A method and composition for extending the lifespan of an individual and delaying the onset of age-related disease is provided. The method includes the administration of an effective dose of oxaloacetate, wherein the oxaloacetate acts to mimic the cellular conditions obtained under caloric restriction to provide similar benefits. The invention further includes methods and compositions for reducing the incidence or treatment of cancer. Compositions and methods for reducing body fat by administering an effective amount of oxaloacetate are likewise provided. Compositions for DNA repair in UV damaged cells is provided are also provided. Similarly, a method for treating a hang-over comprising administering an effective amount of oxaloacetate is disclosed.

Claims:

1. Use of an effective lifespan-extending amount of a composition selected from the group consisting of oxaloacetate, oxaloacetic acid, an oxaloacetate salt, alpha-ketoglutarate and aspartate for the manufacture of a medicament for extending the lifespan of an organism.

2. The use of claim 1, wherein said composition is formulated for oral administration.

3. The use of claim 1, wherein said composition is formulated with a buffer.

4. The use of claim 1, wherein said organism is a mammal.

5. The use of claim 4, wherein said mammal is a human.

6. The use of claim 1, wherein said compound is topically administered.

7. The use of claim 1, wherein said compound is administered parenterally.

8. A method of mimicking the beneficial health effect of caloric restriction without reducing caloric intake, comprising: administering an effective amount of a compound selected from the group consisting of oxaloacetate, oxaloacetic acid, and an oxaloacetate salt.

9. The method of claim 8, wherein said beneficial health effect is selected from the group consisting of weight loss, improvement of cardiac function, and extension of life span.
10. The method of claim 8, wherein said compound is formulated for oral administration.

11. The method of claim 8, wherein said compound further includes a buffer.

12. The method of claim 8, wherein said organism is a mammal.

13. The method of claim 12, wherein said mammal is a human.

14. The method of claim 8, wherein said compound is administered parenterally.

15. The method of claim 8, further comprising administering a therapeutic agent selected from the group consisting of an antibacterial, an antifungal, a chemotherapeutic agent, an antihistamine, protein, enzyme, hormone, non-steroidal anti-inflammatory, an immuno-stimulatory compound, and a steroid.

16. The method of claim 15, wherein said therapeutic agent is administered separately from said compound.

17. The method of claim 15, wherein said therapeutic agent is administered substantially contemporaneously with said compound.

18. A composition for treating symptoms of skin aging, comprising: an effective amount of a compound selected from the group consisting of oxaloacetate, oxaloacetic acid, an oxaloacetate salt, alpha-ketoglutarate, and aspartate; and a pharmaceutically effective carrier formulated for topical delivery.

19. The composition of claim 18, wherein said pharmaceutically acceptable carrier is selected from the group consisting of a cream, a soap, a shampoo, a conditioner, an ointment, a lotion, a gel, a salve, and an aerosol spray.

20. The composition of claim 18, further comprising a second beneficial agent selected from the group consisting of an emollient, sunscreen, moisturizer, and buffer.

21. The composition of claim 18, wherein said symptoms of skin aging are selected from the group consisting of rhytids, wrinkles, jowls, sun damage, dull appearance of skin, loss of skin taughtness, keratosis, hyperpigmentation, melasma, and skin discoloration.

22. The composition of claim 18, further comprising a lipophilic agent, wherein said lipophilic agent acts to modify the rate of absorption of said composition.

23. Use of a composition selected from the group consisting of oxaloacetate, oxaloacetic acid, an oxaloacetate salt, alpha-ketoglutarate, and aspartate in the manufacture of a medicament for reducing the signs of skin aging, wherein said composition is formulated for topical administration with a pharmaceutically acceptable carrier.

24. A method for protecting DNA and enhancing DNA damage repair from sun exposure, comprising topically administering an effective amount of a composition comprising a compound selected from the group consisting of oxaloacetate, oxaloacetic acid, an oxaloacetate salt, alpha-ketoglutarate and aspartate.

25. An improved animal chow formulation comprising a compound selected from the group consisting of oxaloacetate, oxaloacetic acid, an oxaloacetate salt, alpha-ketoglutarate, and aspartate, wherein said chow increases the life span of said animal.

26. A method for increasing the activity of the Sir2 gene and/or at least one gene homologue by administering an effective amount of a compound selected from the group consisting of oxaloacetate, oxaloacetic acid, an oxaloacetate salt, alpha-ketoglutarate and aspartate.

27. The method of claim 26, wherein said compound is administered orally.
28. The method of claim 26, wherein said compound is formulated with a buffer.

29. The method of claim 26, wherein said organism is a mammal.

30. The method of claim 29, wherein said mammal is a human.

31. The method of claim 26, wherein said compound is topically administered.

32. The method of claim 26, wherein said compound is administered parenterally.

33. The method of claim 26, wherein said gene homologue is Sirt1.

34. Use of a pharmaceutically effective amount of a composition selected from the group consisting of oxaloacetate, oxaloacetic acid, an oxaloacetate salt, alpha-ketoglutarate and aspartate in the manufacture of a medicament for reducing the incidence of cancer.

35. The use of claim 34, further comprising administering a chemotherapeutic agent selected from the group consisting of cyclophosphamide, chlorambucil, melphalan, estramustine, iposphamide, prednimustin, busulphan, iotepa, carmustin, lomustine, methotrexate, azathioprine, mercaptopurine, thioguanine, cytarabine, fluorouracil, vinblatine, vincristine, vindesine, etoposide, teniposide, dactinomucin, doxorubicin, mitomycin, epirubicin, bleomycin, mitomycin, cisplatin, carboplatin, procarbazine, amacrine, mitoxantrone, tamoxifen, nilutamid, and aminoglutemide.

36. The use of claim 34, wherein said composition is administered orally.

37. The use of claim 34, wherein said composition is administered parenterally.

38. The use of claim 35, wherein said chemotherapeutic agent is administered prior to administering said compound.

39. The use of claim 35, wherein said chemotherapeutic agent is administered after administering said compound.

40. The use of claim 35, wherein said chemotherapeutic agent is administered substantially contemporaneously with said compound.

41. The use of claim 34, wherein said cancer is selected from the group consisting of primary and metastatic malignant solid tumor disease and a hematological malignancy.

42. The use of claim 41, wherein said hematological malignancy is selected from the group consisting of acute and chronic myelogenous leukemia, acute and chronic lymphatic leukemia, multiple myeloma, Waldenstrom's macroglobulinemia, hairy cell leukemia, myelodisplastic syndrome, polycytaemia vera, and essential thrombocytosis.

43. Use of a pharmaceutically effective amount of a composition selected from the group consisting of oxaloacetate, oxaloacetic acid, an oxaloacetate salt, alpha-ketoglutarate and aspartate in the manufacture of a medicament for treating a disease associated with aging, wherein said composition is formulated with a pharmaceutically acceptable carrier.

44. The use of claim 43, wherein said disease is selected from the group consisting of osteoporosis, bone loss, arthritis, stiffening joints, cataracts, macular degeneration, and heart disease.

45. The use of claim 43, wherein said disease is a neurodegenerative disease selected from the group consisting of Alzheimer disease and Parkinson's Disease.

46. Use of a pharmaceutically effective amount of a compound selected from the group consisting of oxaloacetate, oxaloacetic acid, an oxaloacetate salt, alpha-ketoglutarate and
aspartate in the manufacture of a medicament for reducing the symptoms associated with over-consumption of alcohol.

47. The use of claim 46, wherein said symptoms are selected from the group consisting of headache, poor sense of overall well-being, diarrhea, loss of appetite, shakiness, fatigue, and nausea.

48. A composition of matter formulated for topical administration comprising a compound selected from the group consisting of oxaloacetate, oxaloacetic acid, an oxaloacetate salt, alpha-ketoglutarate, and aspartate, and at least one of a second therapeutic agent selected from the group consisting of a sunscreen, a cosmetic carrier, a vitamin.

51. A therapeutic composition comprising a compound selected from the group consisting of oxaloacetate, oxaloacetic acid, an oxaloacetate salt, alpha-ketoglutarate and aspartate and a therapeutic agent.

52. A method of increasing the ratio of NAD+ to NADH in a cell, comprising: administering an effective dose of a compound selected from the group consisting of oxaloacetate, oxaloacetic acid, an oxaloacetate salt, alpha-ketoglutarate, and aspartate, wherein said increase in the ratio of NAD+ to NADH in said cell results in mimicking the beneficial health effect of caloric restriction without reducing caloric intake.

53. A method of mimicking caloric restriction in a specific tissue of an animal comprising administering a compound selected from the group consisting of oxaloacetate, oxaloacetic acid, an oxaloacetate salt, alpha-ketoglutarate and aspartate, wherein said mimicking is localized to the area of administration of said compound.

Description:

BACKGROUND OF THE INVENTION

[0001]1. Field of the Invention

[0002]The present invention pertains to a method of extending life span in organisms and delays the onset and many of the complications associated with age-related diseases, including cancer. More particularly, the invention relates to the administration of a chemical agent to upregulate and downregulate the expression (i.e. gene activation) of the same beneficial genes that are activated in caloric restriction. The genes are activated by mimicking the same intracellular conditions as are seen in caloric restriction, but without the need to reduce caloric intake. Compositions and methods to prolong life and protect an organism from age-related diseases are likewise provided.

[0003]2. Description of the Related Art

[0004]Many attempts have been made to extend life span in single cell organisms and multicellular animals. These attempts have included various nutritionally-based interventions, vitamin supplements, antioxidant supplements, exercise, hormonal, pharmaceutical and other paradigms (Lane, M. et al. Nutritional Modulation of aging in nonhuman primates, 1999 The Journal of Nutrition, Health & Aging, Vol. 3, No. 2 pp 69-76). While these attempts sometimes result in better health, in the last 70 years, only activation of beneficial genes has caused an increase in lifespan. Three methods of beneficial gene activation have been proven to extend mean and maximal lifespan: 1) gene activation by calorie restriction (CR); 2) certain types of animals receiving genetic engineering (the artificial addition or deletion of genes); and 3) the use of chemicals that activate the Sir2 gene by lowering the Michaelis constants, K_m, of the Sir-2 enzymes for the co-substrate NAD+. CR is the limitation of total calories derived from carbohydrates, fats, or proteins to a level 25% to 60% below that of control animals fed ad libitum (Koubova et al, How does calorie restriction work? 2003 Genes & Development. Vol. 17 pp 212-221). Success in extending lifespan with gene activation by CR includes a wide range of different organisms including yeast, rotifers, guppies, spiders, fruit flies, hamsters, rats, mice

[0005]It is significant that CR works on such a wide range of organisms, from the single celled to very complex (including primates). The wide range of success of CR indicates that the process of life extension is based on the effects within the individual cells of the organisms, and that the process allowing life span extension is preserved across species. In rodents, the extension in life span can approach 50% (Koubova et al.). This lifespan comes at a price, however, as the organism needs to be fed at least 25% less calories than it would normally consume.


[0007]The benefits of CR are not due to dietary antioxidants, as single agents or combinations of antioxidants do not produce an increase in lifespan or delay tumorigenesis and other age related disease. Instead, CR works due to signaling changes that activate gene expression that reduce cellular proliferation or increase apoptosis. Multiple genes involved in the electron transport chain, immune response, protein turnover and protein synthesis are changed in CR (Lee, et al., The impact of α-Lipoic Acid, Coenzyme Q10, and Caloric Restriction on Life Span and Gene Expression Patterns in Mice, Free Radical Biology & Medicine, Vol. 36, No. 8, pp. 1043-1057, 2004). Masteimak et al shows that genes related to insulin and insulin growth factor 1 (IGF1) are altered including PPARα, a gene suggested to play an important role in metabolic
control and the accumulation and preservation of fat storage cells. (Masternak, et. al., Divergent Effects of Caloric Restriction on Gene Expression in Normal and Long-Lived Mice, Journal of Genontology, 2004, Vol. 59A, No. 8, 784-788). The activity of FOXO genes have also been shown to change under caloric restriction (Daitolku, et al., Silent information regulator 2 potenates Foxo1-mediated transcription through its deacetylase activity, PNAS, Jul. 6, 2004).

Within the last decade, it has been determined that the Silenced Information Regulator 2 (Sir2) gene in yeast and worms (Sir2.1 in worms, SIRT1 in humans) is also one of the genes that regulates lifespan and is activated in CR. Mutant worms and yeast with extra copies of Sir2 or Sir2.1 live longer, while mutations in the Sir2 gene severely reduce lifespan. See, e.g. Tissenbaum et al. Other animals contain similar genes or homologues to the Sir2 gene, including humans (the SIRT1 gene). CR creates a set of conditions in the cell that signals the activation of beneficial genes to lengthen lifespan and delay the onset of age-related disease. The activation of Sir2 by CR is one pathway to increased lifespan. CR also stimulates other genes that increase lifespan independent of Sir2 in a parallel pathway. Kaeberlein et al, “Sir2-Independent Life Span Extension by Calorie Restriction in Yeast” 2004, PloS Biology: 2: 9: e296: 1381-1387

It has been shown that activation of Sir2 can activate or silence other genes and proteins, including FOXO type genes. Also, the activation of the Sir2 gene (SIRT1 in humans) normally turned on in CR blunted the protein PPAR gamma that activated fat-storage genes, so that fat cells would shed fat and prevented cells from differentiating into fat cells. See, e.g. Picard et al. supra. This would explain the low amounts of fat seen in mammals under CR.

Lin et al. determined that the internal cellular signaling condition generated by CR to activate beneficial genes is the increase in NAD+/NADH (oxidized and reduced nicotinamide adenine dinucleotide) ratios within the cell as compared to non-CR conditions. Lin, S. et al, Calorie restriction extends yeast life span by lowering the level of NADH. 2004 Genes & Development Vol. 18 pp 12-16. Lin also noted that NAD+levels in cells remain constant between CR and non-CR conditions, while the reduced form of NAD+, NADH, is significantly lowered in CR (up to 50%), which allows activation of at least one beneficial gene, the Sir2 type gene. High levels of NADH are an inhibitor of the Sir2 gene.

Lin’s study showed at least one of the intracellular requirements for signaling the activation of beneficial genes resulting in increased longevity and health benefits found during CR. The study used recombinant genetic modifications to achieve the increase the ratio of NAD+/NADH, (without the restriction in calories) and thereby “mimic” caloric restriction results of increased lifespan and general improvement in health. The important characteristic shown was that calories did not have to be reduced, but rather that beneficial genes need to be activated within the individual cells in order to achieve the same benefits of CR.

In other studies by Horitz, Wood and Lamming, researchers have discovered an alternate pathway for increasing life span that is distinct from CR and genetic engineering to increase the NAD+/NADH ratio to stimulate at least one beneficial gene. Instead of inserting genes to modify the NAD+/NADH ratio or to add additional copies of a beneficial gene by genetic engineering, they instead used chemical agents to lower the substrate-binding affinity between NAD+ and Sir2 allowing the Sir2 (SIRT1 in humans) to activate more readily. Howitz et al., Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan, Nature 425: 191-196; Wood et al., Sirtuin activators mimic caloric restriction and delay ageing in metazoans, Nature, Volume 430, 5 Aug. 2004; Lamming et al., Small molecules that regulate lifespan: evidence for xenohormesis, Molecular Microbiology, 2004, Vol. 53(4), 1003-1009. The chemical agents discovered are polyphenols and include the compound resveratrol. The polyphenols are found in plants, but are not part of the natural chemical makeup of mammals including humans. The polyphenols are very specific in the activation of the Sir2 gene and its homologues. The Sir2 gene extends lifespan, but does not activate all of the beneficial genes activated by CR. As a result of this, the Sir2 activating polyphenols produce a lower increase in lifespan extension than does CR. Kaeberlein et al, “Sir2-Independent Life Span Extension by Calorie Restriction in Yeast” 2004, PloS Biology: 2: 9: e296: 1381-1387.

Changing the inter-cellular binding potential with chemical agents or using genetic
engineering to increase lifespan, reduce fat accumulation, and delay cancer and age related
disease and improve overall health is a marvelous achievement. As a caution, however, genetic
ingineering is hardly a well-understood field, and is unlikely to help increase the lifespan of
humans any time in the near future. Using chemical agents to lower the binding affinity of
certain enzymes in order to stimulate Sir2 or Sirt1 (in humans) is also an uncertain path, as
there is no long-term determination of risks. Additionally, the Sir2 or Sirt1 gene is only one of the
genomes that can be activated to increase lifespan, and produces a modest increase, whereas
activation of more beneficial genes can result in longer increases in lifespan. Finally, what if the
application of the foreign chemicals such as Resveratrol cause harm in some isolated area
of the human body?

[0014]The only long-term studies performed to extend lifespan, reduce body fat and delay
cancer and other age-related conditions focused on actual caloric restriction. The studies, done
since the 1930's, have shown the many benefits of caloric restriction, with the only noted
potential disadvantages being that organisms took longer before they were of age to reproduce,
and the organisms tended to be smaller than non-calorie restricted organisms.

[0015]We have been taught that the intercellular conditions seen in CR to activate beneficial
genomes include an increase in the NAD+/NADH ratio, which acts as a switching mechanism for
the cell. To lower overall risk, it would be better to stimulate the same set of beneficial genomes
seen in CR by using the identical signaling method for the genes involved with CR. It would be
beneficial to activate other life-extending genes in addition to or besides the Sir2 gene.
Moreover, it would be of great benefit to find chemical agents that increase the NAD+/NADH
ratio. Chemical agents that increase NAD+/NADH could provide a proven safe pathway (70
years of research) for lifespan expansion and the delay in the onset of age-related diseases. It
would also be beneficial if the activation agent to increase the NAD+/NADH ratio was a
chemical that is already found in mammals including humans, rather than introducing foreign
compounds with unknown long-term results.

[0016]Due to the wide variety of chemical reactions available to the cell, each cell reacts in a
manner to conserve the NAD+/NADH ratio. It is, in effect, a buffered response. It is especially
difficult to increase the ratio. However, ethanol can decrease the NAD+/NADH ratio, which
results in higher triglycerides and "fatty liver" disease.

[0017]Finding a compound to increase the NAD+/NADH ratio to activate beneficial genes is not
trivial. One reason for this is due to the difficulty in directly measuring the NAD+/NADH ratio with
current technology. Instead of measuring NAD+/NADH directly, the ratio is inferred indirectly by
the measurement of the pyruvate/lactate ratio. Typically, when the amount of pyruvate to lactate
increases, NAD+/NADH increases.

[0018]Thus, one method of increasing the NAD+/NADH ratio in the cells would be to increase
the amount of pyruvate into the cell. In gluconeogenesis, pyruvate can be converted to glucose
and converts a NADH to NAD+, which will increase the NAD+/NADH ratio. Also, under
anaerobic conditions, pyruvate is converted to lactate by the enzyme lactate dehydrogenase.
The conversion of pyruvate to lactate under anaerobic conditions again converts a NADH to
NAD+. There are reports of an increase in the NAD+/NADH ratio with the injection of pyruvate
into rats. Work done by Ido on the study of blood flow in the retina and visual cortex show that
NADH levels in the cytosol can be dropped by 50%, doubling the NAD+/NADH ratio. Ido, et al,
NADH augments blood flow in physiologically activated retina and visual cortex, PNAS, Jan. 13,
2004, Vol. 101, no. 2 pp 653-658. Despite this reported temporary change in the ratio, no
extension of lifespan occurs with pyruvate because pyruvate also penetrates the inner
mitochondrial membrane and preferentially engages in lowering the NAD+/NADH ratio through
the Citric Acid Cycle. The ratio, temporarily raised by pyruvate, is then lowered when the
pyruvate is processed through the Citric Acid Cycle. The typical cell buffers against increases in
the NAD+/NADH ratio.

[0019]Anderson, et al. also had difficulty in using chemical agents to increase the NAD+/NADH
ratio and activate beneficial genes. Anderson used acetaldehyde, known to reduce NADH in
cells, but did not see any increase in the activity of beneficial genes. There is also some debate
that changing the NAD+/NADH ratio will activate beneficial genes at all. Based on his work,
Anderson teaches, "variations in NADH are unlikely to affect the activity of Sir2 or SIRT1" (beneficial genes) Anderson et al., Yeast Life-Span Extension by Calorie Restriction Is Independent of NAD Fluct. . . , Science 2003 302: 2124-2126.

[0020]There is a current need to create intracellular conditions similar to CR (i.e. increase of the NAD+/NADH ratio) with a Caloric Restriction "mimic" chemical that would allow beneficial genes to be implemented. Thus, the benefits of increased lifespan, lower cancer rates, lower body fat content and the delay in age-related disease without the heavy restrictions of diet imposed by CR or by genetic modification of the individual organism can be realized. The preference would be to have the chemical agents be currently part of human metabolism. The present invention provides such a chemical and method for the novel activation of beneficial genes.

SUMMARY OF THE INVENTION

[0021]The disclosure of the invention relates to methods and compositions for extending the lifespan and delaying the onset of age-related disease in an individual in need thereof. In one aspect of the invention, a method for extending the lifespan of an organism is provided. The method includes administering an effective amount of a compound such as oxaloacetate, oxaloacetic acid, an oxaloacetate salt, or its metabolic precursors alpha-ketoglutarate or aspartate. The compound can be administered orally, topically, and/or parenterally. Advantageously, the compound is formulated with a buffer. Optionally, the organism is a mammal. In one aspect of the invention, the mammal is a human.

[0022]In another aspect of the invention, a method of mimicking the beneficial health effect of caloric restriction without reducing caloric intake is described, wherein the method includes administering an effective amount of oxaloacetate, oxaloacetic acid, or an oxaloacetate salt. The beneficial health effect of caloric restriction can include weight loss, improvement of cardiac function, reversal of diabetes, and extension of life span. Advantageously, the compound is formulated for oral, parenteral, or topical administration. In a further aspect of the invention, the compound can include a buffer. Optionally, the method can include the step of administering a therapeutic agent such as an antibacterial, an antifungal, a chemotherapeutic agent, an anti-histamine, protein, enzyme, hormone, non-steroidal anti-inflammatory, an immuno-stimulatory compound, or a steroid. The therapeutic agent can be administered separately from the compound or substantially contemporaneously with the compound.

[0023]In another aspect of the invention, a composition for treating symptoms of skin aging is described. The composition can include an effective amount of oxaloacetate, oxaloacetic acid, an oxaloacetate salt, alpha-ketoglutarate, or aspartate, and a pharmaceutically effective carrier. Advantageously, the pharmaceutically effective carrier can be a cream, a soap, a shampoo, a conditioner, an ointment, a lotion, a gel, a salve, or an aerosol spray. Optionally, the composition can include a second beneficial agent such as an emollient, sunscreen, moisturizer, and/or buffer. The composition can be useful in treating symptoms of skin aging such as rhytids, wrinkles, jowls, sun damage, dull appearance of skin, loss of skin taughtness, keratosis, hypelpigmentation, melasma, and skin discoloration. The composition can further include a lipophilic agent, wherein the lipophilic agent acts to modify the rate of absorption of the composition.

[0024]A method for reducing the signs of skin aging is likewise provided. The method includes topically administering an effective amount of oxaloacetate, oxaloacetic acid, an oxaloacetate salt, alpha-ketoglutarate, or aspartate and a pharmaceutically acceptable carrier.

[0025]In yet another aspect of the invention, a method for protecting DNA and enhancing DNA damage repair from sun exposure is described. The method includes topically administering an effective amount of oxaloacetate, oxaloacetic acid, an oxaloacetate salt, alpha-ketoglutarate and aspartate and a pharmaceutically acceptable carrier.

[0026]In still another aspect of the invention, an improved animal chow formulation for increasing the life span of an animal is described, wherein the animal chow includes oxaloacetate, oxaloacetic acid, an oxaloacetate salt, alpha-ketoglutarate, or aspartate.
A method for activating beneficial genes and gene homologues by administering an effective amount of oxaloacetate, oxaloacetic acid, an oxaloacetate salt, alpha-ketoglutarate or aspartate is likewise described. The Oxaloacetate administration induces the change in expression of 356 genes in liver tissue in a similar manner as expressed by animals under Calorie Restriction. The change in these genes was sufficient to induce increases in health span and life span. The oxaloacetate can be administered orally, topically, or parenterally and is advantageously formulated with a buffer.

In another aspect of the invention, a method for reducing the incidence of cancer, treating cancer, and increasing the effectiveness of cancer treatment is described. The method includes administering to an individual in need thereof a pharmaceutically effective amount of a compound such as oxaloacetate, oxaloacetic acid, an oxaloacetate salt, alpha-ketoglutarate and aspartate. Optionally, the method can include the administration of a chemotherapeutic agent such as cyclophosphamide, chlorambucil, melphalan, estramustine, iphosphamide, prednimustin, busulphan, tiotepa, carmustin, lomustine, methotrexate, azathioprine, mercaptopurine, thioguanine, cytarabine, fluorouracil, vinblastine, vincristine, vindesine, etoposide, teniposide, dacitomomucin, doxorubin, dunorubicine, epirubicine, bleomycin, nitomycin, cisplatin, carboplatin, procarbazine, amacrine, mitoxantron, tamoxifen, nilutamid, oraminoglutemide. The compound can be administered orally, topically, or parenterally. In some aspects of the invention, the chemotherapeutic agent is administered prior to administering the oxaloacetate compound. In other aspects, the chemotherapeutic agent is administered after or substantially contemporaneously with administering the compound. The cancer can be primary or metastatic malignant solid tumor disease or a hematological malignancy. If the cancer is a hematological malignancy, it may include acute and chronic myelogenous leukemia, acute and chronic lymphatic leukemia, multiple myeloma, Waldenstrom's macroglobulinemia, hairy cell leukemia, myelodisplastic syndrome, polycytaemia vera, and essential thrombocytosis.

In another aspect of the invention, a method of treating a disease associated with aging is described. The method includes administering a pharmaceutically acceptable amount of a compound selected from the group consisting of oxaloacetate, oxaloacetic acid, an oxaloacetate salt, alpha-ketoglutarate and aspartate, wherein the compound is formulated with a pharmaceutically acceptable carrier. A disease associated with aging can include osteoporosis, bone loss, arthritis, stiffening joints, cataracts, macular degeneration, diabetes, inflammation and heart disease. Optionally, the disease can be a neurodegenerative disease such as Alzheimer disease or Parkinson's disease.

In another aspect of the invention, a method of reducing the symptoms associated with over-consumption of alcohol is provided. The method includes identifying an individual suffering from over-consumption of alcohol and administering a pharmaceutically effective amount of oxaloacetate, oxaloacetic acid, an oxaloacetate salt, alpha-ketoglutarate or aspartate. Symptoms of over-consumption of alcohol include, for example, headache, poor sense of overall well-being, diarrhea, loss of appetite, shakiness, fatigue, and nausea.

In another aspect of the invention, a composition of matter formulated for topical administration is described, wherein the composition includes oxaloacetate, oxaloacetic acid, an oxaloacetate salt, alpha-ketoglutarate, or aspartate, and a sunscreen.

In yet another aspect of the invention, a cosmetic composition formulated for topical administration is provided, wherein the cosmetic composition includes a compound such as oxaloacetate, oxaloacetic acid, an oxaloacetate salt, alpha-ketoglutarate or aspartate, and a cosmetic carrier.

In still another aspect of the invention, a composition of matter formulated for oral administration is disclosed, wherein the composition includes oxaloacetate, oxaloacetic acid, an oxaloacetate salt, alpha-ketoglutarate or aspartate and a vitamin.

In another aspect of the invention, a therapeutic composition is disclosed, wherein the composition includes a compound such as oxaloacetate, oxaloacetic acid, an oxaloacetate salt, alpha-ketoglutarate or aspartate and a therapeutic agent.
BRIEF DESCRIPTION OF THE DRAWINGS

[0036] FIG. 1 is a schematic representation of the effect of oxaloacetate supplementation on increasing the ratio of NAD+/NADH to create a biological diode.

[0037] FIG. 2 is a graph illustrating the effect of oxaloacetate supplementation on extending the life span of the C. elegans nematode.

[0038] FIG. 3 is a graph illustrating the effect of Splitomycin (a selective Sir2 inhibitor) and oxaloacetate supplementation on the lifespan of the C. elegans nematode.

[0039] FIG. 4 is a graph illustrating the effect of oxaloacetate supplementation on extending the life span of the D. melanogaster fruit fly.

[0040] FIG. 5 is a graph illustrating the effect of oxaloacetate supplementation on extending the life span of the D. melanogaster fruit fly when the fly is placed under stress.

[0041] FIG. 6 is a graph illustrating the effect of oxaloacetate supplementation on the reduced weight gain of older C57B1/6 type mice.

[0042] FIG. 7 is a graph illustrating the effect of oxaloacetate supplementation on the reduced weight gain of younger C57BL/6 type mice.

[0043] FIG. 8 is a graph illustrating the effect of oxaloacetate supplementation on extending the life span of C57B1/6 type mice.

[0044] FIG. 9 is graph indicating the overlap between the change in gene expression between mice that are calorie restricted and mice that are supplemented with oxaloacetate versus a control group of mice fed ad libitum.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[0045] The present invention relates to compositions and methods for extending lifespan and treating disorders associated with aging in an individual in need thereof. The present invention is based, in part, on the surprising discovery that the administration of oxaloacetate and chemical precursors, including alpha-ketoglutarate and aspartate, results in a dose dependent lifespan increase in average population life span of up to 36% and up to 40% increase in maximal life span over similar populations of multi-cell control organisms including simple animals such as the nematode C. elegans, the more complicated fly D. melanogaster and in complex mammals. Without being bound to a particular theory, it is believed that external cellular contact with an oxaloacetate compound or its precursors, and subsequent transfer of the oxaloacetate into the cell, leads to metabolic signaling changes that activate beneficial genes that increase the lifespan of organisms. As used herein, the term "oxaloacetate" includes oxaloacetate, its salts, and chemical precursors of oxaloacetate including, without limitation, alpha-ketoglutarate and aspartate. The phrase "individual in need thereof" refers to any multi-cellular organism that would benefit from the life-extending and/or anti-aging effects of oxaloacetate. An individual includes, without limitation, any vertebrate or invertebrate susceptible to oxaloacetate administration. Exemplary vertebrates include fish, amphibians, reptiles, birds, and mammals such as humans, primates, canines, felines, or other animals.

[0046] The present invention is based, in part, on the observation that oxaloacetate introduced into a cell cannot cross the inner membrane of the mitochondria [23]. The additional oxaloacetate introduced into the cytosol is reduced to malate by the enzyme malate dehydrogenase. This reaction also converts NADH into NAD+, increasing the NAD+/NADH ratio. The malate formed by the introduction of additional oxaloacetate to the cytosol can cross the mitochondria membrane through an exchange for α-ketoglutarate. Once in the mitochondria, the malate can be converted back into oxaloacetate by way of the Citric Acid Cycle. Conversion of the malate back into oxaloacetate would generate NADH from NAD+, and lower the NAD+/NADH ratio, as occurs in pyruvate which prevents the increase in lifespan. The lowering
of the NAD+/NADH ratio, however, does not occur with the addition of oxaloacetate in the cytosol, because the Gibbs Free Energy, delta G, is highly positive (+29.7) for the reaction of malate to oxaloacetate. Under normal conditions, the only reason the reaction of malate to oxaloacetate proceeds at all in the Citric Acid Cycle is due to the energy gained due to the conversion of oxaloacetate to acetyl CoA in the mitochondria (delta G of -32.2) and the energy of the other intermediates of the Citric Acid Cycle. Because the oxaloacetate added to the cytosol cannot penetrate the mitochondrial membrane, there is no additional oxaloacetate in the mitochondria to power the reaction of malate to oxaloacetate in the Citric Acid Cycle. Thus, the ratio of NAD+/NADH stays high with the addition of oxaloacetate to the cytosol. In effect, an electron biological diode is created by the addition of oxaloacetate to the cytosol, the inability of oxaloacetate to penetrate the mitochondria, and the high delta G of the reaction of malate back into oxaloacetate in the mitochondria. FIG. 1 illustrates the effect of oxaloacetate supplementation on increasing the ratio of NAD+/NADH. As indicated in FIG. 1, the supplementation of extra-cellular oxaloacetate acts to penetrate the cell membrane but is precluded from penetrating the inner mitochondrial membrane. The maintained increase in the NAD+/NADH ratio is a signaling effect that starts the increase in the expression of beneficial genes and the increase in associated beneficial proteins along with the decrease in the expression of non-beneficial genes and the decrease in associate non-beneficial proteins. By keeping the NAD+/NADH ratio higher than would normally occur, oxaloacetate effectively mimics the effect of CR and facilitates the regulation of genes to produce beneficial repairs, reduce the incidence of cancer and other age-related disease, block fat production, reduce apoptosis, and increase the overall lifespan of an organism.

The increase in NAD+/NADH ratio by oxaloacetate allows the activation of beneficial genes, which results in the same benefits as those seen in CR because the signaling mechanism is similar. The beneficial genes upregulated in CR include the following classes of genes: lipid catabolism and activation of the oxidative stress response; regulation of central metabolic pathways including SAM and urea cycles; regulation of hormonal pathways; DHEA and insulin/igf signaling; genome instability and apoptosis. Reviews of these gene types can be found in works by Bauer (Bauer, et al, "Starvation response in mouse liver shows strong correlation with lifespan prolonging processes", Physiologicaly Genomics, Feb. 3, 2004, 10.1152/physiolgenomics.00203.2003), Cao (Cao, et al, "Genomic profiling of short- and long-term caloric restriction effects in the liver of aging mice", Proc Natl Acad Sci 98: 10630-10635, 2001), and Lee (Lee, et al., The impact of α-Lipoic Acid, Coenzyme Q10, and Caloric Restriction on Life Span and Gene Expression Patterns in Mice, Free Radical Biology & Medicine, Vol. 36, No. 8, pp. 1043-1057, 2004) hereby incorporated by reference in their entireties. The inventor shows that mice subjected to calorie restriction results in the change of levels of gene expression in 1,763 genes in liver tissue as compared to a control group fed freely. Mice fed oxaloacetate but allowed to eat freely resulted in a change of expression in 765 genes. Because these are pooled results, many of the changes in gene expression are due to individual variations within the mice. However, when genes that are changed from the control group are commonly expressed by both the calorie restricted mice and the oxaloacetate administered mice, these genes can be considered as the driving reason for similarities in physical changes as compared to the control group fed freely that they are compared against. The physical changes documented include the decrease in body weight, an increase in health span and resistance to disease, and an increase in lifespan. 363 genes showed a common change from the control mice. Of these 363 genes, 357 show either an upregulation of the expression of the gene or a down regulation in the expression of the gene in the same direction away from the control group. It is apparent that these 357 genes expressed in similar fashion as compared to the control are responsible for the positive changes in lifespan (98% of all genes changed in common as compared to the control group). Homologues of these genes expressed in other animals will have a similar physical effect. Note that there may be other genes expressed in other tissues other than the liver that undoubtedly also assist in lifespan extension, however the liver is one of the key organs for the regulation of metabolism, that through calorie restriction has shown to be critical to increases in mammalian lifespan and health span.

The beneficial genes activated and non-beneficial genes down-regulated in the liver tissue are shown in Tables 1 and 2.

**TABLE-US-00001** **TABLE 1 Directional Analysis of Gene Expression comparison of Calorie...
Restricted Mice and Oxaloacetae Mice to Control Mice change in Gene Activity Expressed by Oxaloacetae and CR Mice Versus Control Mice Expression for Genes Shown to Change Commonly Affymatrix Mouse Genome 430 2.0 Array CR to C OX to C Gene Movement in Gene Symbol Gene Title Affymatrix No. Signal Log Ratio Change Signal Log Ratio Change Same Direction? Aacs acetocetol-CoA synthetase 8056 1.5 -0.2 D NO Abcg2 ATP-binding cassette, sub-family G (WHITE), member 2 7165 -0.5 D -0.3 D YES Abhd6 abhydrolase domain containing 6 3498 -0.3 D -0.4 D YES Acaad 1 acetol-Coenzyme A acyltransferase 1 1341 -0.3 D -0.3 D YES Acp1 acid phosphatase 1, soluble 6975 -1.1 D -0.9 D YES Actb actin, beta, cytoplasmic 46 -0.3 D -0.2 D YES Actg actin, gamma, cytoplasmic 174 -0.4 D -0.5 D YES Adn adipsin 2262 -1.1 D -0.7 D YES Ahcy1 S-adenosylhomocysteine hydrolase-like 1 11090 -0.6 D -0.4 D YES AI746432 expressed sequence AI746432 8752 -0.3 D -0.3 D YES AI746432 expressed sequence AI746432 19500 -0.4 D -0.5 D YES Akr1d1aldo-keto reductase1 family, member D1 21474 -0.7 D -0.5 D YES Aldh3a2 aldehyde dehydrogenase family 3, subfamily A2 171 -0.3 D -0.4 D YES Anxa5 annexin A5 9826 -0.7 D -0.4 D YES Aof1 amine oxidase, flavin containing 1 27011 -0.7 D -0.7 D YES Aox1 aldehyde oxidase 1 3830 -0.7 D -0.4 D YES Ap3s2 adator-related protein complex 3, sigma 2 subunit 3760 -0.3 D -0.4 D YES Ap3s2 adaptor-related protein complex 3, sigma 2 subunit 17618 -0.2 D -0.3 D YES Apoa4 apolipoprotein A-IV 2156 -0.7 D -0.8 D YES Apoa4 apolipoprotein A-IV 14772 -0.7 D -1 D YES Asb13 ankyrin repeat and SOCS box-containing protein 13 3796 -0.9 D -0.7 D YES Asb13 ankyrin repeat and SOCS box-containing protein 13 17635 -0.8 D -0.3 D YES Atg6v1 ATGase, H+, transporting, lysosomal 50/57 kDa, V1 subunit H 221 -0.4 D -0.5 D YES Bpt1 bisphosphate 3'-nucleotidase 1 17387 -0.6 D -0.7 D YES Bpr17 brain protein 17 3141 -1.1 D -0.4 D YES Btf3 basic transcription factor 3 8098 -0.4 D -0.5 D YES Btg1 B-cell translocation gene 1, anti-proliferative 10342 -0.9 D -0.3 D YES Btg2 B-cell translocation gene 2, anti-proliferative 16448 -0.8 D -0.4 D YES C330018j07Rik RIKEN cdna C330018j07 gene 11390 -0.6 D -0.6 D YES Cald1 caldesmon 1 9027 -0.4 D -0.4 D YES Calm1 calmodulin 1 21261 -0.5 D -0.3 D YES Car14 carbonic anhydrase 14 18852 -0.4 D -0.6 D YES Cbxb1 chromobox homolog 1 (Drosophila HP1 beta) 13736 -0.5 D -0.4 D YES Ccng1 cyclin G1 5086 -0.9 D -0.3 D YES Cct6a chaperonin subunit 6a (zeta) 21805 -0.5 D -0.2 D YES Cdl151 CD151 antigen 21861 -0.8 D -0.5 D YES Cdl163 CD163 antigen 3539 -1 D -0.5 D YES Cds6 CD36 antigen 7425 -1.1 D -0.4 D YES Cds6 CD36 antigen 19010 -1.2 D -0.7 D YES Cds6 CD36 antigen 19011 -1.3 D -0.8 D YES Cd59a antigen 3105 -0.3 D -0.3 D YES Cd59a antigen 12906 -0.5 D -0.3 D YES Cdc42 cell division cycle 42 homolog (S. cerevisiae) 119 -0.4 D -0.5 D YES Ces3 carboxylesterase 3 14412 -0.5 D -0.4 D YES Chc1I chromosome condensation 1-like 784 -0.7 D -0.5 D YES Chpt1 choline phosphotransferase 1 10405 -0.6 D -0.4 D YES Chpt1 choline phosphotransferase 1 14436 -0.4 D -0.3 D YES Cklls6 chemokine-like factor super family 6 8051 -0.3 D -0.5 D YES Cml5 camello-like 5 9070 -1.3 D -0.7 D YES Cnp1b cellular nucleic acid binding protein 15347 -0.9 D -0.5 D YES Cnn3 calprolin 3, acidic 21628 0.5 I -0.4 D YES Co3a31 procollagen, type III, alpha 1 12142 -1.1 D -0.7 D YES Crys crystallin, zeta 15627 -0.7 D -0.7 D YES Cugbp2 CUG triplet repeat, RNA binding protein 2 19281 -0.5 D -0.9 D YES Cxadr coxsackievirus and adenovirus receptor 39334 -0.5 D -0.4 D YES Cyp17a1 cholesteryl P450, family 17, subfamily a, polypeptide 1 1412 -1.1 D -0.4 D YES Cyp2b20 cytochrome P450, family 2, subfamily b, polypeptide 20 6516 -1 D -0.3 D YES Cyp2b20 cytochrome P450, family 2, subfamily b, polypeptide 20 9904 -1.1 D -0.3 D YES Cyp2b20 cytochrome P450, family 2, subfamily b, polypeptide 20 19914 -0.8 D -0.3 D YES Cyp2b9 cytochrome P450, family 2, subfamily b, polypeptide 9 3985 -2.5 D -0.1 D YES Cyp3c38 cytochrome P450, family 2, subfamily c, polypeptide 38 20628 -0.9 D -0.6 D YES Cyp2j5 cytochrome P450, family 2, subfamily j, polypeptide 5 1926 -0.2 D -0.3 D YES Cyp2j5 cytochrome P450, family 2, subfamily j, polypeptide 5 1927 -0.7 D -0.5 D YES Cyp4a10 cytochrome P450, family 4, subfamily a, polypeptide 3 15692 1.6 I 0.6 I YES D10Erd641e DNA segment, Chr 10, ERATO Doi 641, expressed 17514 -0.5 D -0.2 D YES D11Erd175e DNA segment, Chr 11, ERATO Doi 175, expressed 1000 -0.3 D -0.3 D YES D11Erd672e DNA segment, Chr 11, ERATO Doi 672, expressed 15860 -0.8 D -0.5 D YES D19Wsu12e DNA segment, Chr 19, Wayne State University University, 12 expressed 13728 -0.7 D -0.3 D YES D430028G21Rik RIKEN cdna D430028G21 gene 15861 -0.5 D -0.8 D YES D5300020C15Rik RIKEN cdna D5300020C15 gene 8006 -0.8 D -0.7 D YES D630002G06 hypothetical protein D630002G06 19762 -1.3 D -0.5 D YES D8Wsu49e DNA segment, Chr 8, Wayne State University 49, expressed 14498 -0.4 D -0.7 D YES Ddc dopa decarboxylase 10474 -1.1 D -0.4 D YES Desr developmentally and sexually retarded with transient immune abnormalities 27378 0.9 0.7 I YES Desr developmentally and
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rat (fragments) (LOC232993), 17655 -3.6 D -4.2 D YES mRNA -- -- 18738 -1.9 D -1.2 D YES -- Mus musculus transcribed sequences 21421 -1.2 D -0.5 D YES -- Mus musculus similar to glucosamine-6-phosphate deaminase (LOC381691), mRNA 21459 -0.8 D -0.6 D YES -- Mus musculus similar to Cytochrome c, somatic (LOC384146), mRNA 21852 -0.5 D -0.3 D YES -- Mus musculus transcribed sequences 22028 -0.4 D -0.4 D YES -- -- 22122 -0.4 D -0.4 D YES -- Mus musculus adult male liver tumor cDNA, RIKEN full-length enriched library, 27836 -0.7 D -0.3 D YES clone: C730049014 product:unknown EST, full insert sequence -- Mus musculus transcribed sequences 30047 -0.4 D -0.6 D YES -- -- 31861 -0.5 D -0.6 D YES -- Mus musculus transcribed sequences 31941 -0.7 D -0.5 D YES -- -- 38789 -0.6 D -0.5 D YES -- Mus musculus transcribed sequences 38815 -2.9 D -0.7 D YES -- Mus musculus adult male corpora quadrigemina cDNA, RIKEN full-length enriched 41400 -1.0 D -0.8 D YES library, clone: B230114P17 product:unknown EST, full insert sequence -- Mus musculus similar to cadherin 19, type 2 preproprotein (LOC227485), mRNA 42276 -1.4 D -0.8 D YES -- -- 43216 -0.7 D -0.5 D YES -- Mus musculus transcribed sequences 43312 -2.1 D -2.1 D YES -- Mus musculus transcribed sequences 45080 -1.8 D -0.7 D YES 0610012D09Rik RIKEN cDNA 0610012D09 gene 15468 -0.5 D -0.5 D YES 0610012H03Rik RIKEN cDNA 0610012H03 gene 39151 -0.4 D -0.5 D YES 0610016O18Rik RIKEN cDNA 0610016O18 gene 39221 -0.6 D -0.6 D YES 0610033L19Rik RIKEN cDNA 0610033L19 gene 10852 -0.5 D -0.6 D YES 0610039N19Rik RIKEN cDNA 0610039N19 gene 8975 -0.5 D -0.7 D YES 1110028A07Rik RIKEN cDNA 1110028A07 gene 19615 -0.6 D -0.2 D YES 1110067D22Rik RIKEN cDNA 1110067D22 gene 19440 -1.8 D -0.6 D YES 1200015F23Rik RIKEN cDNA 1200015F23 gene 12422 -0.4 D -0.4 D YES 1300014I06Rik RIKEN cDNA 1300014I06 gene 23234 -0.2 D -0.3 D YES 1600032L17Rik...
RIKEN cDNA 1600032L17 gene 23279 -0.8 D -1.7 D YES 1700124F02Rik RIKEN cDNA 1700124F02 gene 19675 0.61 -0.3 D YES 1810011O10Rik RIKEN cDNA 1810011O10 gene 19542 -0.7 D -0.4 D YES 1810023F06Rik RIKEN cDNA 1810023F06 gene 9379 -1.5 D -0.6 D YES 1810029G24Rik RIKEN cDNA 1810029G24 gene 22643 -0.5 D -0.3 D YES 1810044O22Rik RIKEN cDNA 1810044O22 gene 17020 -0.6 D -0.3 D YES 2010004N24Rik RIKEN cDNA 2010004N24 gene 12995 -0.6 D -0.7 D YES 2010306G19Rik RIKEN cDNA 2010306G19 gene 13304 -0.8 D -0.3 D YES 2310075C12Rik RIKEN cDNA 2310075C12 gene 16799 -0.6 D -0.4 D YES 2310076L09Rik RIKEN cDNA 2310076L09 gene 9196 -0.4 D -0.6 D YES 2310076L09Rik RIKEN cDNA 2310076L09 gene 32934 -0.4 D -0.4 D YES 2400006A19Rik RIKEN cDNA 2400006A19 gene 7955 -0.5 D -0.3 D YES 2410003B16Rik RIKEN cDNA 2410003B16 gene 27074 -1.2 D -0.4 D YES 2410013I23Rik RIKEN cDNA 2410013I23 gene 8383 -0.6 D -0.5 D YES 2510004L01Rik RIKEN cDNA 2510004L01 gene 5268 -1.8 D -0.9 D YES 2510004L01Rik RIKEN cDNA 2510004L01 gene 14650 -2.1 D -0.6 D YES 2510006C20Rik RIKEN cDNA 2510006C20 gene 3469 -0.5 D -0.6 D YES 2600017P15Rik RIKEN cDNA 2600017P15 gene 12858 -0.4 D -0.4 D YES 2610030H06Rik RIKEN cDNA 2610030H06 gene 27347 -0.6 D -0.5 D YES 2610207I16Rik RIKEN cDNA 2610207I16 gene 11115 -0.7 D -0.7 D YES 2610318G18Rik RIKEN cDNA 2610318G18 gene 9041 -0.8 D -0.7 D YES 2990026G05Rik RIKEN cDNA 2990026G05 gene 15394 -0.6 D -0.4 D YES 3300001H21Rik RIKEN cDNA 3300001H21 gene 13914 -0.4 D -0.5 D YES 3930402F23Rik RIKEN cDNA 3930402F23 gene 2566 -1.3 D -1 D YES 4631422C05Rik RIKEN cDNA 4631422C05 gene 15552 -0.4 D -0.4 D YES 4733401N12Rik RIKEN cDNA 4733401N12 gene 22855 -0.9 D -0.3 D YES 4833411K15Rik RIKEN cDNA 4833411K15 gene 21763 -0.4 D -0.6 D YES 4833439L19Rik RIKEN cDNA 4833439L19 gene 16276 -0.2 D -0.4 D YES 4930469P12Rik RIKEN cDNA 4930469P12 gene 3391 -0.4 D -0.3 D YES 4931406C07Rik RIKEN cDNA 4931406C07 gene 21195 -0.5 D -0.5 D YES 4933433D23Rik RIKEN cDNA 4933433D23 gene 5094 1.6 I 0.7 I YES 5033421K01Rik RIKEN cDNA 5033421K01 gene 10711 -1.3 D -1D YES 5730494M16Rik RIKEN cDNA 5730494M16 gene 44727 -2.2 D -1.2 D YES 5730494N06Rik RIKEN cDNA 5730494N06 gene 20857 -0.3 D -0.3 YES 5830413E08Rik RIKEN cDNA 5830413E08 gene 12475 -1.2 D -0.6 D YES 6030440G05Rik RIKEN cDNA 6030440G05 gene 15466 -0.7 D -0.3 D YES 6300587F24Rik RIKEN cDNA 6300587F24 gene 41583 -0.8 D -0.6 D YES 6430628I05Rik RIKEN cDNA 6430628I05 gene 8245 -0.8 D -0.4 D YES 6430628I05Rik RIKEN cDNA 6430628I05 gene 15190 -0.8 D -0.3 D YES 6530411B15Rik RIKEN cDNA 6530411B15 gene 8392 -0.5 D -0.5 D YES 9030624L02Rik RIKEN cDNA 9030624L02 gene 11396 -0.7 D -0.3 D YES 9130009C22Rik RIKEN cDNA 9130009C22 gene 10535 -0.6 D -0.3 D YES 9130019P20Rik RIKEN cDNA 9130019P20 gene 39136 2.1 0.6 YES 9630015D15Rik RIKEN cDNA 9630015D15 gene 27052 -0.6 D -0.3 D YES A430056A10Rik RIKEN cDNA A430056A10 gene 7814 -2.6 D -1.5 D YES A63002509Rik RIKEN cDNA A63002509 gene 30037 -1 D -1.2 D YES A930009M04Rik RIKEN cDNA A930009M04 gene 15355 -0.8 D -0.5 D YES AW539457 expressed sequence AW539457 26927 -1.8 D -0.9 D YES BC005632 cDNA sequence BC005632 148 -0.3 D -0.2 D YES BC023754 cDNA sequence BC023754 11102 0.8 10.5 YES BC035295 cDNA sequence BC035295 27518 1.1 11.3 YES Mice Fed Oxaloacetate with Genes Moving in Same Direction as Calorie Restricted Mice 356 Mice Fed Oxaloacetate with Genes Moving in Opposite Direction as Calorie Restricted Mice 7 Percentage of Mice Fed Oxaloacetate with Genes Moving in Same Direction as Calorie Restricted Mice 98.1% Gene Ontology Biological Process Gene Ontology Cellular Component Gene Ontology Molecular Function Pathway InterPro 8152 // metabolism // inferred from -- 3824 // catalytic activity // inferred from -- IPR000873 // AMP-dependent sequence or structural similarity sequence or structural similarity // 30729 // synthetase and ligase // IPR005914 // 6.2.1.16; acetoacetate-CoA ligase Acetoacetyl-CoA synthase activity; 1.03e-132 // extended:Unknown 6810 // transport // inferred from electronic 5887 // integral to plasma membrane // 166 // nucleotide binding // inferred from -- IPR003439 // ABC transporter // annotation inferred from electronic annotation // 16020 sequence or structural similarity // 4009 // IPR006162 // Phosphopantetheine // membrane // inferred from sequence or ATP-binding cassette (ABC) transporter attachment site // IPR003593 // AAA structural similarity // 16021 // integral to activity // inferred from electronic ATPase membrane // traceable author statement annotation // 5524 // ATP binding // inferred from electronic annotation

6725 //aromatic compound metabolism // 16021 // integral to membrane // traceable 3824 // catalytic activity // inferred from -- IPR000073 // Alpha/beta hydrolase inferred from sequence or structural author statement sequence or structural similarity or structural similarity // 16787 // fold // IPR003089 //
6730 // one-carbon compound metabolism // extracellular space // traceable 4089 //
carbonate dehydratase activity // -- IPR001148 // Carbonic anhydrase, // inferred from electronic
annotation author statement // 16021 // integral to inferred from electronic annotation // 8270
eukaryotic membrane // traceable author statement // zinc ion binding // inferred from electronic
annotation // 16829 // lyase activity // inferred from electronic annotation 6333 // chromatin
assembly/disassembly // 785 // chromatin // inferred from sequence 3682 // chromatin binding //
inferrerd from Gene_Trap_Resource_2-IPR000953 // Chromo // IPR008251 // inferred from
sequence or structural or structural similarity // 5634 // nucleus // sequence or structural
similarity // 5515 // 04-02_Named_Genes Chromo shadow similarity inferred from electronic
annotation // 5654 protein binding // inferred from physical // nucleoplasm // inferred from
sequence or interaction structural similarity // 5720 // nuclear heterochromatin // inferred from
sequence or structural similarity // 5721 // centric heterochromatin // inferred from direct assay
74 // regulation of cell cycle // inferred from 5634 // nucleus // inferred from electronic 16538 //
cyclin-dependent protein kinase -- IPR006671 // Cyclin, N-terminal electronic annotation // 910
// cytokinesis // annotation regulator activity // inferred from electronic domain // IPR006670 //
Cyclin inferred from electronic annotation // 7049 annotation // cell cycle // inferred from
electronic annotation // 7067 // mitosis // inferred from electronic annotation 6457 // protein
folding // inferred from -- 3754 // chaperone activity // inferred from Gene_Trap_Resource_2-
IPR002423 // Chaperonin Cpn60/TCP- electronic annotation electronic annotation // 5524 //
ATP 04-02_Named_Genes 1 // IPR001844 // Chaperonin Cpn60 binding // inferred from
electronic // IPR002194 // Chaperonin TCP-1 annotation IPR008950 // GroEL-like
chaperone, ATPase -- 5866 // plasma membrane // inferred from -- -- IPR00301 //
CD9/CD37/CD63 electronic annotation // 16021 // integral to antigen // IPR00830 //
membrane // traceable author statement Peripherin/rom-1 // IPR008952 // Tetraspanin -- 5615
// extracellular space // traceable 4872 // receptor activity // inferred from -- IPR001190 //
Speract/scavenger author statement // 16020 // membrane // electronic annotation // 5044 //
scavenger receptor inferred from sequence or sequence structural activity // inferred from
sequence similarity // 16021 // integral to membrane or structural similarity // traceable author
statement 6810 // transport // inferred from electronic 5764 // lysosome // inferred from electronic
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// annotation // 7155 // cell adhesion // annotation // 5886 // plasma membrane // electronic
annotation // 5194 // cell IPR005428 // Adhesion molecule inferred from electronic annotation
inferred from electronic annotation // 16020 adhesion molecule activity // inferred from CD36 //
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integral to membrane // traceable author statement 6810 // transport // inferred from electronic
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electronic annotation // 5194 // cell IPR005428 // Adhesion molecule inferred from electronic
annotation inferred from electronic annotation // 16020 adhesion molecule activity // inferred from
CD36 // membrane // inferred from electronic sequence or structural similarity annotation //
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traceable -- Gene_Trap_Resource_2- IPR003632 // Cell-surface glycoprotein author statement
// 5886 // plasma 04-02_Named_Genes Ly-6/CD59 // IPR001526 // CD59 membrane // inferred
from electronic antigen annotation -- 5615 // extracellular space // traceable --
Gene_Trap_Resource_2- IPR003632 // Cell-surface glycoprotein author statement // 5886 //
plasma 04-02_Named_Genes Ly-6/CD59 // IPR001526 // CD59 membrane // inferred from
electronic antigen annotation 910 // cytokinesis // inferred from electronic 30175 // filopodium
inferred from 3924 // GTPase activity // traceable author G13_Signaling_Pathway IPR001806 //
Ras GTPase superfamily annotation // 7015 // actin filament sequence or structural similarity
statement // 3925 // small monomeric //// IPR003578 // Ras small GTPase, organization //

traceable 4497 // monooxygenase activity // inferred -- IPR001128 // Cytochrome P450 //
electronic annotation author statement // 5783 // endoplasmic from electronic annotation //
16491 // IPR002401 // E-class P450, group I // reticulum // inferred from electronic
oxidoreductase activity // inferred from IPR008071 // E-class P450, CYP2J annotation // 5792 //
microsome // inferred electronic annotation // 16712 // from electronic annotation // 16020 //
oxidoreductase activity, acting on paired membrane // inferred from electronic donors, with
incorporation or reduction of annotation molecular oxygen, reduced flavin or flavoprotein as one
donor, and incorporation of one atom of oxygen // inferred from electronic annotation -- 5783 //
endoplasmic reticulum // inferred 4497 // monooxygenase activity // inferred -- IPR001128 //
Cytochrome P450 // from sequence or structural similarity // from electronic annotation //
16491 // IPR002401 // E-class P450, group I // 16021 // integral to membrane // traceable
oxidoreductase activity // inferred from IPR002402 // E-class P450, group II author statement
electronic annotation // 18685 // alkan-1- monooxygenase activity // inferred from sequence or structural
similarities // 6118 // electron transport // inferred from 5783 // endoplasmic reticulum //
inferred 4497 // monooxygenase activity // inferred -- IPR001128 // Cytochrome P450 //
electronic annotation // 8203 // cholesterol from electronic annotation // 5782 // from electronic
annotation // 8123 // IPR002403 // E-class P450, group IV metabolism // inferred from electronic
microwave // inferred from electronic cholesterol 7-alpha-monooxygenase annotation
annotation // 16020 // membrane // inferred activity // inferred from electronic from electronic
annotation // 16021 // annotation // 16491 // oxidoreductase integral to membrane // traceable
author activity // inferred from electronic statement annotation -- -- -- -- -- 6412 // protein
biosynthesis // inferred from 5840 // ribosome // inferred from sequence 3735 // structural
constituent of ribosome // -- IPR004038 // Ribosomal protein sequence or structural similarity or structural
similarity inferred from sequence or structural L7AE // inferred from sequence or structural
IPR008071 // E - class P450, group II author statement // 5782 // cytochrome P450 // group //
from electronic annotation // 18685 // from sequence or structural similarity // 6118 //
electron transport // inferred from -- 5489 // electron transporter activity // -- IPR013577 //
Eukaryotic protein of sequence or structural similarity inferred from sequence or structural
unknown function DUF953 // similarity IPR006662 // Thioredoxin type domain // IPR006663 //
Thioredoxin domain 2 -- -- -- -- -- IPR007484 // Peptidase M28 -- -- -- -- -- 7165 // signal
transduction -- inferred from 5615 // extracellular space // traceable 4673 // protein-histidine
kinase activity // -- IPR003594 // ATP-binding region, sequence or structural similarity author
statement // 5739 // mitochondrion // inferred from sequence or structural ATPase-like //
IPR005467 // Histidine inferred from sequence or structural similarity // 4740 // [pyruvate kinase
similarity dehydrogenase (lipooamide)] kinase activity // inferred from sequence or structural
similarities // 5524 // ATP binding // inferred from sequence or structural similarity // 16301 //
kinase activity // inferred from sequence or structural similarity // 16740 // transferase activity //
inferred from sequence or structural similarity -- 5215 // sugar_tr, transporter activity; 3.3e-06 -
- IPR005828 // General substrate -- extended.inferred from electronic transporter // IPR007114 //
Major annotation facilitator superfamily -- 5615 // extracellular space // traceable --
Gene_Trapping Resource_2- IPR000413 // Integrins alpha chain author statement // 16021 //
transduct // 0- membrane // traceable author statement 02_IMAGE_and_RIKEN_cDNAs 6520 //
amino acid metabolism // inferred -- 4058 // aromatic-L-amino-acid Catecholamine_Biosynthesis
IPR002129 // Pyridoxal-dependent from electronic annotation // 42423 // dehydrogenase activity
activity inferred from dehydrogenase catecholamine biosynthesis // inferred from electronic
annotation // 16829 // lyase electronic annotation activity // inferred from electronic annotation //
16831 // carboxylyase activity // inferred from electronic annotation -- 5622 // intracellular // inferred from
3677 // DNA binding // inferred from -- sequence or structural similarity sequence or structural
similarities -- 5622 // intracellular // inferred from 3677 // DNA binding // inferred from --
sequence or structural similarity sequence or structural similarity -- 8415 // acyltransferase
activity // inferred -- IPR007130 // Dicarboxylglycerol from electronic annotation // 16740 //
acyltransferase // IPR006662 // transferase activity // inferred from Thioredoxin type domain
electronic annotation -- 5615 // extracellular space // traceable 4800 // thyroxine 5'-deiodinase
activity // -- IPR000643 // L-iodothyronine deiodinase author statement // 16021 // integral to
inferred from electronic annotation // 16491 // IPR008261 // L-iodothyronine membrane // inferred from
electronic // oxidoreductase activity // inferred from deiodinase, active site annotation
electronic annotation // 16787 // hydrolase activity // inferred from electronic annotation -- 5856 //
cytoskeleton // inferred from 3777 // actin binding // inferred from -- IPR001715 // Calponin-like
actin- electronic annotation // 45202 // synapase // electronic annotation // 5198 // structural
binding // IPR001202 // inferred from direct assay molecule activity // inferred from electronic
WW/Rsp5/WWP domain // IPR002017 annotation // 5509 // calcium ion binding // Spectrin
inferred from // Glutathione S-transferase, N- electronic annotation terminal // IPR003081 // Glutathione S-transferase, Mu class 5978 // glycogen biosynthesis // inferred -- 3824 // catalytic activity // inferred from -- IPR008631 // Glycogen synthase from sequence or structural similarity sequence or structural similarity // 4373 // glycogen (starch) synthase activity // inferred from sequence or structural similarity // 16740 // transferase activity // inferred from sequence or structural similarity // 16757 // transferase activity, transferring glycosyl groups // inferred from sequence or structural similarity 6334 // nucleosome assembly // inferred 786 // nucleosome // inferred from 3677 // DNA binding // inferred from -- // from sequence or structural similarity sequence or structural similarity // 5634 // sequence or structural similarity 7001 // chromosome organization and nucleus // inferred from direct assay // biogenesis (sensu Eukarya) // inferred from 5694 // chromosome // inferred from sequence or structural similarity sequence or structural similarity 8152 // metabolism // inferred from -- // oxidoreductase activity // inferred -- IPR002198 // Short-chain electronic annotation from electronic annotation dehydrogenase/reductase SDR // IPR002347 // Glucose/ribitol dehydrogenase 6118 // electron transport // inferred from 5777 // peroxisome // inferred from 16491 // oxidoreductase activity // inferred -- IPR000262 // FMN-dependent alpha- electronic annotation /// 6605 // protein electronic annotation from electronic annotation /// 3973 // hydroxy acid dehydrogenase /// targeting // inferred from sequence or 1.1.3.15; (S)-2-hydroxy-acid oxidase IPR008259 // FMN-dependent alpha- structural similarity activity; 4.5.26-95 // extended:Unknown hydroxy acid dehydrogenase, active site // IPR003009 // FMN-related compound-binding core 1505 // regulation of neurotransmitter levels -- 8168 // methyltransferase activity // inferred -- // traceable author statement from sequence or structural similarity // 8170 // N-methyltransferase activity // inferred from direct assay // 16740 // transferase activity // inferred from sequence or structural similarity // 46539 // 2.1.1.8; histamine N-methyltransferase activity; 3.8e-134 // extended:Unknown 6355 // regulation of transcription, DNA - 5634 // nucleus // inferred from direct assay 3676 // nucleic acid binding // inferred from -- IPR005004 // RNA-binding region dependent // inferred from sequence or // 19013 // viral nucleocapsid // inferred sequence or structural similarity // 3677 // RNP-1 (RNA recognition motif) // structural similarity // 6397 // mRNA from electronic annotation // 30529 // DNA binding // inferred from sequence or IPR006535 // HnRNP R and Q splicing processing // inferred from sequence or ribonucleoprotein complex // inferred from structural similarity // 3723 // RNA binding factor structural similarity electronic annotation // inferred from sequence or structural similarity 6693 // prostaglandin metabolism // inferred -- 3824 // catalytic activity // inferred from -- IPR002198 // Short-chain from sequence or structural similarity // sequence or structural similarity // 4667 // dehydrogenase/reductase SDR // 8152 // metabolism // inferred from prostaglandin-D synthase activity // inferred IPR002347 // Glucose/ribitol sequence or structural similarity from sequence or structural similarity // dehydrogenase 5489 // electron transporter activity // inferred from sequence or structural similarity // 16404 // 15- hydroxyprostaglandin dehydrogenase (NAD+) activity // inferred from sequence or structural similarity // 16491 // oxidoreductase activity // inferred from electronic annotation

IPR006115 // 6-phosphogluconate branch // inferred from sequence or electron transporter activity // inferred from dehydrogenase, NAD binding domain structural similarity sequence or structural similarity // 8114 /// IPR006183 // 6-phosphogluconate phosphogluconate 2-dehydrogenase dehydrogenase /// IPR006184 // 6-activity // inferred from electronic phosphogluconate-binding site /// annotation /// 16491 // oxidoreductase IPR008927 // 6-phosphogluconate activity // inferred from sequence or dehydrogenase, C-terminal-like /// structural similarity IPR006113 // 6-phosphogluconate dehydrogenase, decarboxylating 6098 // pentose-phosphate shunt // inferred -- 4616 // phosphogluconate dehydrogenase Pentose phosphate IPR006114 // 6-phosphogluconate from sequence or structural similarity /// (decarboxylating) activity // inferred from pathway /// dehydrogenase, C-terminal /// 9051 // pentose-phosphate shunt, oxidative sequence or structural similarity /// 5489 // Pentose_Phasphate_Pathway IPR006115 // 6-phosphogluconate branch // inferred from sequence or electron transporter activity // inferred from dehydrogenase, NAD binding domain structural similarity sequence or structural similarity /// 8114 /// IPR006183 // 6-phosphogluconate phosphogluconate 2-dehydrogenase dehydrogenase /// IPR006184 // 6-activity // inferred from electronic phosphogluconate-binding site /// annotation /// 16491 // oxidoreductase IPR008927 // 6-phosphogluconate activity // inferred from sequence or dehydrogenase, C-terminal-like /// structural similarity IPR006113 // 6-phosphogluconate dehydrogenase, decarboxylating 6098 // pentose-phosphate shunt // inferred -- 4616 // phosphogluconate dehydrogenase Pentose phosphate IPR006114 // 6-phosphogluconate from sequence or structural similarity /// (decarboxylating) activity // inferred from pathway /// dehydrogenase, C-terminal /// 9051 // pentose-phosphate shunt, oxidative sequence or structural similarity /// 5489 // Pentose_Phasphate_Pathway IPR006115 // 6-phosphogluconate branch // inferred from sequence or electron transporter activity // inferred from dehydrogenase, NAD binding domain structural similarity sequence or structural similarity /// 8114 /// IPR006183 // 6-phosphogluconate phosphogluconate 2-dehydrogenase dehydrogenase /// IPR006184 // 6-activity // inferred from electronic phosphogluconate-binding site /// annotation /// 16491 // oxidoreductase IPR008927 // 6-phosphogluconate activity // inferred from sequence or dehydrogenase, C-terminal-like /// structural similarity IPR006113 // 6-phosphogluconate dehydrogenase, decarboxylating 45210 // FasL biosynthesis // inferred from -- ---- -- --- IPR001849 // Pleckstrin-like direct assay -- 16021 // integral to membrane // inferred 5509 // calcium ion binding // inferred from -- IPR005552 // Scramblase from electronic annotation electronic annotation -- -- -- -- -- 5615 // extracellular space // traceable 5044 // scavenger receptor activity // -- IPR001190 // Speract/scavenger author statement /// 16020 // membrane // traceable author statement /// 5515 -- receptor /// IPR000210 // BTB/POZ inferred from sequence or structural protein binding // inferred from physical domain similarity interaction 910 // cytokinesis // inferred from sequence 8287 // protein serine/threonine 158 // protein phosphatase type 2A activity G13_Signaling_Pathway IPR006186 // Serine/threonine-specific or structural similarity // 5977 // glycogen phosphatase complex // inferred from // inferred from sequence or structural protein phosphatase and bis(5- metabolism // inferred from sequence or electronic annotation similarity /// 163 // protein phosphatase type nucleosyl-tetraphosphatase // structural similarity 1 activity // inferred from sequence or IPR004843 // Metallo- structural similarity /// 4722 // protein phosphoesterase serine/threonine phosphatase activity // inferred from electronic annotation /// 8420 // CTD phosphatase activity // inferred from sequence or structural similarity /// 15071 // protein phosphatase type 2C activity // inferred from sequence or structural similarity /// 16787 // hydrolase activity // inferred from sequence or structural similarity /// 17018 // myosin phosphatase activity // inferred from sequence or structural similarity /// 30145 // manganese ion binding // inferred from electronic annotation 6139 // nucleobase, nucleoside, nucleotide 5783 // endoplasmic reticulum // inferred 5507 // copper ion binding // inferred from -- IPR000817 // Prion protein and nucleic acid metabolism // traceable from direct assay /// 5794 // Golgi direct assay author statement /// 6878 // copper ion apparatus // inferred from direct assay /// homeostasis // traceable author statement 5886 // plasma membrane // inferred from // 6979 // response to oxidative stress // direct assay /// 45121 // lipid raft // inferred from direct assay from direct assay 6139 // nucleobase, nucleoside, nucleotide 5783 // endoplasmic reticulum // inferred 5507 // copper ion binding // inferred from -- IPR000817 // Prion protein and nucleic acid metabolism // traceable from direct assay /// 5794 // Golgi direct assay author statement /// 6878 // copper ion apparatus // inferred from direct assay /// homeostasis // traceable author statement 5886 // plasma membrane // inferred from // 6979 // response to oxidative stress // direct assay /// 45121 // lipid raft // inferred from direct assay from direct assay 30163 // protein catabolism // inferred from 5634 // nucleus // inferred


Nitrilase/cyanide electronic annotation author statement electronic annotation // 16810 //
hydrolase hydrolase and apolipoprotein N-activity, acting on carbon-nitrogen (but not acyltransferase peptide) bonds // inferred from electronic annotation 6807 // nitrogen metabolism // inferred from 5615 // extracellular space // traceable 16787 // hydrolase activity // inferred from -- IPR003010 //
hydrolase hydrolase and apolipoprotein N-activity, acting on carbon-nitrogen (but not acyltransferase peptide) bonds // inferred from electronic annotation 6807 // nitrogen metabolism // inferred from 5615 // extracellular space // traceable 16787 // hydrolase activity // inferred from -- IPR003010 //
factor activity // inferred from sequence or structural similarity -- -- -- -- -- -- -- IPR008151 //
Phytoene dehydrogenase-related protein -- 5615 // extracellular space // traceable -- -- author statement // 16021 // integral to membrane // traceable author statement -- -- -- -- -- 5529 // sugar binding // inferred from -- IPR001079 // Galectin, galactose- sequence or structural similarity binding lectin // IPR008985 // Concanavalin A-like lectin/glucoanase -- -- -- -- -- -- --
IPR001683 // Phox-like -- -- -- -- IPR005036 // Putative phosphatase regulatory subunit 9116 //
nucleoside metabolism // inferred -- 3824 // catalytic activity // inferred from -- IPR000845 //
Pyruvate and other from sequence or structural similarity sequence or structural similarity // 16740 //
phosphorylases, family 1 // transferase activity // inferred from IPR010059 // Uridine
phosphorylase, electronic annotation // 16757 // eukaryotic transferase activity, transferring
glucosyl groups // inferred from electronic annotation // 4850 // 2.4.2.3; uridine phosphorylase
activity; 2.59e-118 // extended:inferred from electronic annotation -- -- -- -- -- 5615 //
extracellular space // traceable -- -- IPR009311 // Interferon-induced 6-16 author statement //
16021 // integral to membrane // traceable author statement 6465 // signal peptide processing
inferred 5783 // endoplasmic reticulum // inferred 8233 // peptidase activity // inferred from
-- IPR000508 // Peptidase S26, signal from electronic annotation // 6508 // from electronic
annotation // 5792 // electronic annotation // 16787 // hydrolase peptidase l // IPR001733 //
Peptidase proteolysis and peptidolysis // inferred from microsome // inferred from electronic
annotation // inferred from electronic S26B, eukaryotic signal peptidase electronic annotation
annotation // 16020 // membrane // inferred annotation from electronic annotation // 16021 //
terminated // inferred from electron transport // inferred from 5739 // mitochondrion // inferred from
-- -- IPR001199 // Cytochrome b5 sequence or structural similarity sequence or structural similarity // 16021 //
terminated // traceable author statement -- 19867 // outer membrane // inferred from sequence or structural
similarity 6790 // sulfur metabolism // inferred from 5615 // extracellular space // inferred from
4065 // aroclor oxidase activity // inferred from -- IPR000917 // Sulfatase sequence or structural similarity // 8152 //
sequence or structural similarity // 5783 // sequence or structural similarity // 8449 // metabolism // inferred from sequence or endoplasmic reticulum // inferred from N-
acetylglucosamine-6-sulfatase activity // structural similarity electronic annotation // 5794 //
Golgi inferred from sequence or structural apparatus // inferred from electronic similarity // 8484 //
sulfuric ester hydrolase annotation activity // inferred from sequence or structural similarity //
16787 // hydrolase activity // inferred from electronic annotation -- -- -- -- -- IPR001623 // Heat
shock protein DnaJ, N-terminal // IPR002939 // Chaperone DnaJ, C-terminal // IPR003095 //
Heat shock protein DnaJ // IPR008971 // HSP40/DnaJ peptide-binding -- 5615 // extracellular
space // traceable -- -- IPR007947 // CD164 related protein author statement // 16021 // integral
to membrane // traceable author statement -- -- -- -- -- -- IPR004279 // Perilipin -- -- -- -- -- IPR004279 //
Perilipin -- 5829 // cytosol // inferred from electronic -- -- -- -- -- IPR000717 // Proteasome component
annotation region PCI // IPR008941 // TPR-like -- -- -- -- -- IPR007110 // Immunoglobulin-like--
IPR003598 // Immunoglobulin C-2 type 6928 // cell motility // inferred from -- 5198 // structural
molecule activity // -- -- IPR008273 // Cellular retinaldehyde-binding sequence or structural similarity
inferred from sequence or structural binding/triple function, N-terminal // similarity IPR001251 //
Cellular retinaldehyde-binding/triple function, C-terminal // IPR000535 // Major sperm protein
(MSP) domain // IPR008962 // PapD-like -- 5615 // extracellular space // traceable 3824 //
catalytic activity // inferred from -- -- IPR007197 // Radical SAM // author statement sequence or
structural similarity IPR006638 // Elongator protein 3/MiaB/NifB -- 5615 // extracellular space
traceable 3824 // catalytic activity // inferred from -- -- IPR007197 // Radical SAM // author
statement sequence or structural similarity IPR006638 // Elongator protein 3/MiaB/NifB -- -- -- -- --
IPR006840 // ChaC-like protein 6118 // electron transport // inferred from 5739 // mitochondrion
inferred from 3995 // acyl-CoA dehydrogenase activity // -- -- IPR006090 // Acyl-CoA electronic
annotation electronic annotation inferred from electronic annotation // 16491 // dehydrogenase,
C-terminal // // oxidoreductase activity // inferred from IPR006091 // Acyl-CoA electronic
annotation dehydrogenase, central domain // IPR006092 // Acyl-CoA dehydrogenase, N-
terminal // IPR006089 // Acyl-CoA dehydrogenase // IPR009075 // Acyl-CoA dehydrogenase
C-terminal-like // IPR009100 // Acyl-CoA dehydrogenase, middle and N-terminal -- -- -- -- --
8152 // metabolism // inferred from -- 5498 // sterol carrier activity // inferred from -- IPR002198 //
Short-chain sequence or structural similarity sequence or structural similarity // 16491 //
dehydrogenase/reductase SDR // oxidoreductase activity // inferred from IPR002347 //
Glucose/ribitol electronic annotation dehydrogenase // IPR003033 // Sterol-binding -- -- -- -- --
6461 // protein complex assembly // 16020 // membrane // inferred from -- -- IPR003780 //
Cytochrome oxidase inferred from sequence or structural sequence or structural similarity //
hydrolase activity // inferred from electronic annotation // -- -- -- -- -- -- IPR00801 // Complex 1 LYR protein -- -- -- -- 6810 // transport // inferred from electronic 5743 // mitochondrial inner membrane // 5488 // binding // inferred from sequence or -- IPR001993 // Mitochondrial substrate annotation inferred from sequence or structural similarity carrier // IPR002030 // Mitochondrial similarity // 16020 // membrane // inferred brown fat uncoupling protein // from electronic annotation // 16021 // IPR002067 // Mitochondrial carrier integral to membrane // inferred from protein // IPR002113 // Adenine electronic annotation nucleotide translocator 1 7264 // small GTPase mediated signal 5795 // Golgi stack // inferred from 3925 // small monomeric GTPase activity // -- IPR001806 // Ras GTPase superfamily transduction // inferred from sequence or GTPase activity inferred from sequence small GTPase, Rab type structural similarity or structural similarity 5525 // GTP binding // inferred from electronic annotation -- -- -- -- -- -- IPR008590 // Eukaryotic protein of unknown function DUF872 // IPR008994 // Nucleic acid-binding OB- fold -- -- Gene Trap Resource_2 -- 04-02_IMAGE_and_RIKEN_cDNAs -- 5856 // Band_41; cytoskeleton; 1.2e-19 // -- IPR000299 // Band 4.1 // IPR009065 extended:Unknown // FERM 6397 // mRNA processing // inferred from 5622 // intracellular // inferred from 8565 // protein transporter activity // inferred -- IPR002075 // Nuclear transport factor sequence or structural similarity 6406 // sequence or structural similarity 5634 // from sequence or structural similarity 2 (NTF2) mRNA-nucleus export // inferred from nucleus // inferred from sequence or sequence or structural similarity 6606 // structural similarity protein-nucleus import // inferred from sequence or structural similarity 6810 // transport // inferred from sequence or structural similarity 6886 // intracellular protein transport // inferred from sequence or structural similarity 15031 // protein transport // inferred from sequence or structural similarity 6917 // induction of apoptosis // inferred 5635 // nuclear membrane // inferred from 16506 // apoptosis activator activity // -- -- from direct assay // 8632 // apoptotic direct assay // 5783 // endoplasmic inferred from direct assay program // inferred from direct assay reticulum // inferred from direct assay 16021 // integral to membrane // traceable author statement 6917 // induction of apoptosis // inferred 5635 // nuclear membrane // inferred from 16506 // apoptosis activator activity // -- -- from direct assay 8632 // apoptotic direct assay // 5783 // endoplasmic inferred from direct assay program // inferred from direct assay reticulum // inferred from direct assay 16021 // integral to membrane // traceable author statement -- 5615 // extracellular space // traceable -- -- author statement -- 5615 // extracellular space // 3824 // catalytic activity // inferred from -- IPR002018 // Carboxylesterase, type B author statement sequence or structural similarity // 16787 // // IPR000379 // hydrolase activity // inferred from electronic Esterase/lipase/thioesterase annotation // 16789 // carboxylic ester hydrolase activity // inferred from sequence or structural similarity -- -- 3676 // nucleic acid binding // inferred from -- IPR001410 // DEAD/DEAH box sequence or structural similarity 4386 // helicase // IPR001650 // Helicase, C- helicase activity // inferred from sequence terminal or structural similarity 5524 // ATP binding // inferred from sequence or structural similarity 8026 // ATP dependent helicase activity // inferred from sequence or structural similarity 16787 // hydrolase activity // inferred from electronic annotation -- -- -- -- IPR010916 // TONB Box N terminus -- -- -- -- IPR002114 // HPrel // serine phosphorylation site -- -- -- -- IPR002097 // Profilin/allergen -- -- 4842 // HECT; ubiquitin-
TABLE - US-00002

<table>
<thead>
<tr>
<th>Table Title</th>
<th>Table Content</th>
</tr>
</thead>
</table>
| TABLE 1 1.7 Fold Cut-Off in Either Calorie Restricted or Oxaloacetate Gene Expression Directional Analysis of Gene Expression Comparison of Calorie Restricted Mice and Oxaloacetate Mice to Control Mice Change in Gene Activity Expressed by Oxaloacetate and CR Mice Versus Control Mice Expression for Genes Shown to Change Commonly Affymatrix Mouse Genome 430 2.0 Array CR to EX Ox to C Gene Symbol Gene Title Affymatrix No. Signal Log Ratio Change Signal Log Ratio Change Ge Direction? Cyp2b9 cytochrome P450, family 2, subfamily b, polypeptide 9 3985 -2.5 -D 1 D YES Dgat2l1 diacylglycerol O-acetyltransferase 2-like 1 3899 -1.8 -D 1.4 D YES Fabp4 fatty acid binding protein 4, adipocyte 19390 -1.7 -D 1.4 D YES Fabp5 fatty acid binding protein 5, epidermal 417 1.2 1.8 I YES Foxq1 forkhead box Q1 6994 1.1 I 2.1 I YES Foxq1 forkhead box Q1 30006 1.9 I 2.2 I YES Lift1 interferon-induced protein with tetratricopeptide repeats 1 18910 -2 D -0.5 D YES Lcn2 lipocalin 2 12006 -1.8 -D 0.7 D YES Lgal1 lectin, galactose binding, soluble 1 3968 -1.7 -D -0.5 D YES LOC209387 tripartite motif protein 30-like 22024 -1.9 -D 0.4 -D YES Ly6d lymphocyte antigen 6 complex, locus D 1325 -3.4 -D 2.2 D YES Saa1 serum amyloid A 1 18915 -1.9 -D -0.5 D YES Saa2 serum amyloid A 2 3470 -1.8 -D 0.3 D YES Saa2 serum amyloid A 2 17502 -1.9 D -D 0.5 D YES Serpin4-ps1 serine (or cysteine) proteinase inhibitor, clade a, member 4, 35241 3.6 I 1.8 I YES pseudogene 1 Serpinb1a serine (or cysteine) proteinase inhibitor, clade B, member 4, 1a 713 -1.7 D -D 1.2 D YES Socsc2 suppressor of cytokine signaling 2 17285 2.8 I 0.8 I YES Trim2 tripartite motif protein 2 16727 -1.7 D -0.4 D YES Tubb2 tubulin, beta 2 11606 -2.7 D -1 D YES Ucp2 uncoupling protein 2, mitochondrial 16364 -1.7 D -0.5 D YES Usp18 ubiquitin specific protease 18 2586 -1.8 D -0.8 D YES -- Mus musculus transcribed sequence with weak similarity to protein 15668 -2.6 D -0.9 D YES sp: P32456 (H. sapiens) GBP2_HUMAN Interferon-induced guanylate- binding protein 2 (Guanine nucleotide-binding protein 2) -- Mus musculus similar to cytochrome P450 2B4-fragments) 17655 -3.6 D -4.2 D YES (LOC232993), mRNA -- -- 18738 -1.9 -D 1.2 D YES -- Mus musculus transcribed sequences 38815 -2.9 -D 0.7 D YES -- Mus musculus transcribed sequences 43312 -2.1 D 0.1 D YES Mus musculus transcribed sequences 45080 -1.8 D -0.7 D YES 1110067D22Rik RIken cDNA 1110067D22 gene 19440 -1.8 D -0.6 D YES 1600032L17Rik RIken cDNA 1600032L17 gene 23279 -0.8 -D 1.7 D YES 251004L01Rik RIken cDNA 251004L01 gene 5268 -1.8 D -0.9 D YES 251004L01Rik RIken cDNA 251004L01 gene 14650 -2.1 D 0.6 D YES 4933433D23Rik RIken cDNA 4933433D23 gene 5094 1.6 I 0.7 I YES 5730494M16Rik RIken cDNA 5730494M16 gene 44727 -2.2 -D 1.2 D YES 9130019P20Rik RIken cDNA 9130019P20 gene 39136 2 I 0.6 I YES A430056A10Rik RIken cDNA A430056A10 gene 7814 -2.6 -D 1.5 D YES AW539457 expressed sequence AW539457 26927 -1.8 D 0.9 D YES Microbial Oxaloacetate with Genes Moving in Same Direction as Calorie Restricted Mice (1.7 Fold Cut Off) 36 Mice Fed Oxaloacetate with Genes Moving in Opposite Direction as Calorie Restricted Mice (1.7 Fold Cut Off) 0 Percentage of Mice Fed Oxaloacetate with Genes Moving in Same 100% Direction as Calorie Restricted Mice Gene Ontology Biological Process Gene Ontology Cellular Component Gene Ontology Molecular Function Pathway InterPro 6118 // electron transport // inferred from 5783 // endoplasmic reticulum // inferred from 4497 // monoxygenase activity // inferred from -- IPR001128 // electron annotation electronic annotation // 5792 // microsome // electronic annotation // 16491 // oxidoreductase Cytochrome P450 // inferred from electronic annotation // 16020 // activity // inferred from electronic annotation // IPR002401 // E-class membrane // inferred from electronic annotation 16712 // oxidoreductase activity, acting on P450, group I // paired donors, with incorporation or reduction of IPR008068 // E-class molecular oxygen, reduced flavin or flavoprotein P450, CYP2B as one
Table 1 shows a gene expression directional analysis indicating that 98% of the genes that changed expression from the control group and were commonly expressed also in the oxaloacetate supplemented group and Calorie Restricted group moved in the same direction (up regulated or down regulated). Table 1 further illustrates that both calorie restriction and the supplementation of oxaloacetate in the diet causes changes in gene expression as compared to the expression genes in a control group fed as much food as they desired (fed ad libitum). It documents the 363 genes that change in common expression from the control group, and the directional analysis of the change in gene expression for oxaloacetate and calorie restricted mice as compared to the control group.

Table 2 shows increases and decreases in gene expression of commonly expressed changed genes of 1.7 fold in either oxaloacetate supplemented mice or calorie restricted mice as compared to a control group. Table 2 demonstrates that genes expressed by either the calorie restricted group of mice or the oxaloacetate supplemented group of mice that resulted in a 1.7 or greater fold increase (or decrease) in expression as compared to the control group.

Both average lifespan and maximal lifespan of the individual are substantially increased with the application of excess oxaloacetate to the organism. However, unlike CR, there is no need for reduction in caloric intake. It is interesting to note that in the liver no increase in the Sir2 gene in the mice was observed, however this does not exclude the increase of Sir2 gene in other tissues. In our C. elegans experiments represented in FIG. 3, Sir2 (and their homologues, such as Sir1 in humans) was shown to add approximately a 10% increase in lifespan over control animals. Other beneficial genes, turned on in CR, operate in a parallel but independent pathway to the Sir2 type genes are also activated by oxaloacetate and increase lifespan by greater amounts, as much as up to an additional 26%. Between the activation of the Sir2 type genes
and the other beneficial genes, a total increase of up to 36% in lifespan over control animals has been shown to be obtainable.

[0051]In one embodiment of the invention, a nutritional supplement comprising an effective dose of oxaloacetate is provided to extend the lifespan of an individual and to reduce the onset of age-related disease. Oxaloacetate acts to reduce NADH levels in the cytosol of the cell, which increases the NAD+/NADH ratio to levels seen during CR, but without a restriction in calories or genetic modification of the individual. External cellular contact with an oxaloacetate compound, and subsequent transfer of the oxaloacetate into the cell, leads to metabolic changes that increase the NAD/NADH ratio and activate beneficial genes. The oxaloacetate is converted to malate by interaction with cytosolic malate dehydrogenase. In this conversion, NADH is converted to NAD+, which increases the intercellular NAD+/NADH ratio. The present invention is based, in part, on the surprising and novel discovery that the increase in NAD+/NADH ratio signals the activation of the beneficial genes that then act upon the cell to increase lifespan and reduce the onset of age related disease.

[0052]In another embodiment of the current invention, methods for extending lifespan and reducing the onset of age-related disease is provided comprising administering an effective dose of oxaloacetate to an individual in need thereof. As used herein, the phrase "age-related disease" refers to any number of conditions attributable to advance in age. These conditions include, without limitation, osteoporosis, bone loss, arthritis, stiffening joints, cataracts, macular degeneration, and heart disease including atherosclerosis and dyslipidemia. The phrase "age related disease" further encompasses neurodegenerative diseases such as Alzheimer Disease and related disorders, Parkinson's Disease, and cancer. It has been observed that mammals which undergo CR have a reduced incidence of neurological disorders, including Alzheimer disease. The addition of excess oxaloacetate to the cytosol of the cell results in the same signaling mechanism in the cells as CR to turn on the necessary beneficial genes necessary for neurological protection.

[0053]Also included within the meaning of "age-related disease" are cosmetic concerns such as loss of skin firmness and elasticity as well as increase wrinkle depth and pore sizes associated with aging. "Age-related disease" further includes other symptoms associated with aged skin such as wrinkles, rhytids, sun damage, dull appearance of the skin, sagging skin, jowls, keratosis, melasma, and hyperpigmentation.

[0054]Oxaloacetate can be used to protect DNA and enhance DNA repair in skin and other tissues subjected to ultra violet (UV) light. Unlike a sunscreen that blocks UV, oxaloacetate enhances the repairs to DNA in a similar fashion as occurs in CR (Lipman et al.) because addition of excess oxaloacetate mimics the same intercellular signaling conditions as exist in CR. CR has been shown to enhance repairs to DNA in many studies as per mechanisms outlined in references 36, 37, 38, 39 40 and 44. One advantage of using oxaloacetate to mimic CR rather than utilizing CR is that oxaloacetate can be localized on the skin, to create "localized CR" conditions in just the contacted skin, while all other forms of CR take place in the entire organism. Thus, oxaloacetate can be combined with a UV sunblock or cosmetic to produce on-going enhancement of DNA repair to skin cells from UV damage. Reduction in this damage and repair of the damage leads to maintaining younger looking skin for a longer period of time, leads to reduced incidences of skin cancer and may be used as a treatment or assist in the treatment of skin cancer.

[0055]Oxaloacetate can act in another pathway to extend lifespan and reduce the onset of age related disease by interfering with insulin signaling. Insulin-like signaling has been shown to also control aging, metabolism and development. Mutations in the daf-2 gene and age-1 phosphoinositide 3-kinase gene of Caenorhabditis elegans have lead to increases in lifespan. The insulin-like signaling pathway may also be affected by caloric restriction to retard aging. Oxaloacetate has been shown to block at least a portion of the insulin signaling pathways by Kahn (Kahn, et al, Insulin Increases NADH/NAD+ Redox State, Which Stimulates Guanylate Cyclase in Vascular Smooth Muscle, American Journal of Hypertension, Ltd. 2002, Vol. 15, 273-279). NADH is also important to insulin signaling as shown by MacDonald (MacDonald, et al, Histochemical Evidence for Pathways Insulin Cells Use to Oxidize Glycolysis-Derived NADH, Metabolism, Vol. 51, No. 3 (March), 2002, pp 318-321), and oxaloacetate added to the cytosol
reduces the availability of NADH.

[0056]A third pathway to extend lifespan and reduce the onset of age related disease by oxaloacetate may be in the protective effects of oxaloacetate to mitochondrial DNA. Yamamoto, et al (Yamamoto et al, "Effect of alpha-ketoglutarate and Oxaloacetate on brain mitochondrial DNA damage and seizures induced by kainic acid in mice", Toxicology Letters: 2003: 143: 115-122) showed that both Oxaloacetate and its precursor alpha-ketoglutarate protected brain mitochondrial DNA from damage in mice. Yamamoto failed to teach, however, if this protection could increase lifespan or reduce the onset of age related disease, as was discovered in this invention.

[0057]Oxaloacetate can be administered alone to extend lifespan and reduce the onset of age related disease or in combination with another therapeutic agent. As used herein, the term "therapeutic agent" is a broad term that includes antibacterial agents, antiviral agents, anti-fungals, chemotherapeutics, antigens, proteins, enzymes, hormones, non-steroidal anti-inflammatory drugs, immunostimulatory compounds such as cytokines, and steroids. Suitable antibiotics include, without limitation, amoxicillin, ampicillin, bacampicillin, carbenicillin indanyl, mezlocillin, peperacillin, ticarcillin, amoxicillin-clavulanic acid, ampicillin-sulbactam, benzylpenicillin, cloxacillin, dicloxacillin, methicillin, oxacillin, penicillin G, penicillin V, piperacillin+oxacillin, ticarcillin+clavulanic acid, ticarcillin+chloramphenicol, nafcillin, cefadroxil, cefazolin, cephalixin, cephalothin, cephradine, cefaclor, cefamandol, cefonicid, cefotetan, cefoxitin, cefprozil, cefetetan, cefuroxime, cefuroxime axetil, clindamycin, cefditoren, cefditex, cefixime, cefixime, cefotaxime, cefpodoxime proxetil, cefazolin, cefotaxime, ceptriaxone, cefepime, azithromycin, clarithromycin, clindamycin, erythromycin, lincomycin, troleandomycin, cinoxacin, ciproflaxacin, enoxacin, gatifloxacin, grepafloxacin, levoflaxacin, lomefloxacin, moxifloxacin, nalidixic acid, norfloxacin, ofloxacin, sparfloxacin, trovafloxacin, oxolinic acid, gemifloxacin, norfloxacin, imipenem cilastatin, meropenem, aztreonam, amikacin, gentamicin, kanamycin, neomycin, netilmicin, streptomycin, tobramycin, paeromomycin, teicoplanin, vancomycin, demeclocycline, doxycycline, minocycline, oxtetracycline, tetracycline, chlorotetracycline, mafenide, silver sulfadiazine, sulfacetamide, sulfadiazine, sulfamethoxyazole, sulfasalazine, sulfa-sulfisoxazole, trimethoprim-sulfamethoxazole, sulfamethizole, rifabutin, rifampin, rifapentine, linezolid, quinopristin+dalfopristin, chloramphenicol, colistimethate, fosfomycin, isoniazid, methenamine, metronidazole, mupirocin, nitrofurantoin, nitrofurazone, novobiocin, spectinomycin, trimethoprim, colistin, cycloserine, capreomycin, ethionamide, pyrazinamide, para-aminosalicylic acid, and erythromycin ethylsuccinate+sulfisoxazole. Examples of suitable chemotherapeutic agents include an anticancer agent like cyclophosphamide, chlorambucil, melphalan, estramustine, ifosfamide, prednimustin, busulfan, lomustine, melphalan, methotrexate, azathioprine, mercaptopurine, thioguanine, cytarabine, fluorouracil, vinblastine, vincristine, vindesine, etoposide, teniposide, dactinomycin, doxorubicin, dunnorubicin, epirubicin, bleomycin, mitomycin, cisplatin, carboplatin, procarbazine, amacrine, mitoxantrone, tamoxifen, nilutamid, or aminoglutethimide. Immunostimulatory compounds include, without limitation, a vaccine adjuvant, a vaccine, a cytokine like IL-1, IL-2, IL-12, IL-15, IFN-α, IFN-β, or IFN-γ, or a flavonoid like flavone acetic acids and xantheneone-4-acetic acids.

[0058]When administered in combination with another therapeutic agent, oxaloacetate can be administered separately or as a single formulation with the antibiotic. If administered separately, oxaloacetate should be given in a temporally proximate manner with the antibiotic. In one embodiment, the therapeutic agent and oxaloacetate are given within one week of each other. In another embodiment, the therapeutic agent and oxaloacetate are given within twenty-four hours of each other. In yet another embodiment, the therapeutic agent and oxaloacetate are given within one hour of each other. The administration can be by oral, local, or by systemic injection or infusion. Other methods of administration may also be suitable as will be appreciated by one of skill in the art.

[0059]Methods and compositions are described herein for the modulation of body weight, reduction of fat, treatment of obesity, reduction in cellulite accumulation. In addition to the activation of beneficial CR genes, the administration of oxaloacetate results in the reduction of fat, despite the amount of food consumed, as the differentiation of cells into fat cells is blocked by the activation of Sir2 (Sir1 in humans), which also causes current fat cells shed their fat.
Activation of the human Sirt1 gene that is normally activated in CR blunts the protein PPAR gamma that activates fat storage genes. Sirt1 activation also causes formed fat cells to shed their fat. In some embodiments of the invention, a method of treating obesity via the administration of an effective dose of oxaloacetate is provided. Obesity, defined as an excess of body fat relative to lean body mass, also contributes to a host of other diseases including, without limitation, increased incidences of coronary artery disease, stroke, and diabetes. Hence, the administration of oxaloacetate would be beneficial not only for treating obesity but also for treating the diseases associated with obesity. In one embodiment, a method of treating a non-obese individual for the reduction of unwanted body fat is provided comprising administering an effective dose of oxaloacetate.

[0060]In another embodiment of the current invention, oxaloacetate can be used to reduce the incidence of cancer, or to stop the spread of cancer to non-cancerous cells. Mammals that undergo CR have up to a 40% lower cancer rate. CR has been shown to enhance the repair of DNA (Lipman et al, "The influence of dietary restriction on DNA repair in rodents: a preliminary study", Mech Ageing Dev 1989: 48: 135-43; Weraarchakul et al, "The effect of aging and dietary restriction on DNA repair", Exp Cell Res 1989; 181: 197-204; Licastro et al, "Effect of dietary restriction upon the age-associated decline of lymphocyte DNA repair activity in mice", Age 1988: 11: 48-52; Srivastava et al, "Decreased fidelity of DNA polymerases and decreased DNA excision repair in aging mice: Effects of caloric restriction", Biochem Biophys Res Commun 1992: 182: 712-21; Tilley et al, "Enhanced unscheduled DNA synthesis by secondary cultures of lung cells established from calorically restricted aged rats", Mech Ageing Dev 1992: 63": 165-76), which may be one reason for the reduction in cancer rates. Addition of excess oxaloacetate to the cytosol of the cell results in the same signaling mechanism in the cells as CR to turn on the necessary beneficial genes necessary for cancer protection. Malignancies against which the treatment may be directed include, but are not limited to, primary and metastatic malignant solid tumor disease, and hematological malignancies such as acute and chronic myelogenous leukemia, acute and chronic lymphatic leukemia, multiple myeloma, Waldenstrom's macroglobulinemia, hairy cell leukemia, myelodisplastic syndrome, polycythaemia vera, and essential thrombocytosis.

[0061]Methods and compositions for countering the effects of alcohol consumption are disclosed herein. The administration of oxaloacetate results in an increase in NAD+/NADH ratio, which can quickly counter the effects of alcohol consumption and reduce the symptoms associated with over-indulgence of alcohol. These symptoms include, without limitation, headache, poor sense of overall well-being, diarrhea, loss of appetite, shakiness, fatigue, and nausea. Other symptoms include decreased reaction times, less ability to concentrate, lower managerial skills, and increased risk for injury, even after some of the more obvious hangover symptoms are gone and alcohol can no longer be detected in the blood. The method includes identifying an individual who has consumed amounts of alcohol and administering to that individual an effective amount of oxaloacetate. As used herein, an effective amount of oxaloacetate includes from about 0.5 mg to about 75 mg of oxaloacetate per kg of weight. In a preferred embodiment, an effective amount of oxaloacetate is between about 2 mg to about 40 mg of oxaloacetate per kg of body weight. As will be described in greater detailed below, in a preferred embodiment, oxaloacetate can be administered via injection or ingestion.

Pharmaceutical Preparations and Methods of Administration

[0062]Oxaloacetate can be administered to an individual at therapeutically effective doses to prolong lifespan and/or treat or ameliorate age related diseases and body weight disorders. As used herein, "oxaloacetate" includes oxaloacetic acid, the salt of the acid, or oxaloacetate in a buffered solution as well as mixtures thereof. The term similarly includes oxaloacetate precursors such as alpha-ketoglutarate and aspartate.

[0063]A therapeutically effective dose refers to that amount of oxaloacetate sufficient to result in the desired effect such as the prolongation of life span, treatment of age-related disorders, and/or amelioration of symptoms of body weight disorders.

Effective Dose
Toxicity and therapeutic efficacy of oxaloacetate can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD$_{50}$ (the dose lethal to 50% of the population) and the ED$_{50}$ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD$_{50}$/ED$_{50}$. The LD$_{50}$ of alpha-ketoglutarate for mice is above 5 g/kg of body weight. The LD$_{50}$ of oxaloacetate is above 5 g/kg of body weight. Oxaloacetate has a very low toxicity, as would be expected from a chemical involved in the Citric Acid Cycle of every cell.

Toxicity studies of oxaloacetate run in Japan in 1968 on rats indicates that levels of oxaloacetate at 83 mg/kg of body weight caused changes in pancreatic islets. Some islets were decreased in size and hyperemic, alpha cells being atrophic, while beta cells were hypertrophic and stained densely. At lower doses, 41 mg/kg of body weight, the pancreas of the rates only demonstrated proliferation and hyperplasia of the islet cells. The liver, hypophysis, adrenals and gonadal glands showed no particular changes (Yoshikawa, Kiyohiko, "Studies on Anti-diabetic Effect of Sodium Oxaloacetate", Tohoku Journal of Experimental Medicine, 1968, volume 96, pp. 127-141). Further studies by the inventor using oxaloacetate in mice at a dosage 300% to 50,000% greater than proposed in humans indicated negative toxicity--the mice live longer than the control group. The safety of this compound in reasonable amounts is assured because the compound is a human metabolite.

In clinical studies examining the effect of oxaloacetate on diabetes in humans, 21 diabetic patients received 100 mg to 1,000 mg (2-10 mg/kg of body weight). There were no negative side effects. Fasting blood glucose levels dropped an average of 24% in the patients and urine glucose levels dropped in 19 out of the 21 patients (Yoshikawa).

An example of an effective dose of oxaloacetate administered by an intravenous injection is from between about 0.5 mg to about 1 g of oxaloacetate for each kg of body weight. In a preferred embodiment, the effective dose of oxaloacetate is between about 2.0 mg and about 40 mg for each kg of body weight. The effective dose can be administered in multiple injections over several hours, or continuously. Effective oral dosing would likewise range from about 0.5 mg to about 1 g of oxaloacetate for each kg of body weight with the preferred effective dosage range between about 2 mg to about 40 mg of oxaloacetate for each kg of body weight. For example, an adult male weighing approximately, 80 kg would be administered between about 150 mg to about 3.5 g of oxaloacetate orally per day. Dermally, topical formulations comprising concentrations of about 0.5 to 16 mM of oxaloacetate are effective. CR studies indicate that restricting calories every-other-day yields the same beneficial results as daily CR. Similarly, in some embodiments, oxaloacetate can be administered every-other-day, as once the genes are activated, the effect lasts for at least a two-day period of time. In other embodiments, oxaloacetate is administered 3 times per day after each meal.

Formulations

Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients. Thus, oxaloacetate and its physiologically acceptable salts and solvates may be formulated for administration by inhalation or insufflation (either through the mouth or the nose) or oral, buccal, topical, transdermal, parenteral, or rectal administration. In the case of inhalation, the administration of oxaloacetate will provide aging benefits directly to lung tissue, even if the dosage of oxaloacetate administered is less than is needed to benefit the entire organism. Inhalation of oxaloacetate will delay the on-set of age-related diseases of the lungs and will provide protection from lung diseases.

Oxaloacetate is acidic. The acidity is unlikely to affect organisms that ingest the compound in beneficial amounts as the interior conditions of the stomach are also very acidic. The acidity may affect other tissues, including but not limited to the skin or lungs, that may benefit from the direct application of oxaloacetate. Therefore, in another embodiment, a composition of matter can be created by mixing oxaloacetate with a buffer solution or a base or used as a salt of oxaloacetate so the delivered compound is not caustic. This will enable higher concentrations of oxaloacetate to be delivered safely to the organism, especially if the
oxaloacetate is not delivered by oral ingestion.

[0070] For oral administration, the pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g., pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycollate); or wetting agents (e.g., sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate.

[0071] While the absorption of oxaloacetate from the digestive tract will increase the entire organism’s oxaloacetate levels, the immediate contact of oxaloacetate to the cells in the digestive tract will preferentially be in contact with the digestive tract cells, allowing the reduction in age-related diseases such as colon cancer, even if the ingested amounts of oxaloacetate are insufficient to provide benefit to the entire organism.

[0072] Preparations for oral administration may be suitably formulated to give controlled release of the active compound. For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner. For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of e.g. gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[0073] For the protection of DNA from UV exposure and to enhance DNA repair of the UV damage, also for the treatment of rhytids or skin wrinkles, a preferred method of administration of oxaloacetate is by topical application. The topical pharmaceutical and cosmetic compositions of the present invention maybe made into a wide variety of product types. These include, but are not limited to lotions, creams, beach oils, gels, sticks, sprays, ointments, pastes, mousses and cosmetics. These product types may comprise several types of pharmaceutical or cosmetic carrier systems including, but not limited to solutions, emulsions, gels and solids. The topical pharmaceutical and cosmetic compositions of the present invention formulated as solutions typically include a pharmaceutically-acceptable aqueous or organic solvent. The terms “pharmaceutically-acceptable aqueous solvent” and “pharmaceutically-acceptable organic solvent” refer to a solvent which is capable of having dissolved therein the anti-wrinkle oxaloacetate, and possesses acceptable safety properties (e.g., irritation and sensitization characteristics). One example of a suitable pharmaceutically acceptable aqueous solvent is distilled water. Examples of a suitable pharmaceutically acceptable organic solvent include, for example, monohydric alcohols, such as ethanol, and polyhydric alcohols, such as glycols. If the topical pharmaceutical and cosmetic compositions of the present disclosure are formulated as an aerosol and applied to the skin as a spray-on, a propellant is added to a solution composition.

[0074] In one embodiment, topical pharmaceutical and cosmetic compositions of the present invention further comprise a suitable amount of a topical pharmaceutical and cosmetically-acceptable emollient. As used herein, “emollients” refer to materials used for the prevention or relief of dryness, as well as for the protection of the skin. Wide varieties of suitable emollients are known and may be used herein. Sagarin, Cosmetics, Science and Technology, 2nd Edition, Vol. 1, pp. 32-43 (1972), contains numerous examples of suitable materials. Examples of
classes of useful emollients include hydrocarbon oils and waxes such as mineral oil, petrolatum, paraffin, ceresin, ozokerite, microcrystalline wax, polyethylene, and perhydrovosphenole; silicone oil, such as dimethyl polysiloxanes, methylphenyl polysiloxanes, and water-soluble and alcohol-soluble silicone glycol copolymers. Other suitable emollients include triglyceride esters such as vegetable and animal fats and oils including castor oil, safflower oil, cotton seed oil, corn oil, olive oil, cod liver oil, almond oil, avocado oil, palm oil, sesame oil, and soybean oil; acetylgllyceride esters, such as acetylated monoglycerides; ethoxylated glycerides, such as ethoxylated glycerylmonostearate; alkyl esters of fatty acids including methyl, isopropyl, and buty1 esters of fatty acids, alkyl esters including hexyl laurate, iso-hexyl laurate, iso-hexyl palmitate, isopropyl palmitate, decyl oleate, isodecyl oleate, hexadecyl stearate, decyl stearate, isopropyl istearate, diisopropyl adipate, disooxyhexyl adipate, di-hexyl decyl adipate, di-isopropyl sebacate, lauryl lactate, myristyl lactate, and cetyl lactate; and alkenyl esters of fatty acids such as oleyl myristate, oleyl stearate, and oleyl oleate. Other suitable classes of emollients include fatty acids such as pelargonic, lauric, myristic, palmitic, stearic, iso-stearic, hydroxystearic, oleic, linoleic, ricinoleic, arachidic, behenic, and erucic acids; fatty alcohols such as lauryl, myristyl, cetyl, hexadecyl, stearyl, iso-stearyl, hydroxy stearyl, oleyl, ricinoleyl, behenyl, and erucyl alcohols, as well as 2-octyl dodecanol; fatty alcohol ethers; ethoxylated fatty alcohols; ether-esters such as fatty acid esters of ethoxylated fatty acids; lanolin and derivatives including lanolin oil, lanolin wax, lanolin alcohols, lanolin fatty acids, isopropylene, ethoxylated lanolin, ethoxylated lanolin alcohols, ethoxylated cholesterol, propoxylated lanolin alcohols, acetylated lanolin, acetylated lanolin alcohols, lanolin alcohols linoleate, lanolin alcohols recinoleate, acetate of lanolin alcohols recinoleate, acetate of lanolin alcohols recinoleate, acetate of ethoxylated alcohols esters, hydrogenolysis of lanolin, ethoxylated hydrogenated lanolin, ethoxylated sorbitol lanolin, and liquid and semisolid lanolin absorption bases are illustrative of emollients derived from lanolin; polyhydric alcohols and polyether derivatives such as propylene glycol, dipropylene glycol, polypropylene glycols 2000 and 4000, poloxymethylene poloxypolypropylene glycols, poloxymethylene poloxymethylene glycols, glycerol, sorbitol, ethoxylated sorbitol, hydroxypropylsorbitol, poloxymethylene glycols 200-6000, methoxy poloxymethylene glycols 350, 550, 750, 2000 and 5000, poly[ethylene oxide] homopolymers (100,000-5,000,000), polyalkylene glycols and derivatives, hexylene glycol (2-methyl-2,4-pentanediol), 1,3-butylene glycol, 1,2,6-hexanetriol, ethoxylated glycol USP (2-ethyl,3-hexamadiol), C15-C18 vicinal glycol, and polyoxypropylene derivatives of trimethylolpropane; polyhydric alcohol esters such as ethylene glycol mono- and di-fatty acid esters, diethylene glycol mono- and di-fatty acid esters, poloxymethylene glycol (200-6000) mono- and di-fatty acid esters, propylene glycol mono- and di-fatty esters, polypropylene glycol 2000 monoooleate, poloxymethylene glycol 2000 monostearate, ethoxylated propylene glycol monostearate, glyceryl mono- and di-fatty acid esters, polyglycerol poly-fatty acid esters, ethoxylated glyceryl monostearate, 1,3-butylene glycol monostearate, 1,3-butylene glycol diesterate, poloxymethylene polyol fatty acid ester, sorbitan fatty acid esters, and poloxymethylene sorbitan fatty acid esters; wax esters such as beeswax, spermaceti, myristyl myristate and stearly stearate; beeswax derivatives, e.g., poloxymethylene sorbitol beeswax; vegetable waxes including carnauba and candelilla waxes; and phospholipids, such as lecithin and derivatives; sterols including, for example, cholesterol and cholesterol fatty acid esters; amides such as fatty acid amides, ethoxylated fatty acid amides and solid fatty acid alkanolamides. Particularly useful emollients which provide skin conditioning are glycerol, hexanetriol, butanetriol, lactic acid and its salts, urea, pyrrolidone carboxylic acid and its salts, amino acids, guanidine, diglycerol and triglycerol.

Alternatively, the composition can be formulated as a lotion. A lotion can be made from a solution carrier system. In some embodiments, the lotion includes from about 1% to about 20%, for example, from about 5% to about 10%, of an emollient; and from about 50% to about 90%, for example, from about 60% to about 80% of water.

Another type of product that may be formulated from a solution carrier system is a cream or ointment. An ointment can comprise a simple base of animal or vegetable oils or semi-solid hydrocarbons (oleaginous). Ointments can also include addition ointment bases which absorb water to form emulsions. Optionally, the ointment carriers are water soluble. An ointment can include from about 2% to about 10% of an emollient plus from about 0.1% to about 2% of a thickening agent. Examples of suitable thickening agents include: cellulose derivatives (e.g., methyl cellulose and hydroxy propylmethylcellulose), synthetic high molecular weight polymers (e.g., carboxymethyl polymer and polyvinyl alcohol), plant hydrocolloids (e.g., karaya gum and
tragacanth gum), clay thickeners (e.g., colloidal magnesium aluminum silicate and bentonite), and carboxyvinyl polymers (CARBOPOLS®; sold by B. F. Goodrich Company, such polymers are described in detail in Brown, U.S. Pat. No. 2,798,053, issued Jul. 2, 1975). A more complete disclosure of thickening agents useful herein can be found in Sagarin, Cosmetics, Science and Technology, 2nd Edition, Vol. 1, pp. 72-73 (1972). If the carrier is formulated as an emulsion, from about 1% to about 10%, for instance, from about 2% to about 5%, of the carrier system comprises an emulsifier. Suitable emulsifiers include nonionic, anionic or cationic emulsifiers. Exemplary emulsifiers are disclosed in, for example, McCutcheon's Detergents and Emulsifiers, North American Edition, pages 317-324 (1986). Preferred emulsifiers are anionic or nonionic, although other types can also be employed.

[0077]Single emulsion skin care preparations, such as lotions and creams, of the oil-in-water type and water-in-oil type are well known in the cosmetic arts and are useful in the present embodiments. Multiphase emulsion compositions, such as the water-in-oil-in-water type are also useful in the present embodiments. In general, such single or multiphase emulsions contain water, emollients and emulsifiers as essential ingredients. Triple emulsion carrier systems comprising an oil-in-water-in-silicone fluid emulsion composition are also useful in the present embodiments.

[0078]Another emulsion carrier system useful in the topical pharmaceutical and cosmetic compositions of the present disclosure is a microemulsion carrier system. Such a system preferably comprises from about 9% to about 15% squalane; from about 25% to about 40% silicone oil; from about 8% to about 20% of a fatty alcohol; from about 15% to about 30% of polyoxyethylene sorbitan mono-fatty acid (commercially available under the trade name Tweens) or other nonionics; and from about 7% to about 20% water. This carrier system is combined with the therapeutic agents described above.

[0079]If the topical pharmaceutical and cosmetic compositions of the present disclosure are formulated as a gel or a cosmetic stick, a suitable amount of a thickening agent, as disclosed supra, is added to a cream or lotion formulation. The topical pharmaceutical and cosmetic compositions of the present disclosure may also be formulated as makeup products such as foundations, blush, and lipstick and can contain conventional cosmetic adjuvants, such as dyes, opacifiers, pigments and perfumes. Foundations are solution or lotion-based with appropriate amounts of thickeners such as algin, xanthan gum, cellulose gum, cocamide DEA, guar gum lanolin alcohol, paraffin, and propylene glycol, pigments including ultramarine blue, titanium dioxide, and carmine, colorants such as FD&C Red No. 40 and FD&C Yellow No. 5, moisturizers, and fragrance. Optionally, the foundation can include a sunscreen agent. The topical pharmaceutical and cosmetic compositions of the present disclosure may contain, in addition to the aforementioned components, a wide variety of additional oil-soluble materials and/or water-soluble materials conventionally used in topical compositions, at their established levels. Various water-soluble materials may also be present in the compositions of this invention. These include humectants, such as glycerol, sorbitol, propylene glycol, alkoxylated glucose and hexanetriol, ethyl cellulose, polyvinylalcohol, carboxymethyl cellulose, vegetable gums and clays such as VEEGUM® (magnesium aluminum silicate, R. T. Vanderbilt, Inc.); proteins and polypeptides, preservatives such as the methyl, ethyl, propyl and butyl esters of hydroxybenzoic acid (Parabens®--Mallinckrodt Chemical Corporation), EDTA, methylisothiazolinone and imidazolidinyl ureas (Germall 115®--Sutton Laboratories); and an alkaline agent such as sodium hydroxide or potassium hydroxide to neutralize, if desired, part of the fatty acids or thickener which may be present.

[0080]In some embodiments, oxaloacetate can be formulated as a hair product such as a shampoo or conditioner. Use of these products would then have a dual benefit to the user including a reduction in skin aging and delay in the onset of age-related skin disease including cancer. Oxaloacetate, when applied topically to the hair, can also prevent or reduce hair loss and hair graying.

[0081]The topical pharmaceutical and cosmetic compositions of the present disclosure can also include a safe and effective amount of a penetration enhancing agent. Other conventional skin care product additives may also be included in the compositions of the present invention. For example, collagen, elastin, hydrolysates, primrose oil, jojoba oil, epidermal growth factor,
soybean saponins, mucopolysaccharides, and mixtures thereof may be used. Various vitamins can also be included in the compositions of the present invention. For example, Vitamin A, and derivatives thereof, Vitamin B2, biotin, pantothenic, Vitamin D, and mixtures thereof can be used.

[0082] In some embodiments, the composition comprising oxaloacetate is incorporated into anti-wrinkle skin cleaning compositions. The skin cleaning compositions comprise a cosmetically acceptable surfactant in addition to oxaloacetate. The term "cosmetically-acceptable surfactant" refers to a surfactant that is not only an effective skin cleanser, but also can be used without undue toxicity, irritation, allergic response, and the like. Furthermore, the surfactant should be capable of being commingled with the anti-wrinkle components in a manner such that there is no interaction that would substantially reduce the efficacy of the composition for treating wrinkles in mammalian skin. In addition to the cosmetically effective amounts of the active ingredients, the skin cleaning compositions of the present disclosure contain from about 1% to about 90%, preferably from about 5% to about 10%, of a cosmetically-acceptable surfactant. The physical form of the skin cleansing compositions is not critical. The compositions can be, for example, formulated as toilet bars, liquids, pastes, or mousses. Toilet bars are most preferred since this is the form of cleansing agent most commonly used to wash the skin. The surfactant component of the disclosed compositions is selected from the group consisting of anionic, nonionic, zwitterionic, amphoteric and ampholytic surfactants, as well as mixtures of these surfactants. Such surfactants are well known to those skilled in the detergency art. The cleaning compositions of the present disclosure can optionally contain, at their art-established levels, materials which are conventionally used in skin cleansing compositions.

[0083] Other skin care products for the treatment of skin wrinkles may contain combinations of additional active ingredients. Such combinations include, for example, sunscreens and sunblocks. Optimum regulation of skin wrinkling resulting from exposure to U.V. light can be obtained by using a combination of oxaloacetate together with sunscreens or sunblocks. Useful sunblocks include, for example, zinc oxide and titanium dioxide. Photo damage is a predominant cause of skin wrinkling. Thus, for purposes of wrinkle prevention, the combination of the disclosed compositions with a UVA and/or UVB sunscreen would be most desirable. The inclusion of sunscreens in compositions of the present invention will provide immediate protection against acute UV damage. Thus, the sunscreen will prevent further wrinkle formation caused by UV radiation, while the anti-wrinkle agents treat existing wrinkles and skin atrophy, and enhances DNA repair in the cells of the skin.

[0084] A wide variety of conventional sunscreening agents are suitable for use in combination with the anti-wrinkle formulations. Sagarin, et al., at Chapter VII, pages 189 et seq., of Cosmetics Science and Technology, disclose numerous suitable agents. Specific suitable sunscreening agents include, for example: p-aminobenzoic acid, its salts and its derivatives (ethyl, iso-butyl, glyceryl esters; p-dimethylaminobenzoic acid); anthranilates (i.e., o-aminobenzoates; methyl, menthyl, phenyl, benzyl, phenylethyl, linyl, terpinyl, and cyclohexenyl esters); salicylates (amyl, phenyl, benzyl, menthyl, glyceryl, and dipropylene glycol esters); cinnamic acid derivatives (methyl and benzyl esters, α-phenyl cinnamonicil; butyl cinnamyl pyruvate); dihydroxycinnamic acid derivatives (umbelliferone, methyl umbelliferone, methylacetoumbelliferone); trihydroxycinnamic acid derivatives (esculetin, methylesculetin, daphnin, and the glucosides, esculin and daphnin); hydrocarbons (diphenylbutadiene, stilbene); dibenzalacetone and benzalacetophenone; naphtholsulfonates (sodium salts of 2-naphthol-3,6-disulfonic and of 2-naphthol-6,8-disulfonic acids); dihydroxynaphthoic acid and its salts; o- and p-hydroxybiphenylsulfonates; (7-hydroxy, 7-methyl, 3-phenyl); diazoles (2-acetyl-3-bromoindazole, phenyl benzoxazole, methyl naphthoxazole, various aryl benzothiazoles); quinone salts (bisulfate, sulfate, chloride, oleate, and tannate); quinoline derivatives (8-hydroxyquinoline salts, 2-phenylquinoline); hydroxy- or methoxy-substituted benzophenones; uric and vilouric acids; tannic acid and its derivatives (e.g., hexaethyl ether); (butyl carbotol)(6-propyl piperonate)ether; hydroquinone; benzophenones (oxybenzene, sulisobenzene, dioxybenzene, benzoresorcinol, 2,2',4,4'-tetrahydro-2'-benzophenone, 2,2'-dihydroxy-4,4'-dimethoxybenzophenone, octabenzone; 4-isopropyl dibenzoyl-methane; butylmethoxy dibenzoyl methane; etocrylene; and 4-isopropyl-dibenzoylethane). Mixtures of sunscreen compounds may be used to optimize the desired sunscreen properties of the formulation. A safe and effective amount of sunscreen may be used in the compositions of the present
invention. The sun-screening agent must be compatible with the anti-wrinkle agents. Generally the composition may comprise from about 1% to about 20%, preferably from about 2% to about 10%, of a sun-screening agent. Exact amounts will vary depending upon the sunscreen chosen and the desired Sun Protection Factor (SPF). An agent may also be added to any of the compositions of the present invention to improve the skin substantivity of those compositions, particularly to enhance their resistance to being washed off by water, or rubbed off.

[0085]In yet a further embodiment of the current invention, the oxaloacetate delivered topically can be mixed with a penetration enhancing agent such as dimethylsulfoxide (DMSO), combinations of sucrose fatty acid esters with a sulfoxide or phosphoric oxide, or eugenol, that allows faster migration of the oxaloacetate into the dermal tissues and then further into deeper cellular tissues, including cellulite tissues where stimulation of the Sirt1 gene will cause a reduction of fat tissues.

[0086]In one embodiment, the disclosed compounds are administered through a topical delivery system. Implantable or injectable polymer matrices, and transdermal formulations, from which active ingredients are slowly released are also well known and can be used in the disclosed methods. The controlled release components described above can be used as the means to delivery the disclosed compounds. The compositions can further include components adapted to improve the stability or effectiveness of the applied formulation, such as preservatives, antioxidants, skin penetration enhancers and sustained release materials. Examples of such components are described in the following reference works hereby incorporated by reference: Martindale—The Extra Pharmacopoeia (Pharmaceutical Press, London 1993) and Martin (ed.), Remington's Pharmaceutical Sciences.

[0087]Controlled release preparations can be achieved by the use of polymers to complex or absorb oxaloacetate. The controlled delivery can be exercised by selecting appropriate macromolecule such as polyesters, polyamino acids, polyvinylpyrrolidone, ethylenevinyl acetate, methylcellulose, carboxymethylcellulose, and protamine sulfate, and the concentration of these macromolecule as well as the methods of incorporation are selected in order to control release of active compound.

[0088]Hydrogels, wherein the oxaloacetate is dissolved in an aqueous constituent to gradually release over time, can be prepared by copolymerization of hydrophilic mono-olefinic monomers such as ethylene glycol methacrylate. Matrix devices, wherein the oxaloacetate is dispersed in a matrix of carrier material, can be used. The carrier can be porous, non-porous, solid, semi-solid, permeable or impermeable. Alternatively, a device comprising a central reservoir of the oxaloacetate compound surrounded by a rate controlling membrane can be used to control the release of oxaloacetate. Rate controlling membranes include ethylene-vinyl acetate copolymer or butylene terephthalate/polytetramethylene ether terephthalate. Use of silicon rubber depots are also contemplated.

[0089]In another embodiment, transdermal patches, steady state reservoirs sandwiched between an impervious backing and a membrane face, and transdermal formulations, can also be used to deliver oxaloacetate. Transdermal administration systems are well known in the art. Occlusive transdermal patches for the administration of an active agent to the skin or mucosa are described in U.S. Pat. Nos. 4,573,996, 4,597,961 and 4,839,174, which are hereby incorporated by reference. One type of transdermal patch is a polymer matrix in which the active agent is dissolved in a polymer matrix through which the active ingredient diffuses to the skin. Such transdermal patches are disclosed in U.S. Pat. Nos. 4,839,174, 4,908,213 and 4,943,435, which are hereby incorporated by reference. In one embodiment, the steady state reservoir carries doses of oxaloacetate in doses from about 2 mg to 40 mg per day.

[0090]Present transdermal patch systems are designed to deliver smaller doses over longer periods of time, up to days and weeks. A rate-controlling outer microporous membrane, or micropockets of the disclosed oxaloacetate dispersed throughout a silicone polymer matrix, can be used to control the release rate. Such rate-controlling means are described in U.S. Pat. No. 5,676,969, which is hereby incorporated by reference. In another embodiment, the oxaloacetate is released from the patch into the skin of the patient in about 20-30 minutes or less.
These transdermal patches and formulations can be used with or without use of a penetration enhancer such as dimethylsulfoxide (DMSO), combinations of sucrose fatty acid esters with a sulfoxide or phosphoric oxide, or eugenol. The use of electrolytic transdermal patches is also within the scope of the methods disclosed herein. Electrolytic transdermal patches are described in U.S. Pat. Nos. 5,474,527, 5,336,168, and 5,328,454, the entire contents of which are hereby incorporated by reference.

Oxaloacetate may be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. The injected oxaloacetate can be mixed with other beneficial agents prior to injection including but not limited to antibiotics and other medications, saline solutions, blood plasma, and other fluids. Immediate contact of elevated levels of oxaloacetate with the vascular system cells will result in the reduction in age-related diseases such as hardening of the arteries, even if the amounts of oxaloacetate are insufficient to provide age-related benefits to the entire organism. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulation agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

Oxaloacetate may also be formulated in rectal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, oxaloacetate may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

In yet still another embodiment, oxaloacetate can be mixed with animal foods to increase the life span and general health of pets and other animals. Oxaloacetate can either be formulated as part of the animal food or administered separately as a supplement to the animal’s food. As those skilled in the art know, dry pet foods, typically dry dog foods, normally contain protein, fat, fiber, non-fiber carbohydrates, minerals, vitamins and moisture components. For example, as major ingredients, there are typically one or two cereal grains, generally corn, wheat and/or rice. In addition, for a protein source they may contain poultry meal, by-product meat, meat and bone meal, or other animal or fish meal by-products. At times as well, grain protein supplements such as corn gluten, soybean meal or other oil seed meals can be added. In addition to an effective amount of oxaloacetate of between about 0.01% to 0.1% by weight of the chow, animal chow of the present invention additionally includes the following: typical nutrient content in the food dry matter includes crude protein from 14% to 50%, usually 20% to 25%; crude fat from 5% to 25%; and crude fiber usually is present in the range of from about 3% to 14%, usually about 5% to 7%. The important point is not the precise formulation of the pet food, since many conventional and satisfactory ones for use in conjunction with the present invention are available on the market. Rather, the key to success is that a sufficient amount of oxaloacetate component be added to pet food rations, whichever formulation is used, to provide the oxaloacetate activity level at the ranges necessary to increase life span, reduce body weight, and treat age-related disorders.

EXAMPLES

Particular aspects herein can be more readily understood by reference to the following
examples, which are intended to exemplify the teachings herein, without limiting their scope to the particular exemplified embodiments.

Example 1

[0098] The Caenorhabditis elegans nematode worm is commonly used as a test animal because its genetics are well understood and the basic cellular energy pathways are well conserved throughout all animal species, including humans. A test with these well-understood worms on metabolic energy pathways is a test on the animal kingdom. The conservation of the energy pathways is a reason that CR has worked in all animal species tested.

[0099] C. elegans (N2 wild type, from Carolina Biological Supply) was used as a test organism to assess the addition of oxaloacetate therapy (from Sigma Aldrich Company) applied to the cells to extend lifespan and to act as a caloric restriction "mimic". The oxaloacetate was mixed in a concentration of 16 mM into the nematode growth agar (from Carolina Biological Supply Company) on eight experimental plates on which the C. elegans resides. A similar set of eight control plates with the same nematode growth agar was also prepared. Both the eight oxaloacetate plates and eight control plates included 80 μM of 5’fluorodeoxyuridine (from Sigma Aldrich Company) to eliminate the development of eggs into live progeny. In addition, five "starter plates" were made using nematode growth agar and no 5’fluorodeoxyuridine.

[0100] All plates were streaked with 0.5 ml bacteria Luria broth containing E. coli as a food source for the nematodes. The bacteria were allowed to grow on the plates for several days at 37° C. prior to adding C. elegans.

[0101] Ten C. elegans nematodes were transferred to each of five "starter plates" and were left on the plates for 12 hours to lay eggs. The C. elegans were then removed and the eggs were allowed to grow into young adults for two days. Ten nematodes were then transferred to each control plate and oxaloacetate plate by "picking" worms with a microprobe from the starter plate and moving it onto the target test plates.

[0102] The day after the starter plates have been started with the 10 C. elegans was designated "day 0", and was the estimated time of birth. The C. elegans were kept out of direct sunlight and were raised at a temperature of 21° C. The plates with the C. elegans were counted every day to determine the number of dead nematodes. Nematodes were scored as dead when they no longer respond to a gentle prodding with a probe. Lifespan is defined as the time elapsed from when the nematodes are hatched (lifespan=0) to when they are scored as dead. Nematodes that crawl off the plates or are otherwise lost during the assay have been excluded from calculations. The dead nematodes were removed from the plates each day with microprobe. The total population is the sum of all found dead worms. The counting of the nematodes was continued until all nematodes were dead. The data was then charted and is represented in FIG. 2.

[0103] Data indicated an increase in average lifespan of 23.6% in the nematodes that lived on the oxaloacetate plates as opposed to the control group. Maximal life span was also increased in the oxaloacetate containing plates by 40%.

Example 2

[0104] The experiment in Example 1 was repeated for a range of oxaloacetate concentrations in the nematode growth agar. An increase in lifespan for C. elegans was seen at 2 mM oxaloacetate in the agar, and increased with 4 mM, 6 mM, 8 mM, 10 mM, 12 mM, 14 mM and 16 mM concentrations. The highest increase in lifespan was at 16 mM (approximate 25%) for the concentrations tested, and the lowest increase was a 2 mM (approximately 10%).

Example 3

[0105] The experiment in Example 1 was repeated but additional plates containing splitomycin alone with the agar and splitomycin with oxaloacetate and the agar were also used. Splitomycin
is a selective inhibitor for the Sir2 gene (Sir2a in yeast, Sirt1 in humans). The nematodes on plates with inhibited Sir2 function but no oxaloacetate lived for shorter periods than the control group as reflected in FIG. 3. FIG. 3 illustrates that similar to CR, oxaloacetate upregulates the Silent Information Regulator gene (Sir2) to increase lifespan. It also shows that other beneficial CR activated genes are upregulated and downregulated by oxaloacetate to increase lifespan without Sir2 activation. Nematodes on plates with oxaloacetate lived approximately 36% longer than the control group. Nematodes on plates with inhibited Sir2 function but also included oxaloacetate had an increased lifespan above the control group of 15%. This demonstrates that Sir2 makes up approximately 1/3 of the lifespan increase in CR, while the other CR beneficial genes contribute to as much as 2/3 of the lifespan increase. It also demonstrates that one of the genes activated by oxaloacetate addition is Sir2 (Sir2a in yeast, Sirt1 in humans).

Example 4

[0106] The fruit fly Drosophila melanogaster (Vestigal type, from Carolina Biological Supply) was used as a test organism to assess the addition of oxaloacetate therapy (from Sigma Aldrich Company) applied to the cells to extend lifespan and to act as a caloric restriction "mimic". The fruit fly is more complex than C. elegans, but the metabolic pathways that involve calorie restriction are a basic building block of life, and are conserved throughout the animal kingdom. In the test group of 53 flies in four vials (2 virgin male, 2 virgin female), oxaloacetate was mixed in a concentration of 16 mM into the 15 ml of distilled water used to wet the 15 ml of dry fly food (from Carolina Biological Supply Company). A small amount of yeast was added on top of the fly food. A similar control group of 56 flies in 4 vials (2 virgin male, 2 virgin female) were also prepared, except that no Oxaloacetate was added.

[0107] The flies were kept in a room receiving reflected natural sunlight at a temperature of 21° C. Each of the flies used emerged from the pupae state within 8 hours of each other.

[0108] The day the flies were moved to the test vials was designated "day 0", reflecting the emergence of the fly into adulthood. The flies were kept out of direct sunlight and were raised at a temperature of 21° C. The food vials were prepared every 3 to 4 days and the flies transferred to prevent infestation of mites and other pathogenic microbial organisms. The vials with the flies were counted every day to determine the number of dead vs. living. Flies were scored as dead when they no longer respond to a gentle prodding with a probe. Lifespan is defined as the time elapsed from when the flies enter into adulthood (lifespan=0) to when they are scored as dead. Flies that were lost due to transfer between food vials or were otherwise lost during the assay have been excluded from calculations. The dead flies were removed from the vials each day with microprobe. The total population is the sum of all found dead flies. The counting of the flies was continued until all flies were dead. The data was then charted and is represented in FIG. 4.

[0109] Data indicated an increase in average lifespan of 20% in the flies that lived on the oxaloacetate supplemented vials as opposed to the control group.

Example 5

[0110] The experiment in Example 4 was repeated, except that the food vials were not changed out every 3 to 4 days and 71 flies were supplemented with oxaloacetate and 70 flies were not. This resulted in infestations of microbial pathogens that attacked the flies, placing the flies under "stress". It is well known in the literature that calorie restricted animals survive stress better than the control animals fed an ad libitum diet. The data from the experiment were charted and are represented in FIG. 5.

[0111] Data indicated an increase of both average lifespan under stress, and a maximal increase in lifespan of 30%. The oxaloacetate supplemented group lived significantly longer, indicating an improved resistance to stress (also found in calorie restricted animals).

Example 6

[0112] C57B1/6 type male mice approximately 9 months of age were obtained from Harlan, San
Diego, Calif. as retired breeders. These mice were all born on the same day. The C57B1/6 type mouse is inbred to reduce the genetic variation between individuals. The mice were housed in individual cages and were provided with ad libitum amounts of fortified food pellets (Kadee). The amount of food consumed was weighed daily, while the mice were weighed weekly. After two weeks, a baseline of the average food consumed by the mice was established at 7.1 grams per mouse per day. In addition, after an overnight fast, blood glucose readings for each mouse were recorded for each mouse. At the two-week point the mice were grouped into three equivalent groups (based on initial weight and fasting blood glucose measurements) of I) eight "Control Mice" fed ad libitum; II) eight "Calorie Restricted" mice, initially fed 20% less than the control group for two weeks, then increased to being fed 40% less than the control group, and III) nine "oxaloacetate" supplemented mice that were fed an increasing dose of oxaloacetate in their food, but were allowed to eat freely.

Male C57B1/6 type mice, when allowed to eat freely, gain weight with age in a linear fashion. The mice were weighed weekly for 21 weeks, and the individual weights were recorded and averaged to form a "group average" for each of the three groups. The incremental increase in weight between the "Control" group and the "Oxaloacetate" supplemented group was charted as represented by FIG. 6. The data indicates that control mice increase in weight in a linear fashion, whereas oxaloacetate supplementation decreases the amount of body weight that mice will gain when eating freely. The larger the amount of supplementation of oxaloacetate, the larger the reduction in weight gain in a dose dependent fashion. At 0.4% oxaloacetate in the food, the oxaloacetate mice gained 20% less weight than the control group, even though both groups were allowed to eat freely.

The results of this experiment indicate that oxaloacetate supplementation can be used to reduce body weight in mammals.

Example 7

The experiment in Example 6 was repeated with one month old male mice (as compared to the nine month old mice used in Example 6). As in the previous Example 6, young C57B1/6 type mice grouped by weight into three groups, I) Control; II) Calorie Restricted; and III) Oxaloacetate supplemented. Instead of slowly increasing the dose of oxaloacetate, however, the mice were subjected to a concentration of 0.4% oxaloacetate from the start of the experiment, and calorie restricted mice were immediately placed on a 40% restricted diet. The mice were weighed weekly, and the results were plotted as represented by FIG. 7.

FIG. 7 shows that young mice also respond to oxaloacetate supplementation for reduced weight gain. Note that it took about three weeks for the effect of the oxaloacetate to fully be seen, after which point the weight differential remained constant due to the non-varying dosage.

Example 8

Three mice of each of the three groups of the C57B1/6 mice of Example 6 were sacrificed after 21 weeks. The liver of each animal was extracted and each set of three mice was pooled and analyzed for gene activity as described by Cao et al. The gene expression of the pooled mice group was shown to change in response to supplementation with oxaloacetate and in a similar fashion to mice under CR, as compared to the control group of mice. The CR mice showed a measurable change in the expression of 1,763 genes, while the oxaloacetate mice showed a measurable change in 765. Because each group was a pooling of three mice, there is a variety of individual responses. Changes in the physical results of the group (such as weight loss, increase in health span, and increase in life span) are due to genes expressed in common by the group, not in individual variation. Thus it is important to look at the genes that changed in expression from the control group, but are commonly expressed between the calorie restricted group and the oxaloacetate supplemented group. There were 363 genes expressed in common between the oxaloacetate group and the calorie restricted group that changed in genomic expression when compared to the control group. A directional analysis of these 363 common genes indicated that 98% of the changed genes expressed in common between the oxaloacetate group of mice and the calorie restricted group of mice moved in the same direction (upregulated or down-regulated) as compared to the control group of mice. Thus, the
supplementation of oxaloacetate mimicked the same genomic change in mice as did mice under calorie restriction, for 98% of the genes in common that changed expression from the control group. The oxaloacetate mice, however, did not have to restrict their diets, but ate freely. The 98% similarity in change between the oxaloacetate fed mice and the calorie restricted mice also ties to the fact that the oxaloacetate supplemented mice did not gain as much weight as the control mice (Example 6 and 7 above) and also live longer and are healthier (Example 9 below). Oxaloacetate supplementation mimics the same critical genomic response as calorie restriction (See FIG. 8). Effective doses of oxaloacetate fed to C. elegans and D. melanogaster increase lifespan by 20 to 36%, and minimum increase in lifespan of mice by 10%.

**Example 9**

[0118] The C57B1/6 mice of Example 6 that were not sacrificed in Example 8 were continued to be fed as three separate groups, 1) Control, 2) Calorie Restricted and 3) Oxaloacetate supplemented (at a continuing dose of 0.4% in food by weight). It was noted that the Calorie Restricted group and the Oxaloacetate group was the healthiest group during the experiment, with a portion of the Control group being affected by inflammation characterized by intermittent bouts of itching and redness of the skin. The experiment is in continuation as this application for patent is filed, but initial indications are that the oxaloacetate group is living longer than the control group, and at least equal to the calorie restricted group.

[0119] Lifespan data is represented by FIG. 9, which at the 21st month in their lives, oxaloacetate supplementation increased lifespan of the group by a minimum of 10% (and still continuing) over the control group, and at least equal to (or better) than the calorie restricted group.

**Example 10**

[0120] An obese individual weighing approximately 200 kg is identified. 1,000 mg of oxaloacetate is administered orally for a period of 30 days, taken every other day. The individual does not restrict calories. A reduction of body fat and body weight is observed following treatment.

**Example 11**

[0121] Individuals presenting with age related disorders including heart disease and osteoporosis are identified. Half of the individuals are administered an effective dose of approximately 7 mg of oxaloacetate per kg of body weight, taken every other day over a period of 30 days or more. The other half of the individuals are administered a placebo. Individuals receiving oxaloacetate demonstrate a reduction in symptoms associated with aging including a decrease in fasting glucose levels, fasting insulin levels, triglycerides, Hs-CRP levels, total cholesterol, LDL cholesterol, and systolic and diastolic blood pressure. Additionally, a reduction in the risk for atherosclerosis is also observed as compared with the untreated individuals.

**Example 12**

[0122] Two groups of individuals are exposed to UV radiation from the sun. One group applies an 8 mM concentration of oxaloacetate to the skin, while the control group does not. Both groups have their skin measured for unscheduled DNA synthesis (UDS), to measure the relative rate of DNA repair. The group with oxaloacetate has a significant increase in UDS over the control group.

**Example 13**

[0123] Two groups of individuals are diagnosed with similar types of cancer tumors. One group ingests a dose of 2,000 mg of oxaloacetate per day, whereas the control group does not. The oxaloacetate reduces the amount of glucose available to the tumor, which limits the growth of the tumor in a fashion similar to that seen in calorie restriction. The control group sees no limitation in tumor growth.
The foregoing description details certain embodiments of the invention. It will be appreciated, however, that no matter how detailed the foregoing appears in text, the invention can be practiced in many ways. As is also stated above, it should be noted that the use of particular terminology when describing certain features or aspects of the invention should not be taken to imply that the terminology is being redefined herein to be restricted to including any specific characteristics of the features or aspects of the invention with which that terminology is associated. The scope of the invention should therefore be construed in accordance with the appended claims and any equivalents thereof. Additionally, throughout this application, various publications have been referenced. The disclosures in these publications are incorporated herein by reference in order to more fully describe the state of the art.

Read more: http://www.faqs.org/patents/app/20080279786#ixzz2E2GCedIP