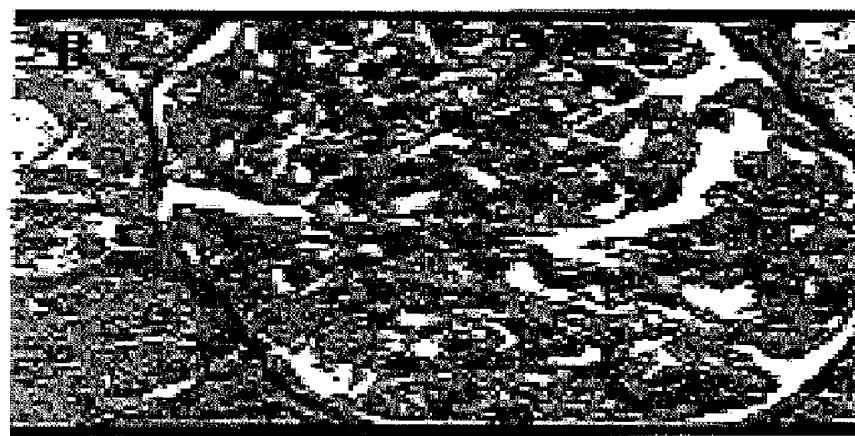


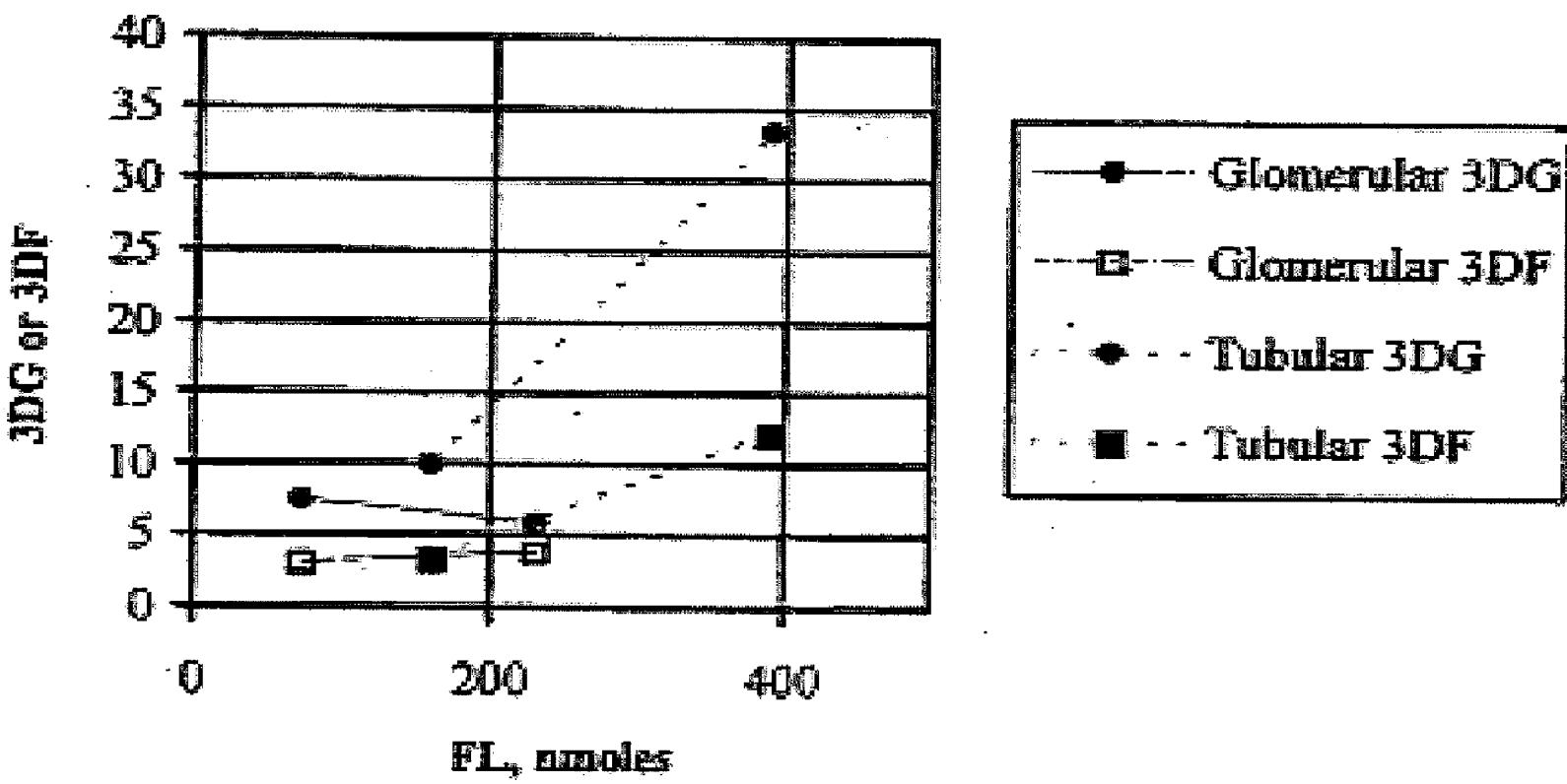
FIG.7B



**FIG.8A**



**FIG.8B**

**Fig. 9**

**FIG 10**

1       mocqllkacdr tatlrafsggp gageisegra ydidaappvfv kvnmtqarq mfegevesle  
6       alrstghtrv pripakvidlp ggysaftvoeh lkmksissqa sklgeqmadi hlytnqklrek  
121      lkcecentvfr tgegaepqyv dkfeghtvrc egfpqmnaw qddowptflar hrldqasqldli  
181      ekdyadrear elwsalqvki pdllfcgleiv pallhgdliws grvaeddvgp iipyipasfyg  
241      lusefetiajal mifggfprstf tayhrikirkka pgidqrilly qlfauylbhwti hfgecryspsa  
301      igtmurilk

**FIG.11**

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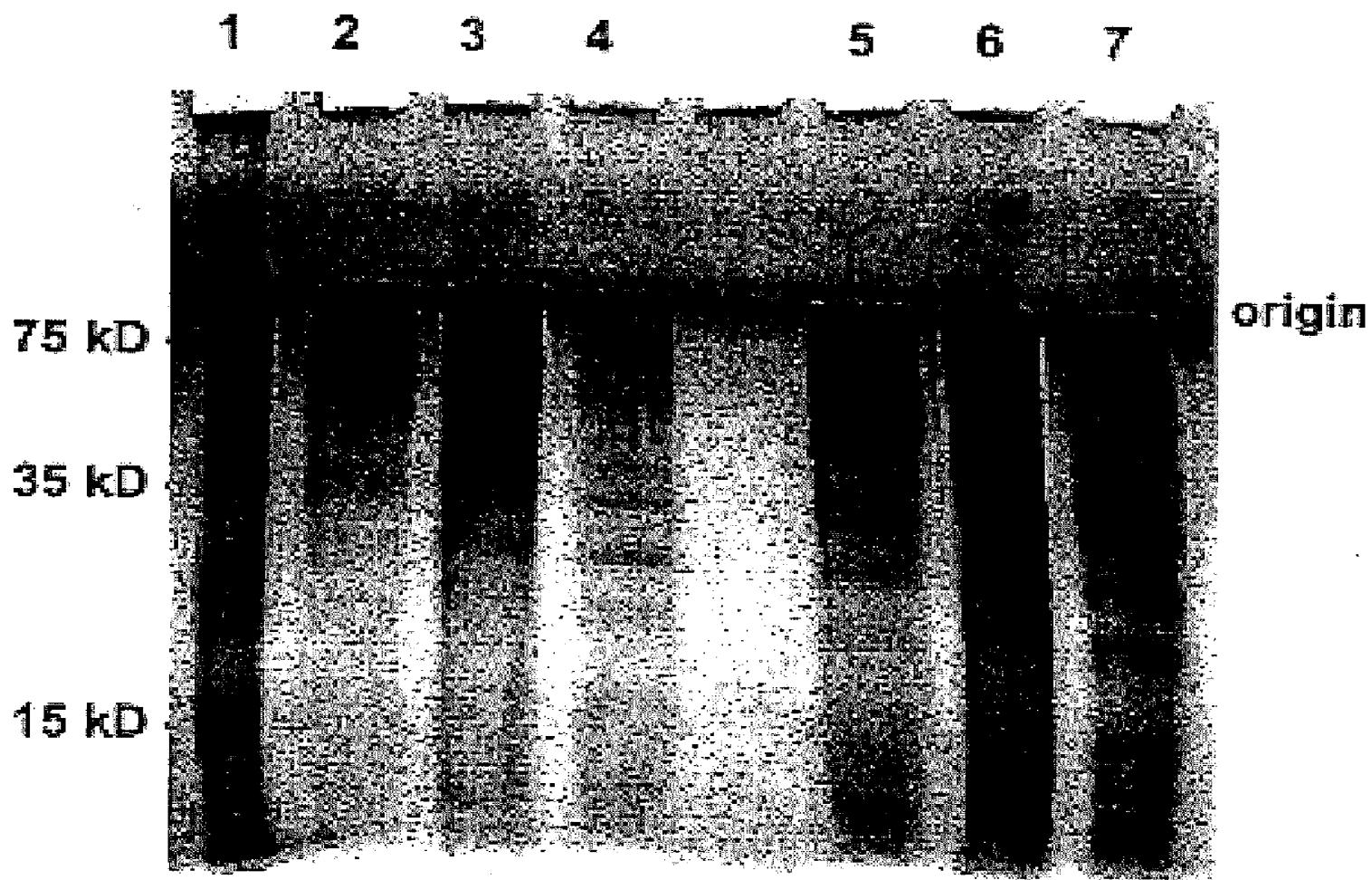


FIG12

Lane  
Primers  
Kidney cDNA  
Skin cDNA

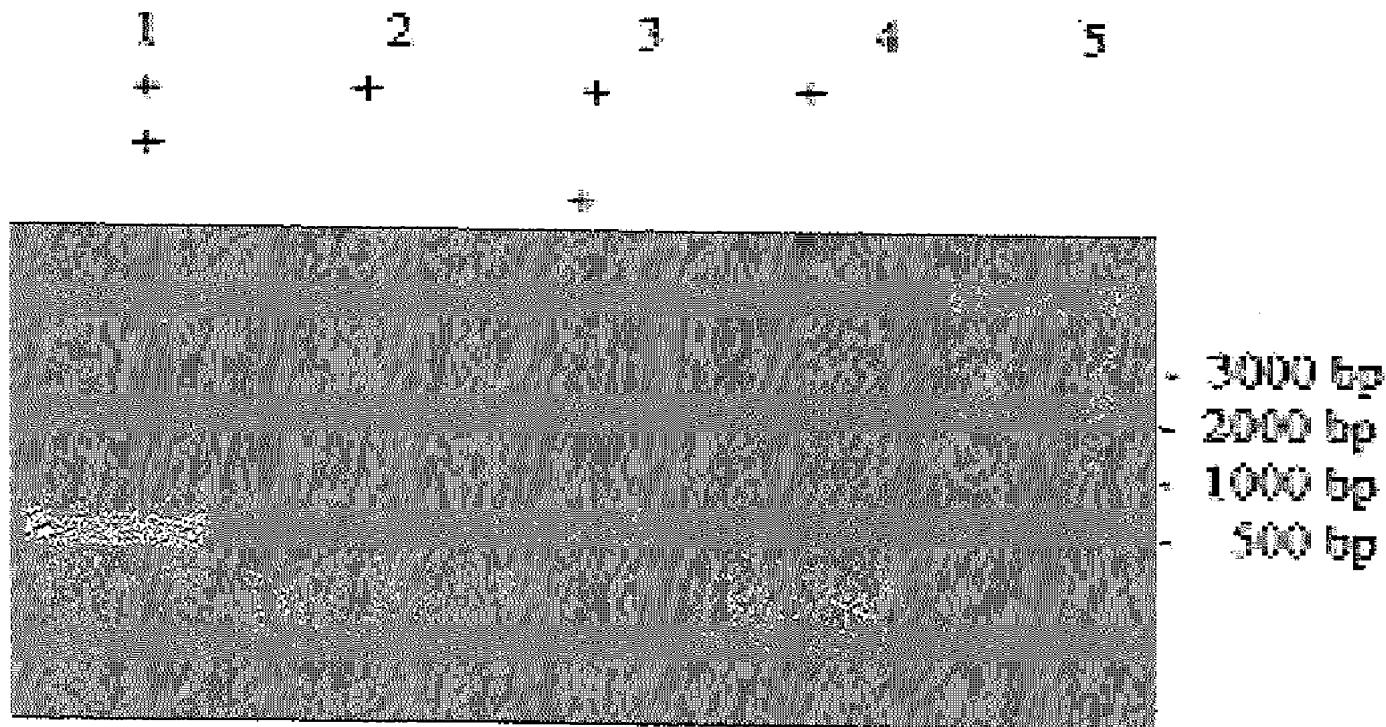
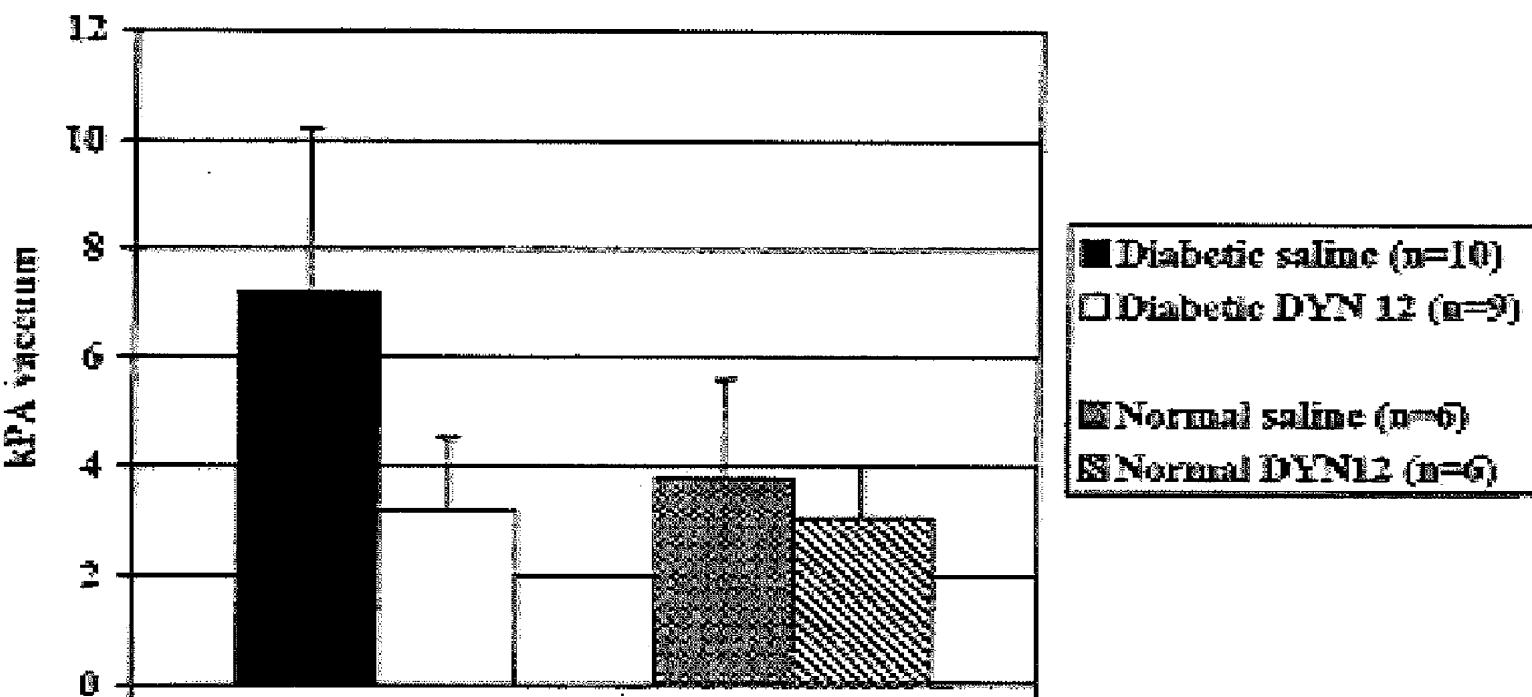
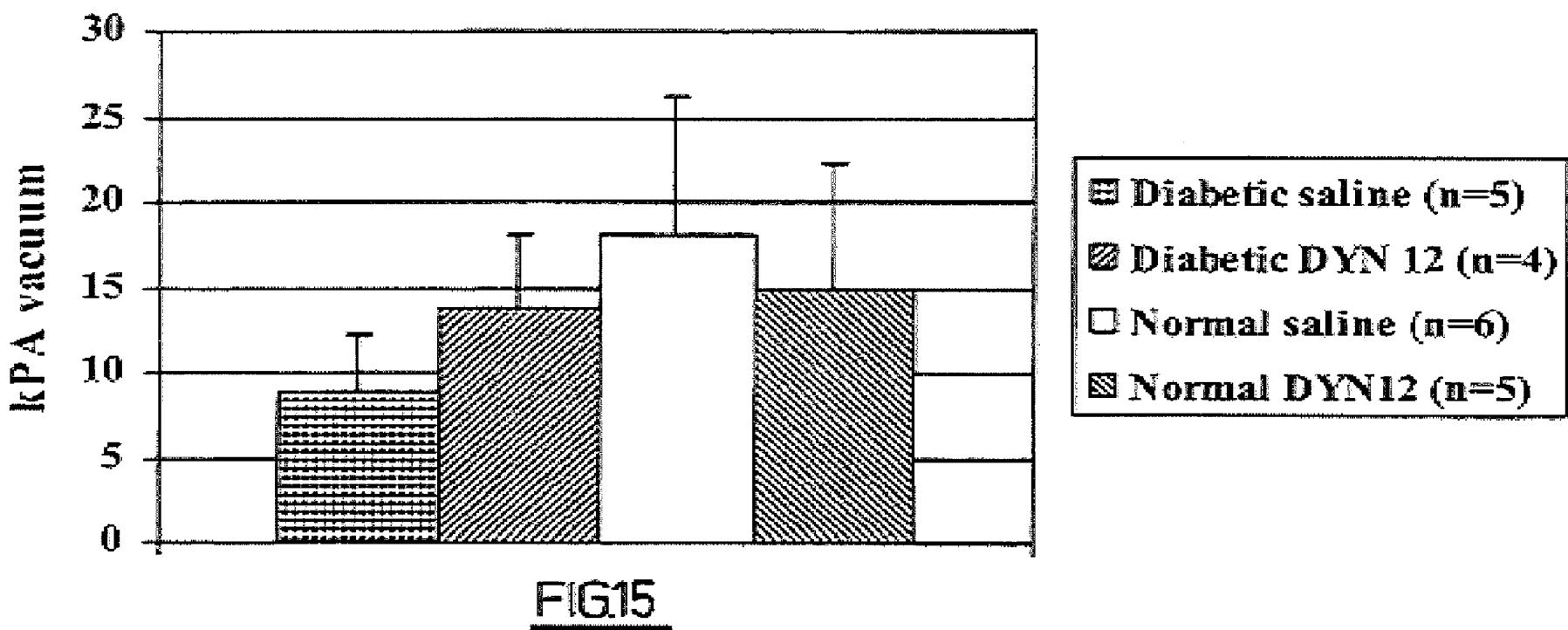
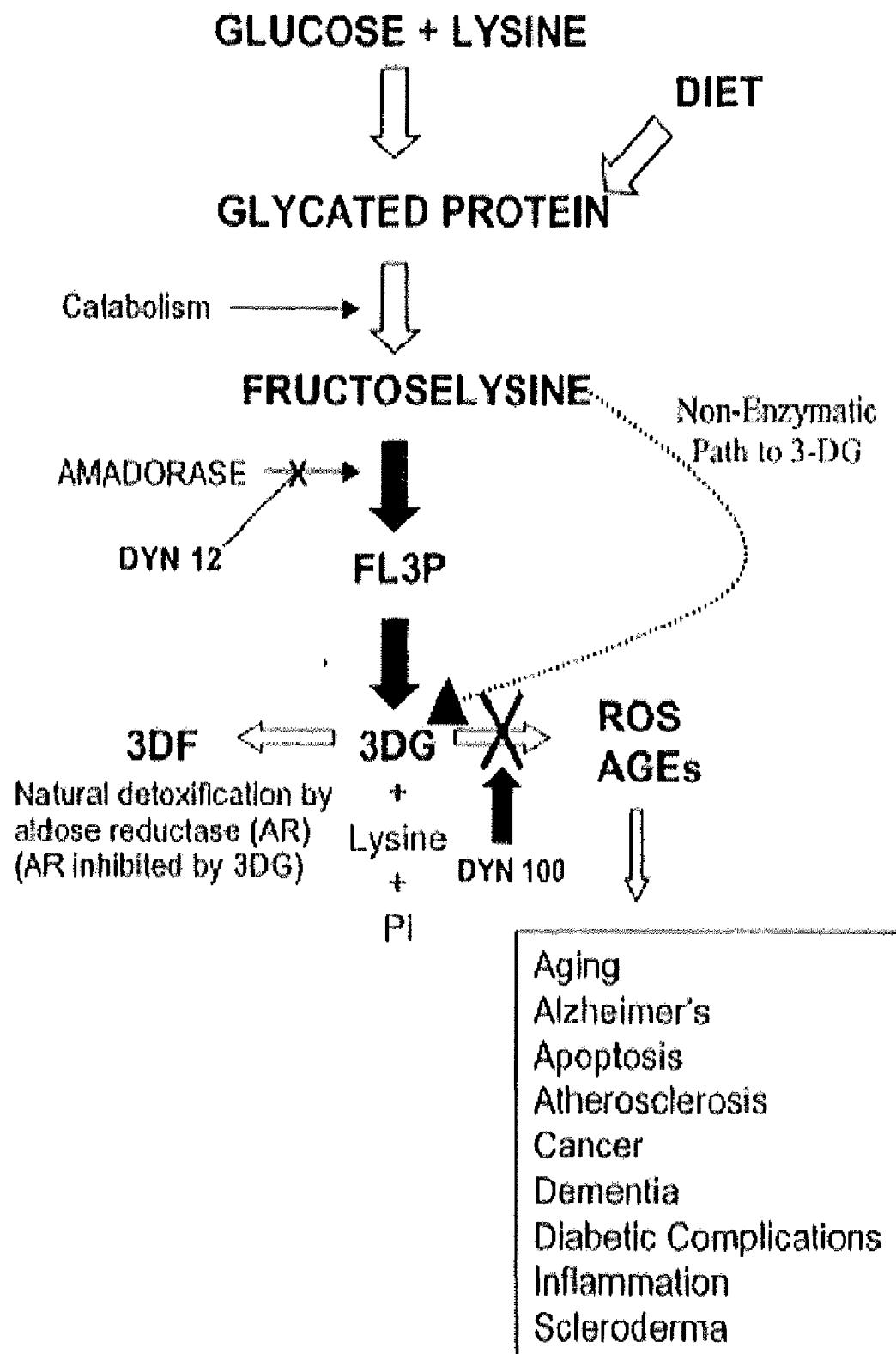


FIG13



**FIG14**





**FIG. 16**

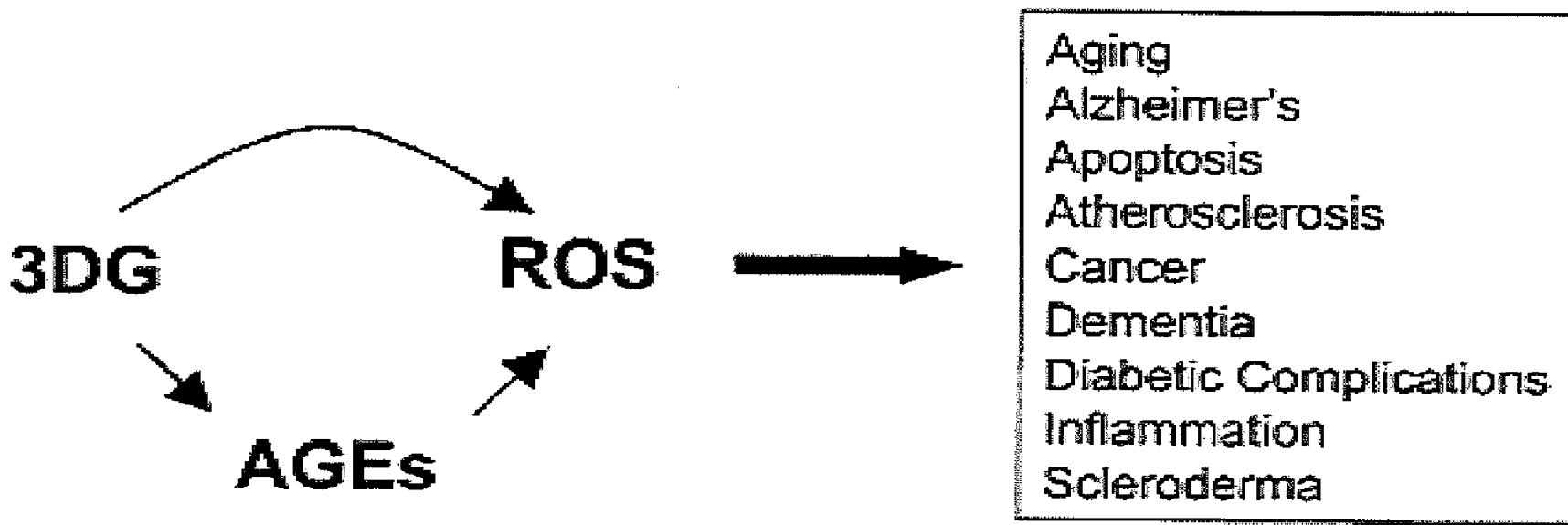
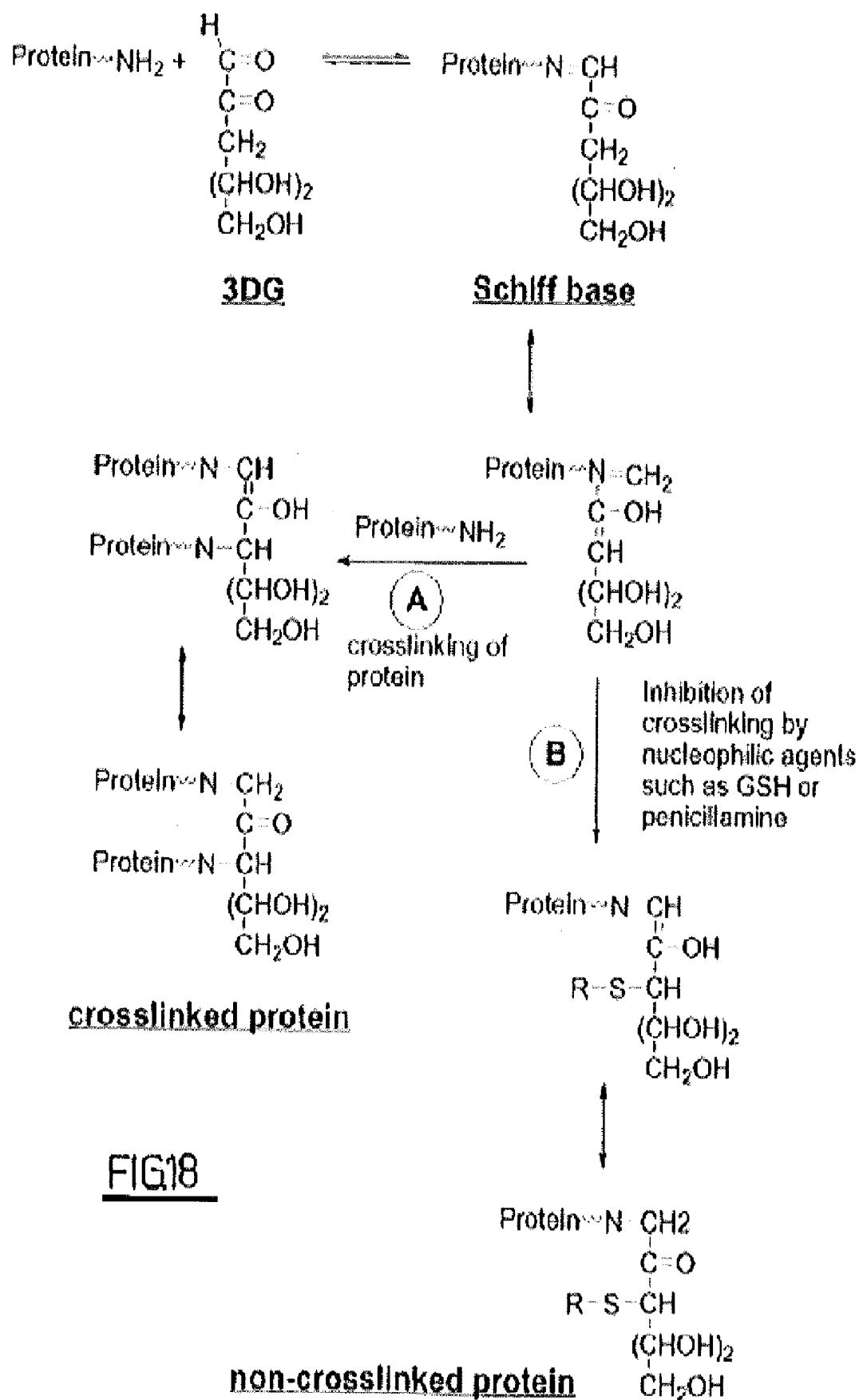


FIG. 17



## TREATMENT OF INFLAMMATORY CONDITIONS

### CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims priority under 35 U.S.C. §119(e) to U.S. Provisional Application No. 60/691, 562 filed Jun. 17, 2005, which application is incorporated by reference herein in its entirety.

### BACKGROUND OF THE INVENTION

[0002] Biological amines react with reducing sugars to form a complex family of rearranged and dehydrated covalent adducts that include many cross-linked structures. Food chemists have long studied this process, referred to as glycation or the Maillard reaction, as a source of flavor, color, and texture changes in cooked, processed, and stored foods. However it is known that this process also occurs slowly in vivo. In a glycation reaction, alpha-dicarbonyl compounds such as deoxyglucosone, methylglyoxal, and glyoxal are more reactive than the parent sugars with respect to their ability to react with amino groups of proteins to form inter- and intramolecular cross-links of proteins, referred to as advanced glycation end products (AGEs or AGE-proteins). The formation of AGE-proteins from sugars is a multi-step process, involving early, reversible reactions with sugars to produce fructose-lysine containing proteins. These modified proteins then continue to react to produce irreversibly modified AGE-proteins. AGE-proteins are not identical to proteins containing glycated-lysine residues, as antibodies raised against AGE-proteins do not react with fructose-lysine.

[0003] The AGEs, which are irreversibly formed, accumulate with aging, atherosclerosis, and diabetes mellitus, and are especially associated with long-lived proteins such as collagens, lens crystallins, and nerve proteins. In the case of diabetic complications, the reactions that lead to AGE-proteins are thought to be kinetically accelerated by the chronic hyperglycemia associated with this disease. It has been shown that long-lived proteins such as collagen and lens crystallins from diabetic subjects contain a significantly greater AGE-protein content than do those from age-matched normal controls. Thus, the unusual incidence of cataracts in diabetics at a relatively early age, as well as the early onset of joint and arterial stiffening and loss of lung capacity observed in diabetics is explained by the increased rate of modification and cross-linking of these structural proteins. Likewise, diabetic retinopathy may be explained by the increased cross-linking of nerve proteins in the eye.

[0004] The alpha-dicarbonyl sugar 3-deoxyglucosone (3DG) is believed to be a key intermediate in the multistep pathway leading to formation of AGE-proteins. 3DG is a potent protein crosslinker and has been shown to be capable of inducing apoptosis, mutations, and formation of active oxygen species.

[0005] Many studies have concentrated on the role of 3DG in diabetes. It has been shown that diabetic humans have elevated levels of 3DG and 3-deoxyfructose (3DF), 3DG's detoxification product, in plasma (Niwa et al., 1993, Biochem. Biophys. Res. Commun. 196:837-843; Wells-Knecht et al., 1994, Diabetes. 43:1152-1156) and in urine (Wells-Knecht et al., 1994, Diabetes. 43:1152-1156), as compared with non-diabetic individuals. Furthermore, diabetics with nephropathy were found to have elevated plasma levels of

3DG compared to non-diabetics (Niwa et al., 1993, Biochem. Biophys. Res. Commun. 196:837-843). A recent study comparing patients with insulin-dependent diabetes mellitus (IDDM) and noninsulin-dependent diabetes mellitus (NIDDM) confirmed that 3DG and 3DF levels were elevated in blood and urine from both types of patient populations (Lal et al., 1995, Arch. Biochem. Biophys. 318:191-199). It has even been shown that incubation of glucose and proteins in vitro under physiological conditions produces 3DG. In turn, it has been demonstrated that 3DG glyicates and crosslinks protein, creating detectable AGE products (Baynes et al., 1984, Methods Enzymol. 106:88-98; Dyer et al., 1991, J. Biol. Chem. 266:11654-11660). The normal pathway for reductive detoxification of 3DG (conversion to 3DF) may be impaired in diabetic humans since their ratio of urinary and plasma 3DG to 3DF differs significantly from non-diabetic individuals (Lal et al., 1995, Arch Biochem. Biophys. 318:191-199).

[0006] Furthermore, elevated levels of 3DG-modified proteins have been found in diabetic rat kidneys compared to control rat kidneys (Niwa et al., 1997, J. Clin. Invest. 99:1272-1280). It has been demonstrated that 3DG has the ability to inactivate enzymes such as glutathione reductase, a central antioxidant enzyme. It has also been shown that hemoglobin-AGE levels are elevated in diabetic individuals (Makita et al., 1992, Science 258:651-653) and other AGE proteins have been shown in experimental models to accumulate with time, increasing from 5-50 fold over periods of 5-20 weeks in the retina, lens and renal cortex of diabetic rats (Brownlee et al., 1994, Diabetes 43:836-841). In addition, it has been demonstrated that 3DG is a teratogenic factor in diabetic embryopathy (Eriksson et al., 1998, Diabetes 47:1960-1966). One pathway for formation of 3DG comprises a reversible reaction between glucose and the  $\epsilon$ -NH<sub>2</sub> groups of lysine-containing proteins, forming a Schiff base (Brownlee et al., 1994, Diabetes 43:836-841). This Schiff base then rearranges to form a more stable ketoamine known as fructoselysine (FL) or the "Amadori product."

[0007] It was initially believed that 3DG production resulted exclusively from subsequent non-enzymatic rearrangement, dehydration, and fragmentation of the fructoselysine containing protein (Brownlee et al., 1994, Diabetes 43:836-841 and Makita et al., 1992, Science 258:651-653). But more recent work has shown that an enzymatic pathway for the production of 3DG also exists and that this pathway produces relatively high concentrations of 3DG in organs affected by diabetes (Brown et al., U.S. Pat. No. 6,004,958). In the enzymatic pathway, a specific kinase (referred to herein as fructoselysine kinase) converts fructose-lysine into fructose-lysine-3-phosphate (FL3P) in an ATP-dependent reaction, and the FL3P then breaks down to form free lysine, inorganic phosphate, and 3DG (Brown et al., U.S. Pat. No. 6,004,958). Methods have also been described for assessing diabetic risk, based on measuring components of the 3DG pathway (WO 99/64561).

[0008] U.S. Pat. No. 6,004,958 describes a class of compounds that inhibits the enzymatic conversion of fructoselysine to FL3P, thereby inhibiting formation of 3DG and other alpha-dicarbonyl sugars produced via this pathway. Specific compounds that are representative of the class have also been described (Brown et al., WO 98/33492). For example, it was disclosed in WO 98/33492 that urinary or plasma 3DG can be reduced by meglumine, sorbitolysine, mannitolysine, and galactitolysine.

[0009] It was also disclosed in WO 98/33492 that diets high in glycated protein are harmful to the kidney and cause a decrease in birth rate. Additionally, the fructoselysine pathway was reported to be involved in kidney carcinogenesis (WO 98/33492) it was further suggested that diet and 3DG may play a role in carcinogenesis associated with the fructoselysine pathway (WO 00/24405; WO 00/62626).

[0010] Once formed, 3DG can be detoxified in the body by at least two pathways. In one pathway, 3DG is reduced to 3-deoxyfructose (3DF) by aldehyde reductase or aldose reductase, and the 3DF is then efficiently excreted in urine (Takahashi et al., 1995, Biochemistry 34:1433; Sato, et al., 1993, Arch. Biochem. Biophys. 307:286-94). Another detoxification reaction oxidizes 3DG to 3-deoxy-2-ketogluconic acid (DGA) by oxoaldehyde dehydrogenase (Fujii et al., 1995, Biochem. Biophys. Res. Comm. 210:852).

[0011] Results of studies to date show that the efficiency of at least one of these enzymes, aldehyde reductase, is adversely affected in diabetes. When isolated from diabetic rat liver, this enzyme is glycated on lysine at positions 67, 84 and 140 and has a low catalytic efficiency when compared with the normal, unmodified enzyme (Takahashi et al., 1995, Biochemistry 34:1433). Since diabetic patients have higher ratios of glycated proteins than normoglycemic individuals they are likely to have both higher levels of 3DG and a reduced ability to detoxify this reactive molecule by reduction to 3DF. It has also been found that overexpression of aldehyde reductase protects PC12 cells from the cytotoxic effects of methylglyoxal or 3DG (Suzuki et al., 1998, J. Biochem. 123:353-357).

[0012] The mechanism by which aldehyde reductase works has been studied. These studies demonstrated that this important detoxification enzyme is inhibited by aldose/aldehyde reductase inhibitors (ARIs) (Barski et al., 1995, Biochemistry 34:11264). ARIs are currently under clinical investigation for their potential to reduce diabetic complications. These compounds, as a class, have shown some effect on short term diabetic complications. However, they lack clinical effect on long term diabetic complications and they worsen kidney function in rats fed a high protein diet. This finding is consistent with the newly discovered metabolic pathway for lysine recovery. For example, a high protein diet will increase the consumption of fructose-lysine, which in turn undergoes conversion into 3DG by the kidney lysine recovery pathway. The detoxification of the resulting 3DG by reduction to 3DF will be inhibited by ARIs therapy. Inhibiting 3DG detoxification will lead to increased 3DG levels, with a concomitant increase in kidney damage, as compared to rats not receiving ARs. This is because inhibition of the aldose reductase by the AR's would reduce availability of aldose reductase for reducing 3DG and 3DF.

[0013] Aminoguanidine, an agent that detoxifies 3DG pharmacologically via formation of rapidly excreted covalent derivatives (Hirsch et al., 1992, Carbohydr. Res. 232:125-130), has been shown to reduce AGE-associated retinal, neural, arterial, and renal pathologies in animal models (Brownlee et al., 1994, Diabetes 43:836-841; Brownlee et al., 1986, Science 232:1629-1632; Ellis et al., 1991, Metabolism 40:1016-1019; Soulis-Liparota et al., 1991, Diabetes 40:1328-1334; and Edelstein et al., 1992, Diabetologia 35:96-97).

[0014] The role of alpha-dicarbonyl sugars and AGE-protein formation in diabetic complications has been extensively studied, as would be understood by the discussion presented

above. But the pathogenic role of alpha-dicarbonyl sugars and AGE-proteins is not limited to diabetes. For example, protein glycation has been implicated in Alzheimer's disease (Harrington et al., Nature, 370: 247 (1994)). In addition, AGE-protein formation in vascular wall collagen appears to be an especially deleterious event, causing crosslinking of collagen molecules to each other and to circulating proteins. This leads to plaque formation, basement membrane thickening, and loss of vascular elasticity (Cerami & Ulrich, 2001, Recent Prog Horm Res: 56:1-21). Increased protein fluorescence is also seen with aging. Some theories trace the aging process to a combination of oxidative damage and sugar-induced protein modification. Thus, a therapy that reduces AGE-protein formation may also be useful in treating other etiologically-similar human disease states, and perhaps slow the aging process.

[0015] In particular, Tobia and Kappler (U.S. Patent Publication No. 2003/0219440 A1) describe the effect of alpha-dicarbonyl sugars and AGE proteins on the condition and aging of skin. US 2003/0219440 reports that 3DG is present in human skin and that the gene encoding the enzyme regulating the synthesis of 3DG is expressed in skin. US 2003/0219440 discloses compositions and methods to inhibit enzymatically induced 3DG synthesis and accumulation in skin, as well as to inhibit 3DG function or increase the rate of detoxification and removal of 3DG from skin. Representative examples of those compositions and methods were purported to reduce collagen crosslinking in vitro and to improve skin elasticity in STZ diabetic rats.

[0016] A link between AGE-proteins and proinflammatory responses has also been established in diseases and disorders in which inflammation is a component. For example, AGEs contribute to kidney disease due to diabetes or aging by means of mesangial cell (MC) receptors, such as the receptor for AGE (RAGE), which promote oxidant-stress-dependent NF- $\kappa$ B activation and inflammatory gene expression (Lu et al., 2004, Proc Natl Acad Sci USA 32: 11767-11772). AGE cross-linking of proteins has been reported to contribute to the pathogenic cascade of cytokine- and interferon- $\gamma$ -mediated inflammation in Alzheimer's disease (Munch et al., 2003, Biochem. Soc. Trans. 31: 1397-1399).

[0017] It has been reported that a common form of AGE-proteins (N- $\epsilon$ (carboxymethyl) lysine (CML)-modified proteins) engage cellular AGE receptors (RAGE) in vitro and in vivo to activate key cell signaling pathways such as the transcription factor NF- $\kappa$ B, with subsequent modulation of gene expression (Kisslinger et al., 1999, J Biol Chem 274: 31740-31749). Those findings linked AGE-RAGE interaction to the development of accelerated vascular and inflammatory complications that typify disorders in which inflammation is an established component. It has also been reported that short exposure of mesothelial cells to even to a single glucose degradation product (e.g., 3DG) results in increased formation of AGEs, enhanced cytotoxic damage and a proinflammatory response, evidenced by increased VCAM-1 expression and elevated production of IL-6 and IL-8 (Welten et al., 2003, Perit Dial Int. 23: 213-221).

[0018] As can be appreciated from the foregoing discussion, the detrimental conditions associated with AGE-proteins and their underlying causative agents, alpha-dicarbonyl sugars, in tissues are many and varied, and include inflammatory diseases and disorders. Though treatments for various inflammatory conditions are available, heretofore they have not been targeted to causative factors such as AGE-proteins

and the compounds that lead to formation of AGE-proteins. Accordingly, a pressing need exists to identify and develop compositions and methods of treating inflammation that are directed to those underlying factors. Additionally, a need exists for the treatment of inflammation-related disorders, such as pain and itch, that are related to the metabolic pathways as described herein. The present invention meets these needs.

#### BRIEF SUMMARY OF THE INVENTION

[0019] The invention includes a method of treating an inflammatory condition in a mammal, the method comprising administering to the mammal a composition comprising an inhibitor of an enzymatic pathway that produces an alpha-dicarbonyl sugar in the mammal, the administration resulting in reduction or elimination of the alpha-dicarbonyl sugar at a site in the mammal, the site being affected by the inflammatory condition, thereby treating the inflammatory condition.

[0020] The invention also includes a method of treating pain in a mammal, the method comprising administering to the mammal a composition comprising an inhibitor of an enzymatic pathway that produces an alpha-dicarbonyl sugar in the mammal, the administration resulting in reduction or elimination of the alpha-dicarbonyl sugar at a site in the mammal, the site being affected by the pain, thereby treating the pain.

[0021] The invention further includes a method of treating itch in a mammal, the method comprising administering to the mammal a composition comprising an inhibitor of an enzymatic pathway that produces an alpha-dicarbonyl sugar in the mammal, the administration resulting in reduction or elimination of the alpha-dicarbonyl sugar at a site in the mammal, the site being affected by the itch, thereby treating the itch.

[0022] In another aspect, the composition is administered to the mammal by a topical, oral, rectal, vaginal, intramuscular, subcutaneous, transdermal or intravenous route, or through the consumption of a nutriceutical product by the mammal.

[0023] In one aspect, a composition comprises an inhibitor of an Amadorase pathway. In another aspect, the composition comprises an inhibitor of fructosamine kinase. In yet another aspect, the composition comprises an inhibitor of the function of an alpha-dicarbonyl sugar. In one embodiment, the alpha-dicarbonyl sugar is 3DG. In yet another aspect, the composition comprises an inhibitor of fructosamine kinase and an inhibitor of the function of an alpha-dicarbonyl sugar. In another embodiment, a single compound can act as an inhibitor of fructosamine kinase and an inhibitor of the function of an alpha-dicarbonyl sugar. In another embodiment, an inhibitor of fructosamine kinase and an inhibitor of the function of an alpha-dicarbonyl sugar are two or more separate compounds. In an aspect of the invention, a composition includes at least two inhibitors or compounds for treatment according to the invention.

[0024] In one aspect of the invention, a composition is used to treat inflammation. In another aspect, a composition is used to treat pain. In yet another aspect, a composition is used to treat itch. In an aspect, a composition is used to treat at least two conditions from the group consisting of inflammation, pain, and itch.

[0025] In one aspect of the invention, administration of a composition results in reduction or elimination of 3DG at the

site in the mammal affected by the inflammatory condition. In an aspect, the mammal is a human.

[0026] In an aspect of the invention, the inflammatory condition is at least one of scleroderma, eczema, an allergic condition, Alzheimer's disease, anemia, angiogenesis, aortic valve stenosis, atherosclerosis, thrombosis, rheumatoid arthritis, osteoarthritis, gout, gouty arthritis, acute pseudogout, acute gouty arthritis, inflammation associated with cancer, congestive heart failure, cystitis, fibromyalgia, fibrosis, glomerulonephritis, inflammation associated with gastro-intestinal disease, inflammatory bowel diseases, kidney failure, glomerulonephritis, myocardial infarction, ocular diseases, pancreatitis, psoriasis, reperfusion injury or damage, respiratory disorders, restenosis, septic shock, endotoxic shock, urosepsis, stroke, surgical complications, systemic lupus erythematosus, polymorphic eruption of pregnancy, transplantation associated arteriopathy, graft vs. host reaction, allograft rejection, chronic transplant rejection, vasculitis.

[0027] In one aspect, a cancer is at least one cancer such as NSCLC, ovarian cancer, pancreatic cancer, breast carcinoma, colon carcinoma, rectum carcinoma, lung carcinoma, oropharynx carcinoma, hypopharynx carcinoma, esophagus carcinoma, stomach carcinoma, pancreas carcinoma, liver carcinoma, gallbladder carcinoma, bile duct carcinoma, small intestine carcinoma, urinary tract carcinoma, kidney carcinoma, bladder carcinoma, urothelium carcinoma, female genital tract carcinoma, cervix carcinoma, uterus carcinoma, ovarian carcinoma, choriocarcinoma, gestational trophoblastic disease, male genital tract carcinoma, prostate carcinoma, seminal vesicles carcinoma, testes carcinoma, germ cell tumors, endocrine gland carcinoma, thyroid carcinoma, adrenal carcinoma, pituitary gland carcinoma, skin carcinoma, hemangiomas, melanomas, sarcomas, bone and soft tissue sarcoma, Kaposi's sarcoma, tumors of the brain, tumors of the nerves, tumors of the eyes, tumors of the meninges, astrocytomas, gliomas, glioblastomas, retinoblastomas, neuromas, neuroblastomas, Schwannomas, meningiomas, solid tumors arising from hematopoietic malignancies, and solid tumors arising from lymphomas.

[0028] In an aspect, the solid tumors arising from hematopoietic malignancies is selected from the group consisting of leukemias, chloromas, plasmacytomas and the plaques and tumors of mycosis fungoides and cutaneous T-cell lymphoma/leukemia.

[0029] In another aspect, a gastro-intestinal disease is selected from the group consisting of aphthous ulcers, pharyngitis, esophagitis, peptic ulcers, gingivitis, periodontitis, oral mucositis, gastrointestinal mucositis, nasal mucositis, and proctitis.

[0030] In another aspect, the inflammatory bowel disease is selected from the group consisting of Crohn's disease, ulcerative colitis, indeterminate colitis, necrotizing enterocolitis, and infectious colitis.

[0031] In an aspect of the invention, the ocular disease is selected from the group consisting of conjunctivitis, retinitis, and uveitis.

[0032] In another aspect, the respiratory disorder is selected from the group consisting of asthma, mononuclear-phagocyte dependent lung injury, idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease, adult respiratory distress syndrome, acute chest syndrome in sickle cell disease, cystic fibrosis.

[0033] In another embodiment of the invention, the pain is at least one of arachnoiditis, arthritis, osteoarthritis, rheuma-

toid arthritis, ankylosing spondylitis, gout, tendonitis, bursitis, sciatica, spondylolisthesis, radiculopathy, burn pain, cancer pain, headaches, migraines, cluster headaches, tension headaches, trigeminal neuralgia, myofascial pain, neuropathic pain, pain associated with diabetic neuropathy, reflex sympathetic dystrophy syndrome, phantom limb pain, post-amputation pain, tendonitis, tenosynovitis, postherpetic neuralgia, shingles-associated pain, central pain syndrome, trauma-associated pain, vasculitis, pain associated with infections, skin tumors, cysts, pain associated with tumors associated with neurofibromatosis, pain associated with strains, bruises, dislocations, fractures, and pain due to exposure to chemicals.

[0034] In another embodiment of the invention, the itch is the result of a condition selected from the group consisting of cutaneous itch, neuropathic itch, neurogenic itch, mixed-type itch, and psychogenic itch.

[0035] In an embodiment of the invention, a composition further comprises a non-steroidal anti inflammatory drug (NSAID). In an aspect, a non-steroidal anti inflammatory drug (NSAID) is selected from the group consisting of ibuprofen (2-(isobutylphenyl)-propionic acid); methotrexate (N-[4-(2,4-diamino-6-pteridinyl-methyl]methylamino]benzoyl)-L-glutamic acid); aspirin (acetylsalicylic acid); salicylic acid; diphenhydramine (2-(diphenylmethoxy)-N,N-dimethylethylamine hydrochloride); naproxen (2-naphthaleneacetic acid, 6-methoxy-9-methyl-, sodium salt, (-)); phenylbutazone (4-butyl-1,2-diphenyl-3,5-pyrazolidinedione); sulindac (2-5-fluoro-2-methyl-1-[[p-(methylsulfonyl)phenyl]methylene]-1H-indene-3-acetic acid; diflunisal (2',4'-difluoro-4-hydroxy-3-biphenylcarboxylic acid; piroxicam (4-hydroxy-2-methyl-N-2-pyridinyl-2H-1,2-benzothiazine-2-carboxamide 1,1-dioxide, an oxicam; indomethacin (1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetic acid); meclofenamate sodium (N-(2,6-dichloro-m-tolyl)anthranilic acid, sodium salt, monohydrate); ketoprofen (2-(3-benzoylphenyl)-propionic acid; tolmetin sodium (sodium 1-methyl-5-(4-methylbenzoyl)-1H-pyrrole-2-acetate dihydrate); diclofenac sodium (2[(2,6-dichlorophenyl)amino]benzenoic acid, monosodium salt); hydroxychloroquine sulphate (2-{[4-[(7-chloro-4-quinolyl)amino]pentyl}ethylamino)ethanol sulfate (1:1); penicillamine (3-mercapto-D-valine); flurbiprofen ([1,1-biphenyl]-4-acetic acid, 2-fluoro-alphamethyl-, (+-)); cetodolac (1-8-diethyl-13,4,9-tetrahydropyrano-[3-4-13]indole-1-acetic acid; mefenamic acid (N-(2,3-xylyl)anthranilic acid; and diphenhydramine hydrochloride (2-diphenylmethoxy-N,N-di-methylethamine hydrochloride).

[0036] In an aspect, the inhibitor of the fructoseamine kinase is an agent that inhibits transcription of a gene encoding the fructoseamine kinase or translation of a mRNA encoding the fructoseamine kinase. In another aspect, the compound is meglumine. In another aspect, the composition further comprises arginine. In an aspect, the result of treatment is greater than the additive result of a treatment using meglumine alone and a treatment using arginine alone.

[0037] In an aspect of the invention, the compound is selected from the group consisting of galactitol lysine, 3-deoxy sorbitol lysine, 3-deoxy-3-fluoro-xylitol lysine, 3-deoxy-3-cyano sorbitol lysine, 3-O-methyl sorbitolysine, sorbitol lysine, mannitol lysine, sorbitol and xylitol. In another aspect, the composition comprises a copper-containing compound. In an aspect, the copper-containing compound is selected from the group consisting of a copper-salicylic acid conjugate, a copper-peptide conjugate, a

copper-amino acid conjugate, and a copper salt. In another aspect, the copper-containing compound is selected from the group consisting of a copper-lysine conjugate and a copper-arginine conjugate.

[0038] In an aspect of the invention, an inhibitor of 3DG chelates 3DG, detoxifies 3DG. In an aspect, the inhibitor is an N-methyl-glucamine-like compound. In another aspect, the inhibitor comprises meglumine. In another aspect, the inhibitor further comprises arginine. In another aspect, the inhibitor of alpha-dicarbonyl sugar function inhibits protein crosslinking. In another aspect, the inhibitor of alpha-dicarbonyl sugar function inhibits formation of reactive oxygen species. In another aspect, the inhibitor of alpha-dicarbonyl sugar function inhibits apoptosis. In another aspect, the inhibitor of alpha-dicarbonyl sugar function inhibits mutagenicity. In another aspect, the inhibitor of alpha-dicarbonyl sugar function inhibits formation of advanced glycation end product modified proteins. In another aspect, the inhibitor is arginine or a derivative or modification thereof.

[0039] The invention also includes method of treating an inflammatory condition in a mammal, the method comprising administering to the mammal a composition comprising an inhibitor of an alpha-dicarbonyl sugar in the mammal, the administration resulting in reduction, elimination or inhibition of the function of the alpha-dicarbonyl sugar at a site in the mammal, the site being affected by the inflammatory condition, thereby treating the inflammatory condition. In an aspect, administration of the composition results in reduction, elimination or inhibition of the function of 3DG at the site in the mammal affected by the inflammatory condition.

[0040] The invention also includes a method of treating pain in a mammal, the method comprising administering to the mammal a composition comprising an inhibitor of an alpha-dicarbonyl sugar in the mammal, the administration resulting in reduction, elimination or inhibition of the function of the alpha-dicarbonyl sugar at a site in the mammal, the site being affected by the pain, thereby treating the pain. In an aspect, administration of the composition results in reduction, elimination or inhibition of the function of 3DG at the site in the mammal affected by the pain.

[0041] The invention also includes a method of treating itch in a mammal, the method comprising administering to the mammal a composition comprising an inhibitor of an alpha-dicarbonyl sugar in the mammal, the administration resulting in reduction, elimination or inhibition of the function of the alpha-dicarbonyl sugar at a site in the mammal, the site being affected by the itch, thereby treating the itch. In an aspect, administration of the composition results in reduction, elimination or inhibition of the function of 3DG at the site in the mammal affected by the itch.

#### BRIEF DESCRIPTION OF THE DRAWINGS

[0042] The foregoing summary, as well as the following detailed description of preferred embodiments of the invention, will be better understood when read in conjunction with the appended drawings. For the purpose of illustrating the invention, there are shown in the drawings embodiments which are presently preferred. It should be understood, however, that the invention is not limited to the precise arrangements and instrumentalities shown. In the drawings:

[0043] FIG. 1 is a schematic diagram depicting the initial step involved in the multi-step reaction leading to crosslinking of proteins.

[0044] FIG. 2 is a schematic diagram which illustrates the reactions involved in the lysine recovery pathway. Fructoselysine (FL) is phosphorylated by a fructosamine kinase such as amadorase to form fructoselysine 3-phosphate (FL3P). FL3P spontaneously decomposes into lysine, Pi, and 3DG (Brown et al., U.S. Pat. No. 6,004,958).

[0045] FIG. 3 is a graph representing a urinary profile showing the variation over time of 3DF, 3DG and FL from a single individual fed 2 grams of FL and followed for 24 hours.

[0046] FIG. 4 is a graph representing 3DF excretion in urine over time from seven volunteers fed 2 grams of fructoselysine.

[0047] FIG. 5 graphically compares 3DF and N-acetyl- $\beta$ -glucosaminidase (NAG) levels in control animals and an experimental group maintained on feed containing 3% glycated protein (Brown et al.).

[0048] FIG. 6 is a graph which demonstrates the linear relationship between 3DF and 3DG levels in urine of rats fed either a control diet or a diet enriched in glycated protein (Brown et al., U.S. Pat. No. 6,004,958).

[0049] FIG. 7, comprising FIG. 7A and FIG. 7B, graphically depicts fasting levels of urinary 3DG in normal subjects and in diabetic patients, plotted against the fasting level of 3DF.

[0050] FIG. 8, comprising FIG. 8A and FIG. 8B, depicts images of photomicrographs illustrating the effects of a diet containing high levels of glycated protein on the kidney. Periodic acid and Schiff (PAS) stained kidney sections were prepared from a rat fed a diet enriched in mildly glycated protein (FIG. 8A) and a rat fed a normal diet (FIG. 8B). In this experiment, non-diabetic rats were fed a diet containing 3% glycated protein for 8 months. This diet substantially elevated levels of FL and its metabolites ( $\geq 3$ -fold in the kidney). FIG. 8A is an image of a photomicrograph of a glomerulus from a rat fed the glycated diet for 8 months. The glomerulus shows segmental sclerosis of the glomerular tuft with adhesion of the sclerotic area to Bowman's capsule (lower left). There is also tubular metaplasia of the parietal epithelia from approximately 9 to 3 o'clock. These sclerotic and metaplastic changes are reminiscent of the pathologies observed in diabetic kidney disease. FIG. 8B is an image from a rat on the control diet for 8 months, comprising a histologically normal glomerulus.

[0051] FIG. 9 is a graphic comparison of 3DG and 3DF levels in glomerular and tubular fractions from rat kidneys after FL feeding.

[0052] FIG. 10 is an image depicting the nucleic acid sequence (SEQ ID NO:1) of human amadorase (fructosamine-3-kinase), NCBI accession number NM\_022158. The accession number for the human gene on chromosome 17 is NT\_010663.

[0053] FIG. 11 is an image depicting the amino acid sequence (SEQ ID NO:2) of human amadorase (fructosamine-3-kinase), NCBI accession number NP\_071441.

[0054] FIG. 12 is an image of a polyacrylamide gel demonstrating the effects of 3DG on collagen crosslinking and the inhibition of 3DG induced crosslinking by arginine. Collagen type I was treated with 3DG in the presence or absence of arginine. The samples were subjected to cyanogen bromide (CNBr) digestion, electrophoresed on a 16.5% SDS Tris-tricine gel, and then the gels were processed using silver stain techniques to visualize the proteins. Lane 1 contains molecular weight marker standards. Lanes 2 and 5 contain 10 and 20  $\mu$ l of the collagen mixture following CNBr digestion. Lanes 3

and 6 contain the collagen mixture treated with 3DG and then digested with CNBr, and loaded at 10 and 20  $\mu$ l respectively. Lanes 4 and 7 contain the mixture of collagen incubated with 5 mM 3DG and 10 mM arginine and then digested with CNBr, and loaded at 10 and 20  $\mu$ l, respectively.

[0055] FIG. 13 is an image of an agarose gel demonstrating that the mRNA for amadorase/fructosamine kinase is present in human skin. RT-PCR was utilized and published amadorase sequences were used as the basis for preparing templates for PCR. Based on the primers used (see Examples) for the PCR reaction, the presence of a 519 bp fragment in the gel indicates the presence of amadorase mRNA. Expression of amadorase, as based on the presence of amadorase mRNA indicated by a 519 bp fragment, was found in the kidney (lane 1) and in the skin (lane 3). No 519 bp fragments were found in the control lanes, which contained primer but no template (lanes 2 and 4). Lane 5 contained DNA molecular weight markers.

[0056] FIG. 14 is a graphic illustration of the effects of DYN 12 (3-O-methylsorbitolysine) treatment on skin elasticity. Diabetic or normal rats were treated with DYN 12 (50 mg/kg daily) or saline for eight weeks and then subjected to skin elasticity tests. The four groups used included diabetic controls (saline injection; solid black bar), diabetics treated with DYN 12 (open bar), normal animal controls (saline injections; stippled bar), and normal animals treated with DYN 12 (cross-hatched bar). Data are expressed in kilopascals (kPa).

[0057] FIG. 15 is graphic illustration of the effects of DYN 12 (3-O-methylsorbitolysine) treatment on skin elasticity. Diabetic or normal rats were treated with DYN 12 (50 mg/kg daily) or saline for eight weeks and then subjected to skin elasticity tests. The four groups used included diabetic controls (saline injection; solid black bar), diabetics treated with DYN 12 (open bar), normal animal controls (saline injections; stippled bar), and normal animals treated with DYN 12 (cross-hatched bar). Data are expressed as averages of the results obtained with each particular group of test subjects. Measurements were taken on the hind leg of the test subjects and were taken on an alert animal restrained by a technician.

[0058] FIG. 16 is a schematic illustration of a novel metabolic pathway in the kidney. The formation of 3DG in the kidney occurs using either endogenous glycated protein or glycated protein derived from dietary sources. By way of the endogenous pathway, the chemical combination of glucose and lysine leads to glycated protein. Alternatively, glycated protein may also be obtained from dietary sources. Catabolism of glycated proteins results in the production of fructoselysine, which is subsequently acted upon by Amadorase. Amadorase, a fructosamine-3-kinase, is part of both pathways. Amadorase phosphorylates fructoselysine to form fructoselysine-3-phosphate, which may then be converted to 3-deoxyglucosone (3DG), producing byproducts of lysine and inorganic phosphate (A very small amount of fructoselysine (<5% total fructoselysine) may be converted to 3DG by way of a non-enzymatic pathway). 3DG may then be detoxified by conversion to 3-deoxyfructose (3DF) or it may go on to produce reactive oxygen species (ROS) and advanced glycation end products (AGEs). As shown in FIG. 16, DYN 12 (3-O-methylsorbitolysine) inhibits the action of Amadorase on fructoselysine, and DYN 100 (arginine) inhibits the 3DG-mediated production of ROS and AGEs.

[0059] FIG. 17 is a schematic illustration of the disease states affected by reactive oxygen species (ROS). 3DG may produce ROS directly, or it may produce advanced glycation end products which go on to form ROS. The ROS are then responsible for advancing various disease states as shown in the figure.

[0060] FIG. 18 is a schematic illustration of both adduct formation and inhibition of adduct formation according to embodiments of the present invention. 3DG can form an adduct with a primary amino group on a protein. Protein-3DG adduct formation creates a Schiff base, the equilibrium of which is depicted in FIG. 18. The protein-3DG Schiff base adduct may go on to form a crosslinked protein, by formation of a second protein-3DG adduct by way of the 3DG molecule involved in the first protein-3DG Schiff base adduct described above, thereby forming a “3DG bridge” between two primary amino groups of a single protein (pathway “A”). Alternatively, such crosslinking may occur between two primary amino groups of separate proteins, forming a “3DG bridge” between two primary amino groups of two separate proteins, resulting in a crosslinked pair of protein molecules. The first protein-3DG Schiff base adduct may be prevented from going on to form such crosslinked proteins as depicted in pathway “A.” For example, such protein crosslinking may be inhibited by nucleophilic agents such as glutathione or penicillamine, as illustrated in FIG. 18 by pathway “B.” Such nucleophilic agents react with the 3DG carbon atom responsible for forming the second Schiff base, preventing that carbon atom from forming a Schiff base protein-3DG adduct and thereby preventing crosslinking of the protein.

[0061] FIG. 19 is a graph depicting the average erythema scores as determined by an expert grader of human volunteers’ SLS-treated skin after treatment with either (i) a base cream (Cream A), (ii) a base cream containing meglumine-HCl and arginine (Cream B) or (iii) with no treatment.

[0062] FIG. 20 is a graph depicting the average erythema scores measured with a chromameter of human volunteers’ SLS-treated skin after treatment with either (i) a base cream (Cream A), (ii) a base cream containing meglumine-HCl and arginine (Cream B) or (iii) with no treatment.

[0063] FIG. 21 is a graph depicting the average transdermal evaporative water loss (TEWL) of human volunteers’ SLS-treated skin after treatment with either (i) a base cream (Cream A), (ii) a base cream containing meglumine-HCl and arginine (Cream B) or (iii) with no treatment.

[0064] FIG. 22, comprising FIGS. 22A-22C, is a series of images illustrating thin sections of skin from a normal individual (FIG. 22A) and from the inflamed area of skin from a person with polymorphic eruption of pregnancy (FIGS. 22B-C). FIGS. 22A-B were stained with a monoclonal antibody to 3DG-imidazolone followed by a fluorescent secondary antibody and FIG. 22C is stained with hematoxylin and eosin.

#### DETAILED DESCRIPTION OF THE INVENTION

[0065] The invention relates generally to compositions and methods of treating deleterious conditions that involve inhibiting the production or effect of alpha-dicarbonyl sugars such as 3DG in the affected tissue and/or removing the sugars from the affected tissue. This is because it has now been discovered, as described in greater detail elsewhere herein, that removal of underlying causative factors of the deleterious conditions results in amelioration of the deleterious conditions. Such deleterious conditions include, but are not limited to, inflammation, pain and itch.

[0066] The invention also relates to the novel discovery, set forth herein for the first time, that compositions comprising both an inhibitor of alpha-dicarbonyl sugar formation and an inhibitor of alpha-dicarbonyl sugar function or effect, together exhibit a synergistic effect in the alleviation of alpha-dicarbonyl sugar-associated conditions, as compared with compositions comprising either type of inhibitor alone. One particularly advantageous combination is the combination of meglumine and arginine for the treatment of alpha-dicarbonyl sugar-associated conditions.

#### DEFINITIONS

[0067] Unless defined otherwise, 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 any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described herein.

[0068] As used herein, each of the following terms has the meaning associated with it in this section.

[0069] The articles “a” and “an” are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

[0070] The term “accumulation of 3DG” or “accumulation of alpha-dicarbonyl sugars” as used herein refers to an detectable increase in the level of 3DG and/or alpha-dicarbonyl sugar over time.

[0071] “Alpha-dicarbonyl sugar,” as used herein, refers to a family of compounds, including 3-Deoxyglucosone, glyoxal, methyl glyoxal and glucosone.

[0072] “Alpha-dicarbonyl sugar associated parameter of wrinkling, aging, disease or disorder of the skin,” as used herein, refers to the biological markers described herein, including 3DG levels, 3DF levels, fructosamine kinase levels, protein crosslinking, and other markers or parameters associated with alpha-dicarbonyl sugar associated wrinkling, aging, diseases or disorders of the skin.

[0073] “3-Deoxyglucosone” or “3DG,” as used herein, refers to the 1,2-dicarbonyl-3-deoxysugar (also known as 3-deoxyhexylulosone), which can be formed via an enzymatic pathway or can be formed via a nonenzymatic pathway. For purposes of the present description, the term 3-deoxyglucosone is an alpha-dicarbonyl sugar which can be formed by pathways including the nonenzymatic pathway described in FIG. 1 and the enzymatic pathway resulting in breakdown of FL3P described in FIG. 2. Another source of 3DG is diet. 3DG is a member of the alpha-dicarbonyl sugar family, also known as 2-oxoaldehydes.

[0074] A “3DG associated” or “3DG related” disease or disorder as used herein, refers to a disease, condition, or disorder which is caused by indicated by or associated with 3DG, including defects related to enhanced synthesis, production, formation, and accumulation of 3DG, as well as those caused by medicated by or associated with decreased levels of degradation, detoxification, binding, and clearance of 3DG.

[0075] “A 3DG inhibiting amount” or an “alpha-dicarbonyl inhibiting amount” of a compound refers to that amount of compound which is sufficient to inhibit the function or process of interest, such as synthesis, formation accumulation and/or function of 3DG or another alpha-dicarbonyl sugar.

[0076] “3-O-methyl sorbitolysine (3-O-Me-sorbitolysine),” is an inhibitor of fructosamine kinases, as described herein. It is used interchangeably with the term “DYN 12”.

[0077] As used herein, “alleviating a disease or disorder symptom,” means reducing the severity of the symptom.

[0078] The term “AGE-proteins” (Advanced Glycation End product modified proteins), as used herein, refers to a product of the reaction between sugars and proteins (Brownlee, 1992, Diabetes Care, 15: 1835; Niwa et al., 1995, Nephron, 69: 438. For example, the reaction between protein lysine residues and glucose, which does not stop with the formation of fructose-lysine (FL). FL can undergo multiple dehydration and rearrangement reactions to produce non-enzymatic 3DG, which reacts again with free amino groups, leading to cross-linking and browning of the protein involved. AGEs also include the products that form from the reaction of 3DG with other compounds, such as lipids and nucleic acids.

[0079] “Amadorase,” as used herein, refers to a fructosamine kinase responsible for the production of 3-DG. More specifically it refers to a protein which can enzymatically convert FL to FL3P, as defined above, when additionally supplied with a source of high energy phosphate.

[0080] The term “Amadori product,” as used herein, refers to a ketoamine, such as, but not limited to, fructoselysine, comprising is a rearrangement product following glucose interaction with the  $\epsilon$ -NH<sub>2</sub> groups of lysine-containing proteins.

[0081] As used herein, “amino acids” are represented by the full name thereof, by the three-letter code corresponding thereto, or by the one-letter code corresponding thereto, as indicated in the following table:

Full Name	Three-Letter Code	One-Letter Code
Aspartic Acid	Asp	D
Glutamic Acid	Glu	E
Lysine	Lys	K
Arginine	Arg	R
Histidine	His	H
Tyrosine	Tyr	Y
Cysteine	Cys	C
Asparagine	Asn	N
Glutamine	Gln	Q
Serine	Ser	S
Threonine	Thr	T
Glycine	Gly	G
Alanine	Ala	A
Valine	Val	V
Leucine	Leu	L
Isoleucine	Ile	I
Methionine	Met	M
Proline	Pro	P
Phenylalanine	Phe	F
Tryptophan	Trp	W

[0082] The term “binding” refers to the adherence of molecules to one another, such as, but not limited to, enzymes to substrates, ligands to receptors, antibodies to antigens, DNA binding domains of proteins to DNA, and DNA or RNA strands to complementary strands.

[0083] “Binding partner,” as used herein, refers to a molecule capable of binding to another molecule.

[0084] The term “biological sample,” as used herein, refers to samples obtained from a living organism, including skin, hair, tissue, blood, plasma, cells, sweat and urine.

[0085] The term “clearance,” as used herein refers to the physiological process of removing a compound or molecule, such as by diffusion, exfoliation, removal via the bloodstream, and excretion in urine, or via other sweat or other fluid.

[0086] A “coding region” of a gene consists of the nucleotide residues of the coding strand of the gene and the nucleotides of the non-coding strand of the gene which are homologous with or complementary to, respectively, the coding region of an mRNA molecule which is produced by transcription of the gene.

[0087] “Complementary” as used herein refers to the broad concept of subunit sequence complementarity between two nucleic acids, e.g., two DNA molecules. When a nucleotide position in both of the molecules is occupied by nucleotides normally capable of base pairing with each other, then the nucleic acids are considered to be complementary to each other at this position. Thus, two nucleic acids are complementary to each other when a substantial number (at least 50%) of corresponding positions in each of the molecules are occupied by nucleotides which normally base pair with each other (e.g., A:T and G:C nucleotide pairs). Thus, it is known that an adenine residue of a first nucleic acid region is capable of forming specific hydrogen bonds (“base pairing”) with a residue of a second nucleic acid region which is antiparallel to the first region if the residue is thymine or uracil. Similarly, it is known that a cytosine residue of a first nucleic acid strand is capable of base pairing with a residue of a second nucleic acid strand which is antiparallel to the first strand if the residue is guanine. A first region of a nucleic acid is complementary to a second region of the same or a different nucleic acid if, when the two regions are arranged in an antiparallel fashion, at least one nucleotide residue of the first region is capable of base pairing with a residue of the second region. Preferably, the first region comprises a first portion and the second region comprises a second portion, whereby, when the first and second portions are arranged in an antiparallel fashion, at least about 50%, and preferably at least about 75%, at least about 90%, or at least about 95% of the nucleotide residues of the first portion are capable of base pairing with nucleotide residues in the second portion. More preferably, all nucleotide residues of the first portion are capable of base pairing with nucleotide residues in the second portion.

[0088] A “compound,” as used herein, refers to any type of substance or agent that is commonly considered a drug, or a candidate for use as a drug, as well as combinations and mixtures of the above, or modified versions or derivatives of the compound.

[0089] As used herein, the terms “conservative variation” or “conservative substitution” refer to the replacement of an amino acid residue by another, biologically similar residue. Conservative variations or substitutions are not likely to significantly change the shape of the peptide chain. Examples of conservative variations, or substitutions, include the replacement of one hydrophobic residue such as isoleucine, valine, leucine or alanine for another, or the substitution of one charged amino acid for another, such as the substitution of arginine for lysine, glutamic for aspartic acid, or glutamine for asparagine, and the like.

[0090] “Detoxification” of 3DG refers to the breakdown or conversion of 3DG to a form which does not allow it to perform its normal function. Detoxification can be brought about or stimulated by any composition or method, including

“pharmacologic detoxification”, or metabolic pathway which can cause detoxification of 3DG.

[0091] “Pharmacologic detoxification of “3DG” or other alpha-dicarbonyl sugars refers to a process in which a compound binds with or modifies 3DG, which in turn causes it to become inactive or to be removed by metabolic processes such as, but not limited to, excretion.

[0092] A “disease” is a state of health of an animal wherein the animal cannot maintain homeostasis, and wherein if the disease is not ameliorated then the animal’s health continues to deteriorate. As used herein, normal aging is included as a disease.

[0093] A “disorder” in an animal is a state of health in which the animal is able to maintain homeostasis, but in which the animal’s state of health is less favorable than it would be in the absence of the disorder. Left untreated, a disorder does not necessarily cause a further decrease in the animal’s state of health.

[0094] As used herein, the term “domain” refers to a part of a molecule or structure that shares common physicochemical features, such as, but not limited to, hydrophobic, polar, globular and helical domains or properties such as ligand binding, signal transduction, cell penetration and the like. Specific examples of binding domains include, but are not limited to, DNA binding domains and ATP binding domains.

[0095] An “effective amount” or “therapeutically effective amount” of a compound is that amount of compound which is sufficient to provide a beneficial effect to the subject to which the compound is administered, or gives the appearance of providing a therapeutic effect as in a cosmetic.

[0096] As used herein, the term “effector domain” refers to a domain capable of directly interacting with an effector molecule, chemical, or structure in the cytoplasm which is capable of regulating a biochemical pathway.

[0097] “Encoding” refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (i.e., rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be referred to as encoding the protein or other product of that gene or cDNA. Unless otherwise specified, a “nucleotide sequence encoding an amino acid sequence” includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. Nucleotide sequences that encode proteins and RNA may include introns.

[0098] The term “floating,” as used herein, refers to bonds of a substituent to a ring structure, such that the substituent can be attached to the ring structure at any available carbon juncture. A “fixed” bond means that a substituent is attached at a specific site.

[0099] The term “formation of 3DG” refers to 3DG which is not necessarily formed via a synthetic pathway, but can be formed via a pathway such as spontaneous or induced breakdown of a precursor.

[0100] As used herein, the term “fragment,” as applied to a protein or peptide, can ordinarily be at least about 3-15 amino acids in length, at least about 15-25 amino acids, at least about 25-50 amino acids in length, at least about 50-75 amino acids in length, at least about 75-100 amino acids in length, and greater than 100 amino acids in length.

[0101] As used herein, the term “fragment,” as applied to a nucleic acid, can ordinarily be at least about 20 nucleotides in length, typically, at least about 50 nucleotides, more typically, from about 50 to about 100 nucleotides, preferably, at least about 100 to about 200 nucleotides, even more preferably, at least about 200 nucleotides to about 300 nucleotides, yet even more preferably, at least about 300 to about 350, even more preferably, at least about 350 nucleotides to about 500 nucleotides, yet even more preferably, at least about 500 to about 600, even more preferably, at least about 600 nucleotides to about 620 nucleotides, yet even more preferably, at least about 620 to about 650, and most preferably, the nucleic acid fragment will be greater than about 650 nucleotides in length.

[0102] The term “fructose-lysine” (FL) is used herein to signify any glycated-lysine, whether incorporated in a protein/peptide or released from a protein/peptide by proteolytic digestion. This term is specifically not limited to the chemical structure commonly referred to as fructose-lysine, which is reported to form from the reaction of protein lysine residues and glucose. As noted above, lysine amino groups can react with a wide variety of sugars. Indeed, one report indicates that glucose is the least reactive sugar out of a group of sixteen (16) different sugars tested (Bunn et al., *Science*, 213: 222 (1981)). Thus, tagatose-lysine formed from galactose and lysine, analogously to glucose is included wherever the term fructose-lysine is mentioned in this description, as is the condensation product of all other sugars, whether naturally-occurring or not. It will be understood from the description herein that the reaction between protein-lysine residues and sugars involves multiple reaction steps. The final steps in this reaction sequence involve the crosslinking of proteins and the production of multimeric species, known as AGE-proteins, some of which are fluorescent. Once an AGE protein forms, then proteolytic digestion of such AGE-proteins does not yield lysine covalently linked to a sugar molecule. Thus, these species are not included within the meaning of “fructose-lysine”, as that term is used herein.

[0103] The term “Fructose-lysine-3-phosphate,” as used herein, refers to a compound formed by the enzymatic transfer of a high energy phosphate group from ATP to FL. The term fructose-lysine-3-phosphate (FL3P), as used herein, is meant to include all phosphorylated fructose-lysine moieties that can be enzymatically formed whether free or protein-bound.

[0104] “Fructose-lysine-3-phosphate kinase” (FL3K), as used herein, refers to one or more proteins, such as amadorase, which can enzymatically convert FL to FL3P, as described herein, when supplied with a source of high energy phosphate. The term is used interchangeably with “fructose-lysine kinase (FLK)” and with “amadorase”.

[0105] The term “FL3P Lysine Recovery Pathway,” as used herein, refers to a lysine recovery pathway which exists in human skin and kidney, and possibly other tissues, and which regenerates unmodified lysine as a free amino acid or as incorporated in a polypeptide chain.

[0106] The term “Glycated Diet,” as used herein, refers to any given diet in which a percentage of normal protein is

replaced with glycated protein. The expressions “glycated diet” and “glycated protein diet” are used interchangeably herein.

[0107] “Glycated lysine residues,” as used herein, refers to the modified lysine residue of a stable adduct produced by the reaction of a reducing sugar and a lysine-containing protein.

[0108] The majority of protein lysine residues are located on the surface of proteins as expected for a positively charged amino acid. Thus, lysine residues on proteins, which come in contact with serum, or other biological fluids, can freely react with sugar molecules in solution. This reaction occurs in multiple stages. The initial stage involves the formation of a Schiff base between the lysine free amino group and the sugar keto-group. This initial product then undergoes the Amadori rearrangement, to produce a stable ketoamine compound.

[0109] This series of reactions can occur with various sugars. When the sugar involved is glucose, the initial Schiff base product will involve imine formation between the aldehyde moiety on C-1 of the glucose and the lysine  $\epsilon$ -amino group. The Amadori rearrangement will result in formation of lysine coupled to the C-1 carbon of fructose, 1-deoxy-1-( $\epsilon$ -aminolysine)-fructose, herein referred to as fructose-lysine or FL. Similar reactions will occur with other aldose sugars, for example galactose and ribose (Dills, 1993, Am. J. Clin. Nutr. 58:S779). For the purpose of the present invention, the early products of the reaction of any reducing sugar and the  $\epsilon$ -amino residue of protein lysine are included within the meaning of glycated-lysine residue, regardless of the exact structure of the modifying sugar molecule.

[0110] “Homologous” as used herein, refers to the subunit sequence similarity between two polymeric molecules, e.g., between two nucleic acid molecules, e.g., two DNA molecules or two RNA molecules, or between two polypeptide molecules. When a subunit position in both of the two molecules is occupied by the same monomeric subunit, e.g., if a position in each of two DNA molecules is occupied by adenine, then they are homologous at that position. The homology between two sequences is a direct function of the number of matching or homologous positions, e.g., if half (e.g., five positions in a polymer ten subunits in length) of the positions in two compound sequences are homologous then the two sequences are 50% homologous, if 90% of the positions, e.g., 9 of 10, are matched or homologous, the two sequences share 90% homology. By way of example, the DNA sequences 3'ATTGCCS' and 3'TATGGC share 50% homology.

[0111] As used herein, “homologous” or homology” are used synonymously with “identity”. The determination of percent identity or homology between two nucleotide or amino acid sequences can be accomplished using a mathematical algorithm. For example, a mathematical algorithm useful for comparing two sequences is the algorithm of Karlin and Altschul (1990, Proc. Natl. Acad. Sci. USA 87:2264-2268), modified as in Karlin and Altschul (1993, Proc. Natl. Acad. Sci. USA 90:5873-5877). This algorithm is incorporated into the NBLAST and(BLAST programs of Altschul, et al. (1990, J. Mol. Biol. 215:403-410), and can be accessed, for example at the National Center for Biotechnology Information (NCBI) world wide web site. BLAST nucleotide searches can be performed with the NBLAST program (designated “blastn” at the NCBI web site), using the following parameters: gap penalty=5; gap extension penalty=2; mismatch penalty=3; match reward=1; expectation value 10.0; and word size=11 to obtain nucleotide sequences homolo-

gous to a nucleic acid described herein. BLAST protein searches can be performed with the XBLAST program (designated “blastn” at the NCBI web site) or the NCBI “blastp” program, using the following parameters: expectation value 10.0, BLOSUM62 scoring matrix to obtain amino acid sequences homologous to a protein molecule described herein. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al. (1997, Nucleic Acids Res. 25:3389-3402). Alternatively, PSI-Blast or PHI-Blast can be used to perform an iterated search which detects distant relationships between molecules (Id.) and relationships between molecules which share a common pattern. When utilizing BLAST, Gapped BLAST, PSI-Blast, and PHI-Blast programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used.

[0112] The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, typically exact matches are counted. The term “induction of 3DG” or “inducing 3DG,” as used herein, refers to methods or means which start or stimulate a pathway or event leading to the synthesis, production, or formation of 3DG or increase in its levels, or stimulate an increase in function of 3DG. Similarly, the phrase “induction of alpha-dicarbonyl sugars”, refers to induction of members of the alpha-dicarbonyl sugar family, including 3DG, glyoxal, methyl glyoxal, and glucosone.

[0113] “Inhibiting 3DG” as described herein, refers to any method or technique which inhibits 3DG synthesis, production, formation, accumulation, or function, as well as methods of inhibiting the induction or stimulation of synthesis, formation, accumulation, or function of 3DG. It also refers to any metabolic pathway which can regulate 3DG function or induction. The term also refers to any composition or method for inhibiting 3DG function by detoxifying 3DG or causing the clearance of 3DG. Inhibition can be direct or indirect. Induction refers to induction of synthesis of 3DG or to induction of function. Similarly, the phrase “inhibiting alpha-dicarbonyl sugars”, refers to inhibiting members of the alpha-dicarbonyl sugar family, including 3DG, glyoxal, methyl glyoxal, and glucosone.

[0114] The term “inhibiting accumulation of 3DG,” as used herein, refers to the use of any composition or method which decreases synthesis, increases degradation, or increases clearance, of 3DG such that the result is lower levels of 3DG or functional 3DG in the tissue being examined or treated, compared with the levels in tissue not treated with the composition or method. Similarly, the phrase “inhibiting accumulation of alpha-dicarbonyl sugars”, refers to inhibiting accumulation of members of the alpha-dicarbonyl sugar family, including 3DG, glyoxal, methyl glyoxal, and glucosone, and intermediates thereof.

[0115] As used herein, an “instructional material” includes a publication, a recording, a diagram, or any other medium of expression which can be used to communicate the usefulness of the peptide of the invention in the kit for effecting alleviation of the various diseases or disorders recited herein. Optionally, or alternately, the instructional material can describe one or more methods of alleviating the diseases or disorders in a cell or a tissue of a mammal. The instructional material of the kit of the invention can, for example, be affixed to a container which contains the identified compound invention or be shipped together with a container which contains

the identified compound. Alternatively, the instructional material can be shipped separately from the container with the intention that the instructional material and the compound be used cooperatively by the recipient.

[0116] An “isolated nucleic acid” refers to a nucleic acid segment or fragment which has been separated from sequences which flank it in a naturally occurring state, e.g., a DNA fragment which has been removed from the sequences which are normally adjacent to the fragment, e.g., the sequences adjacent to the fragment in a genome in which it naturally occurs. The term also applies to nucleic acids which have been substantially purified from other components which naturally accompany the nucleic acid, e.g., RNA or DNA or proteins, which naturally accompany it in the cell. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (e.g., as a cDNA or a genomic or cDNA fragment produced by PCR or restriction enzyme digestion) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence. “Modified” compound, as used herein, refers to a modification or derivation of a compound, which may be a chemical modification, such as in chemically altering a compound in order to increase or change its functional ability or activity.

[0117] The term “mutagenicity” refers to the ability of a compound to induce or increase the frequency of mutation. The term “nucleic acid” typically refers to large polynucleotides.

[0118] The term “oligonucleotide” typically refers to short polynucleotides, generally, no greater than about 50 nucleotides. It will be understood that when a nucleotide sequence is represented by a DNA sequences (i.e., A, T, G, C), this also includes an RNA sequence (i.e., A, U, G, C) in which “U” replaces “T.”

[0119] The term “peptide” typically refers to short polypeptides.

[0120] “Permeation enhancement” and “permeation enhancers” as used herein relate to the process and added materials which bring about an increase in the permeability of skin to a poorly skin permeating pharmacologically active agent, i.e., so as to increase the rate at which the drug permeates through the skin and enters the bloodstream. “Permeation enhancer” is used interchangeably with “penetration enhancer”.

[0121] As used herein, the term “pharmaceutically-acceptable carrier” means a chemical composition with which an appropriate compound or derivative can be combined and which, following the combination, can be used to administer the appropriate compound to a subject.

[0122] As used herein, the term “physiologically acceptable” ester or salt means an ester or salt form of the active ingredient which is compatible with any other ingredients of the pharmaceutical composition, which is not deleterious to the subject to which the composition is to be administered.

[0123] “Polypeptide” refers to a polymer composed of amino acid residues, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof linked via peptide bonds, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof.

[0124] A “polynucleotide” means a single strand or parallel and anti-parallel strands of a nucleic acid. Thus, a polynucleotide may be either a single-stranded or a double-stranded nucleic acid.

[0125] “Primer” refers to a polynucleotide that is capable of specifically hybridizing to a designated polynucleotide template and providing a point of initiation for synthesis of a complementary polynucleotide. Such synthesis occurs when the polynucleotide primer is placed under conditions in which synthesis is induced, i.e., in the presence of nucleotides, a complementary polynucleotide template, and an agent for polymerization such as DNA polymerase. A primer is typically single-stranded, but may be double-stranded. Primers are typically deoxyribonucleic acids, but a wide variety of synthetic and naturally occurring primers are useful for many applications. A primer is complementary to the template to which it is designed to hybridize to serve as a site for the initiation of synthesis, but need not reflect the exact sequence of the template. In such a case, specific hybridization of the primer to the template depends on the stringency of the hybridization conditions. Primers can be labeled with, e.g., chromogenic, radioactive, or fluorescent moieties and used as detectable moieties.

[0126] As used herein, the term “promoter/regulatory sequence” means a nucleic acid sequence which is required for expression of a gene product operably linked to the promoter/regulator sequence. In some instances, this sequence may be the core promoter sequence and in other instances, this sequence may also include an enhancer sequence and other regulatory elements which are required for expression of the gene product. The promoter/regulatory sequence may, for example, be one which expresses the gene product in a tissue specific manner.

[0127] A “constitutive” promoter is a promoter which drives expression of a gene to which it is operably linked, in a constant manner in a cell. By way of example, promoters which drive expression of cellular housekeeping genes are considered to be constitutive promoters.

[0128] An “inducible” promoter is a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a living cell substantially only when an inducer which corresponds to the promoter is present in the cell.

[0129] A “tissue-specific” promoter is a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a living cell substantially only if the cell is a cell of the tissue type corresponding to the promoter.

[0130] A “prophylactic” treatment is a treatment administered to a subject who does not exhibit signs of a disease or exhibits only early signs of the disease for the purpose of decreasing the risk of developing pathology associated with the disease.

[0131] The term “protein” typically refers to large polypeptides.

[0132] Reactive Oxygen Species Various harmful forms of oxygen are generated in the body; singlet oxygen, superoxide radicals, hydrogen peroxide, and hydroxyl radicals all cause tissue damage. A catchall term for these and similar oxygen related species is “reactive oxygen species” (ROS). The term also includes ROS formed by the internalization of AGEs into cells and the ROS that form therefrom.

[0133] “Removing 3-deoxyglucosone,” as used herein, refers to any composition or method, the use of which results in lower levels of 3-deoxyglucosone (3DG) or lower levels of functional 3DG when compared to the level of 3DG or the level of functional 3DG in the absence of the composition. Lower levels of 3DG can result from its decreased synthesis or formation, increased degradation, increased clearance, or any combination of thereof. Lower levels of functional 3DG can result from modifying the 3DG molecule such that it can function less efficient in the process of glycation or can result from binding of 3DG with another molecule which blocks inhibits the ability of 3DG to function. Lower levels of 3DG can also result from increased clearance and excretion in urine of 3DG. The term is also used interchangeably with “inhibiting accumulation of 3DG”. Similarly, the phrase “removing alpha-dicarbonyl sugars”, refers to removal of members of the alpha-dicarbonyl sugar family, including 3DG, glyoxal, methyl glyoxal, and glucosone.

[0134] Also, the terms glycated-lysine residue, glycated protein and glycosylated protein or lysine residue are used interchangeably herein, is consistently with current usage in the art where such terms are art-recognized used interchangeably.

[0135] The term “skin,” as used herein, refers to the commonly used definition of skin, e.g., the epidermis and dermis, and the cells, glands, mucosa and connective tissue which comprise the skin.

[0136] The term “standard,” as used herein, refers to something used for comparison. For example, it can be a known standard agent or compound which is administered and used for comparing results when administering a test compound, or it can be a standard parameter or function which is measured to obtain a control value when measuring an effect of an agent or compound on a parameter or function. “Standard” can also refer to an “internal standard”, such as an agent or compound which is added at known amounts to a sample and which is useful in determining such things as purification or recovery rates when a sample is processed or subjected to purification or extraction procedures before a marker of interest is measured. Internal standards are often but are not limited to, a purified marker of interest which has been labeled, such as with a radioactive isotope, allowing it to be distinguished from an endogenous substance in a sample.

[0137] A “susceptible test animal,” as used herein, refers to a strain of laboratory animal which, due to for instance the presence of certain genetic mutations, have a higher propensity toward a disease disorder or condition of choice, such as diabetes, cancer, and the like.

[0138] “Synthesis of 3DG”, as used herein refers to the formation or production of 3DG. 3DG can be formed based on an enzyme dependent pathway or a non-enzyme dependent pathway. Similarly, the phrase “synthesis of alpha-dicarbonyl sugars”, refers to synthesis or spontaneous formation of members of the alpha-dicarbonyl sugar family, including 3DG, glyoxal, methyl glyoxal, and glucosone, and adducts as disclosed herein.

[0139] “Synthetic peptides or polypeptides” mean a non-naturally occurring peptide or polypeptide. Synthetic peptides or polypeptides can be synthesized, for example, using an automated polypeptide synthesizer. Those of skill in the art know of various solid phase peptide synthesis methods.

[0140] A “therapeutic” treatment is a treatment administered to a subject who exhibits signs of pathology, for the purpose of diminishing or eliminating those signs.

[0141] By “transdermal” delivery is intended both transdermal (or “percutaneous”) and transmucosal administration, i.e., delivery by passage of a drug through the skin or mucosal tissue and into the bloodstream. Transdermal also refers to the skin as a portal for the administration of drugs or compounds by topical application of the drug or compound thereto.

[0142] The term “topical application”, as used herein, refers to administration to a surface, such as the skin. This term is used interchangeably with “cutaneous application”.

[0143] The term to “treat,” as used herein, means reducing the frequency with which symptoms are experienced by a patient or subject or administering an agent or compound to reduce the frequency with which symptoms are experienced.

[0144] As used herein, “treating a disease or disorder” means reducing the frequency with which a symptom of the disease or disorder is experienced by a patient. Disease and disorder are used interchangeably herein. As used herein, the term “wild-type” refers to the genotype and phenotype that is characteristic of most of the members of a species occurring naturally and contrasting with the genotype and phenotype of a mutant.

[0145] Accordingly, the compositions and methods of the present invention are expected to find utility in the treatment of a wide variety of diseases and disorders in which inflammation plays a role. These include, among others, allergic conditions, alzheimer’s disease, anemia, angiogenesis, aortic valve stenosis, arthritis, atherosclerosis, thrombosis, rheumatoid arthritis, osteoarthritis, gout, gouty arthritis, acute pseudogout, acute gouty arthritis, inflammation associated with cancer, congestive heart failure, cystitis, fibromyalgia, fibrosis, glomerulonephritis, inflammation associated with gastro-intestinal disease, inflammatory bowel diseases, kidney failure, glomerulonephritis, myocardial infarction, ocular diseases, pancreatitis, psoriasis, reperfusion injury or damage, respiratory disorders, restenosis, septic shock, inflammatory conditions of the skin, endotoxic shock, urosepsis, stroke, surgical complications, systemic lupus erythematosus, transplantation associated arteriopathy, graft vs. host reaction, allograft rejection, chronic transplant rejection and vasculitis.

[0146] In accordance with particular aspects of the present invention, it has been demonstrated that topical application of composition containing an inhibitor of 3DG production and an inhibitor of 3DG function resulted in decreased redness and irritation associated with razor burn. A topical formulation comprising the same active agents was reported by participants in a skin irritation trial to decrease redness associated with detergent chapping, to accelerate the healing process, and to cause an overall improvement in skin texture as compared with a formulation that did not contain the active agents. In addition, topical application of that composition has been found to decrease inflammation associated with psoriasis, eczema and polycythemia, and to decrease the number and severity of facial acne lesions.

[0147] In view of the foregoing demonstrations by the inventors, inflammatory conditions of the skin are considered particularly amenable to treatment by targeting alpha-dicarbonyl sugar production and function. Inflammatory conditions of the skin contemplated for treatment in accordance with embodiments of the present invention include, but are not limited to: transient inflammation and irritation of skin due to hair removal by shaving, waxing, tweezing, electrolysis, or use of depilatory products; various forms of dermatitis, including seborrheic dermatitis, nummular dermatitis, con-

tact dermatitis, atopic dermatitis, exfoliative dermatitis, perioral dermatitis and stasis dermatitis, to name some common examples; and inflammatory skin diseases or disorders such as psoriasis, folliculitis, rosacea, telangiectasia, acne, impetigo, erysipelas, paronychia, erythrasma, eczema, rash (diaper rash, poison ivy, poison oak) and sunburn, to name a few.

[0148] Also as set forth in the present disclosure, topical application of a composition containing an inhibitor of 3DG production and an inhibitor of 3DG function resulted in decreased pain associated with sinus inflammation. The same formulation was also reported to provide relief from joint swelling, pain and tenderness in arthritic patients when topically applied to the skin overlying the affected joint tissue.

[0149] In view of these demonstrations by the inventors, inflammatory conditions of tissues underlying the skin are also considered particularly amenable to treatment by targeting alpha-dicarbonyl sugar production and function. Inflammatory conditions of underlying tissues include, but are not limited to: sinus pressure and inflammation; joint tissue inflammation associated with various forms of arthritic disease, such as rheumatoid arthritis, osteoarthritis, gout, gouty arthritis, acute pseudogout and acute gouty arthritis.

[0150] Methods of Inhibiting Synthesis, Formation, and Accumulation of 3DG and Other Alpha-dicarbonyl Sugars

[0151] It has been discovered in the present invention that an enzyme which is involved in the enzymatic synthetic pathway of 3DG production is present at high levels in skin (see Example 20). Furthermore, it has also been discovered in the present invention that 3DG is present at high levels in skin (see Example 19). Accordingly, the invention includes compositions and methods which interfere with both enzymatic and nonenzymatic based synthesis or formation of 3DG in skin, and which also interfere with the function of 3DG in skin. 3DG is a member of a family of compounds called alpha-dicarbonyl sugars. Other members of the family include glyoxal, methyl glyoxal, and glucosone. The present invention also relates to compositions and methods for inhibiting accumulation of 3DG and other alpha-dicarbonyl sugars in skin and for inhibiting 3DG dependent or associated skin wrinkling, skin aging, or other skin diseases or disorders, as well as skin wrinkling, skin aging, or other skin diseases and disorders associated with other alpha-dicarbonyl sugars. The invention also includes inhibiting accumulation of 3DG in skin using compositions and methods for stimulating the pathways, or components of the pathways, leading to 3DG detoxification, degradation, or clearance from the skin.

[0152] It should be noted that 3DG is a member of the alpha-dicarbonyl sugar family of molecules. It should also be noted that other members of the alpha-dicarbonyl sugar family can perform functions similar to 3DG, as described herein, and that like 3DG functions, the functions of other members of the alpha-dicarbonyl sugar family are inhabitable as well. Thus, the invention should be construed to include methods of inhibiting synthesis, formation, and accumulation of other alpha-dicarbonyl sugars as well.

[0153] Inhibition of 3DG synthesis, formation, and accumulation in skin can be direct or indirect. For example, direct inhibition of 3DG synthesis refers to blocking an event that occurs immediately prior to or upstream in a pathway of 3DG synthesis or formation, such as blocking amadorase or the conversion of fructose-lysine-3-phosphate (FL3P) to 3DG, lysine, and inorganic phosphate. Indirect inhibition can include blocking or inhibiting upstream precursors, enzymes,

or pathways, which lead to the synthesis of 3DG. Components of an upstream pathway, for example, include the amadorase gene and amadorase mRNA. The invention should not be construed to include inhibition of only the enzymatic and nonenzymatic pathways described herein, but should be construed to include methods of inhibiting other enzymatic and nonenzymatic pathways of 3DG synthesis, formation and accumulation in skin as well. The invention should also be construed to include the other members of the alpha-dicarbonyl sugar family, including glyoxal, methyl glyoxal, and glucosone where applicable.

[0154] Various assays described herein may be used to directly measure 3DG synthesis or levels of 3DG, or assays may be used which are correlative of 3DG synthesis or levels, such as measurement of its breakdown product, 3DF.

[0155] The present invention includes novel methods for the inhibition of 3DG synthesis in skin. Preferably, the skin is mammalian skin, and more preferably, the mammal skin is human skin.

[0156] In one aspect, the inhibitor inhibits an enzyme involved in the synthesis of 3DG. In one embodiment the enzyme is a fructosamine kinase. In yet another embodiment the fructosamine kinase is amadorase, as disclosed in U.S. Pat. No. 6,004,958.

[0157] In yet another aspect of the invention the inhibitor inhibits the nonenzymatic synthesis and formation of 3DG in the skin.

[0158] In one embodiment of the invention, the inhibitor inhibits the accumulation of 3DG in the skin. In one aspect, the 3DG is synthesized or formed in the skin. However, the inhibitor can also inhibit accumulation of 3DG in the skin, where the source of 3DG is other than the skin. In one aspect, the source of the 3DG is dietary, i.e., it is derived from an external source rather than an internal source, and then accumulates in the skin. Thus, this aspect of the invention includes the inhibition of 3DG synthesis or formation in the skin and/or inhibition of accumulation of 3DG in the skin. In the latter case, the source of 3DG may be enzymatic synthesis of 3DG directly in the skin, enzymatic synthesis of 3DG in a tissue other than skin, nonenzymatic synthesis or formation of 3DG in the skin or in a non-skin tissue, or the source of the 3DG may be external, such as, for example, dietary. The methods to be used for inhibiting accumulation of 3DG or other alpha-dicarbonyl sugars via any one of these pathways are more fully described elsewhere herein.

[0159] The present invention also relates to methods and compositions for treating tissues other than skin. As described in detail elsewhere herein, and as will be understood by the skilled artisan when armed with the present disclosure, the methods and compositions of the invention are equally applicable to any tissue in which 3DG exists and can exist. Such tissues include, but are not limited to, kidney and pancreas. Therefore, the compositions and methods of the invention will be understood to be equally applicable to tissues that contain or can contain 3DG.

[0160] In another embodiment of the invention, a method of inhibiting the synthesis, formation, or accumulation of 3DG in the skin, and in other tissues, is useful to prevent inflammation. As set forth in detail elsewhere herein, inhibition of synthesis, formation or accumulation of 3DG contributes to inflammation and inflammatory processes. Therefore, the present invention features a method of diminishing or inhibiting inflammation by inhibiting the synthesis, formation and/or accumulation of 3DG.

[0161] In another embodiment of the invention, a method is provided of using a composition of the invention for the treatment of inflammation or of an inflammation-related condition in a mammal, wherein the inflammatory condition is associated with one or more major organs in the mammal. In one aspect, the mammal is a human. Major organs include, for example, skin, heart, eyes, kidneys, pancreas, lungs, and the circulatory system. In another aspect, a composition of the invention is provided in an oral dosage form to a mammal. Such compositions useful in a method according to the invention are described in detail elsewhere herein. By way of a non-limiting example, such compositions include meglumine and meglumine+arginine.

[0162] In yet another embodiment of the invention, compositions and methods are provided for the treatment of pain in a mammal. Pain is a complicated process that involves interplay between a number of important chemicals, called neurotransmitters, that transmit nerve impulses from one nerve cell to another. There are many different neurotransmitters in the human body, and, in the case of pain, act in various combinations to produce painful sensations in the body. Some chemicals govern mild pain sensations; others control intense or severe pain.

[0163] The body's chemicals act in the transmission of pain messages by stimulating neurotransmitter receptors found on the surface of cells; each receptor has a corresponding neurotransmitter. Receptors function much like gates or ports and enable pain messages to pass through and on to neighboring cells. One brain chemical of special interest to neuroscientists is glutamate. During experiments, mice with blocked glutamate receptors show a reduction in their responses to pain. Other important receptors in pain transmission are opiate-like receptors. Morphine and other opioid drugs work by locking on to these opioid receptors, switching on pain-inhibiting pathways or circuits, and thereby blocking pain.

[0164] Another type of receptor that responds to painful stimuli is called a nociceptor. Nociceptors are thin nerve fibers in the skin, muscle, and other body tissues, that, when stimulated, carry pain signals to the spinal cord and brain. Normally, nociceptors only respond to strong physical stimuli. However, when tissues become injured or inflamed, they release chemicals that make nociceptors much more sensitive and cause them to transmit pain signals in response to even gentle stimuli. This condition is called allodynia, a state in which pain is produced by innocuous stimuli.

[0165] It has been shown herein for the first time that compositions and methods, as set forth herein, are useful to diminish or alleviate pain in a mammal. In one aspect, the mammal is a human. Such a method comprises administering a composition of the invention to a mammal, either topically or orally. Compositions useful in a method of alleviating or diminishing pain according to the present invention are described in detail elsewhere herein. By way of a non-limiting example, such compositions include meglumine and meglumine+arginine.

[0166] Various types of pain treatable by the compositions and methods, as set forth herein, include arachnoiditis; arthritis, such as osteoarthritis, and rheumatoid arthritis; ankylosing spondylitis; gout; tendonitis; bursitis sciatica; spondylolisthesis; radiculopathy; burn pain; cancer pain; headaches; migraines; cluster headaches; and tension headaches; trigeminal neuralgia; myofascial pain; neuropathic pain, including diabetic neuropathy, reflex sympathetic dystrophy syndrome, phantom limb and post-amputation pain; tendonitis;

tenosynovitis; postherpetic neuralgia; shingles-associated pain; central pain syndrome; trauma-associated pain; vasculitis; pain associated with infections, including herpes simplex; skin tumors, cysts; and tumors associated with neurofibromatosis; and pain associated with strains, bruises, dislocations; fractures; and pain due to exposure to chemicals (e.g. exfoliants such as retinoids, carboxylic acids, beta-hydroxy acids, alpha-keto acids, benzoyl peroxide and phenol).

[0167] In still another embodiment of the invention, compositions and methods are provided for the treatment of itch in a mammal. In origin, itch can be cutaneous ("pruritoceptive", e.g. dermatitis), neuropathic (e.g. multiple sclerosis), neurogenic (e.g. cholestasis), mixed (e.g. uraemia) or psychogenic. Although itch of cutaneous origin shares a common neural pathway with pain, the afferent C-fibres subserving itch are a functionally distinct subset: they respond to histamine, acetylcholine and other pruritogens, but are insensitive to mechanical stimuli.

[0168] Different types of itch have responded to various treatments. Histamine is the main mediator for itch in insect bite reactions and in most forms of urticaria, and in these circumstances the itch responds well to H1-antihistamines. However, in most dermatoses and in systemic disease, low-sedative H1-antihistamines are ineffective. Opioid antagonists relieve itch caused by spinal opioids, cholestasis and, possibly, uraemia. Ondansetron relieves itch caused by spinal opioids (but not cholestasis and uraemia). Other drug treatments for itch include rifampicin, colestyramine and 17-alkyl androgens (cholestasis), thalidomide (uraemia), cimetidine and corticosteroids (Hodgkin's lymphoma), paroxetine (paraneoplastic itch), aspirin and paroxetine (polycythaemia vera) and indometacin (some HIV+ patients). Ultraviolet B therapy, particularly narrow-band UVB, has been postulated as a treatment for itch in uraemia. This is because it has been shown herein for the first time that compositions and methods, as set forth herein, are useful to diminish or alleviate itch in a mammal. In one aspect, the mammal is a human. Such a method comprises administering a composition of the invention to a mammal, either topically or orally. Compositions useful in a method of alleviating or diminishing itch according to the present invention are described in detail elsewhere herein. By way of a non-limiting example, such compositions include meglumine and meglumine+arginine.

[0169] In another embodiment, the present invention provides a method for treatment of inflammation, itch, pain, and other diseases or disorders as set forth herein, as well as those that will be apparent from the disclosure, wherein the treatment is by way of a composition comprising two or more compounds, further wherein the combination of compounds results in a synergistic effect of treatment. That is, the result of the treatment with the combination of compounds is greater than the additive effect of the results of treatment with each compound separately.

[0170] In one embodiment of the invention, a method of treating a patient includes treatment with a composition comprising both an inhibitor of alpha-dicarbonyl sugar formation and an inhibitor of alpha-dicarbonyl sugar function or effect, wherein the multiple inhibitors together exhibit a synergistic effect in the alleviation of alpha-dicarbonyl sugar-associated conditions, as compared with compositions comprising either type of inhibitor alone. In a preferred embodiment, a method includes the combination of meglumine and arginine for the treatment of alpha-dicarbonyl sugar-associated conditions.

[0171] While not wishing to be limited by any particular theory, it is noted that arginine not only inactivates 3DG, as set forth in detail elsewhere herein, but arginine also feeds into the nitric oxide pathway and stimulates NO production which causes vasodilation. This complements the anti-oxidative, anti-inflammatory action of meglumine so the effect of meglumine and arginine in combination is greater than the additive effect of treatment with each compound alone.

[0172] Methods of Treating Diabetes

[0173] The invention also relates to compositions and methods for treating diabetes. Diabetes, and in particular, type II diabetes, is associated with damage to the pancreas. Type II diabetes results from a combination of genetic and lifestyle factors. In people genetically predisposed to diabetes, overeating and lack of physical activity lead to insulin resistance with characteristic postprandial hyperglycemia. Obesity is an inflammatory disease characterized by elevated levels of the proinflammatory cytokines TNF-alpha, IL-6 and IL-1, all of which contribute to insulin resistance (rev in Wollen, K. E. and Hotamisligil, G. S. 2005. J. Clin. Invest. 115:1111-1119). In the pre-diabetic BB rat, there are elevated levels of allograft inflammatory factor 1 (AIF) in the pancreas (Chen Z.-W. et al. 1997. PNAS 94:13897-13894). Together, the inflammatory state, elevated lipid levels and oxidative stress state characteristic of 'metabolic syndrome' leads to diminished pancreatic function due to beta cell apoptosis, resulting in Type II diabetes. This condition may be further exacerbated in that diabetics also have increased levels of 3DG, which also leads to release of cytokines, production of inflammatory advanced glycation endproducts (AGEs) and increased oxidative stress.

[0174] Therefore, the present invention provides compositions and methods for treating diabetes. In one embodiment, the invention provides a method comprising administering to a patient a composition as set forth in detail elsewhere herein, wherein the composition alleviates the diabetic condition of the patient. In another embodiment, a method includes administration to a patient a composition as set forth in detail herein, wherein the composition prevents a diabetic condition in a patient predisposed to diabetes. Compositions useful for treating diabetes are described in detail elsewhere herein in greater detail. Examples of such compositions include, but should not be limited to, meglumine and meglumine+arginine.

[0175] Since the pancreas has elevated levels of F3K enzyme activity, this causes elevated levels of fructose lysine 3 phosphate which breaks down into 3DG. Hence the pancreas is making its own 3DG which has an effect locally to destroy beta cells and adversely effect supporting extracellular matrix and vascularization of the pancreas.

[0176] Methods of Removing 3DG from Skin

[0177] The present invention also relates to compositions and methods for removing 3DG and other alpha-dicarbonyl sugars from skin and for inhibiting 3DG dependent or associated skin wrinkling, skin aging, or other skin diseases or disorders, as well as skin wrinkling, skin aging, or other skin diseases and disorders associated with other alpha-dicarbonyl sugars. To this end, the invention includes compositions and methods for inhibiting the production, synthesis, formation, and accumulation of 3DG in skin. The invention also includes compositions and methods for stimulating the pathways, or components of the pathways, leading to 3DG detoxification, degradation, or clearance from the skin.

[0178] Using Compounds to Inhibit 3DG Synthesis

[0179] In one embodiment the invention includes a method of inhibiting 3DG synthesis in the skin of a mammal, said method comprising administering to a mammal an effective amount of an inhibitor of 3DG synthesis, or a derivative or modification thereof, thereby inhibiting 3DG synthesis in the skin of a mammal. Preferably, the mammal is a human.

[0180] In one embodiment, the inhibitor comprises from about 0.0001% to about 15% by weight of the pharmaceutical composition. In one aspect, the inhibitor is administered as a controlled-release formulation. In another aspect the pharmaceutical composition comprises a lotion, a cream, a gel, a liniment, an ointment, a paste, a toothpaste, a mouthwash, an oral rinse, a coating, a solution, a powder, and a suspension. In yet another aspect, the composition further comprises a moisturizer, a humectant, a demulcent, oil, water, an emulsifier, a thickener, a thinner, a surface active agent, a fragrance, a preservative, an antioxidant, a hydrotropic agent, a chelating agent, a vitamin, a mineral, a permeation enhancer, a cosmetic adjuvant, a bleaching agent, a depigmentation agent, a foaming agent, a conditioner, a viscosifier, a buffering agent, and a sunscreen.

[0181] The invention should be construed to include various methods of administration, including topical, oral, intramuscular, and intravenous.

[0182] In one aspect of the invention, the inhibitor of 3DG synthesis is an inhibitor of fructosamine kinase/amadorase. The inhibitor of fructosamine kinase can be a compound such as N-methyl-glucamine and N-methyl-glucamine-like compounds. In one embodiment of the invention, an inhibitor of 3DG synthesis is meglumine.

[0183] In one aspect of the invention, representative inhibitor compounds having the above formula include galactitol lysine, 3-deoxy sorbitol lysine, 3-deoxy-3-fluoro-xylitol lysine, and 3-deoxy-3-cyano sorbitol lysine and 3-O-methyl sorbitolysine. Examples of known compounds that may be used as inhibitors in practicing this invention include, without limitation, meglumine, sorbitol lysine, galactitol lysine, manitol lysine, xylitol and sorbitol. A preferred inhibitor is 3-O-methyl sorbitolysine.

[0184] The compounds of the invention may be administered to, for example, a cell, a tissue, or a subject by any of several methods described herein and by others which are known to those of skill in the art. In one aspect, an inhibitor of the invention which inhibits enzymatic synthesis of 3DG may be synthesized in vitro using techniques known in the art (see Example 8).

[0185] Compositions and Methods Useful for Inhibiting 3DG Function

[0186] The invention, as disclosed herein, relates to the involvement of 3DG in causing various skin diseases and disorders and to methods of inhibiting the function of 3DG in order to alleviate or treat 3DG associated skin diseases and disorders. The invention also relates to the involvement of 3DG in other diseases and disorders, such as gum diseases and disorders. Such gingival diseases and disorders include, but are not limited to, gingivitis, receding gums, and other 3DG or other alpha-dicarbonyl sugar associated gingival diseases and disorders. As described above, inhibition of 3DG function can be direct or indirect. Therefore, 3DG function may be inhibited or caused to decrease using many approaches as described herein. Inhibition of 3DG function may be assayed or monitored using techniques described herein as well as others known to those of skill in the art.

Function can be measured directly or it can be estimated using techniques to measure parameters which are known to be correlative of 3DG function. For example, protein crosslinking and protein production can be measured directly using techniques such as electrophoretic analysis (see FIG. 12 and Examples 7 and 18) as well as other techniques (see Examples 21-24). The invention should be construed to include not only compounds useful for preventing 3DG induced crosslinking of molecules such as collagen, elastin, and proteoglycans, but it should also be construed to include compounds which inhibit crosslinking of other molecules as well. The invention should also be construed to include the use of compounds to modulate other 3DG functions as well, such as apoptosis and formation of reactive oxygen species. It is known that in macrophage-derived cells apoptotic cell death can be induced by methylglyoxal and 3DG (Okado et al., 1996, Biochem. Biophys. Res. Commun. 225:219-224). In yet another aspect of the invention, an inhibitor of 3DG inhibits an active oxygen species (Vander Jagt et al., 1997, Biochem. Pharmacol. 53:1133-1140). The invention should be construed to include other alpha-dicarbonyl sugars as well. 3DG and its detoxification product 3DF can be measured several ways using cell, tissue, blood, plasma, and urine samples (see Examples 4, 5, 6, 14, 15, and 17) and FL, a product produced during the synthesis of 3DG, can also be measured (see Examples 5), as can a precursor, FL3P (see FIGS. 1 and 2 and Examples 1, 2, and 3).

[0187] The invention discloses methods which are useful for inhibiting 3DG function in the skin. Such a method includes administering an effective amount of one or more inhibitors of 3DG function, or modifications or derivatives thereof, in a pharmaceutical composition to a subject.

[0188] In one aspect of the invention the 3DG function inhibitor inhibits protein crosslinking. In another aspect, the inhibitor inhibits formation of advanced glycation end product modified proteins. In yet another aspect, the 3DG function inhibitor comprises a structure of an N-methyl-glucamine-like compound, or is arginine or a derivative or modification thereof.

[0189] In one embodiment, the inhibitor comprises from about 0.0001% to about 15% by weight of the pharmaceutical composition. In one aspect, the inhibitor is administered as a controlled-release formulation. In another aspect the pharmaceutical composition comprises a lotion, a cream, a gel, a liniment, an ointment, a paste, a toothpaste, a mouthwash, an oral rinse, a coating, a solution, a powder, and a suspension. In yet another aspect, the composition further comprises a moisturizer, a humectant, a demulcent, oil, water, an emulsifier, a thickener, a thinner, a surface active agent, a fragrance, a preservative, an antioxidant, a hydroscopic agent, a chelating agent, a vitamin, a mineral, a permeation enhancer, a cosmetic adjuvant, a bleaching agent, a depigmentation agent, a foaming agent, a conditioner, a viscosifier, a buffering agent, and a sunscreen. The invention should be construed to include various methods of administration, including topical, oral, intramuscular, and intravenous.

[0190] It should be understood that compositions and methods for inhibiting pathways, events, and precursors leading to the synthesis or production of 3DG, may inhibit not only 3DG synthesis, but also its accumulation, and ultimately its function. The invention should be construed to include compositions and methods to inhibit all pathways and precursors leading to 3DG synthesis (see FIGS. 1 and 2).

[0191] In another embodiment of the invention, the disclosure provides methods for directly inhibiting function of 3DG which is associated with various skin diseases and disorders. In one aspect, the method of inhibiting 3DG function in skin includes inhibiting 3DG with compounds such as those comprising structural formulas similar to N-methyl-glucamine-like compounds as described herein. Compounds comprising these formulas can bind to 3DG and/or inhibits its function, as described herein. In addition, the invention includes other molecules which can bind to and block 3DG function.

[0192] It should be understood that the compounds described herein are not the only compounds capable of inhibiting 3DG function or of treating a 3DG associated skin disease or disorder or diseases and disorders of other tissues and cells. It will be recognized by one of skill in the art that the various embodiments of the invention as described herein related to inhibition of 3DG function, also encompass other methods and compounds useful for inhibiting 3DG function. It will also be recognized by one of skill in the art that other compounds and techniques can be used to practice the invention. The invention should be construed to include compounds and methods useful not merely for the their ability to inhibit 3DG function and to treat a 3DG associated skin disease or disorder, but should be construed to also include the ability to inhibit the function of other members of the alpha-dicarbonyl sugar family of compounds, including glyoxal, methyl glyoxal and glucosone. The invention should also be construed to include treating 3DG associated diseases and disorders other than those of skin, such as 3DG associated diseases and disorders of the gums.

[0193] In another embodiment, the invention provides multi-component compositions for the inhibition of 3DG and 3DG function. It will be understood by the skilled artisan, in view of the disclosure set forth herein, that certain active components, excipients, additives, adjuvants, and the like, may be added to a composition in order to enhance or otherwise modulate the activity of a compound that inhibits 3DG and/or 3DG function. In one aspect, the invention includes a composition comprising cocoa butter, shea butter, aloe oil, vitamin E, glycerol, water, dimethicone and Natipide II, along with arginine-HCl and meglumine-HCl. As will be understood by the skilled artisan, based on the present disclosure, the ratios and concentrations of the individual components of a composition set forth herein can be adjusted in order to modulate the activity of the composition with respect to 3DG. That is, the assays and methods provided herein can be used to determine the effect of the individual components in a composition based on the disclosure set forth herein.

[0194] In an embodiment, the invention also includes a method of treating an inflammatory condition in a mammal, the method comprising administering to the mammal a composition comprising an inhibitor of an alpha-dicarbonyl sugar in the mammal, the administration resulting in reduction, elimination or inhibition of the function of the alpha-dicarbonyl sugar at a site in the mammal. In an aspect of the invention, administration of the composition results in reduction, elimination or inhibition of the function of 3DG at the site in the mammal affected by the inflammatory condition.

[0195] In an aspect, the 3DG function inhibitor comprises a structure of an N-methyl-glucamine-like compound, or is arginine or a derivative or modification thereof. In yet another aspect, the 3DG function inhibitor comprises the structure of meglumine.

[0196] As described in detail elsewhere herein, "inhibition of 3DG" refers, in part, to any method or technique which inhibits 3DG function, as well as methods of inhibiting the induction or stimulation of the function of 3DG. It also refers, in part, to any composition or method for inhibiting 3DG function by detoxifying 3DG or causing the clearance of 3DG. Inhibition can be direct or indirect. Induction refers, in part, to induction of function of 3DG. Similarly, the phrase "inhibiting alpha-dicarbonyl sugars", refers to inhibiting members of the alpha-dicarbonyl sugar family, including 3DG, glyoxal, methyl glyoxal, and glucosone.

[0197] The skilled artisan will understand that the methods and compositions for treating a patient by way of alpha-dicarbonyl sugar inhibition described herein, including 3DG inhibition, apply equally to the treatment of other diseases or disorders described herein and related to the presence or accumulation of alpha-dicarbonyl sugars, such as, but not limited to 3DG. That is, other diseases or disorders described herein and related to the presence or accumulation of alpha-dicarbonyl sugars, such as, but not limited to 3DG, can be treated with a composition comprising an inhibitor of 3DG. In one aspect of the invention, diseases or disorders described herein and related to the presence or accumulation of alpha-dicarbonyl sugars, such as, but not limited to 3DG, can be treated with a composition consisting of an inhibitor of 3DG.

[0198] In another embodiment, the invention also includes a method of treating pain in a mammal, the method comprising administering to the mammal a composition comprising an inhibitor of an alpha-dicarbonyl sugar in the mammal, the administration resulting in reduction, elimination or inhibition of the function of the alpha-dicarbonyl sugar at a site in the mammal, to treat the pain. In an aspect of the invention, administration of the composition results in reduction, elimination or inhibition of the function of 3DG at the site in the mammal affected by the pain.

[0199] In yet another embodiment, the invention also includes a method of treating itch in a mammal, the method comprising administering to the mammal a composition comprising an inhibitor of an alpha-dicarbonyl sugar in the mammal, the administration resulting in reduction, elimination or inhibition of the function of the alpha-dicarbonyl sugar at a site in the mammal, to treat the itch. In an aspect of the invention, administration of the composition results in reduction, elimination or inhibition of the function of 3DG at the site in the mammal affected by the itch.

[0200] Assays for Testing Inhibition of 3DG and Other Alpha-dicarbonyl Sugar Synthesis, Formation, Accumulation, and Function

[0201] The present disclosure provides a series of assays for identifying inhibitors of 3DG synthesis, formation, accumulation, and function, as well as measuring the effects of the various inhibitors on 3DG synthesis, formation, accumulation, and function. The assays also include those used to measure 3DG degradation, detoxification, and clearance. The assays of the invention include, but are not limited to, HPLC assays, electrophoretic assays, gas chromatographic-mass spectroscopic assays, amino acid analysis, enzyme activity assays, advanced glycation assays, protein crosslinking assays, NMR analysis, ion exchange chromatography, various chemical analyses, various labeling techniques, surgical and gross dissection techniques, RNA isolation, RT-PCR, histologic techniques, various chemical, biochemical, and molecular synthesis techniques, teratogenicity, mutagenicity, and carcinogenicity assays, urine assays, excretion assays,

and a variety of animal, tissue, blood, plasma, cell, biochemical, and molecular techniques. Synthetic techniques may be used to produce compounds, such as: chemical and enzymatic production of FL3P (Examples 1, 2 and 3); polylysine (Example 4); 3-O-methylsorbitol lysine (Example 8); fructosyl spermine (Example 9); and glycated protein diet (Example 13). Other techniques may be used which are not described herein, but are known to those of skill in the art.

[0202] In one embodiment of the invention, standards may be used when testing new agents or compounds or when measuring the various parameters described herein. For example, fructose-lysine is a known modulator of 3DG and 3DF and it can be administered to a group or subject as a standard or control against which the effects of a test agent or compound can be compared. In addition, when measuring a parameter, measurement of a standard can include measuring parameters such as 3DG or 3DF concentrations in a tissue or fluid obtained from a subject before the subject is treated with a test compound and the same parameters can be measured after treatment with the test compound. In another aspect of the invention, a standard can be an exogenously added standard which is an agent or compound that is added to a sample and is useful as an internal control, especially where a sample is processed through several steps or procedures and the amount of recovery of a marker of interest at each step must be determined. Such exogenously added internal standards are often added in a labeled form, i.e., a radioactive isotope.

[0203] Methods for Diagnosing 3DG Associated Skin Diseases or Disorders

[0204] The present invention discloses the presence of 3DG in skin and methods for measuring 3DG levels in the skin and for measuring an enzyme responsible for 3DG synthesis in the skin (see Examples 19 and 20). The invention also encompasses methods which may be used to diagnose changes in 3DG levels in the skin which may be associated with wrinkling, aging, or various other skin diseases or disorders. The invention should not be construed to include only methods for diagnosing 3DG associated skin diseases and disorders, but should be construed to include methods for diagnosing skin diseases and disorders associated with other alpha-dicarbonyl sugars as well. The invention should also be construed to include methods for diagnosing 3DG associated diseases or disorders of other cells and tissues as well, including, but not limited to, gum diseases and disorders.

[0205] In one embodiment of the invention, a patient with skin wrinkling, skin aging, or another skin disease or disorder, may be subjected to a diagnostic test to determine, for example, the levels of 3DG, the functional activity of 3DG, the levels of 3DF, a 3DF/3DG ratio, the amount of amadorase protein or mRNA present, or the levels of amadorase activity in their skin. Such a test is based on the various methods and assays described herein, or known to those of skill in the art. A higher level of 3DG or amadorase, or their activities, or lower levels of 3DF, compared to a non-affected area of skin or to skin of a normal patient, would be an indication that the skin wrinkling, skin aging, or other skin disease or disorder, is associated with 3DG and that a 3DG inhibitor of the present invention would be an appropriate treatment for the problem. The invention should also be construed to include skin diseases and disorders associated with molecules of the alpha-dicarbonyl sugar family other than 3DG.

[0206] In one aspect of the invention, additional markers of 3DG associated skin diseases or disorders can be measured, including, but not limited to, measuring 3DF and FL levels,

crosslinked protein levels, as well as levels of other alpha-dicarbonyl sugars such as glyoxal, methyl glyoxal, and glucosone.

[0207] A multitude of assays for measuring 3DG levels and function, including measuring its precursors, are described throughout the present disclosure (see Examples 1-22). However, the invention should not be construed to include only the assays described herein, but should be construed to include other assays to measure 3DG levels or function, including assays or techniques which are indirect measures of 3DG levels or functional activity. For example, in one aspect of the invention, indirect measurement of 3DG levels and function can be determined by measuring such things as levels of 3DF, protein crosslinking, proteoglycan crosslinking, or any other assay shown to be correlative of 3DG levels.

[0208] In one aspect of the invention, the sample to be used for measuring 3DG levels, etc., is a skin sample. Skin samples may be obtained by methods which include, but are not limited to, punch biopsies, scraping, and blistering techniques.

[0209] In another aspect of the invention, indirect assays for 3DG levels or function in the skin which are correlative of 3DG associated skin diseases or disorders may be used. The assays may include, but are not limited to, assays for measuring 3DG levels or function in other tissues, sweat, blood, plasma, saliva, or urine.

[0210] The invention discloses a method for diagnosing a 3DG or other alpha-dicarbonyl sugar associated skin disease or disorder comprising acquiring a biological sample from a test subject and comparing the level of 3DG or other alpha-dicarbonyl sugar associated parameter of wrinkling, aging, disease, or disorder of the skin with the level of the same parameter in an otherwise identical biological sample from a control subject. The control can be from an unaffected area of the same subject or from a subject not affected by a 3DG or other alpha-dicarbonyl sugar associated skin disease or disorder. A higher level of the parameter in the test subject is an indication that the test subject has a 3DG or other alpha-dicarbonyl sugar associated wrinkling, aging, disease, or disorder of the skin. The parameters which can be measured are described herein or are known to those of skill in the art, and include, but are not limited to, 3DG, protein crosslinking, proteoglycan crosslinking, advanced glycation end product modified proteins, 3DF, fructosamine kinase/amadorase levels and activity, and fructosamine kinase/amadorase mRNA a changes in levels of reactive oxygen species.

[0211] In yet another aspect of the invention, 3DG or other alpha-dicarbonyl sugars may be associated with skin diseases, disorders conditions and the appearance of these diseases, disorders and conditions selected from the group comprising skin aging, photoaging, skin wrinkling, skin cancer, hyperkeratosis, hyperplasia, acanthosis, papillomatosis, dermatosis, hyperpigmentation, rhinophyma, scleroderma, rosacea, and telangiectasia. In another aspect of the invention, 3DG is associated with functions including, but not limited to, protein crosslinking, mutagenicity, teratogenicity, apoptosis, oxidative damage caused by formation of reactive oxygen species, and cytotoxicity. It is understood that 3DG and other alpha-dicarbonyl sugars are associated with functions causing damage to not only proteins, but to lipids and DNA as well. In aspect of the invention, 3DG or other alpha-dicarbonyl sugars may also be associated with diseases and disorders of the skin (including, but not limited to the mucosa), including, but not limited to, gum diseases and disorders, vaginal and anal mucosa diseases, and the like.

[0212] In yet another aspect of the invention, the assays for measuring 3DG levels and function may be used in conjunction with other methods for measuring skin diseases and disorders, such as measuring the thickness or elasticity and/or moisture of the skin. Many of these assays are described herein. One of skill in the art will appreciate that other assays not described herein may be used in conjunction with the 3DG assays to form a complete diagnosis of the type of skin problem involved and whether or not it is a 3DG associated skin problem.

[0213] The invention should not be construed to include diagnosing a skin disease, condition or disorder merely by measuring levels of the alpha-dicarbonyl sugar 3DG, it should also be construed to include measuring levels of other members of the alpha-dicarbonyl sugar family as well, as well as their breakdown products, including, but not limited to, 3-deoxyfructose.

[0214] Thus, the use of a diagnostic assay to determine an association between 3DG and a skin disease or disorder will allow the selection of appropriate subjects before initiating treatment with an inhibitor of 3DG.

[0215] Methods for Inhibiting or Treating 3DG or Other Alpha-Dicarbonyl Sugar Associated Skin Wrinkling, Skin Aging, or Other Skin Disease, Disorder or Condition

[0216] The invention also discloses methods for inhibiting or treating 3DG related skin diseases or disorders. Some examples of 3DG associated diseases or disorders include, but are not limited to, skin cancer, psoriasis, aging, wrinkling, hyperkeratosis, hyperplasia, acanthosis, papillomatosis, dermatosis, rhinophyma, telangiectasia, and rosacea. A cancer or other disease or disorder may belong to any of a group of cancers or other diseases or disorders, which have been described herein, as well as any other related cancer or other disease or disorder known to those of skill in the art.

[0217] The invention should not be construed as being limited solely to these examples, as other 3DG associated diseases or disorders which are at present unknown, once known, may also be treatable using the methods of the invention. One of skill in the art would appreciate that 3DG inhibitors may be used prophylactically for some diseases or disorders of the skin, wherein 3DG is known, or it becomes known, that 3DG is associated with a skin disease or disorder. For example, 3DG inhibitors may be applied to prevent wrinkling or other skin problems in subjects who are exposed to harsh environmental elements such as the sun (photoaging/photodamage), heat, chemicals, or cold. Such problems can be due to damage to proteins or other molecules such as lipids or nucleic acids caused by 3DG or alpha-dicarbonyl sugars.

[0218] One skilled in the art would appreciate, based upon the disclosure provided herein, that the present invention encompasses methods for prevention of the loss of microcirculation and/or neuro-innervation in the aging, sclerodermic and/or diabetic skin since 3DG increases oxidative stress and AGEs and they, in turn, are linked to neuropathy and circulatory dysfunction.

[0219] The present invention also encompasses methods for prevention of hair loss associated with or mediated by loss of microcirculation and/or loss of neuro-innervation in populations of aging, sclerodermic and/or in diabetic individuals. This is because 3DG is a known precursor to the formation of AGEs which are known to be causally connected to the development of neuropathy. Preliminary data demonstrated that diabetic rats treated with DYN 12 and measured for muscle strength while alert had stronger muscle strength than dia-

abetic rats not so treated. This supports the concept that maintenance of nerve conduction and microcirculation that supports nerve innervation is deleteriously affected not only by AGEs, but also 3DG. Similarly, where 3DG would cause blockage of the microcirculation that supports nerve innervation of the hair follicle, the hair follicle will atrophy and die, as is the case in neuropathy. Accordingly, the present invention includes methods for preventing hair loss, where such hair loss is associated with or mediated by the presence of 3DG in the skin proximal to a hair follicle/shaft.

[0220] Similarly, the invention includes methods for prevention of graying of hair. This is because, as discussed previously with regard to hair loss, inhibiting the presence and/or activity of 3DG in skin associated with a hair follicle or shaft can prevent the deleterious effect of 3DG on microcirculation affecting such hair and, in turn, preventing the graying of the hair due to such deleterious effect.

[0221] Thus, one skilled in the art would appreciate, based upon the disclosure provided herein, that the present invention encompasses methods and compositions relating to prevention of hair loss and/or hair graying. Such compositions and methods encompass, but are not limited to, shampoo or other composition that can be applied to hair and skin associated with a hair follicle to administer the compounds of the invention such that formation, accumulation and/or function of 3DG and/or amadorase is inhibited thereby. Based on the disclosure provided herein, the skilled artisan would understand that such compounds include, but are not limited to, meglumine. Further, the formulation of compositions to be applied to hair follicles and the dosage and treatment regimens therefor, are disclosed herein and are also well-known to those in the art.

[0222] The invention encompasses methods for treatment of skin wound healing. This is because ROS are associated with the origination of wounds. Accordingly, the skilled artisan would appreciate, based upon the disclosure provided herein, that any inhibitor of ROS will positively effect wound healing. Given 3DG's role in the originating of ROS, inhibiting ROS by inhibiting the production of 3DG can result in methods useful to prevent and treat wounds. Further support for use of 3DG inhibition in skin as a useful wound healing therapeutic is provided by studies demonstrating that diabetics are especially prone to wound healing problems, since as previously discussed elsewhere herein, diabetics have elevated levels of 3DG and detoxify the 3DG less efficiently than non diabetics. Thus, the surprising finding that 3DG, as well as the enzyme responsible for its enzymatic synthesis, are present in skin makes possible, for the first time, the development of novel therapeutics for promotion of wound healing, especially for diabetics.

[0223] Since 3DG and the pathway for its formation, are present in skin, and are involved in the production of ROS and since ROS are, in turn, involved in inflammation, the skilled artisan would also appreciate that the invention encompasses methods for treating or ameliorating diseases, disorders or conditions associated with mucosal inflammation. Inhibition of 3DG formation, function, and/or accumulation in skin can inhibit mucosal inflammation such that conditions associated with inflammation of the mucosa (e.g., nasal passages, vagina, rectum, mouth cavity, and the like) can be inhibited by such inhibition. For instance, inhibition of 3DG can be used to modulate browning of teeth, inflammation of the mouth, gingivitis, periodontal disease, herpes sores, and the like.

[0224] Further, because inhibiting 3DG can prevent mucosal inflammation and can induce wound healing, such inhibition can also provide a useful therapeutics for the prevention and/treatment of viral, bacterial or fungal infection where the infection is mediated by pathogenic infection via the skin and/or mucosa. Therefore, the present invention includes methods and compositions for prevention or treatment of fungal, viral and bacterial infection by providing an inactivator of amadorase and/or 3DG to a patient in need of such treatment.

[0225] The invention encompasses methods of treating or preventing gingivitis, periodontal diseases, yellowing of the teeth, and the like. This is because the data disclosed herein demonstrate that 3DG is present in saliva, and is present in skin, indicating that it is present in mucosa. Thus, one skilled in the art would appreciate, based upon the disclosure provided herein, that inhibition of 3DG associated with the mucosa in the mouth cavity can inhibit the deleterious effects associated with or mediated by the molecule, including, but not limited to, gingivitis, periodontal disease, and discoloration of the teeth. This is because oxidative stress and AGEs are associated with these conditions and 3DG induces oxidative stress and AGEs. Further, the skilled artisan, armed with the teachings provided herein, would understand that the present invention encompasses methods of treating Wilson's disease, rheumatoid arthritis, progressive systemic sclerosis, fibrotic lung disease, Raynaud's phenomenon, joint contractures, Sjogren's syndrome, and the like. This is because, 3DG causes the induction of reactive oxygen species and reactive oxygen species cause inflammation, diseases associated with inflammation mediated by or associated with ROS can be prevented or treated by inhibition of 3DG. Therefore Wilson's disease, rheumatoid arthritis, progressive systemic sclerosis, fibrotic lung disease, Raynaud's phenomenon, joint contractures, Sjogren's syndrome, and the like, can be treated according to the methods set forth herein relating to inhibiting 3DG and/or amadorase.

[0226] The present invention includes methods of treating breast cancer. This is because, as more fully set forth elsewhere herein, the data disclosed herein demonstrate that 3DG is present in sweat. Because mammary glands are highly specialized sweat glands, the skilled artisan would appreciate, based upon the disclosure provided herein, that inhibition of 3DG in such tissue would provide a beneficial effect given the deleterious effects associated with or mediated by 3DG.

[0227] Inhibiting 3DG in skin, as appreciated by the skilled artisan based upon the disclosure provided herein, can provide useful therapeutics for treatment of breast cancer because 3DG causes oxidative stress and the formation of reactive oxygen and inhibits enzymes that combat oxidative stress. Thus, 3DG depletes the body's defenses against inflammation, in particular, high levels of 3DG present in skin deleteriously depletes the defenses present in the skin and mucosa. Thus, without wishing to be bound by any particular theory, the effects of 3DG are primarily due to its effect on oxidative stress and, in turn, to the entire inflammatory cascade. That is important for breast cancer where it is believed that long term oxidative stress, and not a single point mutation, causes the disease.

[0228] Likewise, one of skill in the art, once armed with the teachings disclosed herein, would understand that where a bodily fluid, such as saliva, sweat, lymph, urine, semen, and blood, comprising 3DG, is produced by or associated with skin, a disease, disorder or condition mediated by the contact

of such fluid with a cell, tissue or organ can be treated by inhibition of 3DG. Such disease, disorder or condition mediated by or associated with 3DG present in a bodily fluid includes, but is not limited to, non-Hodgkins Lymphoma, where sweat comprising 3DG saturates the lymph glands. Further, the invention includes methods of inhibiting formation of 3DG adducts, and/or inactivating these adducts, since these adducts will also contribute to diseases, disorders or conditions associated with 3DG, including those disclosed elsewhere herein. That is, like prevention of formation, accumulation, and/or functioning of 3DG prevents the deleterious effects of the compound relating to aging and disease, and more specifically, to the deleterious effects of 3DG on skin as disclosed elsewhere herein, inhibiting the deleterious effects of 3DG adducts and/or intermediates wherever found will likewise prevent their deleterious effects. The skilled artisan, once armed with the teachings provided herein, would understand that such 3DG adducts/intermediates include, but are not limited to, those depicted in FIG. 18, and that such intermediates/adducts that form from 3DG that will also contribute to aging and disease, wherever found.

[0229] These adducts are heretofore unknown, and the skilled artisan would appreciate, based on their novel disclosure herein, that inhibiting such adducts will inhibit a disease process mediated by or associated therewith, in skin and wherever such adducts are present. Thus, the present invention encompasses inhibiting the synthesis, formation and accumulation of such 3DG adducts, wherever they are detected using detection methods disclosed herein, known in the art, or to be developed in the future.

[0230] The present invention encompasses methods for treating or ameliorating a wide plethora of diseases, which diseases are mediated by or associated with changes in skin due to the interactions of 3DG with proteins in skin, such as, e.g., collagen and elastin, and with the induction of ROS and their subsequent reaction with components of skin. That is, the data disclosed herein demonstrate that 3DG in the skin mediates or is associated with collagen cross-linking and, in turn, with skin thickening, such that preventing the accumulation, formation, function, and/or increasing the clearance of 3DG and/or Amadorase, from the skin can provide a therapeutic benefit for a disease disorder or condition mediated by or associated with such thickening.

[0231] In addition, the present invention encompasses treating or ameliorating a disease, disorder or condition mediated by or associated with, oxidative stress. This is because 3DG induces oxidative stress., i.e., 3DG induces oxidative stress either directly or through the formation of AGEs and therefore 3DG is involved in the inflammatory response. Thus, inhibiting 3DG will treat or prevent a disease, disorder or condition associated with inflammation. Such disease, disorder or condition includes, but is not limited to, gingivitis, periodontal disease, browning/yellowing of teeth, herpes lesions, and scarring since these are mediated by, or associated with, ROS. Accordingly, preventing ROS, such as by, for instance, treatment of the teeth and/or oral tissue (e.g., gums, and the like) with an inhibitor of 3DG, e.g., meglumine, can reduce deleterious effects of ROS in the buccal cavity such as the aforementioned diseases, disorders or conditions.

[0232] The present invention further encompasses treatments that affect the appearance of skin based upon inhibition of 3DG, its adducts/intermediates, as well as inhibition of amadorase and the synthesis of 3DG. Thus, even where the condition, disorder or disease is not treated or ameliorated,

the invention includes methods of treatment that affect the appearance of the skin such that, at the very least, the condition, disorder or disease affects the appearance of the skin to a lesser degree than in the absence of the treatment. These treatments are therefore cosmetic and can produce an improvement in physical appearance.

[0233] The present invention includes methods of treating skin aging related to the loss of skin elasticity. This is because, as more fully set forth elsewhere herein, the data disclosed herein demonstrate, for the first time, that 3DG and the enzyme associated with its synthesis, are present in skin and that inhibition of 3DG can prevent or reverse the loss of skin elasticity associated with its presence in skin. Accordingly, the skilled artisan would appreciate, once armed with the teachings provided herein, that inhibiting 3DG in skin can reduce skin aging such that the present invention provides useful therapeutics for inhibiting skin aging and loss of skin elasticity. The skilled artisan would further understand that skin aging therapeutics encompass, but are not limited, to various treatment procedures well-known in the dermatological and cosmological arts including, but not limited to, skin wraps, exfoliants, masks, and the like, that can be used to effectuate the various treatments disclosed herein.

[0234] The invention encompasses methods of preventing the susceptibility to viral, fungal and bacterial infections especially in oral, rectal and vaginal routes by inhibiting Amadorase and/or by inactivating 3DG. Specifically, susceptibility to infection by, e.g., HIV, papillomavirus and Epstein-Barr virus can be decreased because changes in skin affect receptivity to disease and 3DG induces the formation of ROS and AGEs and also actively interacts with skin proteins, in particular collagen and elastin, therefore they affect the skin such that receptivity is altered.

[0235] One skilled in the art would understand, based upon the disclosure provided herein, that the present invention provides useful therapeutics for a wide plethora of diseases, disorders or conditions associated with 3DG in skin. This is because, inter alia, it is well-known in the art that 3DG mediates formation of ROS, which, in turn, are well-known to be involved in a wide variety of diseases, disorders or conditions as set forth herein.

[0236] The invention also includes methods for inhibiting or treating skin diseases or disorders associated with members of the alpha-dicarbonyl sugar family of compounds other than 3DG.

[0237] In one aspect of the invention, various changes in the skin can be measured following treatment with inhibitors of 3DG. The skin topography can be defined by parameters such as: (a) number of wrinkles; (b) total area of wrinkles; (c) total length of wrinkles; (d) mean length of wrinkles; and (e) mean depth of wrinkles. The type of wrinkles can be determined on the basis of depth, length, and area. These properties can be used when evaluating the changes in skin due to disease or disorder or the effects of a treatment on the skin. The effects of changes in 3DG levels and function on various skin qualities can be determined based on techniques known in the art. Methods to measure skin quality include, but are not limited to, measuring viscoelastic properties with instruments such as a ballistometer, measuring the mechanical/vertical deformation properties of the skin with an instrument such as a cutometer, or measuring changes in skin capacitance resulting from changes in the degree of hydration using a corneometer.

#### Compositions and Methods for Administration

[0238] The invention relates to the administration of an identified compound in a pharmaceutical or cosmetic com-

position to practice the methods of the invention, the composition comprising the compound or an appropriate derivative or fragment of the compound and a pharmaceutically-acceptable carrier. For example, a chemical composition with which an appropriate inhibitor of enzyme dependent or nonenzyme dependent production of 3DG, or inhibitor of 3DG accumulation or function, or stimulator of 3DG removal, detoxification, or degradation, is combined, is used to administer the appropriate compound to an animal. The invention should be construed to include the use of one, or simultaneous use of more than one, inhibitor of 3DG or stimulator of 3DG removal, degradation, or detoxification. When more than one stimulator or inhibitor is used, they can be administered together or they can be administered separately.

[0239] In one embodiment, the pharmaceutical compositions useful for practicing the invention may be administered to deliver a dose of between 1 ng/kg/day and 100 mg/kg/day. In another embodiment, the pharmaceutical compositions useful for practicing the invention may be administered to deliver a dose of between 1 ng/kg/day and 100 g/kg/day.

[0240] In another embodiment of the invention, a pharmaceutical composition is in the form of a liposome crème. In one aspect, a composition comprises 23.9 grams of BIO-CREME Concentrate (BioChemica International Inc.), blended with 2.9 grams cocoa butter, 1.4 grams shea butter, 2.2 grams aloe oil, 1.1 grams vitamin E, 3.7 grams glycerol, 51 grams water, 1.1 grams dimethicone and 10.8 grams Natipide II, along with 1 gram arginine-HCl and 1 gram meglumine-HCl. However, the invention should not be limited to a liposome-based delivery vehicle.

[0241] As will be understood by the skilled artisan, when armed with the disclosure set forth herein, a composition useful in the present invention can include one active ingredient. Alternatively, a composition useful in the present invention can include at least two active ingredients. In one aspect, multiple active ingredients may be active in an additive manner. In another aspect, multiple active ingredients may be active in a synergistic manner. That is, the multiple active ingredients in a composition of the invention may provide a therapeutic effect that is greater than the addition of the therapeutic effects provided by each of the active ingredients alone. By way of a non-limiting example, a composition can comprise both an inhibitor of alpha-dicarbonyl sugar formation and an inhibitor of alpha-dicarbonyl sugar function or effect, together exhibit a synergistic effect in the alleviation of alpha-dicarbonyl sugar-associated conditions, as compared with compositions comprising either type of inhibitor alone. In one embodiment, the combination of meglumine and arginine for the treatment of alpha-dicarbonyl sugar-associated conditions.

[0242] Other pharmaceutically acceptable carriers which are useful include, but are not limited to, glycerol, water, saline, ethanol and other pharmaceutically acceptable salt solutions such as phosphates and salts of organic acids. Examples of these and other pharmaceutically acceptable carriers are described in Remington's Pharmaceutical Sciences (1991, Mack Publication Co., New Jersey).

[0243] The pharmaceutical compositions may be prepared, packaged, or sold in the form of a sterile injectable aqueous or oily suspension or solution. This suspension or solution may be formulated according to the known art, and may comprise, in addition to the active ingredient, additional ingredients such as the dispersing agents, wetting agents, or suspending agents described herein. Such sterile injectable formulations

may be prepared using a non-toxic parenterally-acceptable diluent or solvent, such as water or 1,3-butane diol, for example. Other acceptable diluents and solvents include, but are not limited to, Ringer's solution, isotonic sodium chloride solution, and fixed oils such as synthetic mono- or di-glycerides.

[0244] Pharmaceutical compositions that are useful in the methods of the invention may be administered, prepared, packaged, and/or sold in formulations suitable for oral, rectal, vaginal, parenteral, topical, pulmonary, intranasal, buccal, ophthalmic, or another route of administration. Other contemplated formulations include projected nanoparticles, liposomal preparations, resealed erythrocytes containing the active ingredient, and immunologically-based formulations.

[0245] The compositions of the invention may be administered via numerous routes, including, but not limited to, oral, rectal, vaginal, parenteral, topical, pulmonary, intranasal, buccal, or ophthalmic administration routes. The route(s) of administration will be readily apparent to the skilled artisan and will depend upon any number of factors including the type and severity of the disease being treated, the type and age of the veterinary or human patient being treated, and the like.

[0246] Pharmaceutical compositions that are useful in the methods of the invention may be administered systemically in oral solid formulations, ophthalmic, suppository, aerosol, topical or other similar formulations. In addition to the compound such as heparan sulfate, or a biological equivalent thereof, such pharmaceutical compositions may contain pharmaceutically-acceptable carriers and other ingredients known to enhance and facilitate drug administration. Other possible formulations, such as nanoparticles, liposomes, resealed erythrocytes, and immunologically based systems may also be used to administer compounds according to the methods of the invention.

[0247] Compounds which are identified using any of the methods described herein may be formulated and administered to a mammal for treatment of skin aging, skin wrinkling, and various skin related diseases, disorders, or conditions described herein.

[0248] The invention encompasses the preparation and use of pharmaceutical compositions comprising a compound useful for treatment of various skin related diseases, disorders, or conditions described herein, including skin aging, photoaging, and wrinkling of the skin. The invention also encompasses 3DG associated diseases and disorders other than those of the skin, including, but not limited to, gum diseases and disorders. Such a pharmaceutical composition may consist of the active ingredient alone, in a form suitable for administration to a subject, or the pharmaceutical composition may comprise at least one active ingredient and one or more pharmaceutically acceptable carriers, one or more additional ingredients, or some combination of these. The active ingredient may be present in the pharmaceutical composition in the form of a physiologically acceptable ester or salt, such as in combination with a physiologically acceptable cation or anion, as is well known in the art.

[0249] An obstacle for topical administration of pharmaceuticals is the stratum corneum layer of the epidermis. The stratum corneum is a highly resistant layer comprised of protein, cholesterol, sphingolipids, free fatty acids and various other lipids, and includes cornified and living cells. One of the factors that limits the penetration rate (flux) of a compound through the stratum corneum is the amount of the active substance which can be loaded or applied onto the skin

surface. The greater the amount of active substance which is applied per unit of area of the skin, the greater the concentration gradient between the skin surface and the lower layers of the skin, and in turn the greater the diffusion force of the active substance through the skin. Therefore, a formulation containing a greater concentration of the active substance is more likely to result in penetration of the active substance through the skin, and more of it, and at a more consistent rate, than a formulation having a lesser concentration, all other things being equal.

[0250] The formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with a carrier or one or more other accessory ingredients, and then, if necessary or desirable, shaping or packaging the product into a desired single- or multi-dose unit.

[0251] Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions which are suitable for ethical administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable for administration to animals of all sorts.

[0252] Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and perform such modification with merely ordinary, if any, experimentation. Subjects to which administration of the pharmaceutical compositions of the invention is contemplated include, but are not limited to, humans and other primates, mammals including commercially relevant mammals such as cattle, pigs, horses, sheep, cats, and dogs.

[0253] Pharmaceutical compositions that are useful in the methods of the invention may be prepared, packaged, or sold in formulations suitable for oral, rectal, vaginal, parenteral, topical, pulmonary, intranasal, buccal, ophthalmic, intrathecal or another route of administration. Other contemplated formulations include projected nanoparticles, liposomal preparations, resealed erythrocytes containing the active ingredient, and immunologically-based formulations.

[0254] A pharmaceutical composition of the invention may be prepared, packaged, or sold in bulk, as a single unit dose, or as a plurality of single unit doses. As used herein, a "unit dose" is a discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient which would be administered to a subject or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage.

[0255] The relative amounts of the active ingredient, the pharmaceutically acceptable carrier, and any additional ingredients in a pharmaceutical composition of the invention will vary, depending upon the identity, size, and condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100% (w/w) active ingredient.

[0256] In addition to the active ingredient, a pharmaceutical composition of the invention may further comprise one or more additional pharmaceutically active agents. Particularly

contemplated additional agents include anti-emetics and scavengers such as cyanide and cyanate scavengers.

[0257] Controlled- or sustained-release formulations of a pharmaceutical composition of the invention may be made using conventional technology.

[0258] Formulations suitable for topical administration include, but are not limited to, liquid or semi-liquid preparations such as liniments, lotions, oil-in-water or water-in-oil emulsions such as creams, ointments or pastes, and solutions or suspensions. Topically-administrable formulations may, for example, comprise from about 1% to about 10% (w/w) active ingredient, although the concentration of the active ingredient may be as high as the solubility limit of the active ingredient in the solvent. Formulations for topical administration may further comprise one or more of the additional ingredients described herein.

[0259] Enhancers of permeation may be used. These materials increase the rate of penetration of drugs across the skin. Typical enhancers in the art include ethanol, glycerol monolaurate, PGML (polyethylene glycol monolaurate), dimethylsulfoxide, and the like. Other enhancers include oleic acid, oleyl alcohol, ethoxydiglycol, laurocapram, alkanecarboxylic acids, dimethylsulfoxide, polar lipids, or N-methyl-2-pyrrolidone.

[0260] One acceptable vehicle for topical delivery of some of the compositions of the invention may contain liposomes. The composition of the liposomes and their use are known in the art (for example, see Constanza, U.S. Pat. No. 6,323,219).

[0261] The source of active compound to be formulated will generally depend upon the particular form of the compound. Small organic molecules and peptidyl or oligo fragments can be chemically synthesized and provided in a pure form suitable for pharmaceutical/cosmetic usage. Products of natural extracts can be purified according to techniques known in the art. Recombinant sources of compounds are also available to those of ordinary skill in the art.

[0262] In alternative embodiments, the topically active pharmaceutical or cosmetic composition may be optionally combined with other ingredients such as moisturizers, cosmetic adjuvants, anti-oxidants, chelating agents, bleaching agents, tyrosinase inhibitors and other known depigmentation agents, surfactants, foaming agents, conditioners, humectants, wetting agents, emulsifying agents, fragrances, viscosifiers, buffering agents, preservatives, sunscreens and the like. In another embodiment, a permeation or penetration enhancer is included in the composition and is effective in improving the percutaneous penetration of the active ingredient into and through the stratum corneum with respect to a composition lacking the permeation enhancer. Various permeation enhancers, including oleic acid, oleyl alcohol, ethoxydiglycol, laurocapram, alkanecarboxylic acids, dimethylsulfoxide, polar lipids, or N-methyl-2-pyrrolidone, are known to those of skill in the art. In another aspect, the composition may further comprise a hydrotropic agent, which functions to increase disorder in the structure of the stratum corneum, and thus allows increased transport across the stratum corneum. Various hydrotropic agents such as isopropyl alcohol, propylene glycol, or sodium xylene sulfonate, are known to those of skill in the art. The compositions of this invention may also contain active amounts of retinoids (i.e., compounds that bind to any members of the family of retinoid receptors), including, for example, tretinoin, retinol, esters of tretinoin and/or retinol and the like.

[0263] The topically active pharmaceutical or cosmetic composition should be applied in an amount effective to affect desired changes. As used herein "amount effective" shall mean an amount sufficient to cover the region of skin surface where a change is desired. An active compound should be present in the amount of from about 0.0001% to about 15% by weight volume of the composition. More preferable, it should be present in an amount from about 0.0005% to about 5% of the composition; most preferably, it should be present in an amount of from about 0.001% to about 1% of the composition. Such compounds may be synthetically- or naturally-derived.

[0264] Liquid derivatives and natural extracts made directly from biological sources may be employed in the compositions of this invention in a concentration (w/v) from about 1 to about 99%. Fractions of natural extracts and protease inhibitors may have a different preferred range, from about 0.01% to about 20% and, more preferably, from about 1% to about 10% of the composition. Of course, mixtures of the active agents of this invention may be combined and used together in the same formulation, or in serial applications of different formulations.

[0265] The composition of the invention may comprise a preservative from about 0.005% to 2.0% by total weight of the composition. The preservative is used to prevent spoilage in the case of an aqueous gel because of repeated patient use when it is exposed to contaminants in the environment from, for example, exposure to air or the patient's skin, including contact with the fingers used for applying a composition of the invention such as a therapeutic gel or cream. Examples of preservatives useful in accordance with the invention included but are not limited to those selected from the group consisting of benzyl alcohol, sorbic acid, parabens, imidurea and combinations thereof. A particularly preferred preservative is a combination of about 0.5% to 2.0% benzyl alcohol and 0.05% to 0.5% sorbic acid.

[0266] The composition preferably includes an antioxidant and a chelating agent which inhibit the degradation of the compound for use in the invention in the aqueous gel formulation. Preferred antioxidants for some compounds are BHT, BHA, alphatocopherol and ascorbic acid in the preferred range of about 0.01% to 0.3% and more preferably BHT in the range of 0.03% to 0.1% by weight by total weight of the composition. Preferably, the chelating agent is present in an amount of from 0.01% to 0.5% by weight by total weight of the composition. Particularly preferred chelating agents include edetate salts (e.g. disodium edetate) and citric acid in the weight range of about 0.01% to 0.20% and more preferably in the range of 0.02% to 0.10% by weight by total weight of the composition. The chelating agent is useful for chelating metal ions in the composition which may be detrimental to the shelf life of the formulation. While BHT and disodium edetate are the particularly preferred antioxidant and chelating agent respectively for some compounds, other suitable and equivalent antioxidants and chelating agents may be substituted therefor as would be known to those skilled in the art.

[0267] Controlled-release preparations may also be used and the methods for the use of such preparations are known to those of skill in the art.

[0268] In some cases, the dosage forms to be used can be provided as slow or controlled-release of one or more active ingredients therein using, for example, hydropropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles,

liposomes, or microspheres or a combination thereof to provide the desired release profile in varying proportions. Suitable controlled-release formulations known to those of ordinary skill in the art, including those described herein, can be readily selected for use with the pharmaceutical compositions of the invention. Thus, single unit dosage forms suitable for oral administration, such as tablets, capsules, gelcaps, and caplets, that are adapted for controlled-release are encompassed by the present invention.

[0269] All controlled-release pharmaceutical products have a common goal of improving drug therapy over that achieved by their non-controlled counterparts. Ideally, the use of an optimally designed controlled-release preparation in medical treatment is characterized by a minimum of drug substance being employed to cure or control the condition in a minimum amount of time. Advantages of controlled-release formulations include extended activity of the drug, reduced dosage frequency, and increased patient compliance. In addition, controlled-release formulations can be used to affect the time of onset of action or other characteristics, such as blood level of the drug, and thus can affect the occurrence of side effects.

[0270] Most controlled-release formulations are designed to initially release an amount of drug that promptly produces the desired therapeutic effect, and gradually and continually release of other amounts of drug to maintain this level of therapeutic effect over an extended period of time. In order to maintain this constant level of drug in the body, the drug must be released from the dosage form at a rate that will replace the amount of drug being metabolized and excreted from the body.

[0271] Controlled-release of an active ingredient can be stimulated by various inducers, for example pH, temperature, enzymes, water, or other physiological conditions or compounds. The term "controlled-release component" in the context of the present invention is defined herein as a compound or compounds, including, but not limited to, polymers, polymer matrices, gels, permeable membranes, liposomes, or microspheres or a combination thereof that facilitates the controlled-release of the active ingredient.

[0272] Liquid suspensions may be prepared using conventional methods to achieve suspension of the active ingredient in an aqueous or oily vehicle. Aqueous vehicles include, for example, water, and isotonic saline. Oily vehicles include, for example, almond oil, oily esters, ethyl alcohol, vegetable oils such as arachis, olive, sesame, or coconut oil, fractionated vegetable oils, and mineral oils such as liquid paraffin. Liquid suspensions may further comprise one or more additional ingredients including, but not limited to, suspending agents, dispersing or wetting agents, emulsifying agents, demulcents, preservatives, buffers, salts, flavorings, coloring agents, and sweetening agents. Oily suspensions may further comprise a thickening agent. Known suspending agents include, but are not limited to, sorbitol syrup, hydrogenated edible fats, sodium alginate, polyvinylpyrrolidone, gum tragacanth, gum acacia, and cellulose derivatives such as sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose. Known dispersing or wetting agents include, but are not limited to, naturally-occurring phosphatides such as lecithin, condensation products of an alkylene oxide with a fatty acid, with a long chain aliphatic alcohol, with a partial ester derived from a fatty acid and a hexitol, or with a partial ester derived from a fatty acid and a hexitol anhydride (e.g., polyoxyethylene stearate, heptadecaethyl-

eneoxycetanol, polyoxyethylene sorbitol monooleate, and polyoxyethylene sorbitan monooleate, respectively). Known emulsifying agents include, but are not limited to, lecithin, and acacia. Known preservatives include, but are not limited to, methyl, ethyl, or n-propyl-para-hydroxybenzoates, ascorbic acid, and sorbic acid. Known sweetening agents include, for example, glycerol, propylene glycol, sorbitol, sucrose, and saccharin. Known thickening agents for oily suspensions include, for example, beeswax, hard paraffin, and cetyl alcohol.

[0273] Liquid solutions of the active ingredient in aqueous or oily solvents may be prepared in substantially the same manner as liquid suspensions, the primary difference being that the active ingredient is dissolved, rather than suspended in the solvent. Liquid solutions of the pharmaceutical composition of the invention may comprise each of the components described with regard to liquid suspensions, it being understood that suspending agents will not necessarily aid dissolution of the active ingredient in the solvent. Aqueous solvents include, for example, water, and isotonic saline. Oily solvents include, for example, almond oil, oily esters, ethyl alcohol, vegetable oils such as arachis, olive, sesame, or coconut oil, fractionated vegetable oils, and mineral oils such as liquid paraffin.

[0274] Powdered and granular formulations of a pharmaceutical preparation of the invention may be prepared using known methods. Such formulations may be administered directly to a subject, used, for example, to form tablets, to fill capsules, or to prepare an aqueous or oily suspension or solution by addition of an aqueous or oily vehicle thereto. Each of these formulations may further comprise one or more of dispersing or wetting agent, a suspending agent, and a preservative. Additional excipients, such as fillers and sweetening, flavoring, or coloring agents, may also be included in these formulations.

[0275] A pharmaceutical composition of the invention may also be prepared, packaged, or sold in the form of oil-in-water emulsion or a water-in-oil emulsion. The oily phase may be a vegetable oil such as olive or arachis oil, a mineral oil such as liquid paraffin, or a combination of these. Such compositions may further comprise one or more emulsifying agents such as naturally occurring gums such as gum acacia or gum tragacanth, naturally-occurring phosphatides such as soybean or lecithin phosphatide, esters or partial esters derived from combinations of fatty acids and hexitol anhydrides such as sorbitan monooleate, and condensation products of such partial esters with ethylene oxide such as polyoxyethylene sorbitan monooleate. These emulsions may also contain additional ingredients including, for example, sweetening or flavoring agents.

[0276] As used herein, an "oily" liquid is one which comprises a carbon-containing liquid molecule and which exhibits a less polar character than water.

[0277] A formulation of a pharmaceutical composition of the invention suitable for oral administration may be prepared, packaged, or sold in the form of a discrete solid dose unit including, but not limited to, a tablet, a hard or soft capsule, a cachet, a troche, or a lozenge, each containing a predetermined amount of the active ingredient. Other formulations suitable for oral administration include, but are not limited to, a powdered or granular formulation, an aqueous or oily suspension, an aqueous or oily solution, a paste, a gel, a

toothpaste, a mouthwash, a coating, an oral rinse, or an emulsion. The terms oral rinse and mouthwash are used interchangeably herein.

[0278] A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for oral or buccal administration. Such a formulation may comprise, but is not limited to, a gel, a liquid, a suspension, a paste, a toothpaste, a mouthwash or oral rinse, and a coating. For example, an oral rinse of the invention may comprise a compound of the invention at about 1.4%, chlorhexidine gluconate (0.12%), ethanol (11.2%), sodium saccharin (0.15%), FD&C Blue No. 1 (0.001%), peppermint oil (0.5%), glycerine (10.0%), Tween 60 (0.3%), and water to 100%. In another embodiment, a toothpaste of the invention may comprise a compound of the invention at about 5.5%, sorbitol, 70% in water (25.0%), sodium saccharin (0.15%), sodium lauryl sulfate (1.75%), carbopol 934, 6% dispersion in (15%), oil of spearmint (1.0%), sodium hydroxide, 50% in water (0.76%), dibasic calcium phosphate dihydrate (45%), and water to 100%. The examples of formulations described herein are not exhaustive and it is understood that the invention includes additional modifications of these and other formulations not described herein, but which are known to those of skill in the art.

[0279] A tablet comprising the active ingredient may, for example, be made by compressing or molding the active ingredient, optionally with one or more additional ingredients. Compressed tablets may be prepared by compressing, in a suitable device, the active ingredient in a free-flowing form such as a powder or granular preparation, optionally mixed with one or more of a binder, a lubricant, an excipient, a surface active agent, and a dispersing agent. Molded tablets may be made by molding, in a suitable device, a mixture of the active ingredient, a pharmaceutically acceptable carrier, and at least sufficient liquid to moisten the mixture. Pharmaceutically acceptable excipients used in the manufacture of tablets include, but are not limited to, inert diluents, granulating and disintegrating agents, binding agents, and lubricating agents. Known dispersing agents include, but are not limited to, potato starch and sodium starch glycollate. Known surface-active agents include, but are not limited to, sodium lauryl sulphate. Known diluents include, but are not limited to, calcium carbonate, sodium carbonate, lactose, microcrystalline cellulose, calcium phosphate, calcium hydrogen phosphate, and sodium phosphate. Known granulating and disintegrating agents include, but are not limited to, corn starch and alginic acid. Known binding agents include, but are not limited to, gelatin, acacia, pre-gelatinized maize starch, polyvinylpyrrolidone, and hydroxypropyl methylcellulose. Known lubricating agents include, but are not limited to, magnesium stearate, stearic acid, silica, and talc.

[0280] Tablets may be non-coated or they may be coated using known methods to achieve delayed disintegration in the gastrointestinal tract of a subject, thereby providing sustained release and absorption of the active ingredient. By way of example, a material such as glyceryl monostearate or glyceryl distearate may be used to coat tablets. Further by way of example, tablets may be coated using methods described in U.S. Pat. Nos. 4,256,108; 4,160,452; and 4,265,874 to form osmotically-controlled release tablets. Tablets may further comprise a sweetening agent, a flavoring agent, a coloring agent, a preservative, or some combination of these in order to provide for pharmaceutically elegant and palatable preparation.

[0281] Hard capsules comprising the active ingredient may be made using a physiologically degradable composition, such as gelatin. Such hard capsules comprise the active ingredient, and may further comprise additional ingredients including, for example, an inert solid diluent such as calcium carbonate, calcium phosphate, or kaolin.

[0282] Soft gelatin capsules comprising the active ingredient may be made using a physiologically degradable composition, such as gelatin. Such soft capsules comprise the active ingredient, which may be mixed with water or an oil medium such as peanut oil, liquid paraffin, or olive oil.

[0283] Liquid formulations of a pharmaceutical composition of the invention which are suitable for oral administration may be prepared, packaged, and sold either in liquid form or in the form of a dry product intended for reconstitution with water or another suitable vehicle prior to use.

[0284] A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for rectal administration. Such a composition may be in the form of, for example, a suppository, a retention enema preparation, and a solution for rectal or colonic irrigation.

[0285] Suppository formulations may be made by combining the active ingredient with a non-irritating pharmaceutically acceptable excipient which is solid at ordinary room temperature (i.e., about 20° C.) and which is liquid at the rectal temperature of the subject (i.e., about 37° C. in a healthy human). Suitable pharmaceutically acceptable excipients include, but are not limited to, cocoa butter, polyethylene glycols, and various glycerides. Suppository formulations may further comprise various additional ingredients including, but not limited to, antioxidants, and preservatives.

[0286] Retention enema preparations or solutions for rectal or colonic irrigation may be made by combining the active ingredient with a pharmaceutically acceptable liquid carrier.

As is well known in the art, enema preparations may be administered using, and may be packaged within, a delivery device adapted to the rectal anatomy of the subject. Enema preparations may further comprise various additional ingredients including, but not limited to, antioxidants, and preservatives.

[0287] A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for vaginal administration. Such a composition may be in the form of, for example, a suppository, an impregnated or coated vaginally-insertable material such as a tampon, a douche preparation, or gel or cream or a solution for vaginal irrigation.

[0288] Methods for impregnating or coating a material with a chemical composition are known in the art, and include, but are not limited to methods of depositing or binding a chemical composition onto a surface, methods of incorporating a chemical composition into the structure of a material during the synthesis of the material (i.e., such as with a physiologically degradable material), and methods of absorbing an aqueous or oily solution or suspension into an absorbent material, with or without subsequent drying.

[0289] Douche preparations or solutions for vaginal irrigation may be made by combining the active ingredient with a pharmaceutically acceptable liquid carrier. As is well known in the art, douche preparations may be administered using, and may be packaged within, a delivery device adapted to the vaginal anatomy of the subject.

[0290] Douche preparations may further comprise various additional ingredients including, but not limited to, antioxi-

dants, antibiotics, antifungal agents, and preservatives. As used herein, “parenteral administration” of a pharmaceutical composition includes any route of administration characterized by physical breaching of a tissue of a subject and administration of the pharmaceutical composition through the breach in the tissue. Parenteral administration thus includes, but is not limited to, administration of a pharmaceutical composition by injection of the composition, by application of the composition through a surgical incision, by application of the composition through a tissue-penetrating non-surgical wound, and the like. In particular, parenteral administration is contemplated to include, but is not limited to, subcutaneous, intraperitoneal, intramuscular, intrasternal injection, and kidney dialytic infusion techniques.

[0291] Formulations of a pharmaceutical composition suitable for parenteral administration comprise the active ingredient combined with a pharmaceutically acceptable carrier, such as sterile water or sterile isotonic saline. Such formulations may be prepared, packaged, or sold in a form suitable for bolus administration or for continuous administration. Injectable formulations may be prepared, packaged, or sold in unit dosage form, such as in ampules or in multi-dose containers containing a preservative. Formulations for parenteral administration include, but are not limited to, suspensions, solutions, emulsions in oily or aqueous vehicles, pastes, and implantable sustained-release or biodegradable formulations. Such formulations may further comprise one or more additional ingredients including, but not limited to, suspending, stabilizing, or dispersing agents. In one embodiment of a formulation for parenteral administration, the active ingredient is provided in dry (i.e., powder or granular) form for reconstitution with a suitable vehicle (e.g., sterile pyrogen-free water) prior to parenteral administration of the reconstituted composition.

[0292] The pharmaceutical compositions may be prepared, packaged, or sold in the form of a sterile injectable aqueous or oily suspension or solution. This suspension or solution may be formulated according to the known art, and may comprise, in addition to the active ingredient, additional ingredients such as the dispersing agents, wetting agents, or suspending agents described herein. Such sterile injectable formulations may be prepared using a non-toxic parenterally-acceptable diluent or solvent, such as water or 1,3-butane diol, for example. Other acceptable diluents and solvents include, but are not limited to, Ringer's solution, isotonic sodium chloride solution, and fixed oils such as synthetic mono- or di-glycerides. Other parentally-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form, in a liposomal preparation, or as a component of a biodegradable polymer system. Compositions for sustained release or implantation may comprise pharmaceutically acceptable polymeric or hydrophobic materials such as an emulsion, an ion exchange resin, a sparingly soluble polymer, or a sparingly soluble salt.

[0293] A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for buccal administration. Such formulations may, for example, be in the form of tablets or lozenges made using conventional methods, and may, for example, 0.1 to 20% (w/w) active ingredient, the balance comprising an orally dissolvable or degradable composition and, optionally, one or more of the additional ingredients described herein. Alternately, formulations suitable for buccal administration may comprise a powder or an aerosolized or atomized solution or suspension

comprising the active ingredient. Such powdered, aerosolized, or aerosolized formulations, when dispersed, preferably have an average particle or droplet size in the range from about 0.1 to about 200 nanometers, and may further comprise one or more of the additional ingredients described herein.

[0294] As used herein, "additional ingredients" include, but are not limited to, one or more of the following: excipients; surface active agents; dispersing agents; inert diluents; granulating and disintegrating agents; binding agents; lubricating agents; sweetening agents; flavoring agents; coloring agents; preservatives; physiologically degradable compositions such as gelatin; aqueous vehicles and solvents; oily vehicles and solvents; suspending agents; dispersing or wetting agents; emulsifying agents; demulcents; buffers; salts; thickening agents; fillers; emulsifying agents; antioxidants; antibiotics; antifungal agents; stabilizing agents; and pharmaceutically acceptable polymeric or hydrophobic materials. Other "additional ingredients" which may be included in the pharmaceutical compositions of the invention are known in the art and described, for example in Genaro, ed. (1985, Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa.), which is incorporated herein by reference.

[0295] Typically, dosages of the compound of the invention which may be administered to an animal, preferably a human, will vary depending upon any number of factors, including but not limited to, the type of animal and type of disease state being treated, the age of the animal and the route of administration.

[0296] The compound can be administered to an animal as frequently as several times daily, or it may be administered less frequently, such as once a day, once a week, once every two weeks, once a month, or even less frequently, such as once every several months or even once a year or less. The frequency of the dose will be readily apparent to the skilled artisan and will depend upon any number of factors, such as, but not limited to, the type and severity of the disease being treated, the type and age of the animal, etc.

[0297] It will be recognized by one of skill in the art that the various embodiments of the invention as described above relating to methods of inhibiting 3DG or treating 3DG related diseases or conditions, includes other diseases and conditions not described herein.

#### [0298] Kits

[0299] The present invention should be construed to include kits for inhibiting or stimulating 3DG, treating 3DG associated skin diseases and disorders, kits for measuring 3DG and 3DG related parameters, and kits for diagnosing 3DG associated skin diseases and disorders. The invention should be construed to include kits for alpha-dicarbonyl sugars other than 3DG as well.

[0300] The invention includes a kit comprising an inhibitor of 3DG or a compound identified in the invention, a standard, and an instructional material which describes administering the inhibitor or a composition comprising the inhibitor or compound to a cell or an animal. This should be construed to include other embodiments of kits that are known to those skilled in the art, such as a kit comprising a standard and a (preferably sterile) solvent suitable for dissolving or suspending the composition of the invention prior to administering the compound to a cell or an animal. Preferably the animal is a mammal. More preferably, the mammal is a human.

[0301] The invention also includes a kit comprising a stimulator of 3DG degradation, detoxification, or clearance, or a such a stimulatory compound identified in the invention,

a standard, and an instructional material which describes administering the stimulator or a composition comprising the stimulator or compound to a cell or an animal. This should be construed to include other embodiments of kits that are known to those skilled in the art, such as a kit comprising a standard and a (preferably sterile) solvent suitable for dissolving or suspending the composition of the invention prior to administering the compound to a cell or an animal.

[0302] In accordance with the present invention, as described above or as discussed in the Examples below, there can be employed conventional chemical, cellular, histochemical, biochemical, molecular biology, microbiology and recombinant DNA techniques which are known to those of skill in the art. Such techniques are explained fully in the literature. See for example, Sambrook et al., 1989 Molecular Cloning—a Laboratory Manual, Cold Spring Harbor Press; Glover, (1985) DNA Cloning: a Practical Approach; Gait, (1984) Oligonucleotide Synthesis; Harlow et al., 1988 Antibodies—a Laboratory Manual, Cold Spring Harbor Press; Roe et al., 1996 DNA Isolation and Sequencing: Essential Techniques, John Wiley; and Ausubel et al., 1995 Current Protocols in Molecular Biology, Greene Publishing.

[0303] Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the compounds of the present invention and practice the claimed methods. The following working examples therefore, specifically point out the preferred embodiments of the present invention, and are not to be construed as limiting in any way the remainder of the disclosure.

## EXAMPLES

[0304] The invention is now described with reference to the following Examples. These Examples are provided for the purpose of illustration only and the invention should in no way be construed as being limited to these Examples, but rather should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

### Methods

#### Transdermal Drug Delivery

[0305] There are several advantages to delivering compounds, including drugs or other therapeutic agents, into the body through the skin, a process called transdermal drug delivery. Transdermal drug delivery offers an attractive alternative to injections and oral medications. It provides the capacity for multi day therapy with a single application thereby improving patient compliance. Such delivery would extend the activity of drugs having short half-life through the reservoir of drug present in the delivery system and its controlled release characteristics. Transdermal drug delivery avoids gastrointestinal tract difficulties during absorption caused by enzymes or drug interactions with food. Not only that, it avoids first pass i.e. the initial passage of a drug substance through the systemic and portal circulation. However, applications of transdermal drug delivery are limited to only a few drugs as a result of low skin permeability [Prausnitz, M. R. et al. Current status and future potential of transdermal drug delivery. 2004. Nat Rev Drug Discov 3(2): p. 115-24].

[0306] Transdermal transport of solutes is largely controlled by stratum corneum lipid bilayers. Solute transport in

stratum corneum lipid bilayers, like in other lipid bilayer systems, is highly anisotropic and size-dependent. Specifically, lipid bilayers exhibit strong structural heterogeneity that results in spatial variations in solute partition and diffusion coefficients. As a result, molecules are believed to diffuse across skin following a tortuous pathway within either the tail-group (for hydrophobic molecules) or head-group (for hydrophilic molecules) regions, in which transport between bilayers can occur at bilayer–bilayer interfaces or other sites of structural disorganization [Marrink, S. J. and Berendsen, H. J. Permeation Process of Small Molecules across Lipid Membranes Studied by Molecular Dynamics Simulations. 1996. *J. Phys. Chem.* 100(41): p. 16729-16738].

[0307] A few drugs will penetrate the skin effectively. Nicotine, estrogen, scopolamine, fentanyl, and nitroglycerine are among the few drugs that can be successfully delivered transdermally from patches simply because they are relatively small and potent at small doses of 0.1 mg to 15 mg/day [Kanikkannan, N. et al. Structure-activity relationship of chemical penetration enhancers in transdermal drug delivery. 2000. *Curr Med Chem* 7(6): p. 593-608]. Many other drugs can be delivered only when an additional enhancement system is provided to “force” them to pass through the skin. Among several methods of transdermal drug delivery are electroporation, sonophoresis, iontophoresis, permeation enhancers (cyclodextrins), and liposomes.

[0308] Compounds of this invention can be administered via topical use of any of these transdermal delivery methods.

#### Liposomes

[0309] Liposomes are microscopic, fluid-filled pouches whose walls are made of layers of phospholipids identical to those that make up the cell membranes. They are well known and their structures and properties have been thoroughly researched. Essentially, they are small uni- or multi-lamellar lipid/water structures with diameters in the micron range. Liposomes can be formed from a variety of natural phospholipids, such as cholesterol, stearylamine, or phosphatidylcholines. They can be formulated to incorporate a wide range of materials as a payload either in the water or in the lipid compartments.

[0310] Liposomes are extremely versatile and are variable due to their composition. They can be used to deliver vaccines, proteins (enzymes), nucleotides, plasmids, drugs, or cosmetics to the body. Liposomes can be used as carriers for lipophilic drugs like the anti-tumor and the anti-viral derivatives of AZT [Kamps, J. A. et al. Preparation and characterization of conjugates of (modified) human serum albumin and liposomes: drug carriers with an intrinsic anti-HIV activity. 1996. *Biochim Biophys Acta* 1278(2): p. 183-90]. Insulin can also be delivered via liposomes [Muramatsu, K. et al. The relationship between the rigidity of the liposomal membrane and the absorption of insulin after nasal administration of liposomes modified with an enhancer containing insulin in rabbits. 1999. *Drug Dev Ind Pharm* 25(10): p. 1099-105]. For medical uses as drug carriers, the liposomes can also be injected intravenously and when they are modified with lipids, their surfaces become more hydrophilic and hence the circulation time in the bloodstream can be increased significantly. Such so-called “stealth” liposomes are especially being used as carriers for hydrophilic (water soluble) anti cancer drugs like doxorubicin. Taxantrone and others are especially effective in treating diseases that affect the phagocytes of the immune system because they tend to accumulate

in the phagocytes, which recognize them as foreign invaders [Rentsch, K. M. et al. Determination of mitoxantrone in mouse whole blood and different tissues by high-performance liquid chromatography. 1996. *J Chromatogr B Biomed App* 1679(1-2): p. 185-92]. They have also been used experimentally to carry normal genes into a cell to replace defective, disease-causing genes [Guo, W. and Lee, R. J. Efficient gene delivery using anionic liposome-complexed polyplexes (LPDII). 2000. *Biosci Rep* 20(5): p. 419-32].

[0311] Liposomes are also sometimes used in cosmetics because of their moisturizing qualities. It was found that phospholipids combined with water immediately formed a sphere because one end of each molecule is water soluble, while the opposite end is water insoluble.

#### Sonophoresis

[0312] Sonophoresis or phonophoresis has been widely used in sports medicine since the sixties. Controlled studies in humans *in vivo* have demonstrated absence or mild effects of the technique with the parameters currently used (frequency 1-3 MHz, intensity 1-2 W/cm<sup>2</sup>, duration 5-10 mins, continuous or pulse mode). However, it was demonstrated in 1995 that administration of macromolecules with conserved biological activity was feasible in animals *in vivo* using low frequency ultrasound. This led to new research into this method of transdermal administration [Machet, L. and Boucaud, A. Phonophoresis: efficiency, mechanisms and skin tolerance. 2002. *Int J Pharm* 243(1-2): p. 1-15].

[0313] In this method, a short application of ultrasound is used to permeabilize skin for a prolonged period of time. The enhancement induced by ultrasound is particularly significant at low-frequencies ( $f < 100$  kHz). During this period, ultrasonically permeabilized skin may be utilized for drug delivery. In addition, a sample of interstitial fluid or its components may be extracted through permeabilized skin for diagnostic applications. Detailed studies on drug delivery have been performed using insulin and mannitol as model drugs. Studies on diagnostics were performed using glucose as a model analyte [Mitragotri, S. and Kost, J. Low-frequency sonophoresis: a noninvasive method of drug delivery and diagnostics. 2000. *Biotechnol Prog* 16(3): p. 488-92].

[0314] *In vitro*, *in vivo*, as well as clinical studies have also demonstrated the successful effect of low-frequency ultrasound on transdermal drug delivery and glucose extraction. Mechanistic insights gained through a number of investigations have also been reviewed [Mitragotri, S. and Kost, J. Low-frequency sonophoresis: a review. 2004. *Adv Drug Deliv Rev* 56(5): p. 589-601].

[0315] At the School of Pharmacy, Faculty of Sciences, University of Geneva, a study was done to shed light on the mechanism(s) by which low-frequency ultrasound (20 KHz) enhances the permeability of the skin. The physical effects on the barrier and the transport pathway, in particular, were examined. The amount of lipid removed from the intercellular domains of the stratum corneum following sonophoresis was determined by infrared spectroscopy. Transport of the fluorescent probes nile red and calcein, under the influence of ultrasound, was evaluated by laser-scanning confocal microscopy. The results were compared with the appropriate passive control data and with data obtained from experiments in which the skin was exposed simply to the thermal effects induced by ultrasound treatment. A significant fraction (approximately 30%) of the intercellular lipids of the stratum corneum, which are principally responsible for skin barrier

function, were removed during the application of low-frequency sonophoresis. Although the confocal images from the nile red experiments were not particularly informative, ultrasound clearly and significantly (again, relative to the corresponding controls) facilitated transport of the hydrophilic calcein via discrete permeabilized regions, whereas other areas of the barrier were apparently unaffected. Lipid removal from the stratum corneum is implicated as a factor contributing the observed permeation enhancement effects of low-frequency ultrasound [Alvarez-Roman, R. et al. Skin permeability enhancement by low frequency sonophoresis: lipid extraction and transport pathways. 2003. *J Pharm Sci* 92(6): p. 1138-46].

[0316] The impact of low-frequency sonophoresis appears to be much more important than that of high-frequency sonophoresis, with significant increases in transport into and from the skin following its application. Although the mechanism of action remains incompletely defined, cavitation and thermal processes are strongly implicated [Merino, G. et al. Ultrasound-enhanced transdermal transport. 2003. *J Pharm Sci* 92(6): p. 1125-37].

[0317] In another study, application of low-frequency ultrasound was been shown to increase skin permeability, thereby facilitating delivery of macromolecules (low-frequency sonophoresis). The study sought to determine a theoretical description of transdermal transport of hydrophilic permeants induced by low-frequency sonophoresis. Parameters such as pore size distribution, absolute porosity, and dependence of effective tortuosity on solute characteristics were investigated. Pig skin was exposed to low-frequency ultrasound at 58 kHz to achieve different skin resistivities. Transdermal delivery of four permeants [mannitol, luteinizing hormone releasing hormone (LHRH), inulin, dextran] in the presence and absence of ultrasound was measured. The porous pathway model was modified to incorporate the permeant characteristics into the model and to achieve a detailed understanding of the pathways responsible for hydrophilic permeant delivery. The slopes of the  $\log k_p(p)$  versus  $\log R$  graphs for individual solutes changed with solute molecular area, suggesting that the permeability-resistivity correlation for each permeant is related to its size. The tortuosity that a permeant experiences within the skin also depends on its size, where larger molecules experience a less tortuous path. With the modified porous pathway model, the effective tortuosities and skin porosity were calculated independently. The results of this study showed that low-frequency sonophoresis creates pathways for permeant delivery with a wide range of pore sizes. The optimum pore size utilized by solutes is related to their molecular radii [Tezel, A. et al. Description of transdermal transport of hydrophilic solutes during low-frequency sonophoresis based on a modified porous pathway model. 2003. *J Pharm Sci* 92(2): p. 381-93].

[0318] In vitro experiments with full thickness pig skin to measure enhancements of skin conductivity and drug permeability have been performed and ultrasound was applied to pretreat the skin using a sonicator operating at a frequency of either 20 or 40 kHz. Pitting of aluminum foil was also noted to measure cavitation, which is the principal mechanism of low-frequency sonophoresis. The skin conductivity enhancement was found to be inversely proportional to the distance of the horn from the skin. As the intensity increased, skin conductivity enhancement also increased up to a certain threshold, and then dropped off. The intensities ( $I_{max}$ ) at which maximum enhancement occur are about 14 W/cm<sup>2</sup> for 20

kHz and 1.7 W/cm<sup>2</sup> for 40 kHz. These findings may be useful in optimizing low-frequency sonophoresis. Overall, the dependence of transport on ultrasound parameters is similar to that of aluminum foil pitting. Hence, these results support the role of cavitation in low-frequency sonophoresis [Terahara, T. et al. Dependence of low-frequency sonophoresis on ultrasound parameters; distance of the horn and intensity. 2002. *Int J Pharm* 235(1-2): p. 35-42].

[0319] Enhancement of drug transport via low frequency sonophoresis is thought to be mediated through cavitation, the formation and collapse of gaseous bubbles. It has been hypothesized that the efficacy of low-frequency sonophoresis can be significantly enhanced by provision of nuclei for cavitation. In a particular study, two porous resins, Diaion HP20 and Diaion HP2MG (2MG), were used as cavitation nuclei. The effect of these resins on cavitation using pitting of aluminum foil was measured. 2MG showed a higher efficacy in enhancing cavitation compared with Diaion HP20. 2MG was also effective in enhancing transdermal mannitol transport. These results confirmed that the addition of cavitation nuclei such as porous resins further increases the effect of low-frequency ultrasound on skin permeability [Terahara, T. et al. Porous resins as a cavitation enhancer for low-frequency sonophoresis. 2002. *J Pharm Sci* 91(3): p. 753-9].

#### Electroporation

[0320] Electroporation is the transitory structural perturbation of lipid bilayer membranes due to the application of very short (<1 sec) high voltage pulses. Its application to the skin has been shown to increase transdermal drug delivery by several orders of magnitude. Moreover, electroporation used alone or in combination with other enhancement methods, expands the range of drugs (small to macromolecules, lipophilic or hydrophilic, charged or neutral molecules), which can be delivered transdermally. Molecular transport through transiently permeabilized skin by electroporation results mainly from enhanced diffusion and electrophoresis. The efficacy of transport depends on the electrical parameters and the physicochemical properties of drugs. The *in vivo* application of high voltage pulses is well tolerated but muscle contractions are usually induced. The electrode and patch design is an important issue to reduce the discomfort of the electrical treatment in humans [Denet, A. R. et al. Skin electroporation for transdermal and topical delivery. 2004. *Adv Drug Deliv Rev* 56(5): p. 659-74].

#### Iontophoresis

[0321] Iontophoresis or ElectroMotive Drug Administration (EMDA) is a very effective method of delivering drugs to the affected site that is commonly used in many countries including the USA. Instead of injecting the drug (usually a steroid) directly into the inflamed, iontophoresis spreads a high concentration of drug evenly through the tissue applying a low density electrical current for times ranging from minutes to hours that attracts the ions in the molecules of the drug and drives them through the skin to be absorbed by the inflamed tissue.

[0322] Transdermal iontophoretic delivery of hydrocortisone solubilized in an aqueous solution of hydroxypropyl-beta-cyclodextrin (HP-beta-CyD) has been investigated and compared with chemical enhancement of co-solvent formulations [Chang, S. L. and Banga, A. K. Transdermal iontophoretic delivery of hydrocortisone from cyclodextrin solu-

tions. 1998. *J Pharm Pharmacol* 50(6): p. 635-40]. The passive permeation of hydrocortisone through human cadaver skin was higher when delivered from propylene glycol than when delivered after solubilization in an aqueous solution of HP-beta-CyD. However, the iontophoretic delivery of the 1% hydrocortisone-9% HP-beta-CyD solution was higher than the amount delivered passively by the 1% hydrocortisone-propylene glycol formulation, even if oleic acid was used as a chemical enhancer. Iontophoretic delivery of 1% hydrocortisone with 3% or 15% HP-beta-CyD was lower than that of the 9% HP-beta-CyD solution. These data suggest that free hydrocortisone rather than complexes is predominantly delivered iontophoretically through the skin and the HP-beta-CyD complex serves as a carrier to replenish depletion of hydrocortisone. HP-beta-CyD prevents hydrocortisone from forming a skin reservoir. Iontophoresis provides better enhancement of transdermal delivery of hydrocortisone than the chemical approach when just sufficient HP-beta-CyD is added to solubilize the hydrocortisone [Chang, S. L. and Banga, A. K. Transdermal iontophoretic delivery of hydrocortisone from cyclodextrin solutions. 1998. *J Pharm Pharmacol* 50(6): p. 635-40].

#### Penetration Enhancers

[0323] Another long-standing approach for improving transdermal drug delivery uses penetration enhancers (also called sorption promoters or accelerants), which penetrate into skin to reversibly decrease the barrier resistance. Numerous compounds have been evaluated for penetration enhancing activity, including sulphoxides (such as dimethylsulphoxide, DMSO), Azones (e.g. laurocapram), pyrrolidones (for example 2-pyrrolidone, 2P), alcohols and alkanols (ethanol, or decanol), glycols (for example propylene glycol, PG, a common excipient in topically applied dosage forms), surfactants (also common in dosage forms) and terpenes. Many potential sites and modes of action have been identified for skin penetration enhancers; the intercellular lipid matrix in which the accelerants may disrupt the packing motif, the intracellular keratin domains or through increasing drug partitioning into the tissue by acting as a solvent for the permeant within the membrane. Further potential mechanisms of action, for example with the enhancers acting on desmosomal connections between corneocytes or altering metabolic activity within the skin, or exerting an influence on the thermodynamic activity/solubility of the drug in its vehicle are also feasible [Williams, A. C. and Barry, B. W. Penetration enhancers. 2004. *Adv Drug Deliv Rev* 56(5): p. 603-18].

[0324] Cyclodextrins are cyclic oligosaccharides with a hydrophilic outer surface and a somewhat lipophilic central cavity. Cyclodextrins are able to form water-soluble inclusion complexes with many lipophilic water-insoluble drugs. In aqueous solutions, drug molecules located in the central cavity are in a dynamic equilibrium with free drug molecules. Furthermore, lipophilic molecules in the aqueous complexation media will compete with each other for a space in the cavity. Due to their size and hydrophilicity only insignificant amounts of cyclodextrins and drug/cyclodextrin complexes are able to penetrate into lipophilic biological barriers, such as intact skin. In general, cyclodextrins enhance topical drug delivery by increasing the drug availability at the barrier surface. At the surface the drug molecules partition from the cyclodextrin cavity into the lipophilic barrier. Thus, drug delivery from aqueous cyclodextrin solutions is both diffusion controlled and membrane controlled. It appears that

cyclodextrins can only enhance topical drug delivery in the presence of water [Loftsson, T. and Masson, M. Cyclodextrins in topical drug formulations: theory and practice. 2001. *Int J Pharm* 225(1-2): p. 15-30].

[0325] It is well known that cyclodextrins can enhance the permeation of poorly soluble drugs through biological membranes. However, the permeability will decrease if cyclodextrin is added in excess of the concentration needed to solvate the drug. The effect of cyclodextrins cannot be explained as solely due to increased solubility of the drug in the aqueous donor phase nor can it be explained by assuming that cyclodextrins act as classical permeation enhancers, i.e. by decreasing the barrier function of the lipophilic membrane. Researchers have modeled the effect of cyclodextrins in terms of mixed barrier consisting of both diffusion and membrane controlled diffusion, where the diffusion of the drug in the aqueous diffusion layer is significantly slower than in the bulk of the donor. This diffusion model is described by simple mathematical equation where the properties of the system are expressed in terms of two constants P(M)/Kd and M1/2. Data for the permeation of hydrocortisone through hairless mouse skin in the presence of various cyclodextrins, and cyclodextrin polymer mixtures, were fitted to obtain values for these two constants. The rise in flux with increased cyclodextrin complex concentration and fall with excess cyclodextrin was accurately predicted. Data for the permeation of drugs through semi-permeable cellophane membrane could also be fitted to the equation. It was concluded that cyclodextrins act as permeation enhancers carrying the drug through the aqueous barrier, from the bulk solution towards the lipophilic surface of biological membranes, where the drug molecules partition from the complex into the lipophilic membrane [Masson, M. et al. Cyclodextrins as permeation enhancers: some theoretical evaluations and in vitro testing. 1999. *J Control Release* 59(1): p. 107-18].

#### Example 1

##### Isolation and Identification of FL3P

[0326] The following assays were performed in order to verify that fructose-lysine (FL) could be identified in its phosphorylated state, e.g., FL3P. A <sup>31</sup>P NMR analysis of a perchloric acid extract of diabetic rat kidneys was performed and showed a new sugar monophosphate resonance at 6.24 ppm which is not observed in non-kidney tissue and is present at greatly reduced levels in non-diabetic kidney. The compound responsible for the observed resonance was isolated by chromatography of the extract on a microcrystalline cellulose column using 1-butanol-acetic acid-water (5:2:3) as eluent. The structure was determined by proton 2D COSY to be fructose-lysine 3-phosphate. This was later confirmed by injecting animals with FL, prepared as previously described (Finot and Mauson, 1969, *Hely. Chim. Acta*, 52:1488), and showing direct phosphorylation to FL3P.

[0327] Using FL specifically deuterated in position-3 confirmed the position of the phosphate at carbon-3. This was performed by analyzing the <sup>31</sup>P NMR spectra, both coupled and decoupled. The normal P—O—C—H coupling produces a doublet in FL3P with a J value of 10.3 Hz; whereas P—O—C-D has no coupling and produces a singlet both coupled and decoupled, as was found for 3-deuterated FL3P. A unique property of FL3P is that when treated with sodium borohy-

dride it is converted into two new resonances at 5.85 and 5.95 ppm, which correspond to mannitol and sorbitol-lysine 3-phosphates.

#### Example 2

##### Synthesis of FL3P

[0328] 1 mmol of dibenzyl-glucose 3-phosphate and 0.25 mmol of  $\alpha$ -carbobenzoxy-lysine was refluxed in 50 ml of MeOH for 3 hours. The solution was diluted with 100 ml water and chromatographed on a Dow-50 column (2.5 $\times$ 20 cm) in the pyridinium form and eluted first with water (200 ml) and then with 600 ml buffer (0.1M pyridine and 0.3M acetic acid). The target compound eluted at the end of the water wash and the beginning of the buffer wash. The results demonstrated that removal of the cbz and benzyl blocking groups with 5% Pd/C at 20 psi of hydrogen gave FL3P in 6% yield.

#### Example 3

##### Enzymatic Production of FL3P from FL and ATP and Assay for Screening Inhibitors

[0329] Initially  $^{31}$ P NMR was used to demonstrate kinase activity in the kidney cortex. A 3 g sample of fresh pig kidney cortex was homogenized in 9 ml of 50 mM Tris.HCl containing 150 mM KCl, 5 mM DTT, 15 mM MgCl<sub>2</sub>, pH 7.5. This was centrifuged at 10,000 g for 30 minutes, and then the supernatant was centrifuged at 100,000 g for 60 minutes. Ammonium sulfate was added to 60% saturation. After 1 hour at 4° C. the precipitate was collected by centrifugation and dissolved in 5 ml. of original buffer. A 2 ml aliquot of this solution was incubated with 10 mM ATP and 10 mM of FL (prepared as in Example 1, above) for 2 hours at 37° C. The reaction was quenched with 300  $\mu$ l of perchloric acid, centrifuged to remove protein, and desalts on a column of Sephadex G 10 (5 $\times$ 10 cm).  $^{31}$ P NMR analysis of the reaction mixture detected formation of FL3P.

[0330] Based on the proof of kinase activity thus obtained, a radioactive assay was developed. This assay was designed to take advantage of the binding to Dow-50 cation exchange resin by FL3P. This characteristic of FL3P was discovered during efforts to isolate it. Since most phosphates do not bind to this resin, it was suspected that the bulk of all compounds that react with ATP as well as any excess ATP would not be bound. The first step was to determine the amount of resin required to remove the ATP in the assay. This was accomplished by pipetting the mixture into a suspension of 200 mg of Dow-1 in 0.9 ml H<sub>2</sub>O, vortexing, and centrifuging to pack the resin. From this 0.8 ml of supernatant was pipetted onto 200 mg of fresh dry resin, vortexed and centrifuged. A 0.5 ml volume of supernatant was pipetted into 10 ml of Ecoscint A and counted. Residual counts were 85 cpm. This procedure was used for the assay. The precipitate from 60% ammonium sulfate precipitation of the crude cortex homogenate was redissolved in the homogenate buffer at 4° C. The assay contains 10 mM  $\gamma^{33}$ P-ATP (40,000 cpm), 10 mM FL, 150 mM KCl, 15 mM MgCl<sub>2</sub>, 5 mM DTT in 0.1 ml of 50 mM Tris.HCl, pH 7.5. The relationship between rates of FL3P production and enzyme concentration was determined using triplicate determinations with 1, 2, and 4 mg of protein for 30 minutes at 37° C. Blanks run concurrently without FL were subtracted

and the data recorded. The observed activity corresponds to an approximate FL3P synthesis rate of 20 nmols/hr/mg protein.

#### Example 4

##### Inhibition of the Formation of 3-Deoxyglucosone by Meglumine and Various Polyolysines

[0331] a. General Polyolysine Synthesis:

[0332] The sugar (11 mmoles),  $\alpha$ -carbobenzoxy-lysine (10 mmols) and NaBH<sub>3</sub>CN (15 mmoles) were dissolved in 50 ml of MeOH—H<sub>2</sub>O (3:2) and stirred at 25° C. for 18 hours. The solution was treated with an excess of Dow-50 (H) ion exchange resin to decompose excess NaBH<sub>3</sub>CN. This mixture (liquid plus resin) was transferred onto a Dow-50 (H) column (2.5 $\times$ 15 cm) and washed well with water to remove excess sugar and boric acid. The carbobenzoxy-polyolysine was eluted with 5% NH<sub>4</sub>OH. The residue obtained upon evaporation was dissolved in water-methanol (9:1) and reduced with hydrogen gas (20 psi) using a 10% palladium on charcoal catalyst. Filtration and evaporation yields the polyolysine.

[0333] b. Experimental Protocol for Reduction of Urinary and Plasma 3-Deoxyglucosone by Sorbitolysine, Mannitolysine and Galactitolysine:

[0334] Urine was collected from six rats for three hours. A plasma sample was also obtained. The animals were then given 10  $\mu$ mos of either sorbitolysine, mannitolysine, or galactitolysine by intraperitoneal injection. Urine was collected for another three hours, and a plasma sample obtained at the end of the three hours.

[0335] a. 3-Deoxyglucosone was Measured in the Samples, as Described in Example 5, Below, and Variable Volumes were Normalized to Creatinine. The average Reduction of Urinary 3-Deoxyglucosone was 50% by Sorbitolysine, 35% by Mannitolysine and 35% by Galactitolysine. Plasma 3-Deoxyglucosone was Reduced 40% by Sorbitolysine, 58% by Mannitolysine and 50% by Galactitolysine.

[0336] b. Use of Meglumine to Reduce Urinary 3-Deoxyglucosone:

[0337] Three rats were treated as in b), immediately above, except meglumine (100  $\mu$ mos) was injected intraperitoneally instead of the above-mentioned lysine derivatives. Three hours after the injection the average 3-deoxyglucosone concentrations in the urine were decreased 42%.

#### Example 5

##### Elevation of Urinary FL, 3DG and 3DF in Humans Following Ingestion of Glycated Protein

[0338] a. Preparation of Glycated Protein Containing Food Product:

[0339] 260 g of casein, 120 g of glucose and 720 ml of water were mixed to give a homogeneous mixture. This mixture was transferred to a metal plate and heated at 65° C. for 68 hours. The resulting cake was then pulverized to a coarse powder.

[0340] This powder contained 60% protein as determined by the Kjeldahl procedure.

[0341] b. Measurement of Glycated Lysine Content:

[0342] One gram of the powder prepared as in step a., above, was hydrolyzed by refluxing with 6N HCl for 20 hours. The resulting solution was adjusted to pH 1.8 with NaOH solution and diluted to 100 ml. The fructoselysine

content was measured on an amino acid analyzer as furosine, the product obtained from acid hydrolysis of fructoselysine. In this way, it was determined that the cake contained 5.5% (w/w) fructoselysine.

[0343] c. Experimental Protocol:

[0344] Volunteers spent two days on a fructoselysine-free diet and then consumed 22.5 g of the food product prepared as described herein, thus effectively receiving a 2 gram dose of fructoselysine. Urine was collected at 2 hour intervals for 14 hours and a final collection was made at 24 hours.

[0345] d. Measurement of FL, 3DG and 3DF in Urine:

[0346] FL was measured by HPLC with a Waters 996 diode Array using a Waters C18 Free Amino Acid column at 46° C. and a gradient elution system of acetonitrile-methyl alcohol-water (45:15:40) into acetonitrile-sodium acetate-water (6:2: 92) at 1 ml/min. Quantitation employed an internal standard of meglumine.

[0347] 3DF was measured by HPLC after deionization of the sample. Analyses were performed on a Dionex DX-500 HPLC system employing a PA1 column (Dionex) and eluting with 32 mM sodium hydroxide at 1 ml/min. Quantitation was performed from standard curves obtained daily with synthetic 3DF.

[0348] 3DG was measured by GC-MS after deionization of the sample. 3DG was derivatized with a 10-fold excess of diaminonaphthalene in PBS. Ethyl acetate extraction gave a salt free fraction which was converted to the trimethyl silyl ethers with Tri-Sil (Pierce). Analysis was performed on a Hewlett-Packard 5890 selected ion monitoring GC-MS system. GC was performed on a fused silica capillary column (DB-5,25 mx.25 mm) using the following temperature program: injector port 250° C., initial column temperature 150° C. which is held for 1 minute, then increased to 290° C. at 16° C./minute and held for 15 minutes. Quantitation of 3DG employed selected ion monitoring using an internal standard of U-13C-3DG.

[0349] The results of the experiments described in this example are now presented.

[0350] The graph depicted in FIG. 3 represents production of FL, 3DF, and 3DG in the urine of one volunteer after consuming the glycated protein. The rapid appearance of all three metabolites is clearly evident. Both 3DF and 3DG show a slight elevation even after twenty-four hours.

[0351] The graph shown in FIG. 4 represents the formation of 3DF in each of the members of a seven-person test group. A similar pattern was seen in all cases. As demonstrated in FIG. 4, 3DF excretion peaks about 4 hours after the FL bolus and a slight elevation of 3DF is noticeable even 24 h after the bolus.

#### Example 6

##### Effects of Increased Dietary Uptake of Glycated Proteins

[0352] N-acetyl- $\beta$ -glucosaminidase (NAGase) is an enzyme excreted into the urine in elevated concentration in diabetics. It is thought to be an early marker of tubular damage, but the pathogenesis of increased NAGase in urine is not well understood. The increased urinary output of NAGase in diabetics has been proposed to be due to activation of lysosomes in proximal tubules induced by diabetes with an increased output into the urine rather than destruction of cells.

[0353] Rats were fed a diet containing 3% glycated protein or control feed over several months. The urinary output of

NAGase and 3DF were determined at various times, as indicated in FIG. 5. The amount of 3DG excreted in urine was also determined.

[0354] The results obtained in this example demonstrate that in all comparisons 3DF and NAGase levels are elevated in the experimental group relative to the control. Thus, animals fed glycated protein excrete excess NAGase into their urine, similar to results obtained with diabetics. NAGase output increased by approximately 50% in the experimental group, compared with control animals. The experimental animals also had a five-fold increase in urine 3DF compared with controls. Urinary 3DF was found to correlate extremely well with 3DG, as can be seen in FIGS. 5 and 6.

#### Example 7

##### Electrophoretic Analysis of Kidney Proteins

[0355] Two rats were injected daily with 5  $\mu$ mol of either FL or mannitol (used as a control) for 5 days. The animals were sacrificed and the kidneys removed and dissected into the cortex and medulla. Tissues were homogenized in 5 volumes of 50 mM Tris.HCl containing 150 mM KCl, 15 mM MgCl<sub>2</sub> and 5 mM DTT, pH 7.5. Cellular debris was removed by centrifugation at 10,000 $\times$ g for 15 minutes, and the supernatant was then centrifuged at 150,000 $\times$ g for 70 minutes. The soluble proteins were analyzed by SDS PAGE on 12% polyacrylamide gels as well as on 4-15 and 10-20% gradient gels.

[0356] It was found that in all cases, lower molecular weight bands were missing or visually reduced from the kidney extract of the animal injected with FL when compared with the animal injected with mannitol.

#### Example 8

##### Synthesis of 3-O-Methylsorbitolysine

[0357] 3-OMe glucose (25 grams, 129 mmol) and  $\alpha$ -Cbz-lysine (12 grams, 43 mmol) were dissolved in 200 ml of water-methanol (2:1). Sodium cyanoborohydride (10 grams, 162 mmol) was added and the reaction stirred for 18 days at room temperature. Reaction of  $\alpha$ -Cbz-lysine was monitored by thin layer chromatography on silica gel employing 1-butanol-acetic acid-water (4:1:1) using ninhydrin for visualization. The reaction was complete when no  $\alpha$ -Cbz-lysine remained. The solution was adjusted to pH 2 with HCl to decompose excess cyanoborohydride, neutralized and then applied to a column (5 $\times$ 50 cm) of Dowex-50 (H<sup>+</sup>) and the column washed well with water to remove excess 3-O-meglucose. The target compound was eluted with 5% ammonium hydroxide. After evaporation the residue was dissolved in 50 ml of water-methanol (2:1) and 10% Pd/C (0.5 gram) was added. The mixture was shaken under 20 psi of hydrogen for 1 hr. The charcoal was filtered off and the filtrate evaporated to a white powder (10.7 gram, 77% yield based on  $\alpha$ -Cbz-lysine) that was homogeneous when analyzed by reversed phase HPLC as the phenylisothiocyanate derivative. Elemental analysis: Calculated for C<sub>13</sub>H<sub>28</sub>N<sub>2</sub>O<sub>7</sub>CH<sub>3</sub>OH<sub>2</sub>H<sub>2</sub>O C, 42.86; H, 9.18; N, 7.14. Found: C, 42.94; H, 8.50; N, 6.95.

[0358] Compounds related to the structure of 3-O-methylsorbitolysine, as discussed elsewhere herein, may be made, e.g., by glycation of a selected nitrogen- or oxygen-containing starting material, which may be an amino acid, polyaminoacid, peptide or the like, with a glycation agent, such as

fructose, which may be chemically modified, if desired, according to procedures well known to those skilled in the art.

#### Example 9

##### Additional Assay for FL3P Kinase Activity

[0359] a. Preparation of Stock Solutions:

[0360] An assay buffer solution was prepared which was 100 mM HEPES pH 8.0, 10 mM ATP, 2 mM MgCl<sub>2</sub>, 5 mM DTT, 0.5 mM PMSF. A fructosyl-spermine stock solution was prepared which was 2 mM fructosyl-spermine HCl. A spermine control solution was prepared which was 2 mM spermine HCl.

[0361] b. Synthesis of Fructosyl-Spermine:

[0362] Synthesis of fructosyl-spermine was performed by an adaptation of a known procedure (J. Hodge and B. Fisher, 1963, Methods Carbohydr. Chem., 2:99-107). A mixture of spermine (500 mg), glucose (500 mg), and sodium pyrosulfite (80 mg) was prepared in a molar ratio of 8:4:1 (spermine: glucose:pyrosulfite) in 50 ml of methanol-water (1:1) and refluxed for 12 hours. The product was diluted to 200 ml with water and loaded onto a DOW-50 column (5×90 cm). The unreacted glucose was removed by 2 column volumes of water and the product and unreacted spermine were removed with 0.1M NH<sub>4</sub>OH. Pooled peak fractions of the product were lyophilized and concentration of fructosyl-spermine was determined by measuring the integral of the C-2 fructosyl peak in a quantitative <sup>13</sup>C NMR spectrum of the product (NMR data collected with a 45° pulse, a 10 second relaxation delay and without NOE decoupling).

[0363] c. Kinase Assay to Determine Purification:

[0364] An incubation mixture was prepared including 10 µl of the enzyme preparation, 10 µl of assay buffer, 1.0 µCi of <sup>33</sup>P ATP, 10 µl of fructosyl-spermine stock solution and 70 µl of water and incubated at 37° C. for 1 hour. At the end of the incubation 90 µl (2×45 µl) of the sample was spotted onto two 2.5 cm diameter cellulose phosphate disks (Whatman P-81) and allowed to dry. The disks were washed extensively with water. After drying, the disks were placed in scintillation vials and counted.

[0365] Each enzyme fraction was assayed in duplicate with an appropriate spermine control.

#### Example 10

##### Kidney Pathology Observed in Test Animals on Glycated Protein Diet

[0366] Three rats were maintained on a glycated protein diet (20% total protein; 3% glycated) for 8 months and compared to 9 rats of the same age maintained on a control diet. The glycated protein diet consisted of a standard nutritious diet to which 3% glycated protein had been substituted for nonglycated protein. The glycated protein was made by mixing together casein and glucose (2:1), adding water (2× the weight of the dried material), and baking the mixture at 60° C. for 72 hours. The control was prepared in the same way except that no water was used and the casein and glucose were not mixed prior to baking.

[0367] The primary finding was a substantial increase in damaged glomeruli in the animals on the glycated diet. Typical lesions observed in these animals were segmental sclerosis of the glomerular tuft with adhesion to Bowman's capsule, tubular metaplasia of the parietal epithelium and interstitial fibrosis. All animals on the glycated protein diet, and only one

of the animals on the control diet showed more than 13% damaged glomeruli. The probability of this happening by chance is less than 2%. In addition to the pathological changes observed in the glomeruli, a number of hyalinated casts within tubules were observed. More of these hyalinated casts were found in animals on the glycated diet, although these were not quantitated. Increased levels of NAGase were also observed in the animals on the glycated diet.

[0368] Based on the results of this experiment, the glycated diet appeared to cause the test animals to develop a series of histological lesions similar to those seen in the diabetic kidney.

#### Example 12

##### Carcinogenic Effects of Fructoselysine Pathway

[0369] To investigate the carcinogenic potential of metabolites formed in the fructoselysine pathway, experiments were conducted on a strain of rats with a high susceptibility to kidney carcinomas.

[0370] Four rats were put on a glycated protein diet and three rats on a control diet. After ten weeks on the diet, the animals were sacrificed and their kidneys examined.

[0371] In all four animals on the diet, kidney carcinomas of size greater than 1 mm were found, whereas no lesions this large were found in the control animals. The probability of this happening by chance is less than 2%.

[0372] The data demonstrate that there are elevated 3DG levels, caused by the excess fructoselysine coming from the glycated protein in the diet, in the kidney tubular cells (known to be the cell of origin of most kidney carcinomas), and the 3DG can interact with the cellular DNA, leading to a variety of mutagenic and ultimately carcinogenic events. The possibility exists that this process is important in the development of human cancers in the kidney and elsewhere.

#### Example 13

##### Dietary Effects of Glycated Protein Diet on Renal Cell Carcinoma in Susceptible Rats

[0373] In addition to the experiments described above, experiments were performed to assess the relationship between a glycated protein diet and renal cell carcinoma.

[0374] Twenty-eight rats with a mutation making them susceptible to the development of kidney carcinoma were divided into two cohorts. One cohort was fed a glycated protein diet and the other cohort was on a control diet. The glycated protein diet consisted of a standard nutritious diet to which 3% glycated protein had been added. The glycated protein was made by mixing together casein and glucose (2:1), adding water (2× the weight of the dried material), and baking the mixture at 60° C. for 72 hours. The control was prepared in the same way except that no water was used and the casein and glucose were not mixed prior to baking. Rats were placed on the diets immediately following weaning at three weeks of age and maintained on the diets ad libitum for the next 16 weeks. The animals were then sacrificed, the kidneys fixed, and hematoxylin and eosin sections were prepared.

[0375] The histological samples were examined by a pathologist. Four types of lesions were identified. These include: cysts; very small collections of tumor-like cells, typically less than 10 cells; small tumors, 0.5 mm or less; and tumors greater than 0.5 mm. For the four types of lesions,

more lesions were observed in the animals on the glycated diet than on the control diet, as shown in the following table (Table A).

TABLE A

	CYSTS	$\leq 10$ CELLS	$\leq 0.5$ mm	$> 0.5$ mm	TOTAL
CONTROL	2	9	9	3	23
GLYCATED	9	21	32	6	68

[0376] To summarize the results, the average number of lesions per kidney section was computed for each diet. These were  $0.82 \pm 0.74$  and  $2.43 \pm 2.33$  in the control and glycated diet, respectively. The likelihood of this happening by chance is about 2 in 100,000.

[0377] These results provide strong support for the premise that the effects of the lysine recovery pathway, the discovery of which underlies the present invention, extend to causing mutations, and thus produce a carcinogenic effect as well. These results provide a basis for the development of therapeutic methods and agents to inhibit this pathway in order to reduce cancer in the kidney as well as in other organs where this pathway may have similar effects.

#### Example 14

##### Urinary Excretion of 3-Deoxy-Fructose is Indicative of Progression to Microalbuminuria in Patients with Type I Diabetes

[0378] As set forth herein, serum levels of the glycation intermediate, three deoxy-glucosone (3DG) and its reductive detoxification product, three deoxy-fructose (3DF), are elevated in diabetes. The relationship between baseline levels of these compounds and subsequent progression of microalbuminuria (MA) has been examined in a group of 39 individuals from a prospective cohort of patients at the Joslin Diabetes Center with insulin-dependent diabetes mellitus (IDDM) and microalbuminuria (based on multiple measurements during the two years of baseline starting between 1990-1993) and not on ACE inhibitors.

[0379] Baseline levels of 3DF and 3DG in random spot urines were measured by HPLC and GC-MS. Individuals that progressed to either a higher level of MA or proteinuria in the next four years ( $n=24$ ) had significantly higher baseline levels of log 3DF/urinary creatinine ratios compared to non-progressors ( $n=15$ ) ( $p=0.02$ ).

[0380] Baseline levels determined in this study were approximately  $0.24 \mu\text{mole}/\text{mg}$  of creatinine in the progressors vs. approximately  $0.18 \mu\text{mole}/\text{mg}$  of creatinine ratios in the non-progressors. Baseline 3DG/urine creatinine ratios did not differ between the groups. Adjustment of the baseline level of  $\text{HgA}_{1c}$  (the major fraction of glycosylated hemoglobin) did not substantially alter these findings. These results provide additional evidence of the association between urinary 3DF and progression of kidney complications on diabetes.

[0381] a. Quantification of 3-Deoxyfructose:

[0382] Samples were processed by passing a 0.3 ml aliquot of the test sample through an ion-exchange column containing 0.15 ml of AG 1-X8 and 0.15 ml of AG 50W-X8 resins. The columns were then washed twice with 0.3 ml deionized water, aspirated to remove free liquid and filtered through a 0.45 mm Millipore filter.

[0383] Injections (50  $\mu\text{l}$ ) of the treated samples were analyzed using a Dionex DX 500 chromatography system. A carbopac PA1 anion-exchange column was employed with an eluant consisting of 16% sodium hydroxide (200 mM) and 84% deionized water. 3DF was detected electrochemically using a pulsed amperometric detector. Standard 3DF solutions spanning the anticipated 3DF concentrations were run both before and after each unknown sample.

[0384] b. Measurement of Urine Creatinine:

[0385] Urine creatinine concentrations were determined by the end-point colorimetric method (Sigma Diagnostic kit 555-A) modified for use with a plate reader. Creatinine concentrations were assessed to normalize urine volumes for measuring metabolite levels present therein.

[0386] c. Measurement of Albumin in the Urine:

[0387] To assess albumin levels in the urine of the test subjects, spot urines were collected and immunoephelometry performed on a BN 100 apparatus with the N-albumin kit (Behring). Anti-albumin antibodies are commercially available. Albumin levels in urine may be assessed by any suitable assay including but not limited to ELISA assays, radioimmunoassays, Western, and dot blotting.

[0388] Based on the data obtained in the study of the Joslin Diabetes Center patients, it appears that elevated levels of urinary 3DF are associated with progression to microalbuminuria in diabetes. This observation provides a new diagnostic parameter for assessing the likelihood of progression to serious kidney complications in patients afflicted with diabetes.

#### Example 15

##### 3-O-Methyl Sorbitolysine Lowers Systemic Levels of 3DG in Normal and Diabetic Rats

[0389] A cohort of twelve diabetic rats was divided into two groups of six. The first group received saline-only injections, and the second received injections of 3-O-methyl sorbitolysine (50 mg/kg body weight) in saline solution. The same procedure was conducted on a cohort of twelve non-diabetic rats.

[0390] As summarized in Table B, within one week, the 3-O-methyl sorbitolysine treatment significantly reduced plasma 3DG levels as compared to the respective saline controls in both diabetic and non-diabetic rats.

TABLE B

##### 3-O-Methyl sorbitolysine (3-OMe) reduces plasma 3DG levels in diabetic and non-diabetic rats.

	Diabetic rats	Non-diabetic rats
Saline only	$0.94 \pm 0.28 \mu\text{M}$ (n = 6)	$0.23 \pm 0.07 \mu\text{M}$ (n = 6)
3-OMe	$0.44 \pm 0.10 \mu\text{M}$ (n = 6)	$0.13 \pm 0.02 \mu\text{M}$ (n = 7)
% Reduction	53%	43%
t-test	$p = 0.0006$	$p = 0.0024$

[0391] The ability of 3-O-methyl sorbitolysine to reduce systemic 3DG levels suggests that diabetic complications other than nephropathy (e.g., retinopathy and stiffening of the aorta) may also be controllable by amadorase inhibitor therapy.

#### Example 16

##### Locus of 3-O-Methyl Sorbitolysine Uptake In Vivo is the Kidney

[0392] Six rats were injected intraperitoneally with 13.5 nmoles (4.4 mg) of 3-O-methyl sorbitolysine. Urine was

collected for 3 hours, after which the rats were sacrificed. The tissues to be analyzed were removed and freeze clamped in liquid nitrogen. Perchloric acid extracts of the tissues were used for metabolite analysis. The tissues examined were taken from the brain, heart, muscle, sciatic nerve, spleen, pancreas, liver, and kidney. Plasma was also analyzed.

[0393] The only tissue extract found to contain 3-O-methyl sorbitolysine was that of the kidney. The urine also contained 3-O-methyl sorbitolysine, but plasma did not. The percentage of the injected dose recovered from urine and kidney varied between 39 and 96%, as shown in Table C, below.

TABLE C

Rat #	nmols 3OMeSL* Injected	Nmols 3OMeSL in urine	nmols 3OMeSL in kidneys	total 3OMeSL recovered	% 3OMeSL recovered
2084	13500	2940	10071	13011	96.4
2085	13500	1675	6582	8257	61.2
2086	13500	1778	5373	7151	53.0
2087	13500	2360	4833	7193	53.3
2088	13500	4200	8155	12355	91.5
2089	13500	1355	3880	5235	38.8

\*3-O-methyl sorbitolysine

#### Example 17

##### Amadorase/Fructosamine Kinase Activity Accounts for a Majority of 3DG Production

[0394] Enzymatic production of 3DG was demonstrated in an *in vitro* assay with various key components (10 mM Mg-ATP, partially purified amadorase, 2.6 mM FL) omitted from the reaction in order to assess their importance in 3DG production.

[0395] The results show that 3DG production is 20-fold higher in the presence of kidney extract containing amadorase and its substrates (compare Table D, reactions 1 and 3). Clearly, the vast majority of 3DG production is enzymatically mediated in the presence of amadorase.

TABLE D

Amadorase-dependent production of 3DG after 24 hours					
Reaction	Amadorase	ATP	FL (nM)	FL3P (nM)	3DG (nM)
1	+	+	2.6	0.2	1.58
2	+	-	2.6	0	0.08
3	-	+	2.6	0	0.09
4	-	-	2.6	0	0.08
5	+	+	0	0	0
6	-	+	0	0	0

#### Example 18

##### Effects of 3DG, and Inhibition of 3DG, on Collagen Crosslinking

[0396] Collagen is present at high levels in skin. To this end, it was determined what effect 3DG has on collagen crosslinking.

[0397] Collagen I was incubated in the presence or absence of 3DG *in vitro*. Calf skin collagen Type I (1.3 mg; Sigma) was incubated in 20 mM Na-phosphate buffer, pH 7.25, either alone, with 5 mM 3DG, or with 5 mM 3DG plus 10 mM

arginine, in a total volume of 1 ml at 37° C. for 24 hours and then frozen and lyophilized. The residue was dissolved in 0.5 ml of 70% formic acid and cyanogen bromide was added (20:1, w/w). This solution was incubated at 30° C. for 18 hours. Samples were dialyzed against 0.125 M Tris, pH 6.8, containing 2% SDS and 2% glycerol, in dialysis tubing with a molecular weight cutoff of 10,000. The samples were all adjusted to a volume of 1 ml. The extent of collagen crosslinking was determined by applying equal volumes of sample and analyzing by SDS-PAGE electrophoresis (16.5% Tris-tricine gel), as determined by the effects of 3DG on the migration of collagen.

[0398] It was found that treatment of collagen with 3DG caused the collagen to migrate as if it had a higher molecular weight, which is indicative of crosslinking. The image of the silver-stained gel in FIG. 12 demonstrates that there are fewer high molecular bands in the groups containing collagen alone or collagen plus 3DG plus arginine. There are more high molecular weight bands in the group treated with 3DG, in the absence of a 3DG inhibitor. There appears to be more protein in the sample treated with 3DG alone. Because all three samples started with the same amount of protein, without being bound by theory, it can be concluded that during dialysis fewer peptides escaped from the 3DG treated sample because more crosslinks were produced and higher molecular weight proteins were retained. In other words, there appears to be less protein in the control and 3DG plus arginine groups, because smaller molecular peptides diffused out during dialysis.

#### Example 19

##### Localization of 3DG in Skin

[0399] The invention as described in the present disclosure identifies for the first time the presence of 3DG in skin.

[0400] A mouse skin model was used. One centimeter (1 cm) squares of skin were prepared and subjected to extraction with perchloric acid. 3DG was measured as described above. Six mice were used and the average amount of 3DG detected in the skin was 1.46+/-0.3 µM. This value was substantially higher than the plasma concentrations of 3DG detected in the same animals (0.19+/-0.05 µM). These data, and the data described below in Example 20, suggest that the high levels of 3DG in the skin are due to production of 3DG in the skin.

#### Example 20

##### Localization of Amadorase mRNA in Skin

[0401] Although high levels of 3DG were found in skin (see previous Example), it was not known whether the 3DG was formed locally and whether skin had the ability to produce 3DG enzymatically. The presence of amadorase mRNA was analyzed and was utilized as one measure of the ability of skin to produce the 3DG present in skin (see previous example).

[0402] PolyA+ messenger RNA isolated from human kidney and skin was purchased from Stratagene. The mRNA was used in RT-PCR procedures. Using the published sequence for amadorase (Delpierre et al., 2000, Diabetes 49:10:1627-1634; Szwergold et al., 2001, Diabetes 50:2139-2147), a reverse primer to the 3' terminal end of the gene (bp 930-912) was subjected to RT to create a cDNA template for PCR. This same primer was used along with a forward primer from the middle of the amadorase gene (bp 412-431) to amplify the amadorase gene from the cDNA template. The product of the

PCR should be a 519 bp fragment. Human skin and kidney samples were subjected to RT-PCR and analyzed by agarose gel electrophoresis, as were controls which contained no cDNA templates.

[0403] The results demonstrate that skin does indeed express amadorase mRNA. Subsequent expression of the protein would account for production of 3DG in skin. As expected, a 519 bp product was observed (see FIG. 13). Not only was the 519 bp fragment found in kidney (lane 1), it was also found in skin (lane 3). The 519 bp fragment was not detected in the groups which received no cDNA template (lanes 2 and 4).

#### Example 21

##### Effects of Fructoselysine on Kidney Cells In Vitro

[0404] As described above, a diet high in glycated proteins, e.g., fructoselysine, has a profound effect on metabolism in vivo. Therefore, the effects of fructoselysine were tested directly on kidney cells in vitro.

[0405] The results demonstrate that fructoselysine administered to kidney cells in vitro causes an increase in type IV collagen levels in the cells. Type IV collagen production was measured in mouse mesangial cells. Controls (grown with 10% glucose) produced 300 ng of Type IV collagen per

10,000 cells, whereas fructoselysine treated cells (5 or 10 mM fructoselysine with 10 mM glucose) produced 560 and 1100 ng/10,000 cells.

#### Example 22

##### Inhibition of 3DG by Inhibiting Amadorase mRNA and Protein

[0406] 3DG synthesis may be inhibited by inhibiting the components of the enzymatic pathway leading to its synthesis. This can be done in several ways. For example, the enzyme which leads to the synthesis of 3DG, called amadorase herein (a fructosamine-3-kinase) can be inhibited from acting using a compound as described above, but it can also be inhibited by blocking the synthesis of its message or protein or by blocking the protein itself, other than with a compound, as described above.

[0407] Amadorase mRNA and protein synthesis and function may be inhibited using compounds or molecules such as transcription or translation inhibitors, antibodies, antisense messages or oligonucleotides, or competitive inhibitors.

[0408] Nucleic Acid and Protein Sequences

[0409] The following represents the 988 bp mRNA-derived DNA sequence for amadorase (fructosamine-3-kinase), Accession No. NM\_022158 (SEQ ID NO:1) (see FIG. 10):

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1 cgtaagctt ggcacgaggc catggaggcag ctgtgcgcg ccgagctgcg caccgcgacc
61 ctggggccct tcggcgcccc cggcgccggc tgcatcagcg agggccgagc ctacgacacg
121 gacgcaggcc cagtgttcgt caaagtcaac cgcaggacgc aggccggca gatgttttag
181 ggggaggtgg ccagcctggaa ggcctccgg agcacgggcc tggtgccgggt gcccggcc
241 atgaaggta tcgacactgc gggaggtggg gccgcctttg tcatggagca tttgaagatg
301 aagagcttga gcagtcaagc ataaaaactt ggagagcaga tggcagattt gcatctttac
361 aaccagaagc tcagggagaa gttgaaggag gaggagaaca cagtggccg aagaggttag
421 ggtgtgagc ctcagtatgt ggacaagttc ggcttccaca cggtaacgtg ctgcggcttc
481 atcccgccagg tgaatgagtg gcaggatgac tggccgacct tttcgcccg gcaccggctc
541 caggcgccgc tggacctcat tgagaaggac tatgtgacc gagaggacac agaactctgg
601 tcccggtac aggtgaagat cccggatctg tttgtggcc tagagattgt ccccgcttgc
661 ctccacgggg atctctggc gggaaacgtg gctgaggacg acgtggggcc cattatttac
721 gacccggctt ccttctatgg ccattccgag ttgtactgg caatcgccctt gatgtttggg
781 gggttcccca gatctttctt caccgcctac cacccgaaga tcccccaaggc tccgggcttc
841 gaccagcggc tgctgctcta ccagctgttt aactacctga accactggaa ccacttgggg
901 cgggagtaca ggagcccttc cttgggcacc atgcaaggc tgctcaagta gccccctgt
961 ccctcccttc ccctgtcccc gtccccgt

```

[0410] The following represents the 309 amino acid residue sequence of human amadorase (fructosamine-3-kinase), Accession No. NP\_071441 (SEQ ID NO:2) (see FIG. 11):

```

1 meqlraelr tatlrafggp gacgisegra ydtdagpvfv kvnrrtqarq mfegevasle
61 alrstglvrv prpmkvidlp gggaaafvmeh lkmkslssqa sklgeqmadl hlynqklrek

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-continued

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121 lkeeeentvgr rgegaepqyv dkfgefhtvtc cgfipqvnew qddwptffar hrlqaqlldi
181 ekdyadrear elwsrlqvki pdlfcgleiv pallhgdlws gnvaeddvgp iiydpasfyg
241 hsefelaiala mfgggfprsff tayhrkipka pgfdqrllly qlfnlnhwn hfgreyrsps
301 lgtmrrllk

```

**[0411]** The sequences identified above were submitted by Delpierre et al. (2000, Diabetes 49:16227-1634). The sequence data of Szwergold et al. (2001, Diabetes 50:2139-2147) are in excellent agreement with those of Delpierre et al. For example, the protein sequence deduced by Szwergold et al. (2001, Diabetes 50:2139-2147) is identical with the cloned human fructosamine-3-kinase sequence of Delpierre et al. (2000, Diabetes 49:16227-1634) in 307 of 309 amino acid residues. Thus, reliance on the published sequences of either group should not be a problem, however, to ensure that no problems arise when a sequence of the protein is to be used, only those portions of the sequence which are not different between the two published sequences will be used.

#### Example 23

##### Presence of Alpha-Dicarbonyl Sugars in Sweat

**[0412]** As disclosed herein, alpha-dicarbonyl sugars are present in skin, but their presence in sweat had not been determined. One of the functions of skin is to act as an excretory organ, therefore, it was determined whether alpha-dicarbonyl sugars are excreted in sweat.

**[0413]** Samples of human sweat were analyzed for the presence of 3DG, as described above. Samples from four subjects were obtained and 3DG was determined to be present at levels of 0.189, 2.8, 0.312, and 0.110A, respectively. Therefore, the results demonstrate the presence of 3DG in sweat.

#### Example 24

##### Effects of DYN 12 (3-O-Methylsorbitolysine) on Skin Elasticity

**[0414]** Administration of DYN 12, a small molecule inhibitor of amadorase, reduces 3DG levels in the plasma of diabetic and non-diabetic animals (Kappler et al., 2002, Diabetes Technol. Ther., Winter 3:4:606-609).

**[0415]** Experiments were performed to determine the effects of DYN 12 on the loss of skin elasticity associated with diabetes. To this end, two groups of STZ-diabetic rats and two groups of normal rats were subjected to treatment with DYN 12 or saline. One group of STZ-diabetic rats (n=9) received daily subcutaneous injections of DYN 12 at 50 mg/kg for eight weeks, as did one group of normal rats (n=6). A group of control diabetic rats (n=10) and a group of normal rats (n=6) received saline instead of DYN 12. One rat was removed from the diabetic DYN 12 group after 2 weeks because its blood glucose readings were inconsistent (too low) with other diabetic rats.

**[0416]** A non-invasive procedure based on CyberDERM, Inc. technology utilizing a skin elasticity measurement device was used to test the effects of DYN 12 treatment on skin elasticity. The procedure provides for non-invasive measurement of skin elasticity based upon the amount of vacuum pull required to displace skin. A suction cup probe is adhered to an area of shaved skin in order to form an airtight seal. Then, a

vacuum is applied to the area of the skin inside the suction cup until the skin is displaced past a sensor located inside the probe. Accordingly, the more pressure that is required to displace the skin, the less elastic the skin is.

**[0417]** The data demonstrate that after eight weeks of treatment skin elasticity in diabetic rats treated with DYN 12 was greater than skin elasticity in diabetic animals which were treated with saline. As seen in FIG. 14, the amount of pressure needed to displace the skin of diabetic rats treated with saline (7.2+/-3.0 kPa) was approximately 2 to 2.25 fold higher than the pressure needed to displace the skin of diabetic animals treated with DYN 12 (3.2+/-1.2 kPa). Also, the elasticity value observed in diabetic rats treated with DYN 12 was not statistically different from the value found in non-diabetic rats treated with saline (p=0.39) (Table E). Thus, the result of treatment of diabetic animals with DYN 12, an indirect inhibitor of 3DG, was skin with greater elasticity than skin in diabetic animals which received only saline.

TABLE E

Statistical Analysis and Comparison of Cohort Groups.		
Group 1	Group 2	p value
Diabetic saline	Non-diabetic saline	p = 0.01
Diabetic saline	Diabetic DYN 12	p = 0.001
Diabetic saline	Non-diabetic DYN 12	p = 0.003
Diabetic DYN 12	Non-diabetic DYN 12	p = 0.39
Diabetic DYN 12	Non-diabetic saline	p = 0.26
Non-diabetic saline	Non-diabetic DYN 12	p = 0.20

**[0418]** The above data demonstrate that the administration of DYN 12 to diabetic rats prevents the loss of skin elasticity (e.g., sclerosis and thickening of the basement membrane of the skin) that is typically observed in untreated diabetic rats, which is evidence that the excess 3DG found in diabetics is the cause of the loss of elasticity. The data disclosed herein further indicate that reducing 3DG levels can also serve to maintain skin elasticity in normal individuals.

**[0419]** Skin elasticity measurements were also taken on the test subjects as described above, but without sedating the test animals before measurement. FIG. 15 illustrates skin elasticity measurements taken on the hind leg of the test subjects while the subjects were alert and being restrained by a technician.

**[0420]** In these experiments, the animals were fiercely fighting restraint and the results are different. The diabetic animals without drug treatment showed less ability to "pull away" from the suction cup and therefore show less "resistance to pull". On the other hand, both the diabetic animals receiving drug and the normal animals had a greater capacity to pull away from the suction cup, and both groups of animals demonstrated stiffness and greater muscle tension. This indicates that the inhibition of the enzyme, and most likely, inac-

tivation of 3DG, results in the sparing of microcirculation deterioration and neuro-deterioration that typifies the diabetic condition.

#### Example 25

##### Level of 3DG in Scleroderma Skin

[0421] It has been determined, according to the methods disclosed previously elsewhere herein, that normal skin had the following concentrations of 3DG (data from several subjects): 0.9  $\mu$ M, 0.7  $\mu$ M, and 0.6  $\mu$ M. Several samples of skin from several scleroderma patients were similarly assayed and had the following level of 3DG: 15  $\mu$ M, 130  $\mu$ M, and 3.5  $\mu$ M. Accordingly, these data demonstrate that the level of 3DG in the skin of scleroderma patients is significantly elevated compared with the level of 3DG in the skin of normal humans.

#### Example 26

##### Formulation of a Liposome Cream Delivery System

[0422] 23.9 grams of BioCreme Concentrate from Bio-Chemica International Inc. was blended with 2.9 grams cocoa butter, 1.4 grams shea butter, 2.2 grams aloe oil, 1.1 grams vitamin E, 3.7 grams glycerol, 51 grams water, 1.1 grams dimethicone and 10.8 grams Natipide II containing 1 gram arginine-HCl and 1 gram meglumine-HCl.

#### Example 27

##### Treatment of Psoriasis

[0423] A blinded study was conducted with 9 adult volunteers having 2-10% of their body surface area affected with psoriasis. Between 2 and 4 psoriasis-affected sites for each volunteer were chosen for treatment; only one type of cream was used on each volunteer. The volunteers were divided into 3 groups of 3 volunteers each, and the affected sites on the volunteers in each group were treated with twice daily applications of one of the following creams: 1) A base cream containing salicylic acid (1.9%) ("Cream SA"); 2) A base cream containing salicylic acid (1.9%) and meglumine (5.5%) and arginine (3.8%) ("Cream SAMA"); or 3) A base cream containing meglumine (5.5%) and arginine (3.8%) ("Cream MA").

[0424] An expert grader was used to examine the skin areas. Assessments were made at the beginning of the study and after 3 weeks with respect to:

[0425] A. Erythema (0=no redness, 1=faint redness, 2=red coloration, 3=very bright red coloration, 4=deep red coloration);

[0426] B. Dryness (0=no dryness/scaling, 1=fine scale partially covering lesions, 2=fine to coarse scale covering most or all of the lesions, 3=coarse, non-tenacious scale predominates covering most or all of the lesions, 4=coarse, thick, tenacious scale over most or all lesions, rough surface);

[0427] C. Induration (0=no evidence of plaque elevation, 1=slight but definite plaque elevation, typically edges indistinct or sloped, 2=moderate plaque elevation with rough or sloped edges, 3=marked plaque elevation typically with hard or sharp edges, 4=very marked plaque elevation typically with hard sharp edges); and

[0428] D. Pruritis (0=no itching, 1=slightly bothersome itching, 2=bothersome itching, but no loss of sleep, 3=constant itching causing intense discomfort and loss of sleep).

[0429] The mean values for the expert grader's scores at 0 weeks (beginning of study) and after 3 weeks are shown in Table F. A statistical t-test was used to determine the significance of any difference between the means. Bold values indicate p<0.05. The volunteers treated with the Cream SA exhibited a statistical improvement with respect to erythema, but no statistical improvement with respect to dryness, induration, or pruritis. The volunteers treated with the Cream SAMA exhibited a statistical benefit for erythema, induration and pruritis, and approached significance for dryness. The volunteers treated with the Cream MA exhibited a statistical benefit for dryness, induration, and purities, and exhibited a non-statistical improvement erythema. Cream MA exhibits clear benefits over Cream SA with respect to dryness, induration and pruritis. Cream SAMA provides clear benefits over Cream SA with respect to dryness, induration and pruritis.

TABLE F

Results of Psoriasis study over 3-week time period.

Cream	Erythema			Dryness/Scaling		
	0 week	3 week	p value	0 week	3 week	p value
SA	1.909	1.333	<b>0.008</b>	1.909	1.818	0.290
SAMA	1.917	1.667	<b>0.041</b>	2.000	1.750	<b>0.070</b>
MA	2.100	2.000	0.172	1.900	1.500	<b>0.018</b>

Cream	Induration			Pruritis		
	0 week	3 week	p value	0 week	3 week	p value
SA	1.818	1.545	0.140	1.181	1.100	0.17
SAMA	1.666	1.333	0.052	1.000	0.583	<b>0.008</b>
MA	2.300	1.500	<b>0.005</b>	1.000	0.333	<b>0.004</b>

#### Example 28

##### Identification and Quantitation of Fructoselysine 3-Phosphate(FL3P) in Rat Pancreas

[0430] A 250 g male Sprague-Dawley rat was sacrificed with an overdose of pentobarbital and the pancreas removed and snap frozen in liquid nitrogen. The pancreas was pulverized in liquid nitrogen with 5  $\mu$ mol of phenylphosphonic acid (an internal standard for quantitation) and six volumes of 5% perchloric acid containing 10 mmol/l trans-1,2-diaminocyclohexane-N,N,N',N'-tetraacetic acid. The resultant slurry was centrifuged at 8,000 g at 4° C. for 10 min. The supernatant was neutralized with KOH and was centrifuged again to remove the precipitate of potassium perchlorate. The supernatant was lyophilized to a powder and reconstituted in 1-ml of D<sub>2</sub>O at pH 7.5 for NMR measurement. <sup>31</sup>P-NMR spectra were obtained in a 10-mm probe at 161.98 MHz on a Bruker AM 400 spectrometer using 60° pulses and a 1.5 second repetition time. The spectra were acquired in blocks of 20,000 scans and were referenced to glycerophosphocholine set at 0.49 ppm. Quantitation of the FL3P resonance was determined by integration of peak area, setting the phenylphosphonic acid area equal to 5 umol. FL3P resonates at 6.23 ppm and was identified by spiking with authentic material as well as reduction with sodium borohydride to sorbitolysine 3-phosphate (5.95 ppm) and mannoselysine 3-phosphate (5.85 ppm). The concentration of FL3P in the pancreas was 28  $\mu$ M.

[0431] The therapeutic creams set forth in Experimental Examples 29-36 contained 3-5.5% meglumine and 3-4% arginine as the active ingredients.

Example 29

Psoriasis

[0432] Five adults with psoriasis applied a base cream containing meglumine and arginine and experienced decreased inflammation and dryness.

Example 30

Eczema

[0433] A seven year old girl with eczema used a base cream containing meglumine and arginine and experienced decreased inflammation, itch and dryness.

Example 31

Arthritis

[0434] Two female adults with arthritis used daily application of a base cream containing meglumine and arginine and experienced relief from joint pain, swelling and tenderness.

Example 32

Sinus Headache

[0435] An adult male and female with headaches centered around the facial and forehead areas applied a base cream containing meglumine and arginine to the affected areas. Both experienced pain relief approximately 30 minutes after application.

Example 33

Acne

[0436] An adult woman with facial acne applied a base cream containing meglumine and arginine to affected skin areas and experienced a decrease in number/severity of lesions, and increased skin smoothness and softness.

Example 34

Razor Burn

[0437] Two adult males with facial razor burn applied a base cream containing meglumine and arginine immediately after shaving and experienced a decrease in skin redness.

Example 35

Polycythemia

[0438] A female adult with skin rash due to polycythemia used a base cream supplemented with meglumine and arginine and experienced decreased inflammation and itching.

Example 36

Sodium Lauryl Sulfate Skin Irritation Trial

[0439] A clinical study was performed to determine the effectiveness of a base cream and a base cream containing meglumine and arginine to reduce redness (inflammation) and repair damage to the skin using a sodium lauryl sulfate (SLS) wound healing (irritation amelioration) test. The protocol included self assessments of the study participants,

expert grader assessments and instrument measurements of evaporative water loss and redness. This was a single blind, controlled, randomized study.

[0440] The volar forearms of a group of twelve women volunteers from 18-55 years old were exposed to an irritant solution (0.3 ml of a 0.5% sodium lauryl sulfate solution) at six sites (three sites on each arm) for 18-24 hours. The four sites that were the most irritated were selected for further treatment with a twice-daily application of either a base cream (Product A) or a cream containing 3% meglumine and 3% arginine (Product B) for 7 days. The remaining two sites were not treated. At 1, 2, 3, 4, 7 and 8 days after the SLS application, the skin areas were assessed using a Minolta Chromameter (to measure color intensity), an expert grader (using an 8-point scale), and a DermaLab Modular System with TEWL Probe (to measure water loss).

[0441] On the eighth day of treatment, the participants filled out a self-assessment questionnaire. Responses are set forth in Table G.

TABLE G

Feature of cream	Counts		
	Product A	Product B	Significance test
Quickest healing	2	10	0.038
Least irritating	6	6	ns
Reduced redness	1	11	0.006
Best feeling	2	10	0.038
Skin healed smoothest	2	10	0.038
Skin looked best	2	10	0.038
Overall best	2	10	0.038

Example 37

Wound Healing Trial

[0442] A trial with human volunteers compared the wound-healing properties of a topical preparation as described as described elsewhere herein ("Cream B") to a base cream lacking meglumine-HCl and arginine ("Cream A"). Six sites on the volar forearms (3 on each arm) of 15 female volunteers were exposed on Day 0 to an irritant solution (0.5% sodium lauryl sulfate, SLS) under occlusion for 18-24 hr. On Day 1, the four arm sites with the most similar degree of damage for 12 of the volunteers who experienced a significant irritation effect from the SLS were selected for the treatment phase of the study. Patches were removed and panelists then had the test creams applied to the four selected sites twice daily for 7 days. The other forearm sites were not treated so they could be used as controls.

[0443] The extent of irritation and healing rates were based on clinical observations of an Expert Grader for erythema (using a 10 point scale), instrument measurements using a Minolta Chromameter (to measure redness) and DermaLab Meter (to measure Transdermal Evaporative Water Loss (TEWL)) on day 0 (prior to SLS exposure), and on days 1, 2, 3, 4, 7, and 8. FIGS. 19 and 20 show the average values for assessments of erythema (redness), and FIG. 21 shows the average values for total evaporative water loss (TEWL) at days 1, 2, 3, 4, 7 and 8 after SLS treatment.

[0444] These study results demonstrate that Cream B enhanced the repair of detergent damaged skin. Although there were no clear cut differences in the early stages of the

study, from Day 3 onward there were significant differences between Cream A and Cream B. Cream B was more effective in reducing erythema especially with regard to visual assessments being made by the Expert Grader (FIG. 19). It was also determined that Cream B enhanced the restoration of the stratum corneum barrier which had been disrupted by exposure to SLS more than Cream A (FIG. 21).

#### Example 38

##### Increase In Isoprostane Levels In Rats On A Glycated Diet

[0445] Elevated levels of 3DG resulting from a glycated diet leads to a 2-fold increase in oxidative stress as measured by urinary levels of isoprostane. Isoprostanes are prostaglandin-like molecules that are produced by free radical mediated peroxidation of lipoproteins. Urinary isoprostanes are used as a non-invasive measurement of oxidant stress in vivo.

[0446] Ten rats were fed a diet containing 3% glycated protein for 18 weeks. A control cohort of 10 rats was placed on control chow for the same time period. A competitive ELISA (Oxford Biochemicals) was used to measure urinary isoprostane levels from each rat. Urinary 3DG levels were determined as described in Example 5. All values were normalized to creatinine levels in the urine to control for urine volume. As shown in Table H, isoprostane levels of rats on glycated diet for 18 weeks (increased 3DG levels) were twice the level of that for rats on normal chow. The statistical significance was significance 0.005.

TABLE H

3DG and isoprostane levels in urine of rats on a control or glycated chow diet for 18 weeks.			
	Control Chow (n = 10)	Glycated Chow (n = 10)	t-test
pmoles 3DG/mg creatinine	23.3 ± 3.8	113.5 ± 24.2	p = 4 × 10 <sup>-10</sup>
ng isoprostane/mg creatinine	222 ± 188	496 ± 236	p = 0.005

#### Example 39

##### Immunofluorescent Localization Of 3DG-Imidazolone In Inflamed Skin

[0447] Polymorphic eruption of pregnancy is a skin condition characterized by inflammation, itch and redness that occurs predominantly in the abdominal area of some women in the third trimester of pregnancy.

[0448] Skin biopsies from the inflamed area of an individual with polymorphic eruption of pregnancy and an individual with normal skin were obtained. Thin sections were embedded and prepared for immunofluorescence as follows. Sections were deparaffinized with two treatments of xylene for 10 minutes each, and two treatments with ethanol for 10 minutes each. The sections were blocked with 5% goat serum diluted in PBS for 15 minutes and washed once with PBS. Mouse monoclonal antibody to 3DG-imidazolone (distributed by Cosmo Bio for TransGenic Inc.) was diluted 1:10 in PBS and applied to the sections for 40 minutes at room temperature in a humidified chamber. Sections were washed with PBS three times for 2 minutes each. Cy-2 conjugated goat anti-mouse antibody (Jackson Immunologicals) was diluted 1:50 in PBS and applied to the sections for 40 minutes in a humidified chamber at room temperature. Sections were washed with PBS three times for 2 minutes each. The sections were mounted on slides with VectraShield Mounting Medium (Vectra Laboratories) and viewed with a Nikon E600 fluorescent microscope. The same skin section from the polymorphic eruption of pregnancy sample was then stained with hematoxylin and eosin as follows. The section was stained with hematoxylin for 5 minutes, washed with water for 3 minutes, treated for 30 seconds with 1% acid alcohol (495 ml of 70% ethanol, 5 ml 10M HCl), washed with water for 3 minutes, treated with Scott's buffer 30 (2 g NaHCO<sub>3</sub>, 20 g MgSO<sub>4</sub>·7H<sub>2</sub>O brought to 1 liter with H<sub>2</sub>O) for 30 seconds, washed with tap water for 3 minutes, treated with eosin for 1 minute, 95% ethanol for 1 minute twice, and finally 100% ethanol for 1 minute. Sections were applied to slides with 1 drop of Cryoseal 60 (Richard-Allen Scientific), a cover slip placed over them, and viewed with a brightfield microscope.

[0449] FIG. 22 shows the immunofluorescent patterns from normal skin (FIG. 22A) and skin from an inflamed area of an individual with polymorphic eruption of pregnancy (FIG. 22B). FIG. 22C shows the hematoxylin and eosin staining of the skin section shown in FIG. 22B. Similar patterns of 3DG-imidazolone were obtained with skin sections from a inflamed areas of skin from an individual with scleroderma and an individual with lupus erythematosus.

[0450] These results indicate that conditions in which there is an elevated level of 3DG may be treated using one or more compounds that directly inhibit 3DG. That is, a compound that can reduce the concentration of 3DG at a site, break down or eliminate 3DG, or effectively inhibit the function or activity of 3DG can serve to treat a disease or disorder mediated by an elevated concentration of 3DG. Compounds and methods for such treatment are described in detail elsewhere herein.

[0451] The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety.

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																25
																30

Thr	Asp	Ala	Gly	Pro	Val	Phe	Val	Lys	Val	Asn	Arg	Arg	Thr	Gln	Ala	
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																95

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																120
																125

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																135
																140

Phe	His	Thr	Val	Thr	Cys	Cys	Gly	Phe	Ile	Pro	Gln	Val	Asn	Glu	Trp	
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																155
																160

Gln	Asp	Asp	Trp	Pro	Thr	Phe	Phe	Ala	Arg	His	Arg	Leu	Gln	Ala	Gln	
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Glu Asp Asp Val Gly Pro Ile Ile Tyr Asp Pro Ala Ser Phe Tyr Gly			
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His Ser Glu Phe Glu Leu Ala Ile Ala Leu Met Phe Gly Gly Phe Pro			
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Arg Ser Phe Phe Thr Ala Tyr His Arg Lys Ile Pro Lys Ala Pro Gly			
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Phe Asp Gln Arg Leu Leu Tyr Gln Leu Phe Asn Tyr Leu Asn His			
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Trp Asn His Phe Gly Arg Glu Tyr Arg Ser Pro Ser Leu Gly Thr Met			
290	295	300	
Arg Arg Leu Leu Lys			
305			

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**1-48.** (canceled)

**49.** A method of treating or ameliorating a disease, disorder or condition mediated by or associated with oxidative distress in a mammal, said method comprising administering to said mammal a composition comprising an inhibitor of an enzymatic pathway that produces an alpha-dicarbonyl sugar in said mammal, said administration resulting in reduction or elimination of said alpha-dicarbonyl sugar at a site in said mammal, wherein said site is affected by said oxidative distress, thereby treating said disease, disorder or condition.

**50.** The method of claim **49**, wherein said composition comprises an inhibitor of an Amadorase pathway.

**51.** The method of claim **50**, wherein said composition comprises an inhibitor of fructoseamine kinase.

**52.** The method of claim **49**, wherein said alpha-dicarbonyl sugar is 3DG.

**53.** The method of claim **49**, wherein said mammal is a human.

**54.** The method of claim **49**, wherein said composition is administered to said mammal by a topical, oral, rectal, vaginal, intramuscular, subcutaneous, transdermal or intravenous route, or through consumption of a nutriceutical product by said mammal.

**55.** The method of claim **49**, wherein said disease, disorder or condition is gingivitis, periodontal disease, browning of the teeth, yellowing of the teeth, herpes lesions or scarring.

**56.** The method of claim **49**, wherein said composition further comprises a non-steroidal anti inflammatory drug (NSAID).

**57.** The method of claim **56**, wherein said NSAID is selected from the group consisting of ibuprofen (2-(isobutylphenyl)-propionic acid); methotrexate (N-[4-(2,4-diamino-6-pteridinyl-methyl]methylamino]benzoyl)-L-glutamic acid); aspirin (acetylsalicylic acid); salicylic acid; diphenhydramine (2-(diphenylmethoxy)-N,N-dimethylethyamine hydrochloride); naproxen ((+)-(S)-2-(6-methoxynaphthalen-2-yl)propanoic acid, sodium salt); phenylbutazone (4-butyl-1,2-diphenyl-3,5-pyrazolidinedione); sulindac

{(1Z)-5-fluoro-2-methyl-1-[4-(methylsulfinyl)benzylidene]-1H-indene-3-yl}acetic acid; diflunisal (2',4'-difluoro-4-hydroxy-3-biphenylcarboxylic acid); piroxicam (4-hydroxy-2-methyl-N-2-pyridinyl-2H-1,2-benzothiazine-2-carboxamide 1,1-dioxide); an oxican; indometacin (1-(4-chlorobenzoyl)-5-methoxy-2-methyl-H-indole-3-acetic acid); meclofenamate sodium (N-(2,6-dichloro-m-tolyl)-anthranilic acid, sodium salt, monohydrate); ketoprofen (2-(3-benzoylphenyl)-propionic acid; tolmetin sodium (sodium 1-methyl-5-(4-methylbenzoyl)-1H-pyrrole-2-acetate dihydrate); diclofenac sodium (2-(2-(2,6-dichlorophenylamino)phenyl)acetic acid, monosodium salt); hydroxychloroquine sulphate (2-{[4-[(7-chloro-4-quinolyl)amino]pentyl]ethylamino}ethanol sulfate (1:1); penicillamine (3-mercapto-D-valine); flurbiprofen ((RS)-2-(2-fluorobiphenyl-4-yl)propanoic acid); etodolac ((RS)-2-(1,8-diethyl-4,9-dihydro-3H-pyrano[3,4-b]indol-1-yl)acetic acid); mefenamic acid (N-(2,3-xylyl)anthranilic acid); and diphenhydramine hydrochloride (2-diphenylmethoxy-N,N-di-methylethyamine hydrochloride).

**58.** The method of claim **49**, wherein said inhibitor is meglumine or a salt thereof.

**59.** The method of claim **58**, wherein said composition further comprises arginine or a salt thereof.

**60.** The method of claim **59**, wherein the result of said administration is greater than the additive result of:

(i) an administration of a composition which comprises meglumine or a salt thereof and is substantially free of arginine or a salt thereof; and

(ii) an administration of a composition which comprises arginine or a salt thereof alone and is substantially free of meglumine or a salt thereof.

**61.** The method of claim **49**, wherein said inhibitor is selected from the group consisting of galactitol lysine, 3-deoxy sorbitol lysine, 3-deoxy-3-fluoro-xylitol lysine, 3-deoxy-3-cyano sorbitol lysine, 3-O-methyl sorbitolysine, sorbitol lysine, mannitol lysine, sorbitol and xylitol.

**62.** The method of claim **49**, wherein said composition comprises a copper-containing compound.

**63.** The method of claim **62**, wherein said copper-containing compound is selected from the group consisting of a copper-salicylic acid conjugate, a copper-peptide conjugate, a copper-amino acid conjugate, and a copper salt.

**64.** The method of claim **63**, wherein said copper-amino acid conjugate is selected from the group consisting of a copper-lysine conjugate and a copper-arginine conjugate.

**65.** The method of claim **49**, wherein said composition further comprises an inhibitor of said alpha-dicarbonyl sugar's function.

**66.** The method of claim **65**, wherein said inhibitor of said alpha-dicarbonyl sugar's function is an N-methyl-glucamine-like compound.

**67.** The method of claim **66**, wherein said inhibitor of said alpha-dicarbonyl sugar's function comprises meglumine or a salt thereof.

**68.** The method of claim **66**, wherein said inhibitor of said alpha-dicarbonyl sugar's function comprises arginine or a salt thereof.

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