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(19) **United States**(12) **Patent Application Publication**
Longo(10) **Pub. No.: US 2015/0133370 A1**(43) **Pub. Date: May 14, 2015**(54) **DIETARY COMPOSITIONS AND METHODS
FOR PROTECTION AGAINST
CHEMOTHERAPY, RADIOTHERAPY,
OXIDATIVE STRESS, AND AGING***A61K 31/401* (2006.01)*A23L 1/305* (2006.01)*A61K 31/70* (2006.01)*A61K 38/02* (2006.01)*A61K 45/06* (2006.01)(71) Applicant: **University of Southern California**, Los
Angeles, CA (US)*A61K 31/405* (2006.01)*A61K 31/047* (2006.01)(72) Inventor: **Valter Longo**, Playa del Rey, CA (US)(52) **U.S. Cl.**CPC *A61K 31/4172* (2013.01); *A61K 31/405*(2013.01); *A61K 31/198* (2013.01); *A61K**31/401* (2013.01); *A61K 31/047* (2013.01);*A61K 31/70* (2013.01); *A61K 38/02* (2013.01);*A61K 45/06* (2013.01); *A23L 1/3051* (2013.01);*A23V 2002/00* (2013.01)(21) Appl. No.: **14/497,752**(22) Filed: **Sep. 26, 2014****Related U.S. Application Data**(60) Division of application No. 12/430,058, filed on Apr.
24, 2009, now Pat. No. 8,865,646, which is a continu-
ation-in-part of application No. 12/058,600, filed on
Mar. 28, 2008, now Pat. No. 8,211,700.**Publication Classification**(51) **Int. Cl.***A61K 31/4172* (2006.01)*A61K 31/198* (2006.01)**ABSTRACT**

(57)

The present invention relates to dietary compositions comprising reduced level of methionine, tryptophan, all amino acids, or protein, dietary compositions comprising glycerol as a substitute for monosaccharides, disaccharides, and polysaccharides, and hypocaloric or calorie free diets with reduced level of energy, carbohydrates, or protein. Also disclosed are methods of using these compositions and diets, as well as fasting, to protect subjects against chemotherapy, radiotherapy, oxidative stress, or aging.

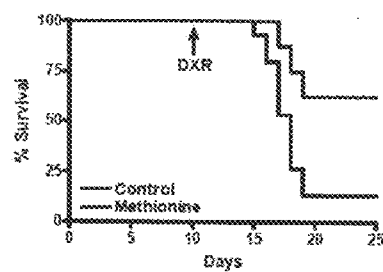


Fig. 1A

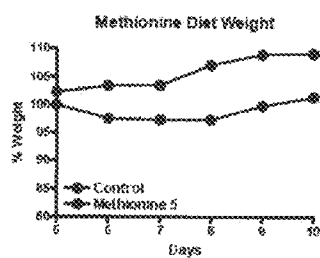


Fig. 1B

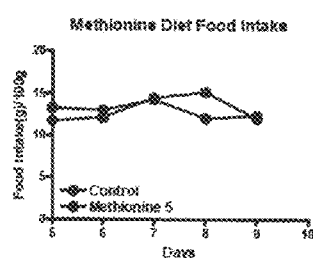


Fig. 1C

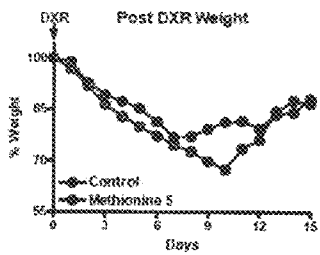


Fig. 1D

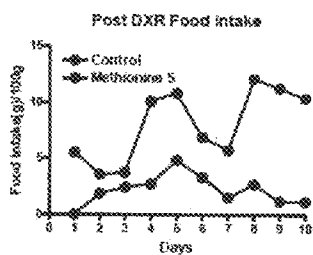


Fig. 1E

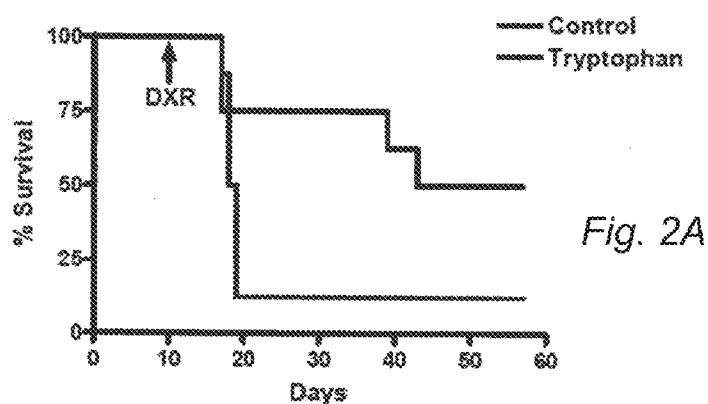


Fig. 2A

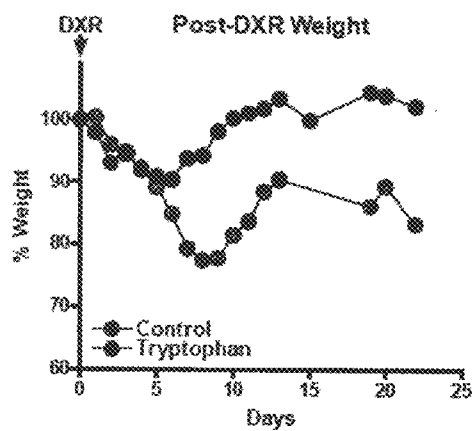


Fig. 2B

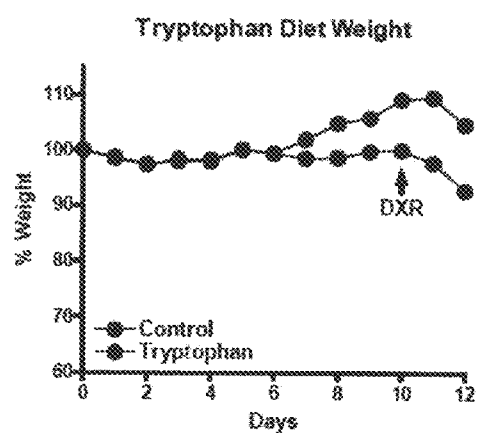


Fig. 2C

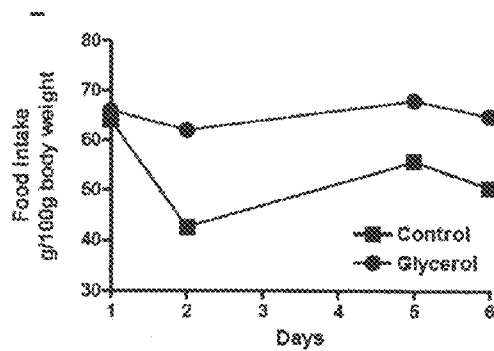


Fig. 3A

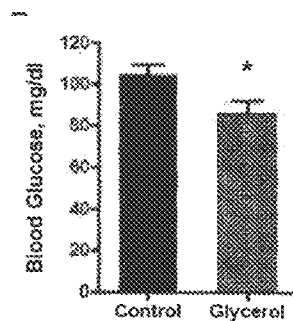


Fig. 3B

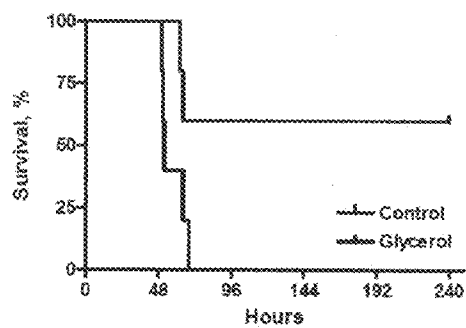


Fig. 3C

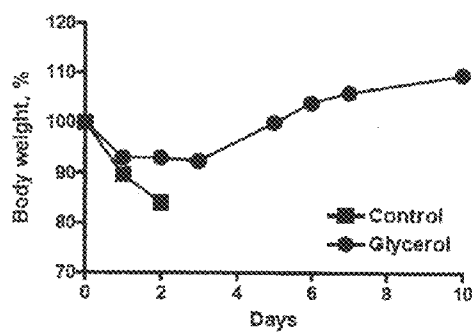


Fig. 3D

FIG. 4A

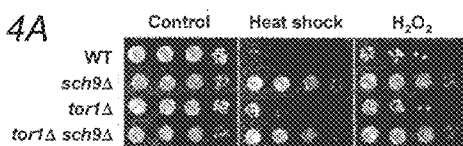


FIG. 4B

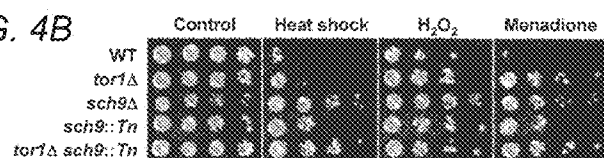


FIG. 4C

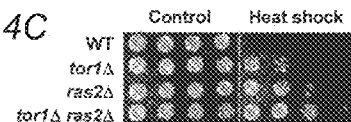


FIG. 4D

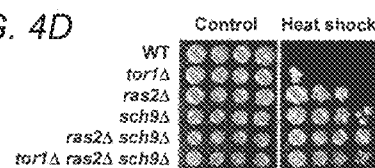


FIG. 4E

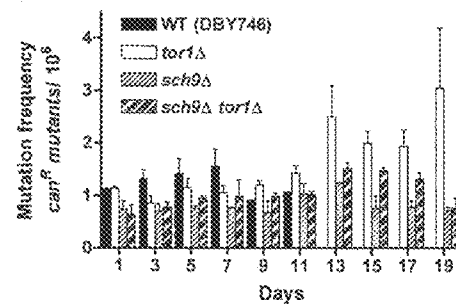


FIG. 4F

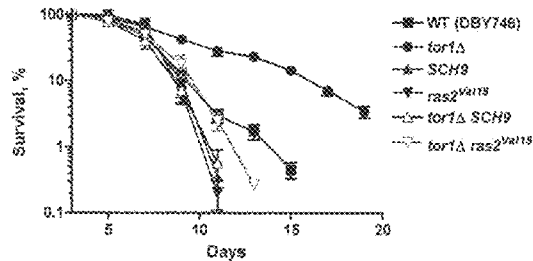


FIG. 4H

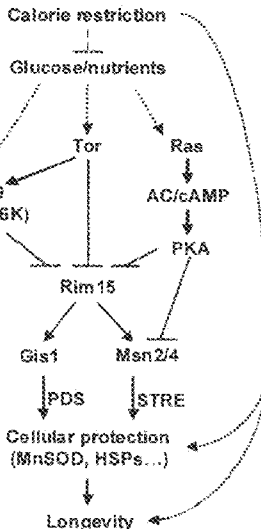
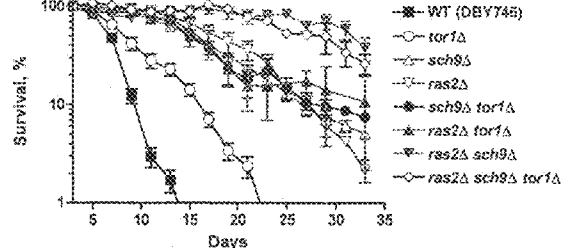


FIG. 4G



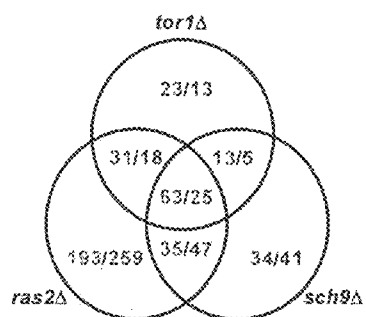


Fig. 5A

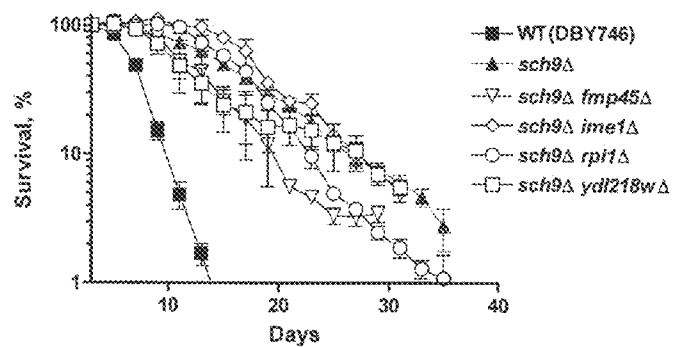
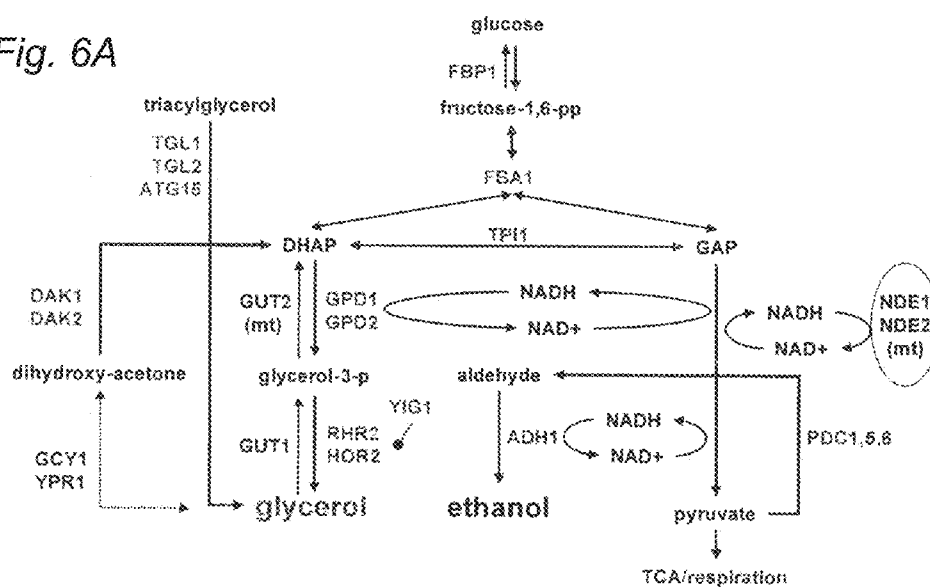


Fig. 5B

Fig. 6A



Expression of glycerol biosynthetic genes			
	<i>sch9Δ</i>	<i>tor1Δ</i>	<i>ras2Δ</i>
<i>GPD1/YDL022W</i>	2.37	2.45	2.75
<i>GPD2/YOL059W</i>	1.42	1.94	2.50
<i>RHR2/YIL053W</i>	1.57	1.22	1.22
<i>HOR2/YER062C</i>	1.59	1.11	1.25
<i>YIG1/YPL201C</i>	0.50	0.54	0.45
<i>GYC1/YOR120W</i>	1.12	1.08	0.85
<i>YPR1/YDR368W</i>	1.11	1.14	1.14
<i>DAK1/YML070W</i>	2.25	1.76	2.51
<i>DAK2/YFL053W</i>	1.88	1.53	19.10

Fig. 6B

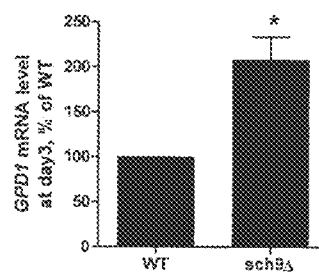


Fig. 6C

Fig. 7A

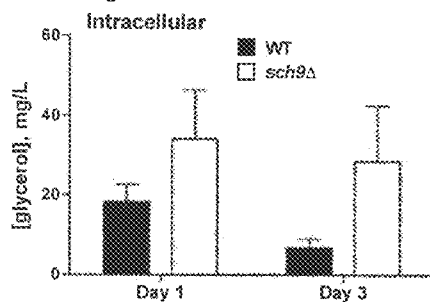


Fig. 7B

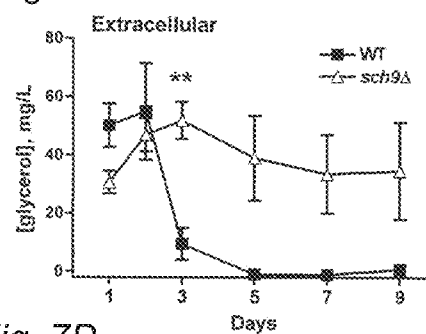


Fig. 7C

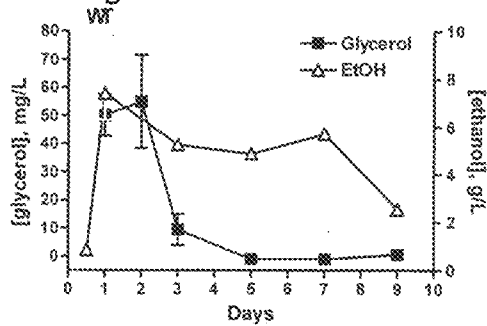


Fig. 7D

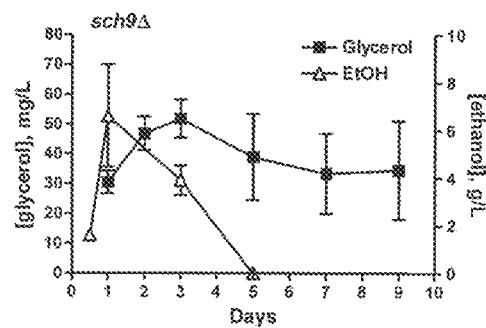


Fig. 7E

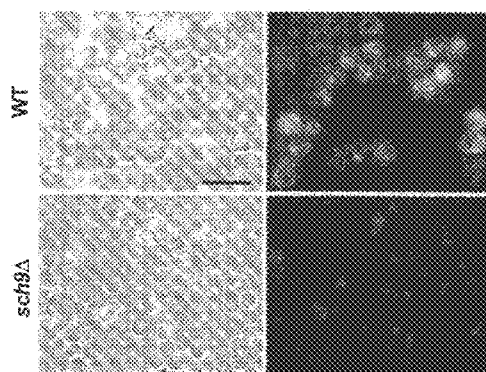


Fig. 8A

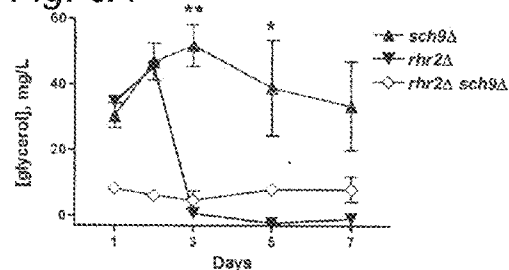


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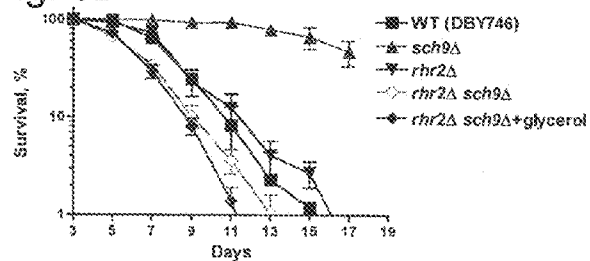


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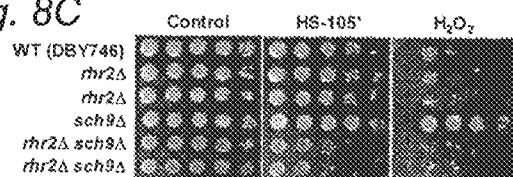


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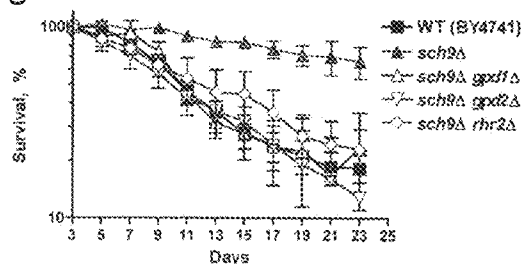


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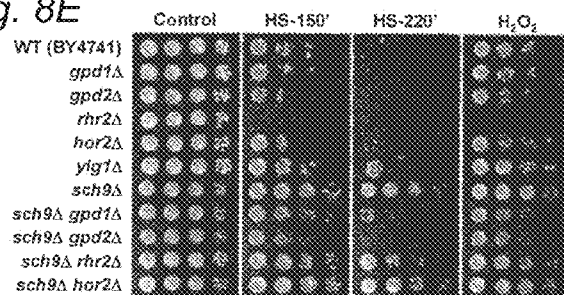


Fig. 9A

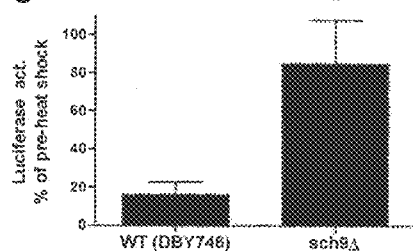


Fig. 9B

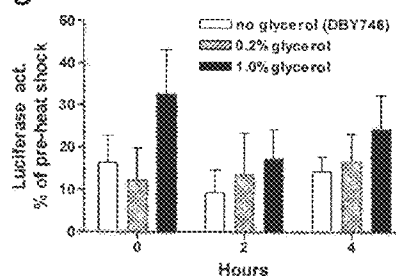


Fig. 9C

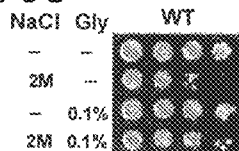


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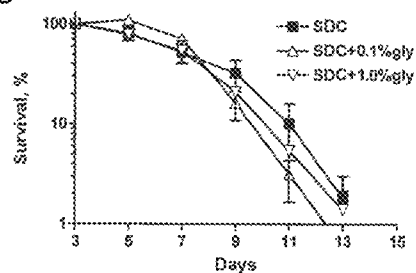


Fig. 9D



Fig. 9F

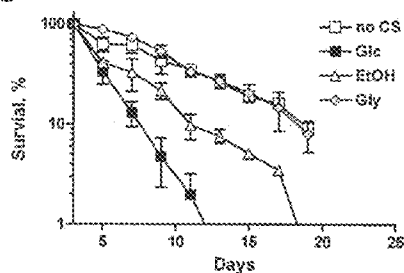


Fig. 9G

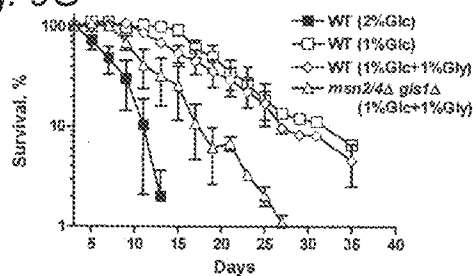


Fig. 9H

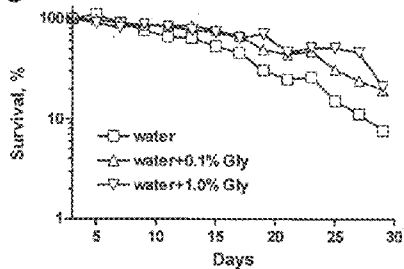


Fig. 9I

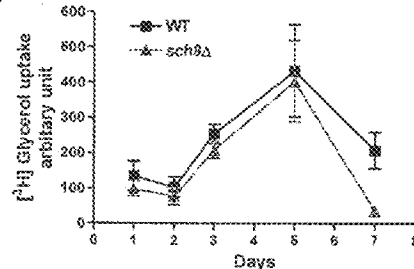


Fig. 10A

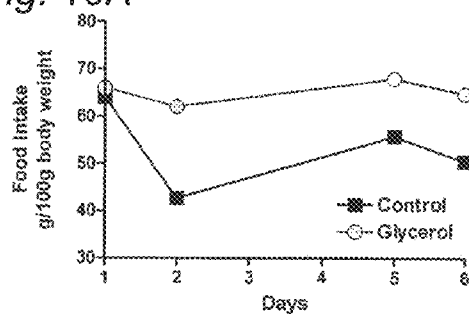


Fig. 10B

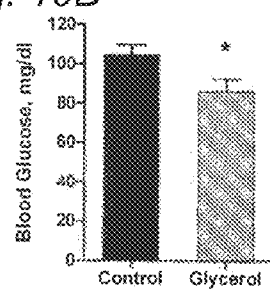


Fig. 10C

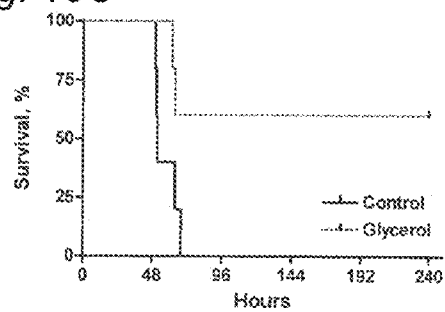
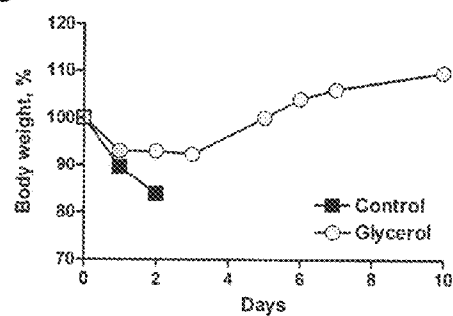


Fig. 10D



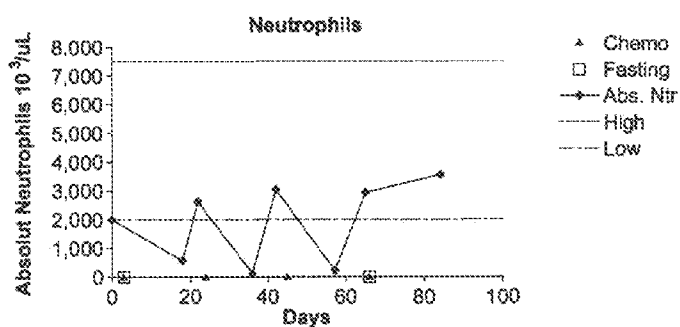


Fig. 11A

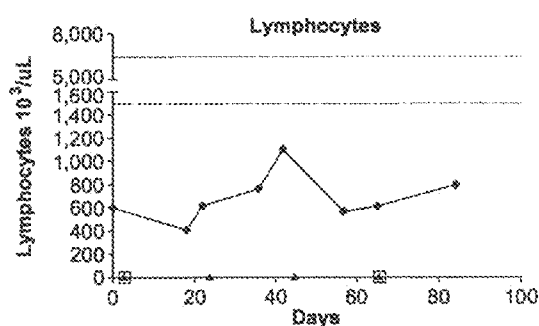


Fig. 11B

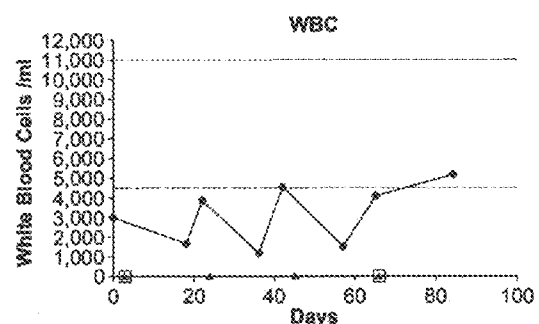


Fig. 11C

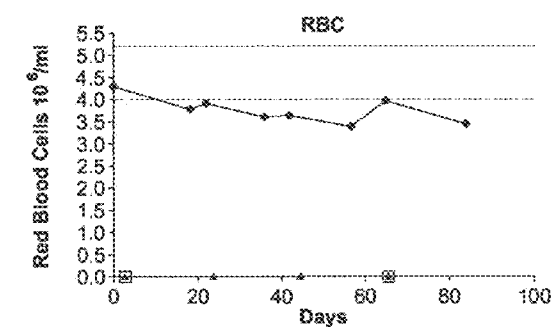


Fig. 11D

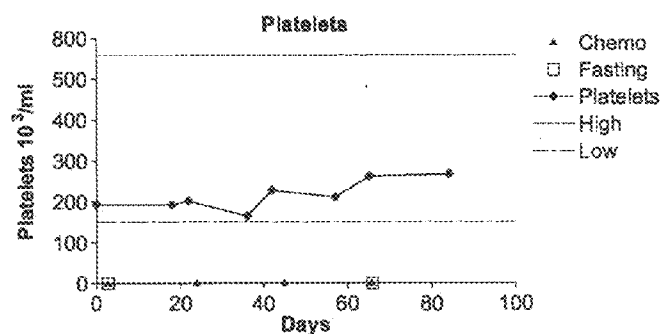


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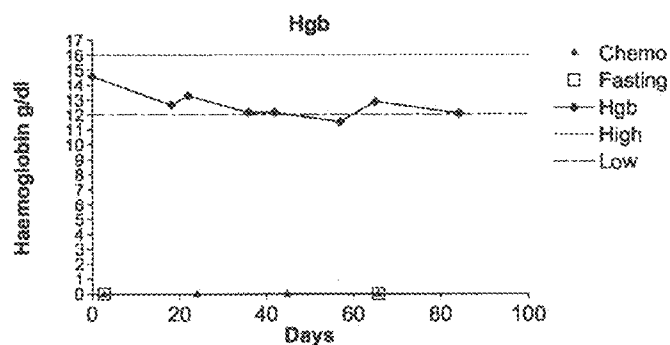


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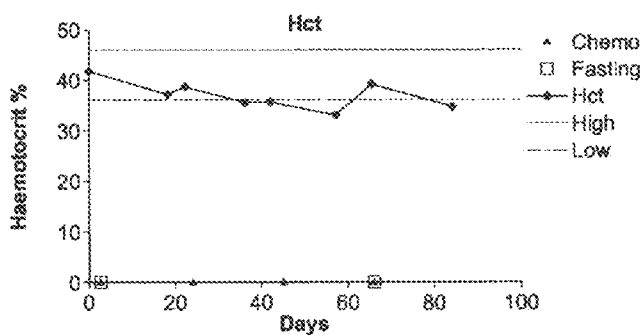


Fig. 11G

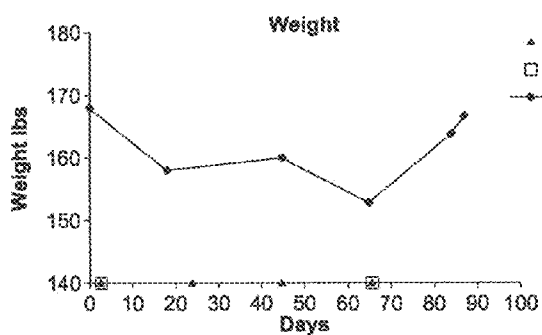
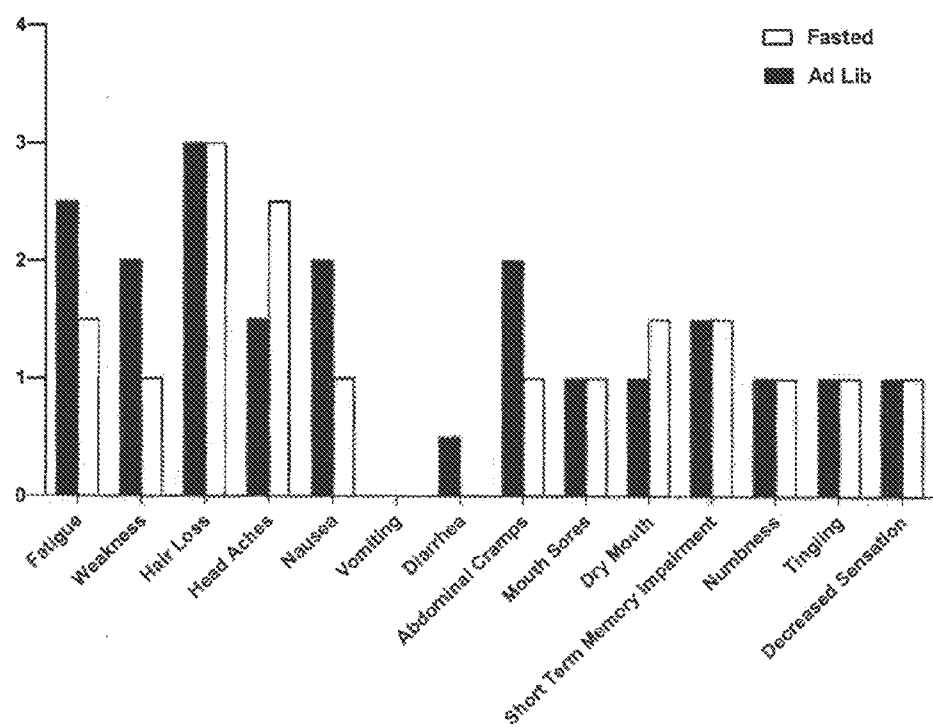
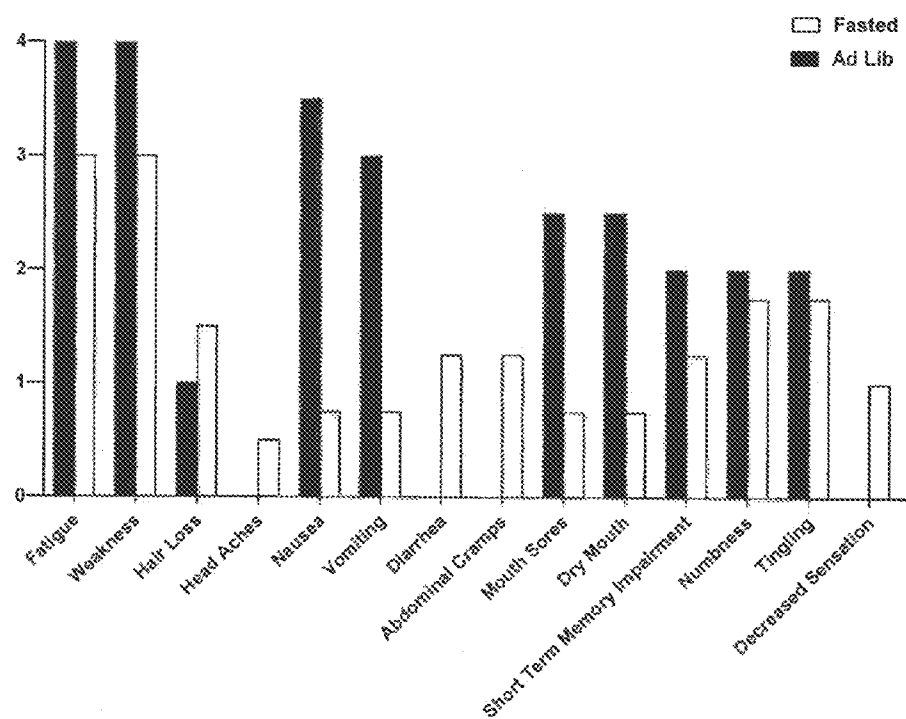


Fig. 11H

*Fig. 12*

*Fig. 13*

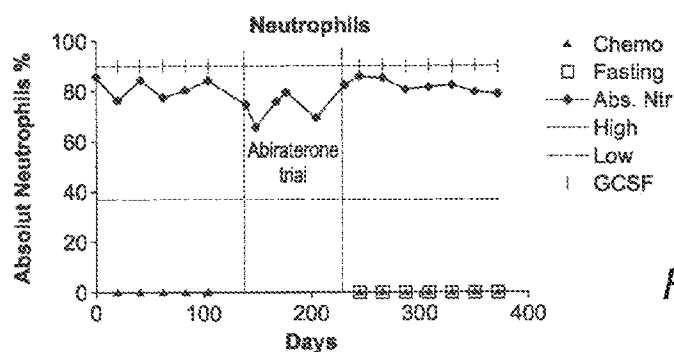


Fig. 14A

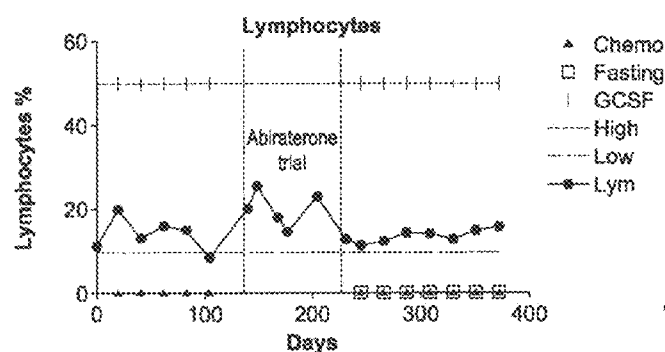


Fig. 14B

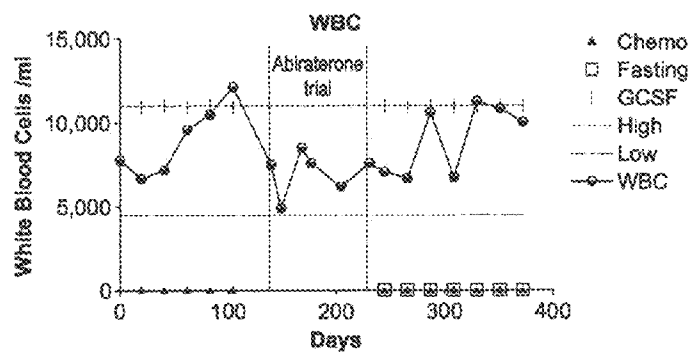


Fig. 14C

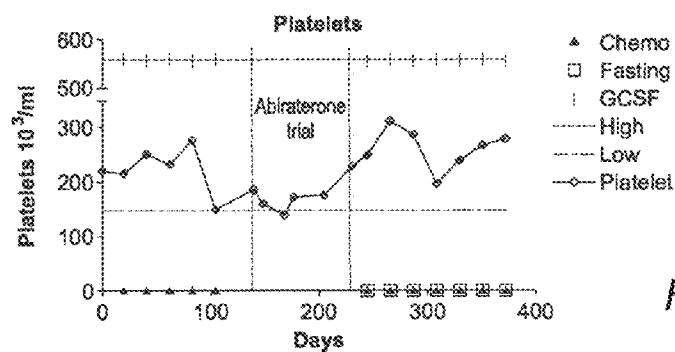


Fig. 14D

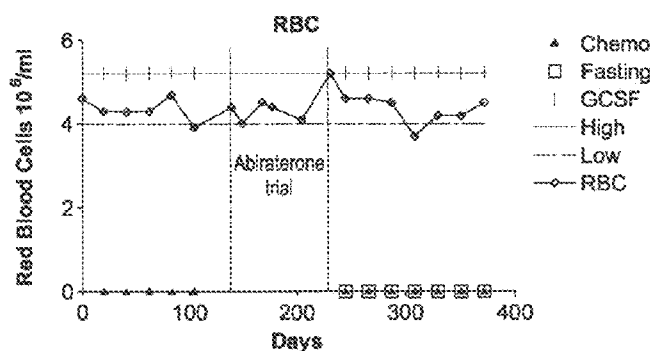


Fig. 14E

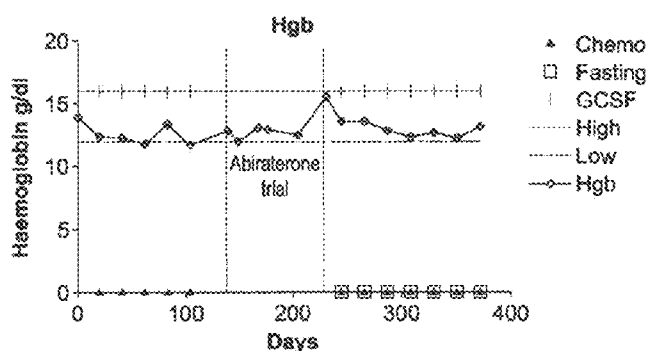


Fig. 14F

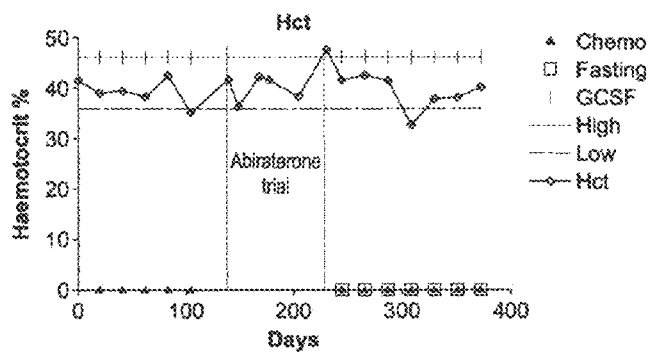


Fig. 14G

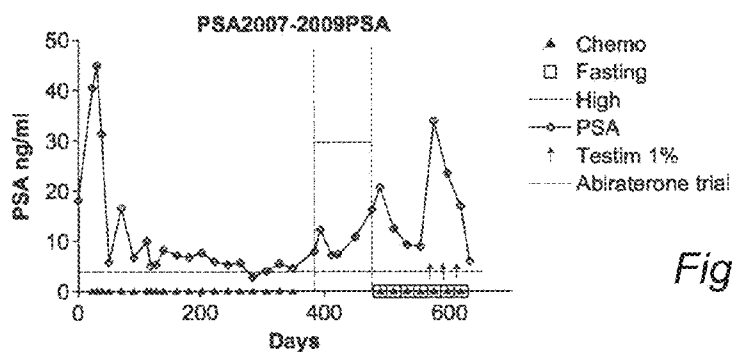
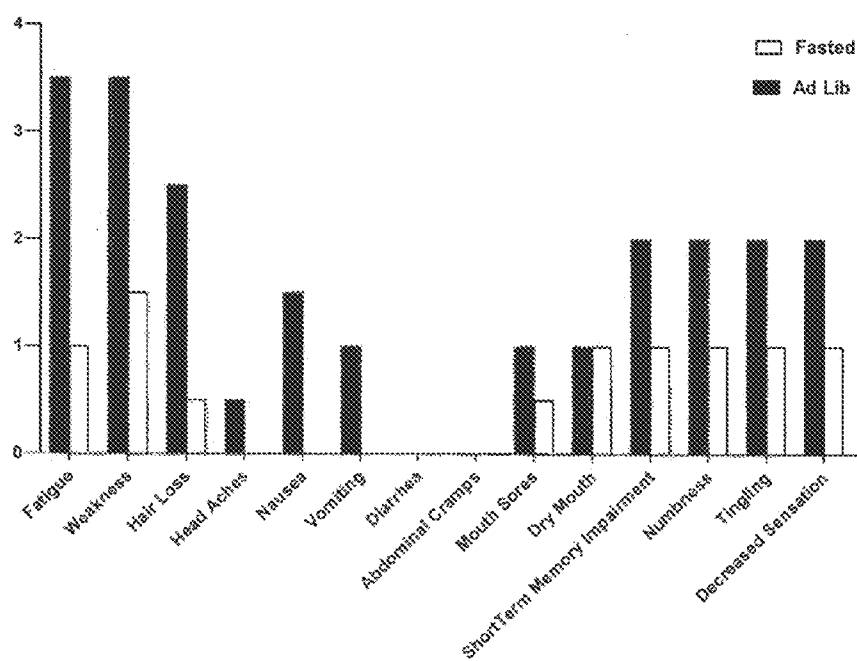


Fig. 14H

*Fig. 15*

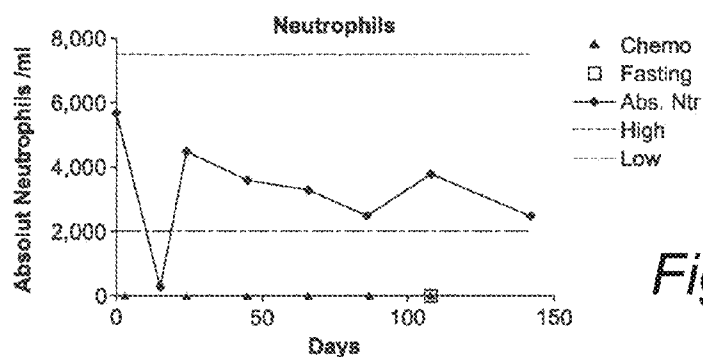


Fig. 16A

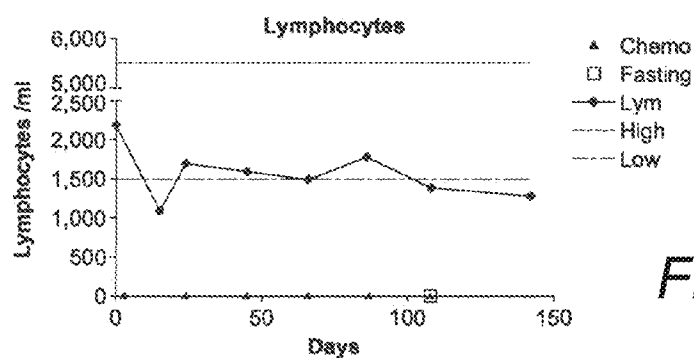


Fig. 16B

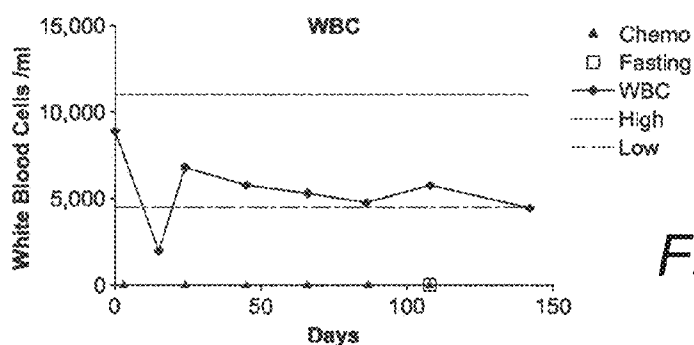


Fig. 16C

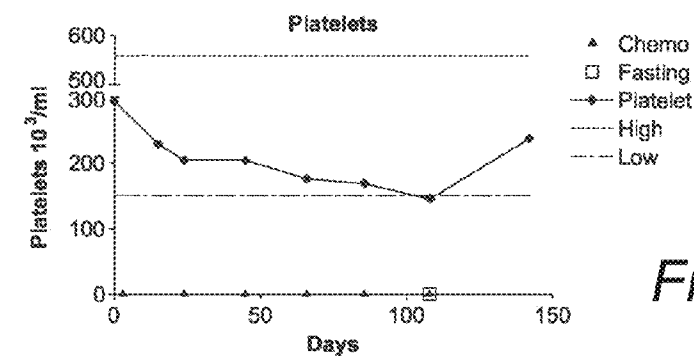


Fig. 16D

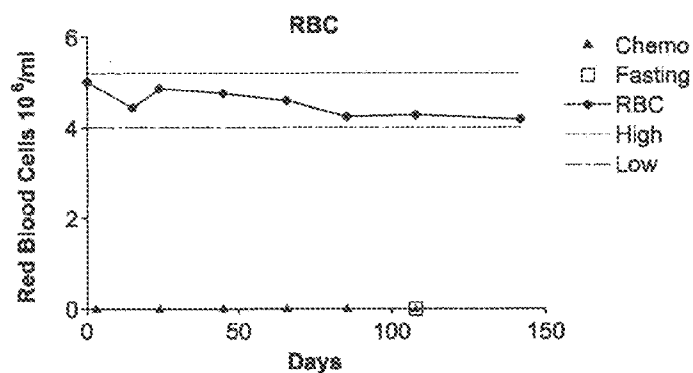


Fig. 16E

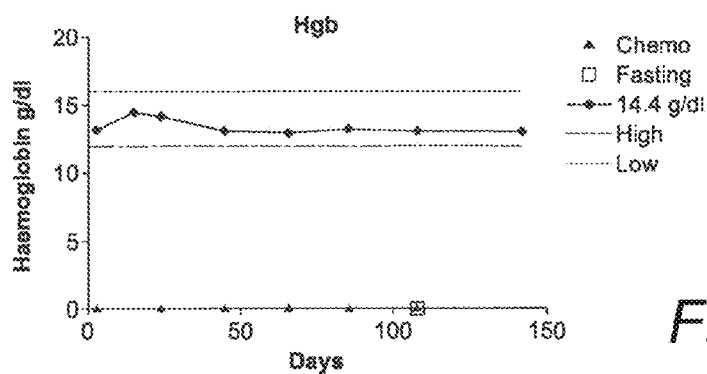


Fig. 16F

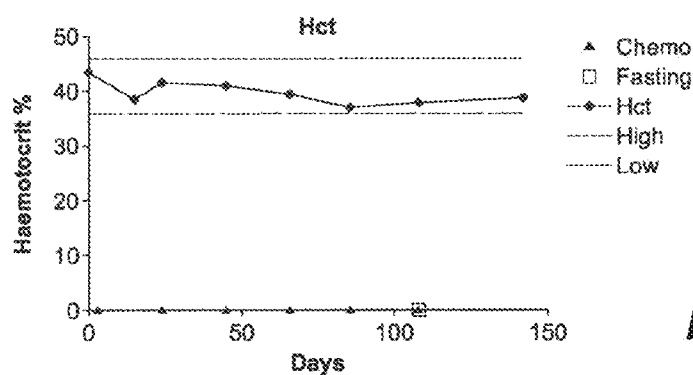


Fig. 16G

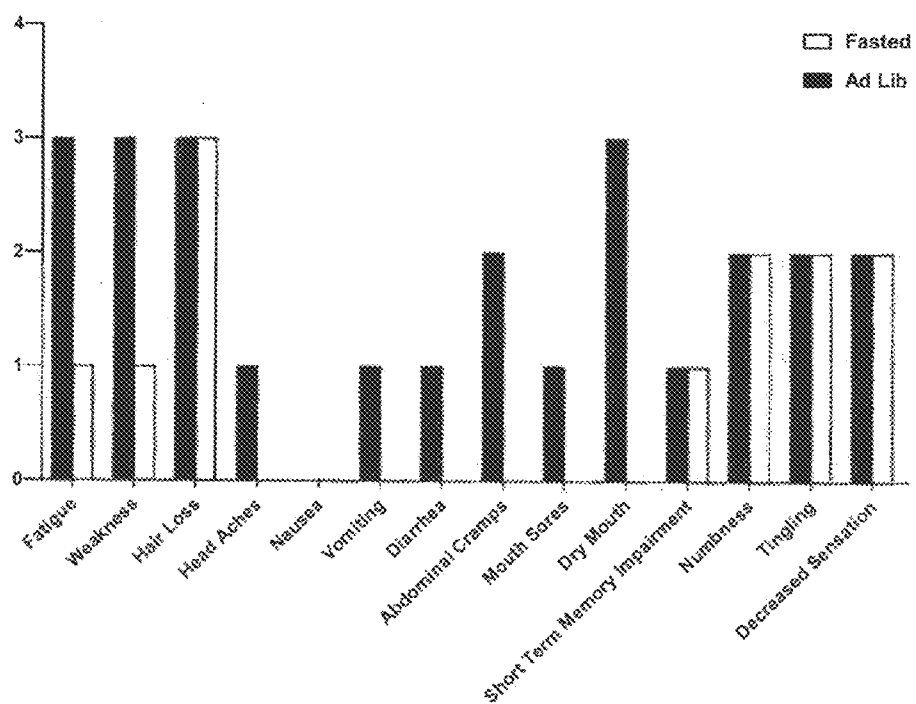


Fig. 17

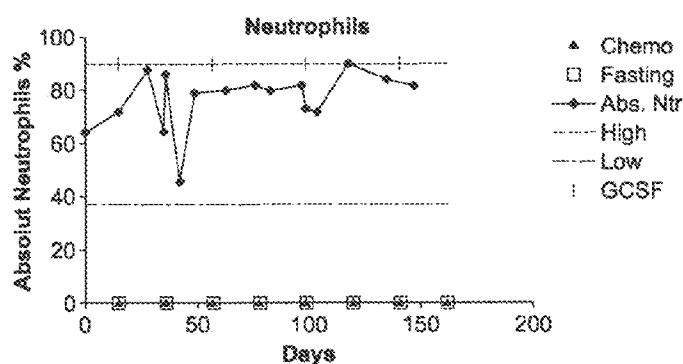


Fig. 18A

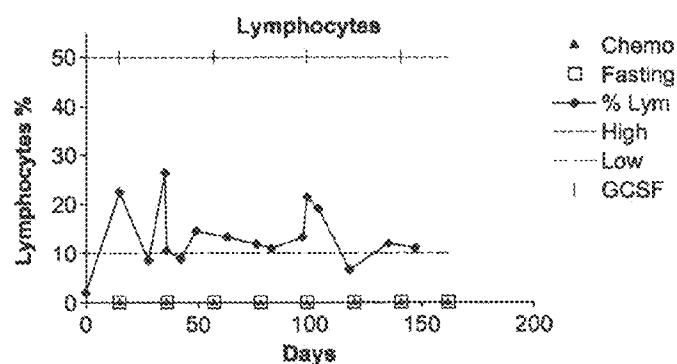


Fig. 18B

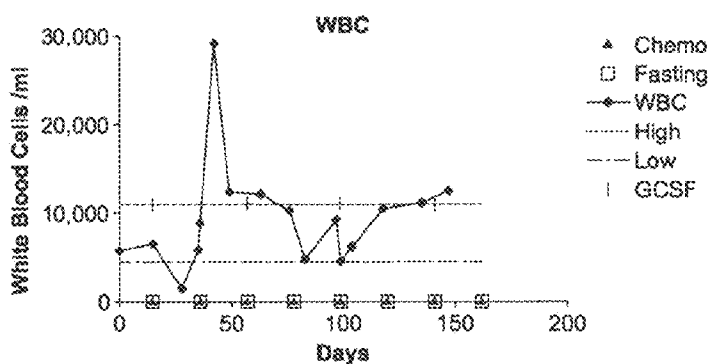


Fig. 18C

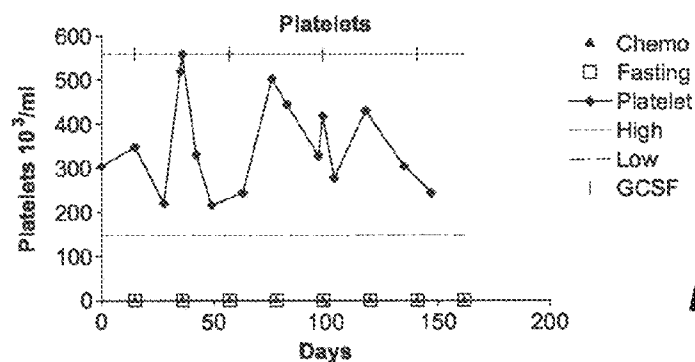


Fig. 18D

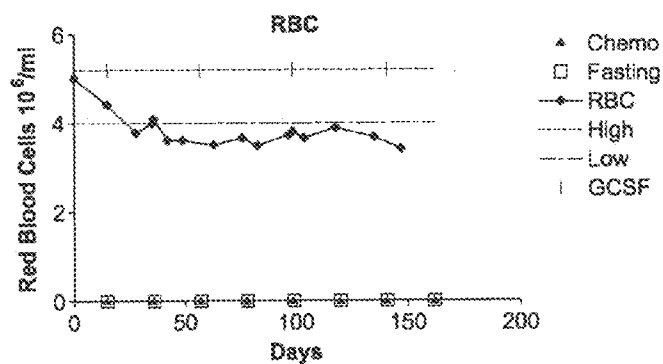


Fig. 18E

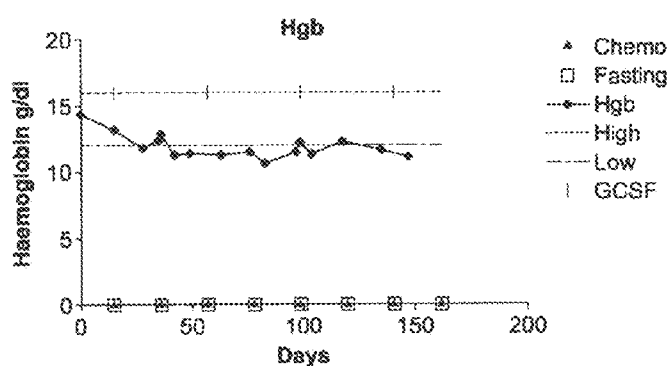


Fig. 18F

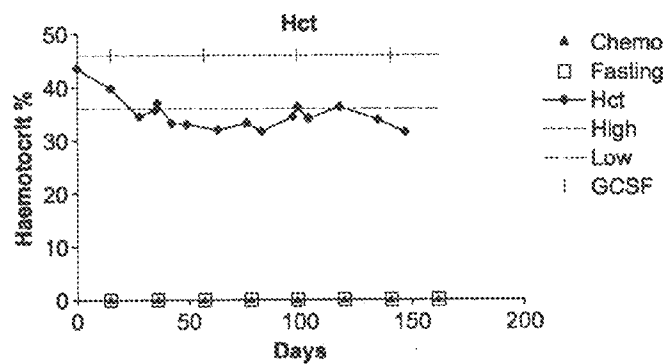


Fig. 18G

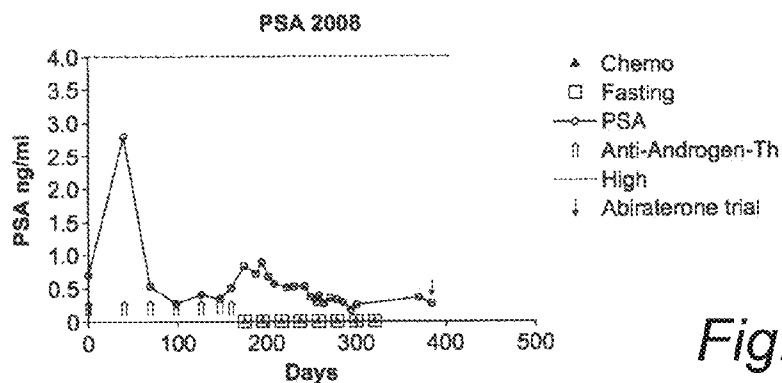


Fig. 18H

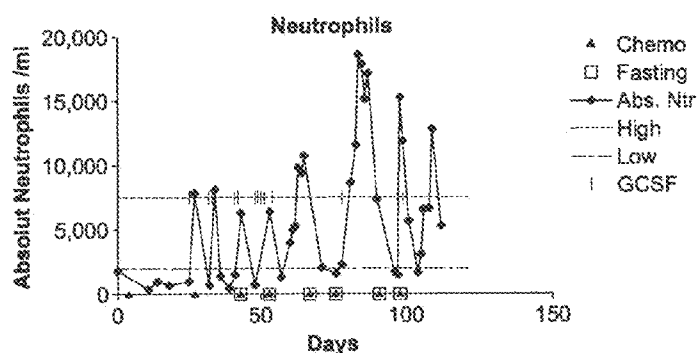


Fig. 19A

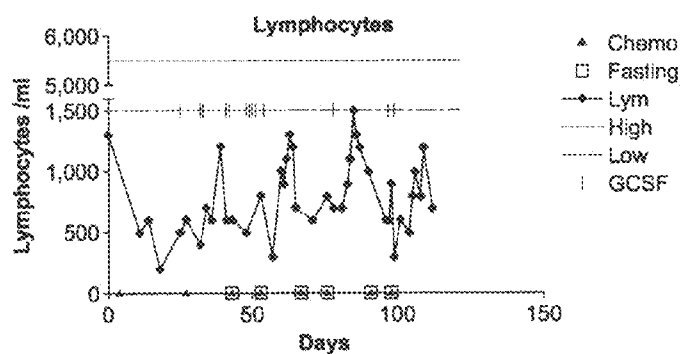


Fig. 19B

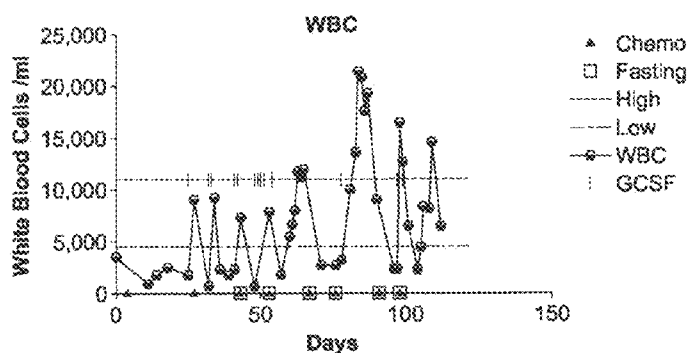


Fig. 19C

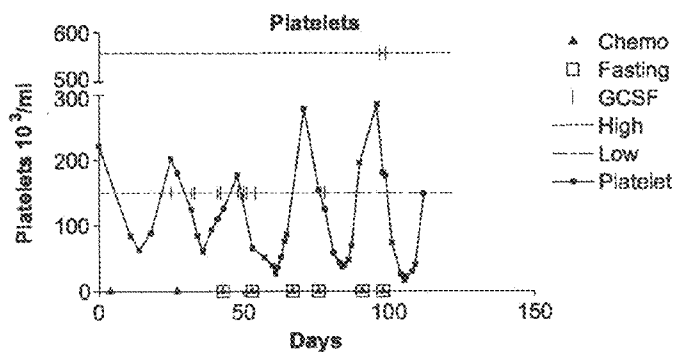


Fig. 19D

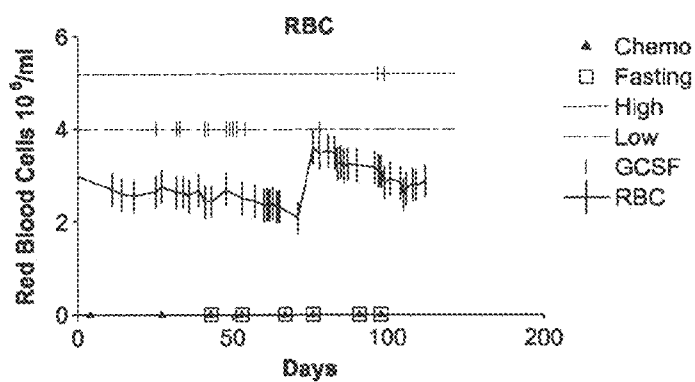


Fig. 19E

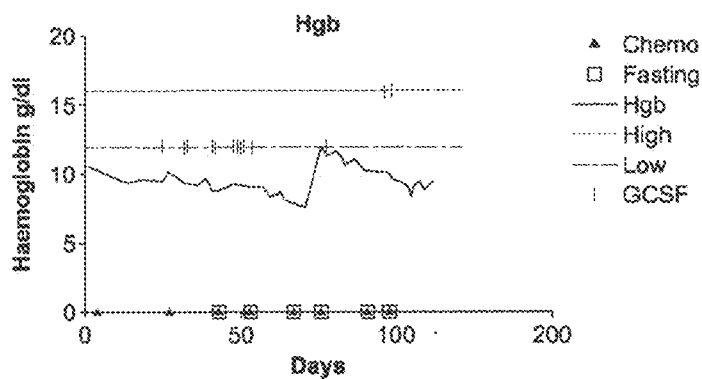


Fig. 19F

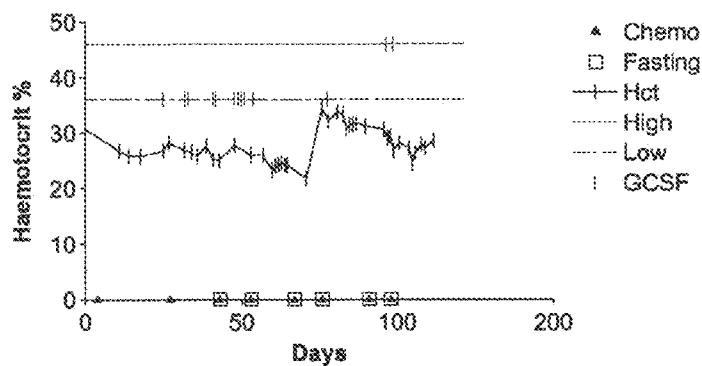


Fig. 19G

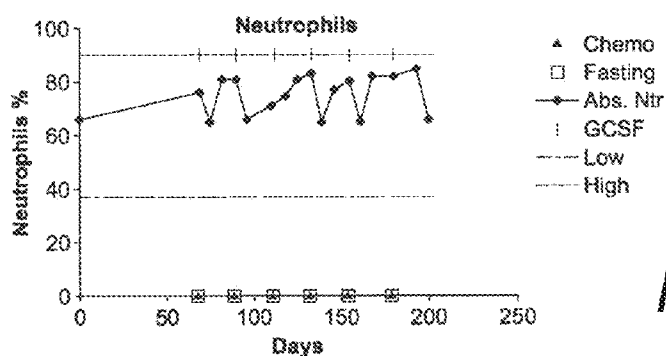


Fig. 20A

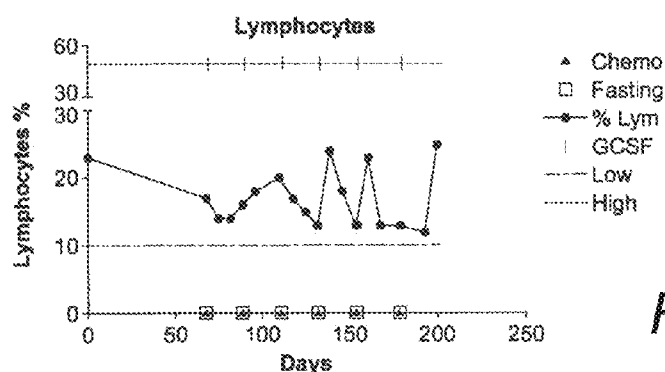


Fig. 20B

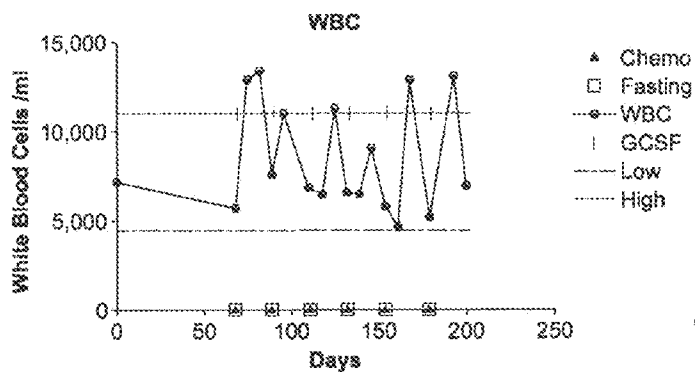


Fig. 20C

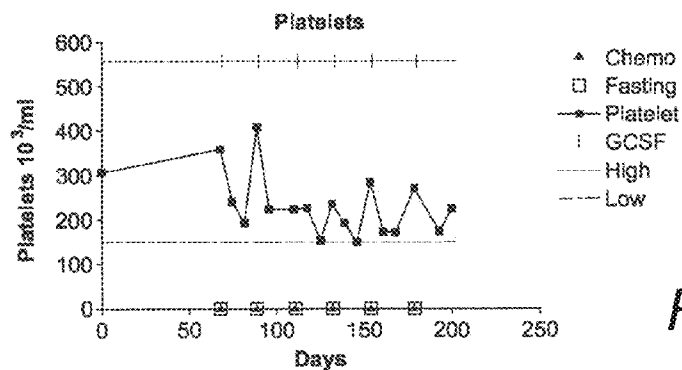


Fig. 20D

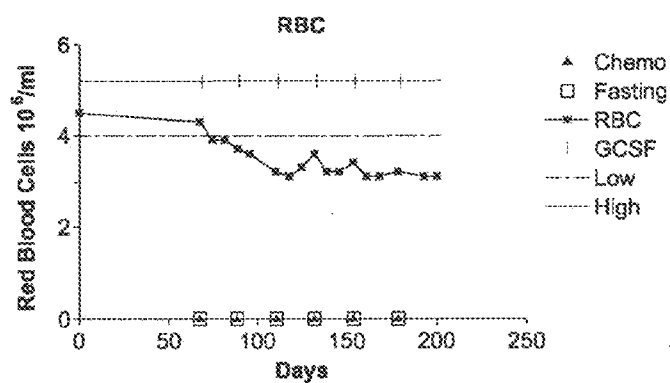


Fig. 20E

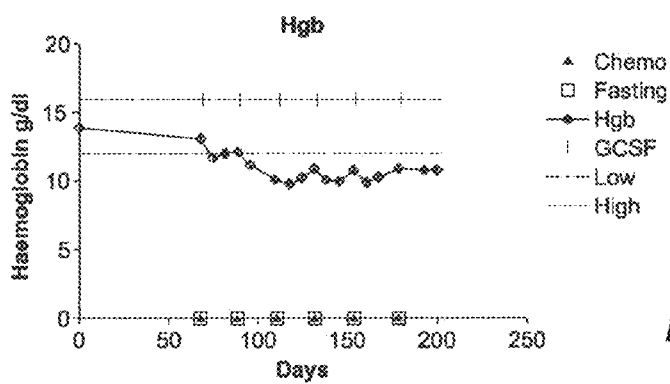


Fig. 20F

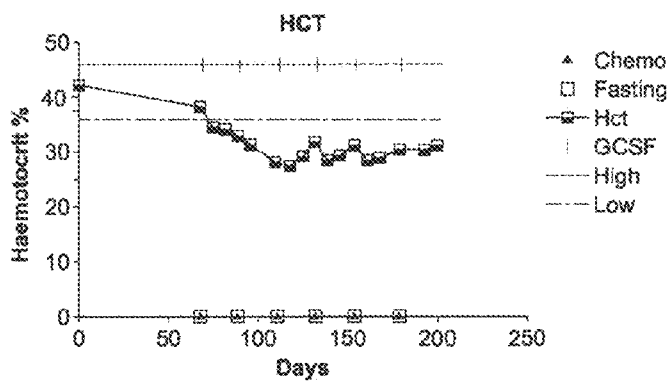


Fig. 20G

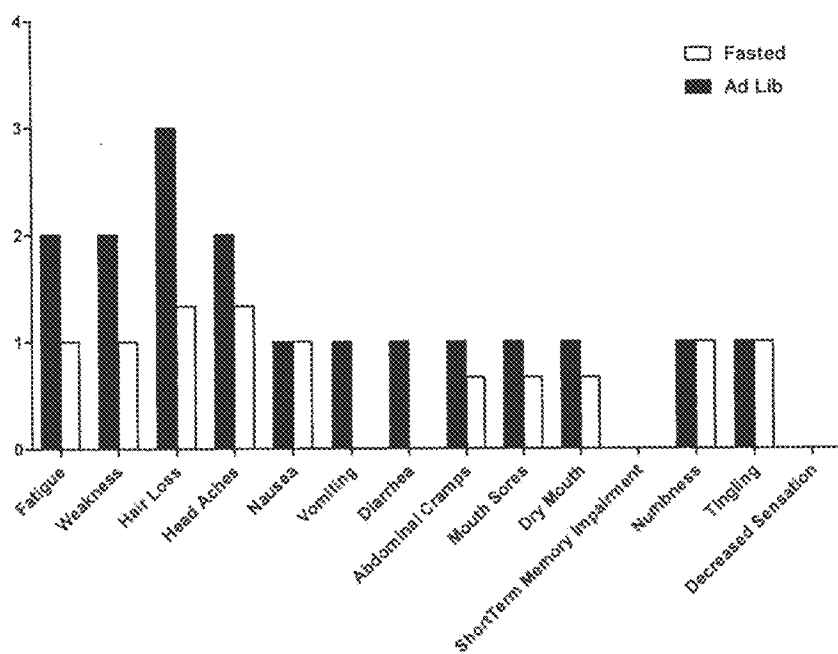


Fig. 21

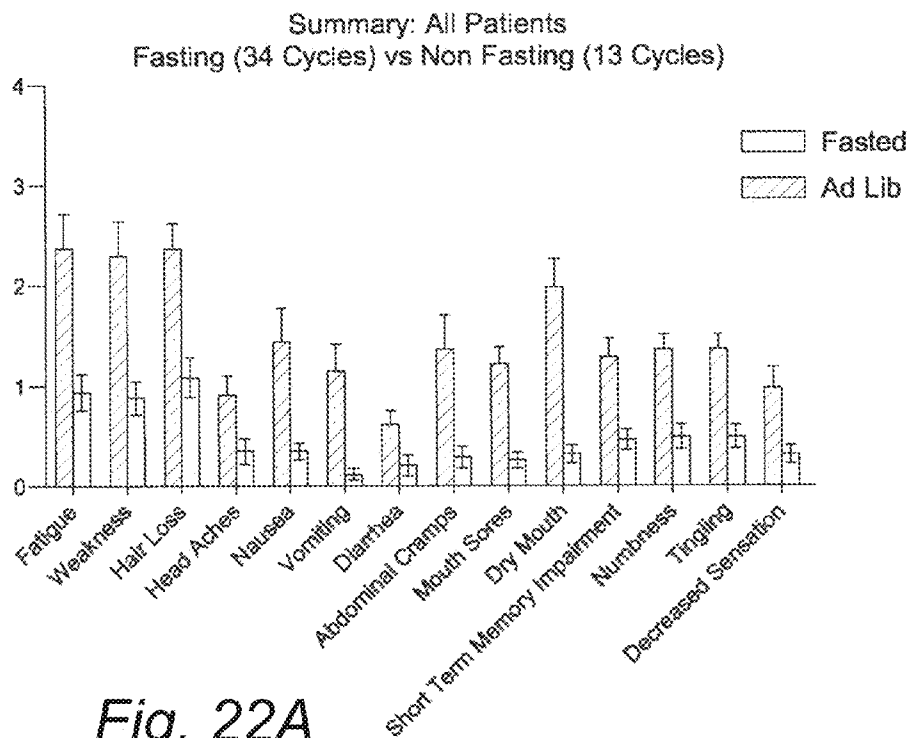


Fig. 22A

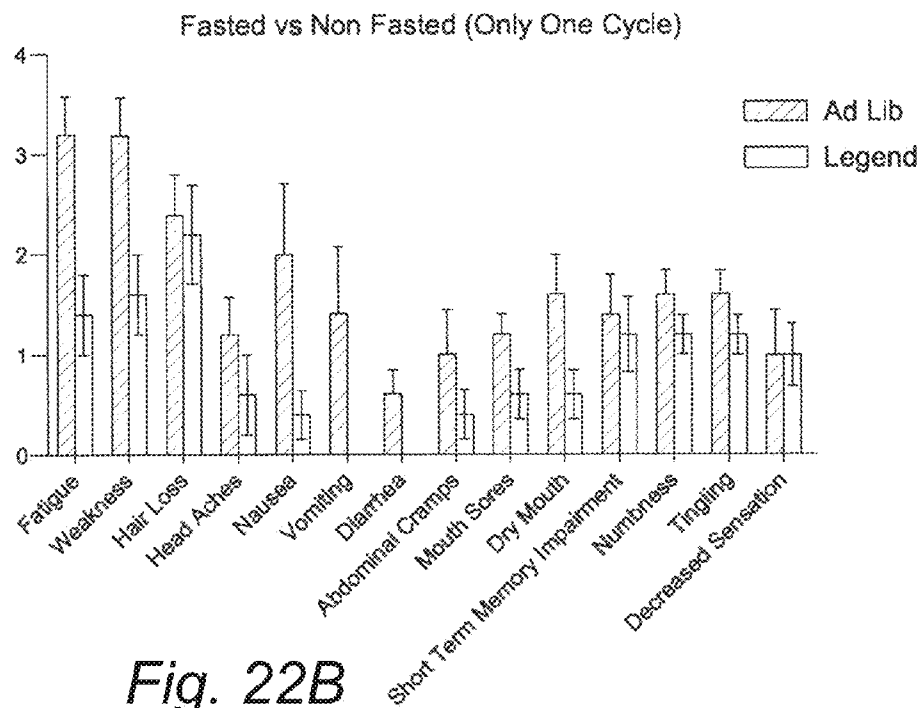


Fig. 22B

Fig. 23A

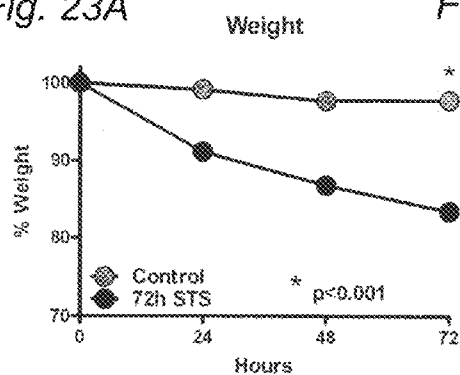


Fig. 23B

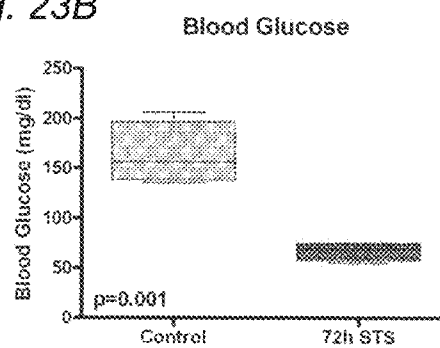


Fig. 23C

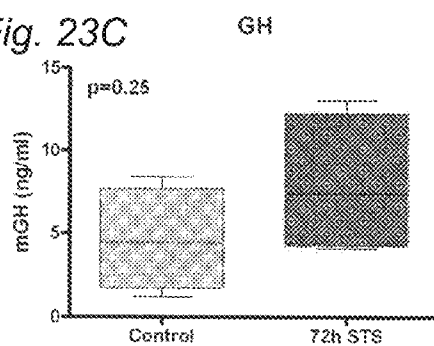


Fig. 23D

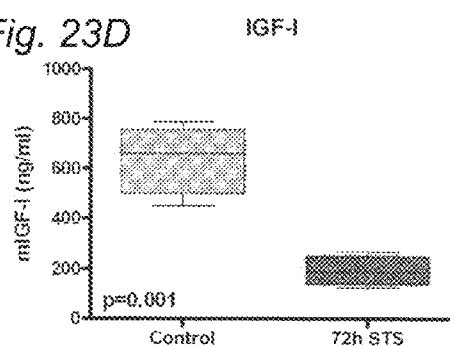


Fig. 23E

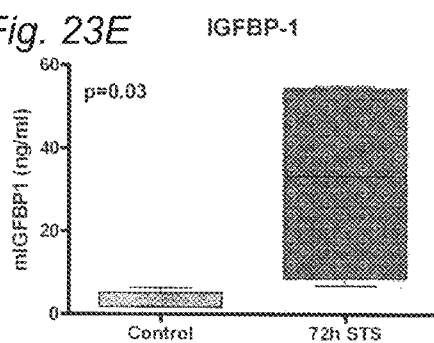
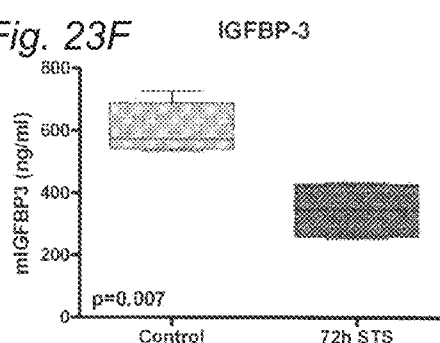


Fig. 23F



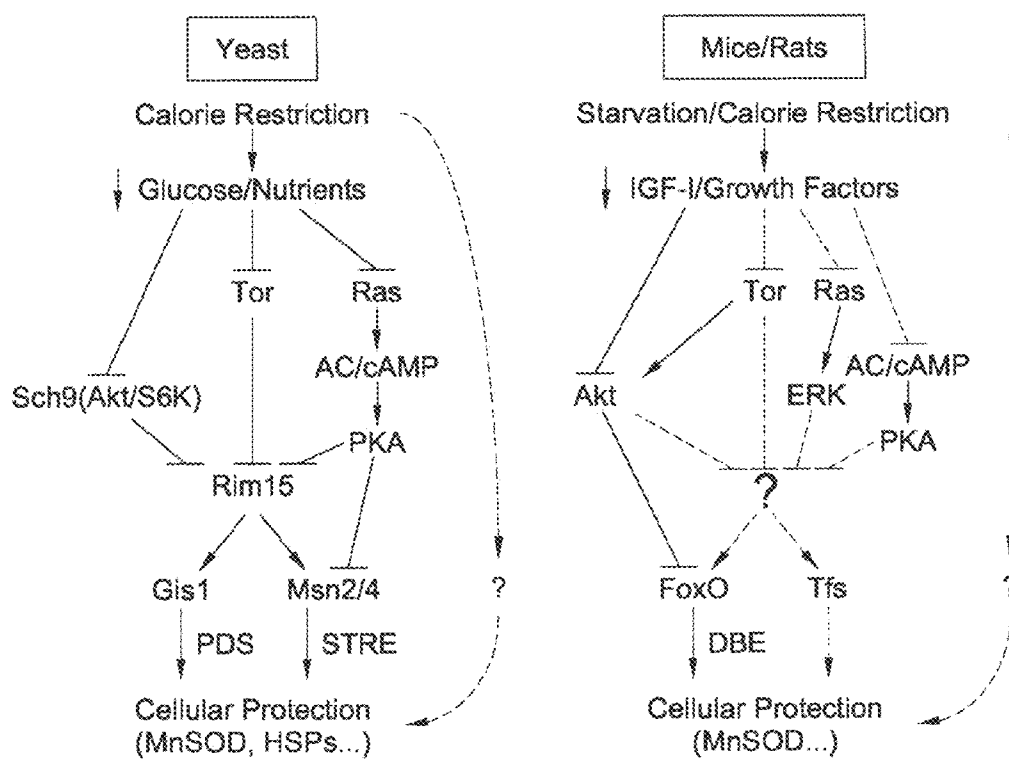


Fig. 24

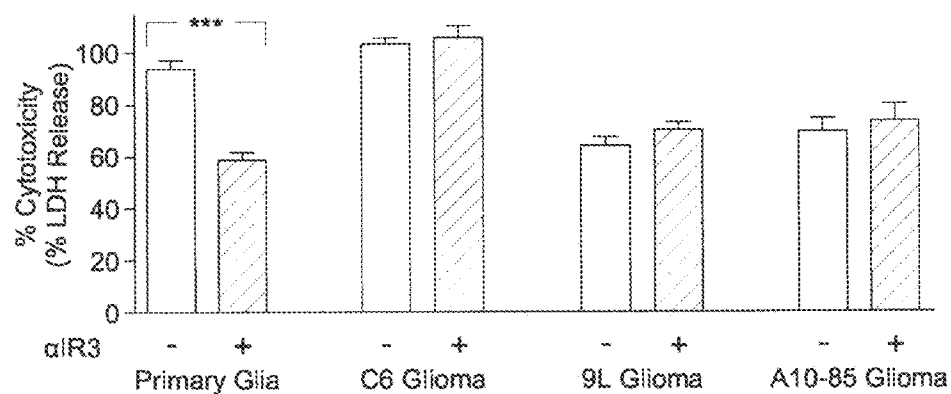


Fig. 25A

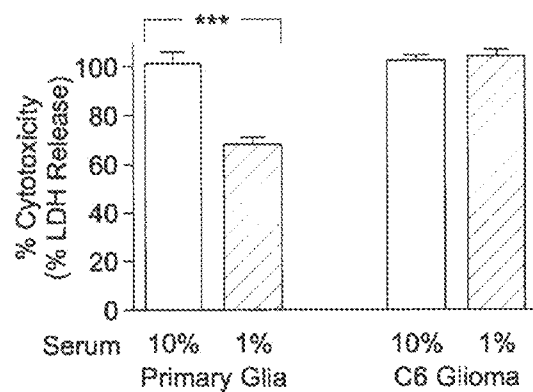


Fig. 25B

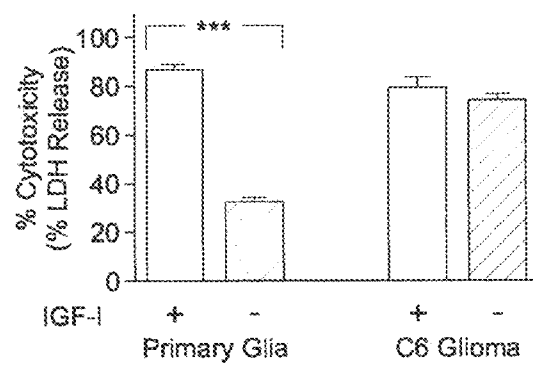


Fig. 25C

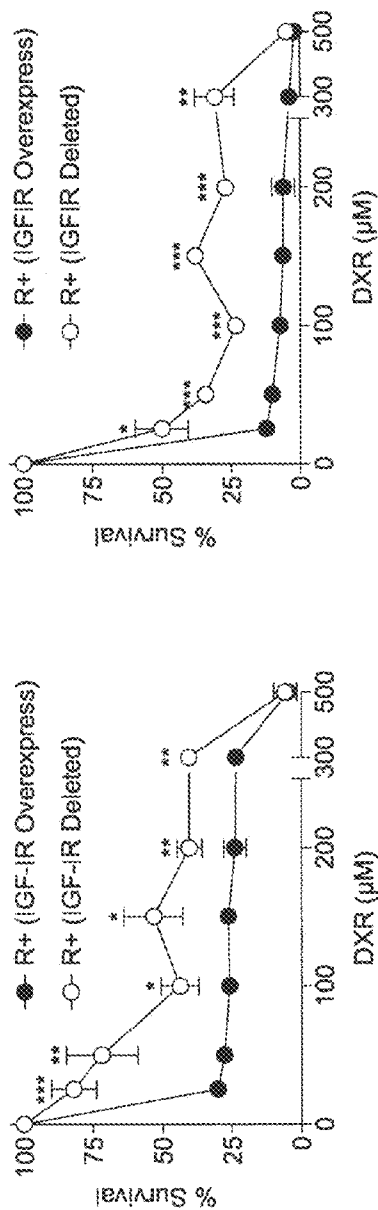


Fig. 26A

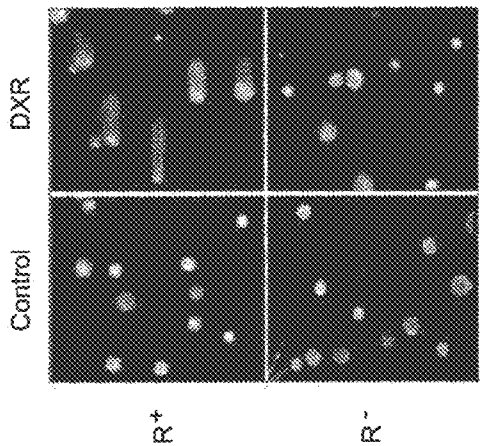


Fig. 26C

Fig. 26B

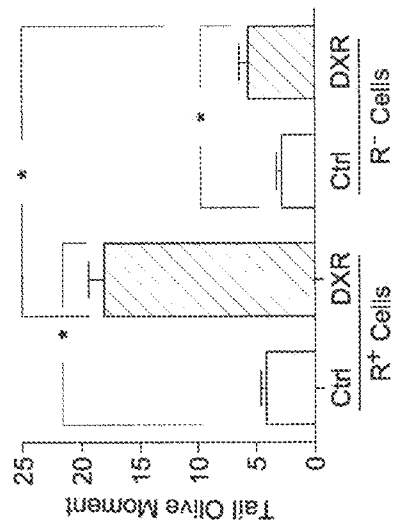


Fig. 26D

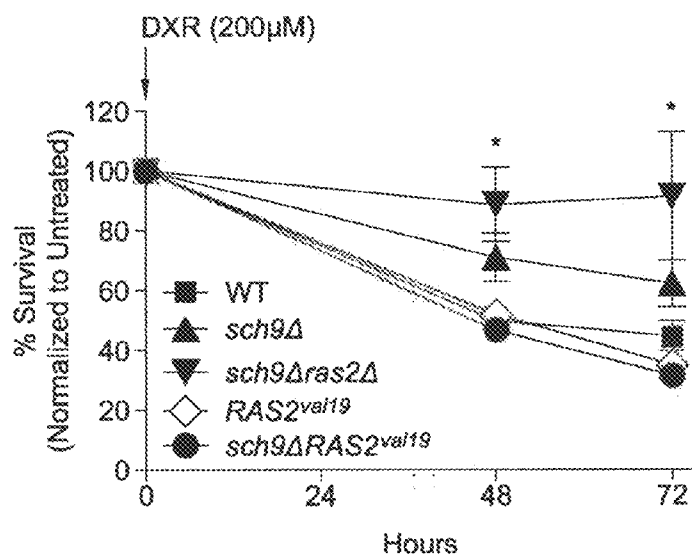


Fig. 27A

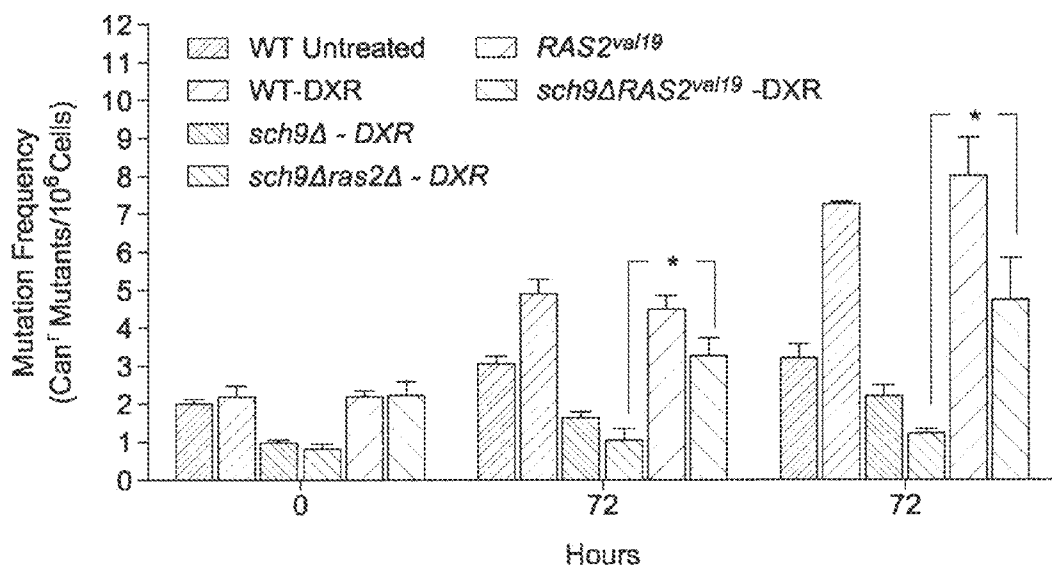


Fig. 27B

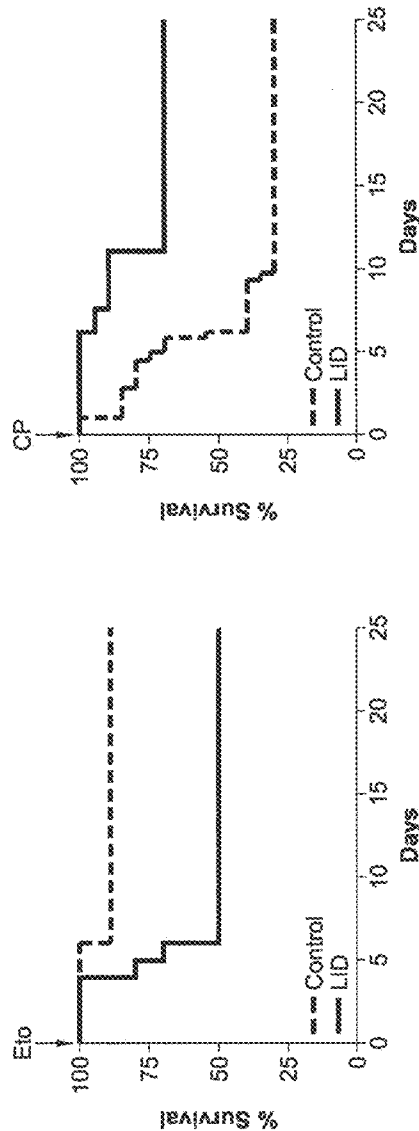


Fig. 28B

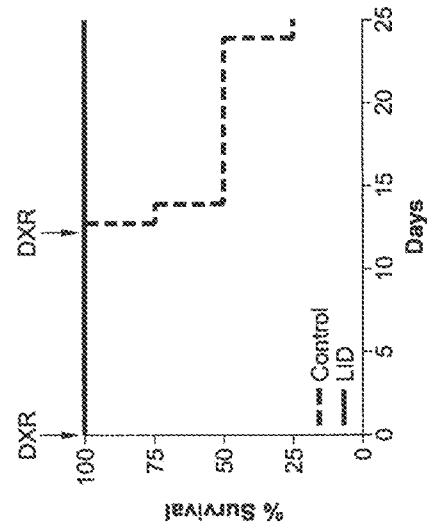
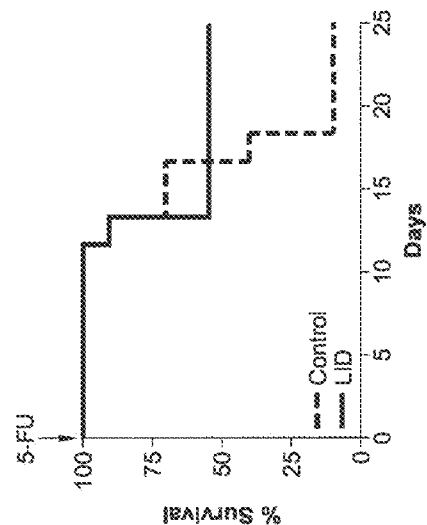
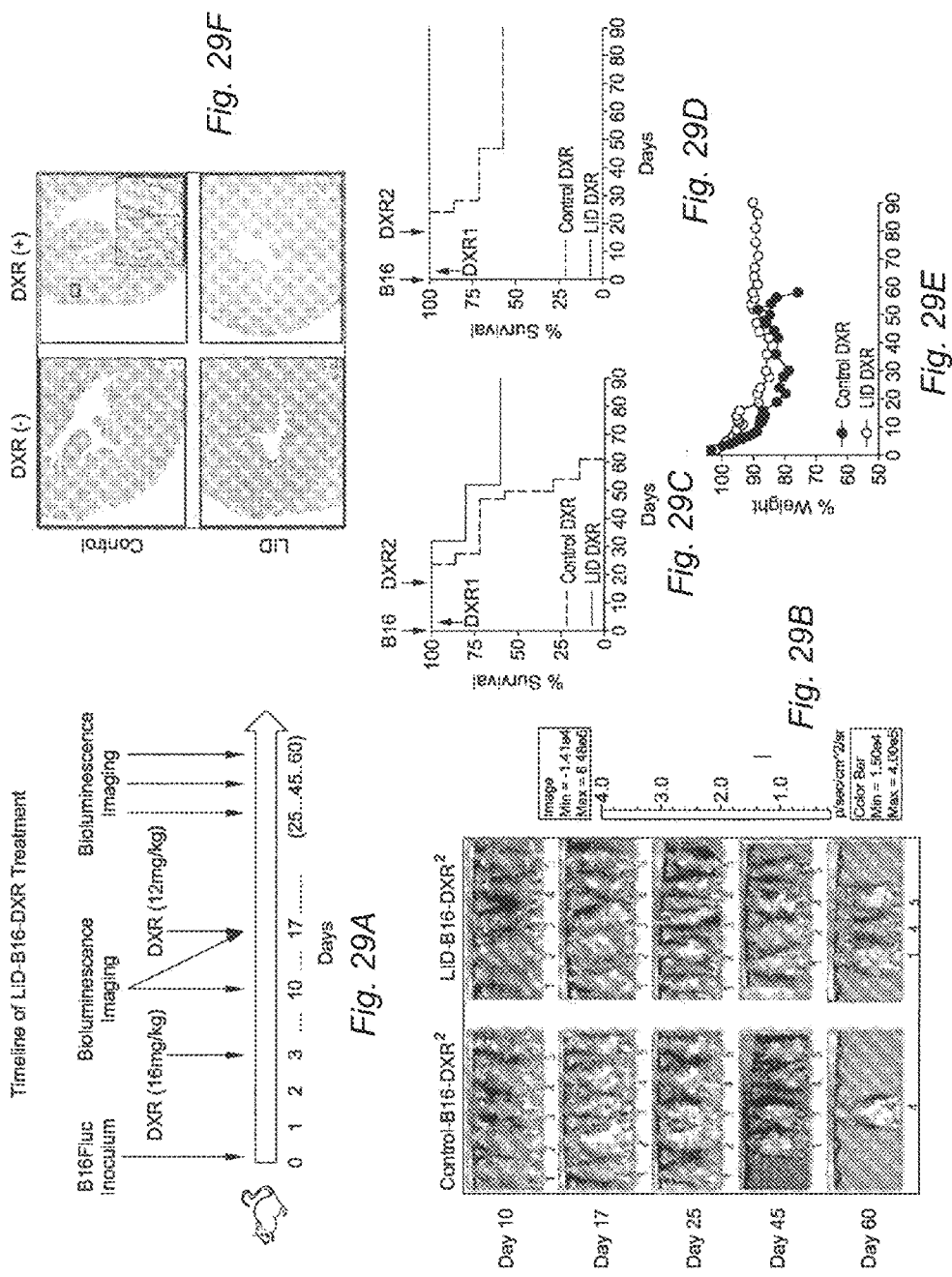


Fig. 28D





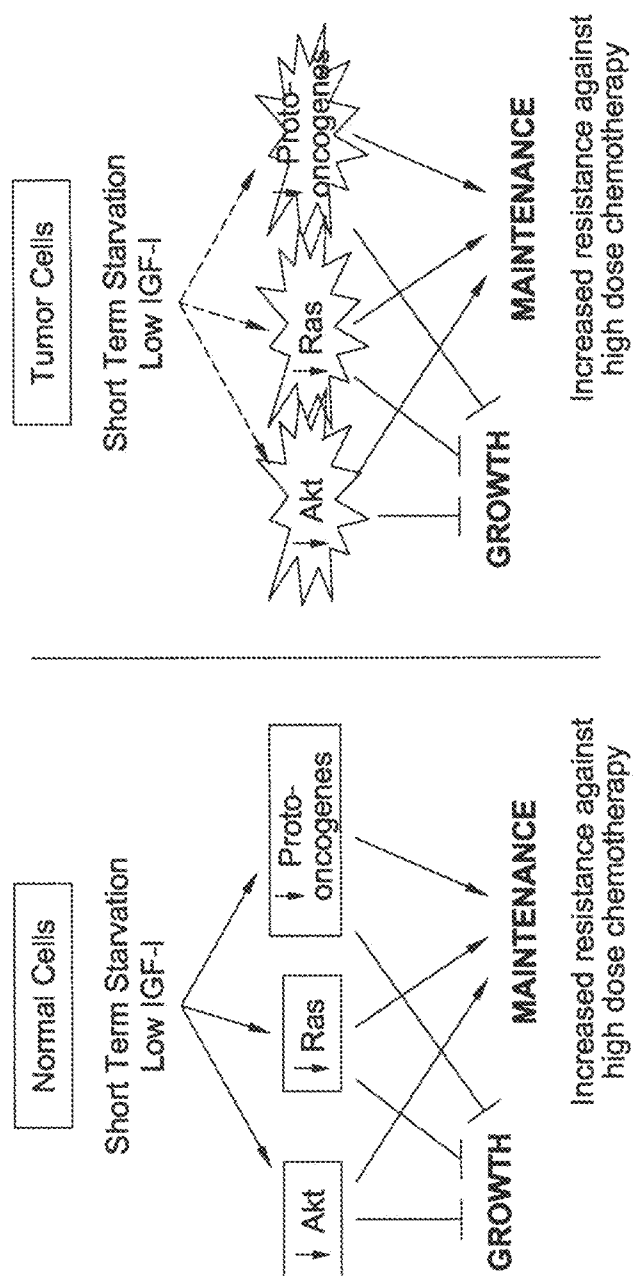


Fig. 30

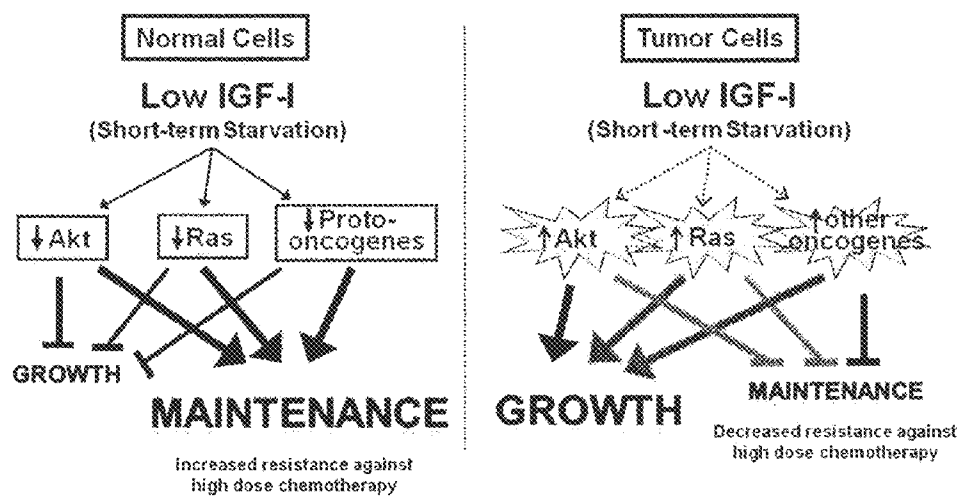


Fig. 31

Fig. 32A

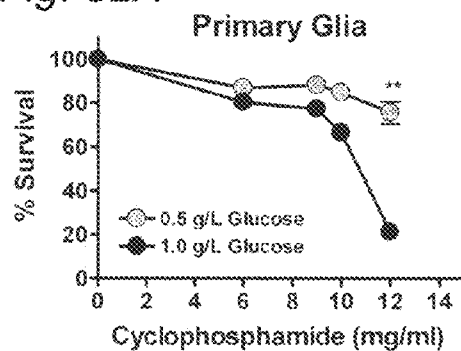


Fig. 32B

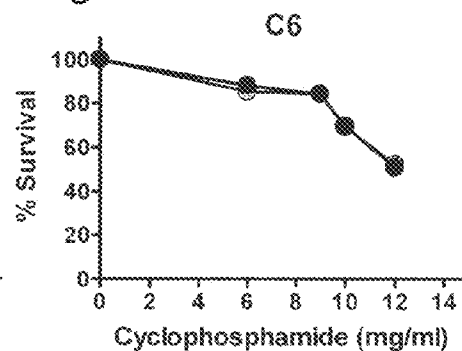


Fig. 32C

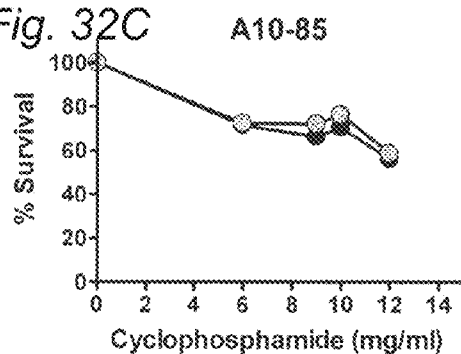


Fig. 32D

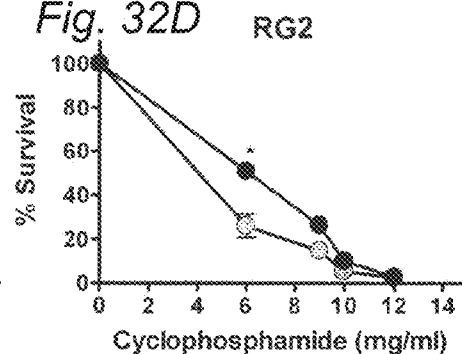


Fig. 32E

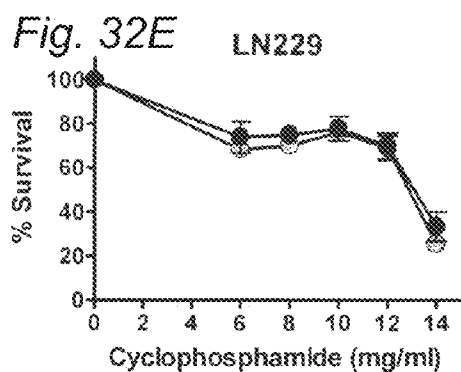
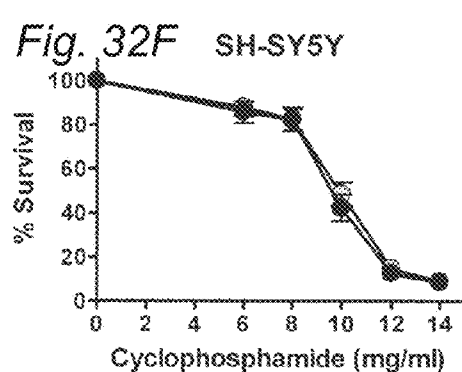
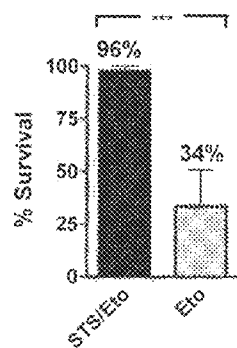
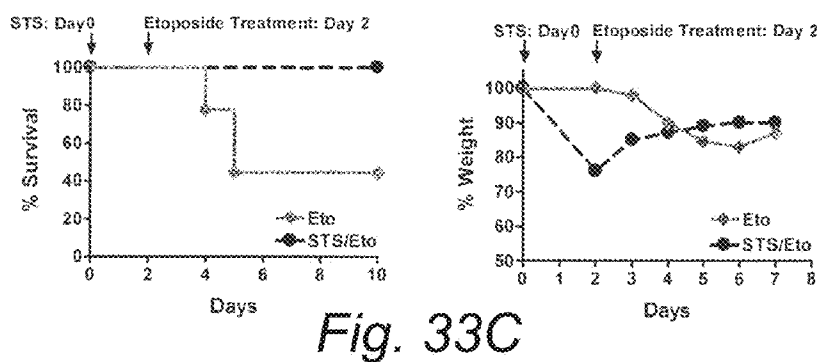
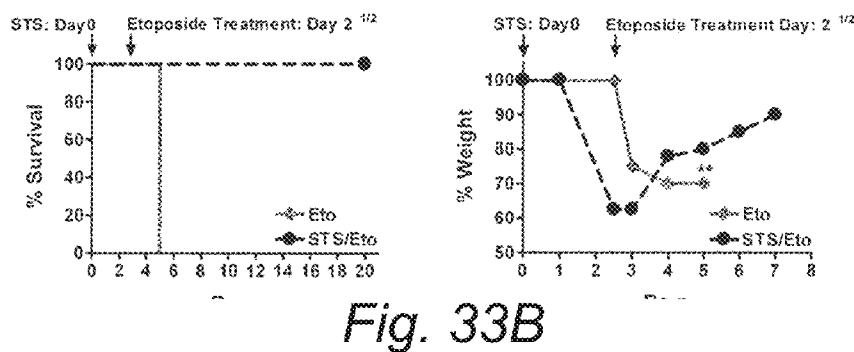
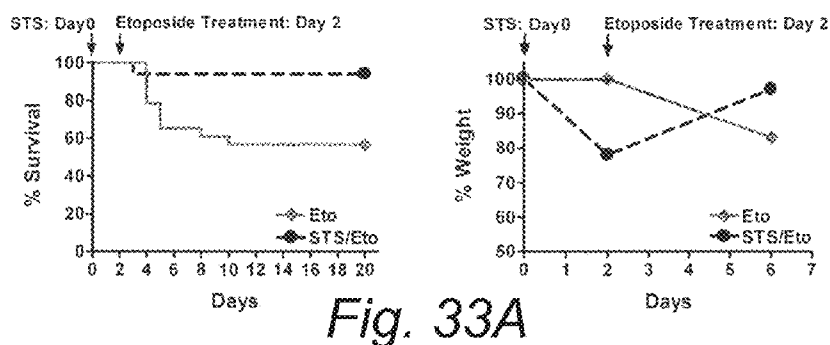
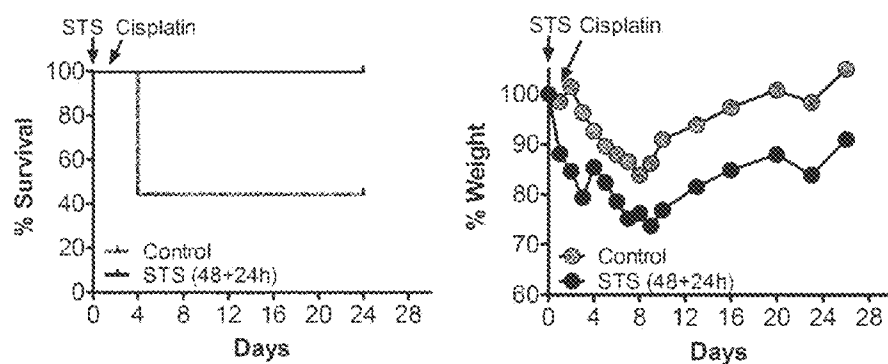
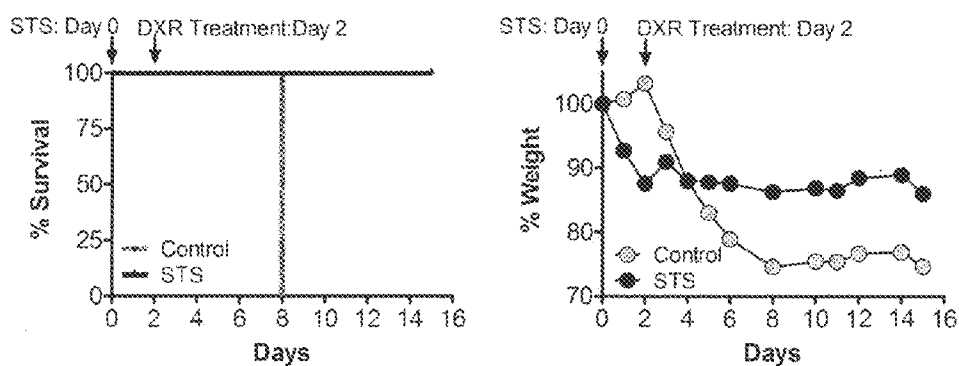


Fig. 32F





*Fig. 33E**Fig. 33F*

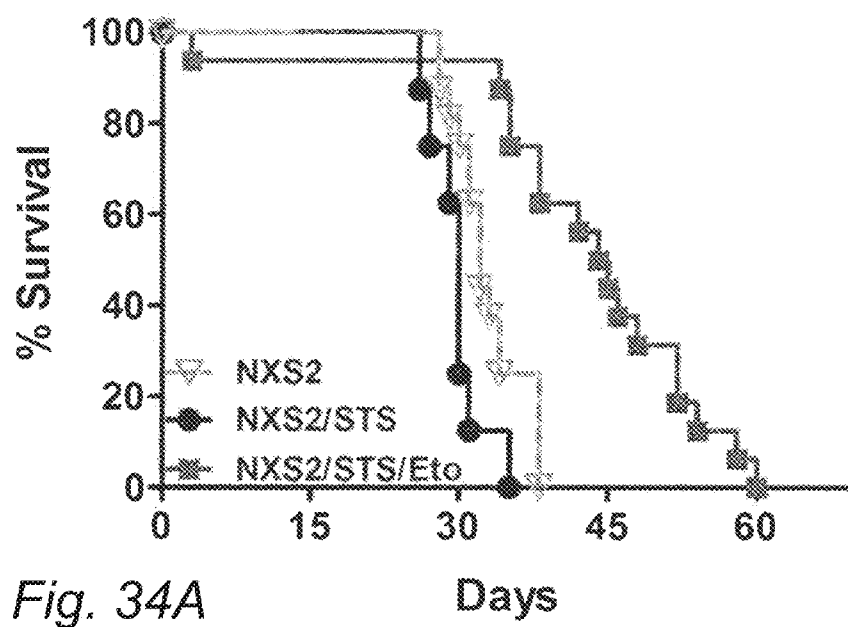


Fig. 34A

Days

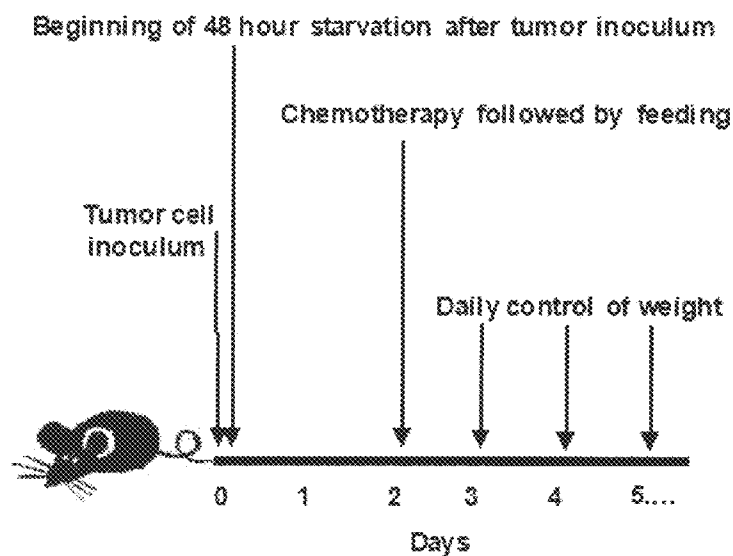
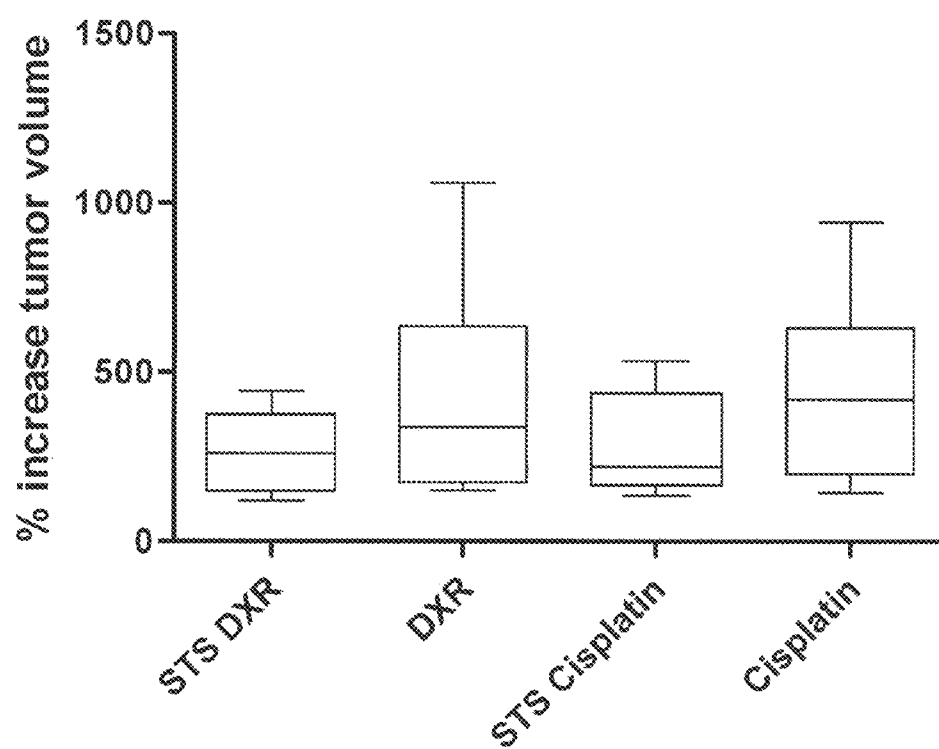


Fig. 34B

*Fig. 34C*

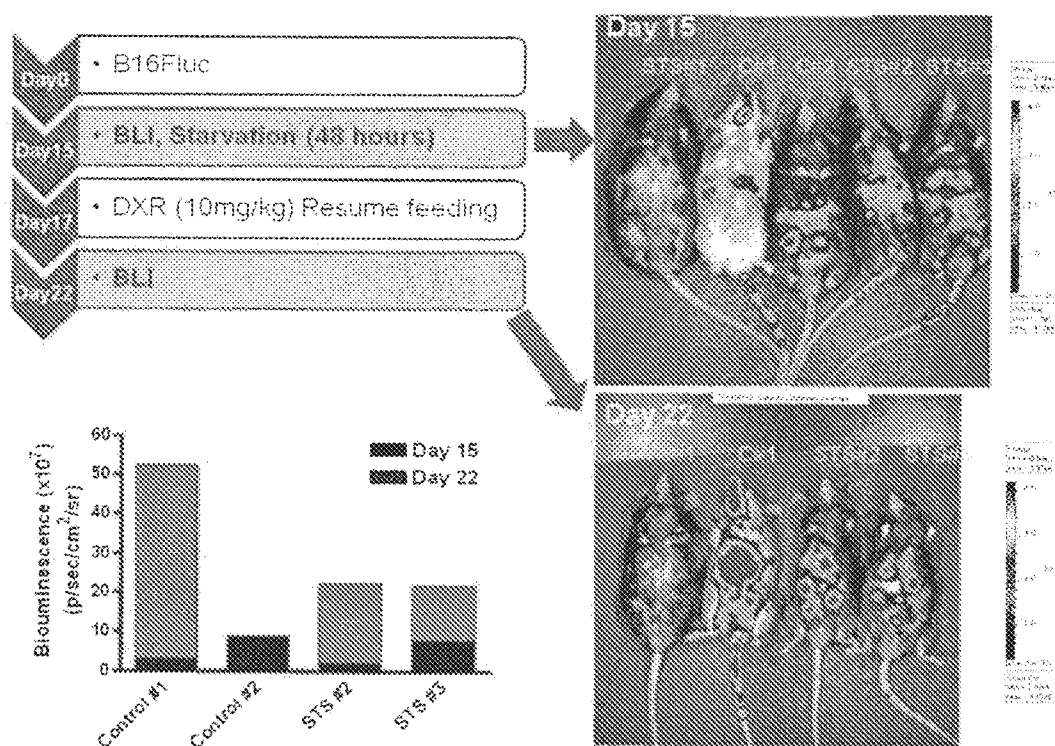


Fig. 35

**DIETARY COMPOSITIONS AND METHODS
FOR PROTECTION AGAINST
CHEMOTHERAPY, RADIOTHERAPY,
OXIDATIVE STRESS, AND AGING**

RELATED APPLICATIONS

[0001] This application is a divisional of U.S. application Ser. No. 12/430,058, filed Apr. 24, 2009, which is a continuation-in-part application of U.S. application Ser. No. 12/058,600, filed Mar. 28, 2008, which claims priority to U.S. Provisional Application Ser. No. 60/908,636, filed Mar. 28, 2007, and U.S. Provisional Application Ser. No. 60,942,561, filed Jun. 7, 2007. The present application also claims priority to U.S. Provisional Application Ser. No. 61/047,680, filed on Apr. 24, 200. The contents of U.S. application Ser. Nos. 12/430,058 and 12/058,600 and U.S. Provisional Application Ser. Nos. 60/908,636, 60/942,561, and 61/047,680 are incorporated herein by reference in their entirety.

FUNDING

[0002] This invention was made with support in part by grants from the National Institutes of Health, AG20642, AG025135, GM075308, and Neurosciences Blueprint. Therefore, the U.S. government has certain rights.

FIELD OF THE INVENTION

[0003] The present invention relates in general to treatment of diseases. More specifically, the invention provides dietary compositions and methods for protection against chemotherapy, radiotherapy, oxidative stress, and aging.

BACKGROUND OF THE INVENTION

[0004] Modern chemotherapy can improve the quality of life of cancer patients via palliation of cancer-related symptoms, and can significantly extend survival in many malignancies as well. However, the inevitable toxic side-effects frequently limit dose intensity and frequency of drugs administration. For instance, the use of doxorubicin or cisplatin can effectively treat many malignancies, but the drug-induced cardiotoxicity and nephrotoxicity, respectively, limit their full potential. Thus, reducing undesired toxicity by selectively protecting normal cells without compromising cancer targeting would prove beneficial to chemotherapy and enhance clinical outcome.

SUMMARY OF THE INVENTION

[0005] The present invention relates to novel dietary compositions and methods useful for protection against chemotherapy, radiotherapy, oxidative stress, and aging.

[0006] Accordingly, in one aspect, the invention features a dietary composition comprising 0-0.2% (by weight) L-methionine, as well as L-tryptophan, L-isoleucine, L-leucine, L-lysine, L-phenylalanine, L-threonine, and L-valine in the amount of at least 0.05% (by weight) each, and no protein. The composition may further comprise one or more amino acids selected from the group consisting of L-alanine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, L-glycine, L-proline, L-serine, L-tyrosine, L-arginine, and L-histidine.

[0007] In another aspect, the invention features a dietary composition comprising 0-0.2% (by weight) L-tryptophan, as well as L-methionine, L-isoleucine, L-leucine, L-lysine,

L-phenylalanine, L-threonine, L-valine in the amount of at least 0.05% (by weight) each, and no protein. The composition may further comprise one or more amino acids selected from the group consisting of L-alanine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, L-glycine, L-proline, L-serine, L-tyrosine, L-arginine, and L-histidine.

[0008] In still another aspect, the invention features a dietary composition comprising L-methionine, L-tryptophan, L-isoleucine, L-leucine, L-lysine, L-phenylalanine, L-threonine, L-valine, L-alanine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, L-glycine, L-proline, L-serine, L-tyrosine, L-arginine, and L-histidine in the amount of 0-0.2% (by weight) each, and no protein.

[0009] In yet another aspect, the invention features a dietary composition comprising glycerol as a substitute for monosaccharides, disaccharides, and polysaccharides.

[0010] Also within the invention is a method of protecting an animal or human against chemotherapy, radiotherapy, oxidative stress, or aging. The method comprises administering a composition of the invention to an animal or human, thereby protecting the animal or human against chemotherapy, radiotherapy, oxidative stress, or aging. The method may further comprise exposing the animal or human to the chemotherapy, radiotherapy, or oxidative stress. In some embodiments, the composition is administered to the animal or human for 3-10 consecutive days prior to the exposing step, 24 hours following the exposing step, or a combination thereof. In some embodiments, the composition is administered every third meal or every 3-10 days to protect the animal or human against aging.

[0011] In addition, the invention features a hypocaloric or calorie free diet comprising dietary materials capable of providing nutrition to a human subject while providing no more than 813-957 kcal total energy, no more than half of which is in carbohydrates if the carbohydrates are present in the dietary materials, wherein the dietary materials include no more than 30-36 g protein. In some embodiments, the dietary materials are capable of providing no more than 700 kcal total energy.

[0012] Moreover, the invention provides a method of protecting an animal or human against chemotherapy, radiotherapy, oxidative stress, or aging by administering to an animal or human a diet capable of providing nutrition while providing no more than 11 kcal energy per kg body weight of the animal or human per day, and no more than 0.4 g protein per kg body weight of the animal or human per day, wherein no more than half of the energy is in carbohydrates if the carbohydrates are present in the diet. In some embodiments, the diet is capable of providing no more than 700 kcal total energy per day. The method may further comprise exposing the animal or human to the chemotherapy, radiotherapy, or oxidative stress. In some embodiments, the diet is administered to the animal or human for 3-10 consecutive days prior to the exposing step, 24 hours following the exposing step, or a combination thereof. In some embodiments, the diet is administered every third meal or every 3-10 days to protect the animal or human against aging.

[0013] The invention further provides a method of protecting an animal or human against chemotherapy. The method comprises fasting an animal or human suffering from cancer for 48-140 hours prior to one round of chemotherapy, 4-56 hours following the chemotherapy, or a combination thereof; and exposing the animal or human to the chemotherapy. In

some embodiments, the animal or human is fasted for no more than 180 hours prior to and following one round of chemotherapy.

[0014] The above-mentioned and other features of this invention and the manner of obtaining and using them will become more apparent, and will be best understood, by reference to the following description, taken in conjunction with the accompanying drawings. The drawings depict only typical embodiments of the invention and do not therefore limit its scope.

BRIEF DESCRIPTION OF THE FIGURES

[0015] FIG. 1A. Graph of % survival.
 [0016] FIG. 1B. Graph of methionine diet body weight %.
 [0017] FIG. 1C. Graph of methionine food intake.
 [0018] FIG. 1D. Graph of post treatment body weight %.
 [0019] FIG. 1E. Graph of post treatment food intake as a function of days. Mice were treated with a low methionine amino acid mix (LMA1) before treatment with doxorubicin.
 [0020] FIG. 2A. Graph of % survival.
 [0021] FIG. 2B. Graph of post treatment body weight %.
 [0022] FIG. 2C. Graph of tryptophan diet body weight % as a function of days. Mice were treated with a low tryptophan amino acid mix (LTA1) before treatment with doxorubicin.
 [0023] FIG. 3A. Graph of food intake as a function of days.
 [0024] FIG. 3B. Graph of blood glucose levels.
 [0025] FIG. 3C. Graph of % survival as a function of time.
 [0026] FIG. 3D. Graph of body weight % as a function of days. Mice were given a glycerol diet before treatment with paraquat.
 [0027] FIG. 4A. Genetic interactions between Sch9, Tor1, and Ras2 in regulating stress resistance and life span. Day 3 wild type (DBY746) and cells lacking Tor1, Sch9, or Ras2 were challenged with heat shock (55° C., 105 min); or oxidative stresses (H₂O₂, 100 mM for 60 min; or menadione, 250 μM for 30 min).
 [0028] FIG. 4B. Genetic interactions between Sch9, Tor1, and Ras2 in regulating stress resistance and life span. Day 3 wild type (DBY746) and cells lacking Tor1, Sch9, or Ras2 were challenged with heat shock (55° C., 75 min) or oxidative stresses (H₂O₂, 100 mM for 60 min; or menadione, 250 μM for 30 min).
 [0029] FIG. 4C. Genetic interactions between Sch9, Tor1, and Ras2 in regulating stress resistance and life span. Day 3 wild type (DBY746) and cells lacking Tor1, Sch9, or Ras2 were challenged with heat shock (55° C., 150 min) or oxidative stresses (H₂O₂, 100 mM for 60 min; or menadione, 250 μM for 30 min).
 [0030] FIG. 4D. Genetic interactions between Sch9, Tor1, and Ras2 in regulating stress resistance and life span. Day 3 wild type (DBY746) and cells lacking Tor1, Sch9, or Ras2 were challenged with heat shock (55° C., 120 min) or oxidative stresses (H₂O₂, 100 mM for 60 min; or menadione, 250 μM for 30 min).
 [0031] FIG. 4E. Genetic interactions between Sch9, Tor1, and Ras2 in regulating stress resistance and life span. (E) Mutation frequency over time measured as can^R mutants per million cells. The average of four experiments is shown. Error bars represent SEM.
 [0032] FIG. 4F. Genetic interactions between Sch9, Tor1, and Ras2 in regulating stress resistance and life span. Chronological survival in minimal complete medium (SDC) of wild type (DBY746), tor1Δ, and mutants overexpressing either SCH9 or constitutively active Ras2 (ras2^{Val19}).

[0033] FIG. 4G. Genetic interactions between Sch9, Tor1, and Ras2 in regulating stress resistance and life span. Chronological survival of wild type (DBY746) and mutants lacking Tor1, Sch9, Ras2 or combinations shown in the graph. The data represent average of at least 4 experiments. Error bars show SEM. For mean life span calculated from non-linear curve fitting see Table 2.

[0034] FIG. 4H. Genetic interactions between Sch9, Tor1, and Ras2 in regulating stress resistance and life span. Longevity regulatory pathways in yeast. The nutrient sensing pathways controlled by Sch9, Tor, and Ras converge on the protein kinase Rim15. In turn, the stress response transcription factors Msn2, Msn4, and Gis1 transactivate stress response genes and enhance cellular protection, which lead to life span extension. Pro-longevity effects of CR are partially mediated by Sch9, Tor, and Ras, and may also require additional yet-to-be identified mechanism(s).

[0035] FIG. 5A. Gene-expression profiles of long-lived mutants. Venn diagram of the number of genes up- or down-regulated more than 2-fold in the tor1Δ, sch9Δ, and ras2Δ mutants, at day 2.5, compared to wild type cells. Microarray analyses were carried out in triplicates. Data represent up/down-regulated genes.

[0036] FIG. 5B. Gene-expression profiles of long-lived mutants. Life span of mutants with deletions of genes most upregulated in long-lived mutants in the sch9Δ background. Three to four independent experiments for each strain were performed. Data represent mean and SEM of pair matched pooled experiments.

[0037] FIG. 6A. Schematic representation of glycerol metabolism. For illustration purpose, genes upregulated more than 20% compared to wild type in all three long-lived mutants are labeled in red; those down-regulated in green.

[0038] FIG. 6B. Fold change in expression levels of glycerol biosynthetic genes in sch9Δ, tor1Δ, and ras2Δ mutants compared to wild type (DBY746) at day 2.5.

[0039] FIG. 6C. Real time quantitative PCR analysis of GPD1 mRNA level in wild type (DBY746) and sch9Δ cells at day 3. Data represent mean and SEM, n=4. *p<0.05, t-test, two-tailed, sch9Δ vs. WT.

[0040] FIG. 7A. Sch9 deficient mutants metabolize ethanol and accumulate glycerol. Intracellular glycerol contents of wild type (DBY746) and cells lacking Sch9 were measured on day 1 and day 3. Data represent mean and SEM of 5 cultures analyzed.

[0041] FIG. 7B. Glycerol concentration in the medium of wild type and sch9Δ cultures. Data represent mean and SEM of 5-7 cultures analyzed. **p<0.01, unpaired t-test, two-tailed, sch9Δ vs. WT.

[0042] FIG. 7C. Glycerol and ethanol concentrations in the medium of wild type cultures. Data represent mean and SEM of 3-5 cultures analyzed.

[0043] FIG. 7D. Glycerol and ethanol concentrations in the medium of wild type sch9Δ cultures. Data represent mean and SEM of 3-5 cultures analyzed.

[0044] FIG. 7E. Nile red staining of neutral lipids of day 1 wild type and sch9Δ mutants. Nile red staining is shown on the right, and phase contrast left. Bar, 10 μm.

[0045] FIG. 8A. Deletion of glycerol biosynthesis genes reverse life span extension and stress resistance associated with deficiency in Sch9. Glycerol concentration in the medium. Data present mean and SEM of 4 cultures analyzed. *p<0.05, **p<0.01, unpaired t-test, two tailed, sch9Δ vs. rhr2Δ sch9Δ.

[0046] FIG. 8B. Deletion of glycerol biosynthesis genes reverse life span extension and stress resistance associated with deficiency in Sch9Life span of wild type (DBY746), sch9Δ, rhr2Δ, and Sch9-deficient mutants lacking Rhr2. Glycerol (1%, final concentration) was added to the one day-old rhr2Δ sch9Δ culture. Data represent mean and SEM of 4-5 cultures analyzed.

[0047] FIG. 8C. Deletion of glycerol biosynthesis genes reverse life span extension and stress resistance associated with deficiency in Sch9Day 3 cells were exposed to heat shock (55° C. for 105 min) or H₂O₂ (150 mM for 60 min). Strains shown are wild type (DBY746), rhr2Δ, sch9Δ, rhr2Δ sch9Δ.

[0048] FIG. 8D. Deletion of glycerol biosynthesis genes reverse life span extension and stress resistance associated with deficiency in Sch9Life span of wild type (BY4741), sch9D, and Sch9-deficient mutants lacking Gpd1, Gpd2, or Rhr2. Data represent mean and SEM of 3 experiments.

[0049] FIG. 8E. Deletion of glycerol biosynthesis genes reverse life span extension and stress resistance associated with deficiency in Sch9Heat shock (55° C.) and oxidative stress (H₂O₂, 500 mM, 60 min) resistance of day 3 mutants lacking glycerol biosynthesis genes.

[0050] FIG. 9A. Effect of glycerol on stress resistance and life span. (A) Day 3 wild type (DBY746) and sch9D mutants expressing bacterial heat-sensitive luciferase were subject to heat stress (42° C. for 60 min). Data represent mean and SEM, n=3. *p<0.05, unpaired t-test, two tailed.

[0051] FIG. 9B. Effect of glycerol on stress resistance and life span. Recovery of luciferase activity after heat stress (42° C. for 60 min) in wild type cells pre-treated with glycerol (with concentrations indicated) for 30 min. Data represent mean and SEM, n=3.

[0052] FIG. 9C. Effect of glycerol on stress resistance and life span. Day 3 wild type cells grown in SDC were washed 3 times with water and exposed to high concentrations of NaCl (2 M) with or without glycerol for 24 hours. The cells were then washed 3 times to remove the salt, serially diluted, and spotted on to YPD plate.

[0053] FIG. 9D. Effect of glycerol on stress resistance and life span. Day 3 wild type and sch9Δ mutants were exposed to high concentration of NaCl (2 and 4 M) for 24 hours.

[0054] FIG. 9E. Effect of glycerol on stress resistance and life span. Chronological survival of wild type cells grown in SDC supplemented with glycerol as indicated. Data represent mean and SEM, n=3.

[0055] FIG. 9F. Effect of glycerol on stress resistance and life span. In situ viability assay. Day 1 SDC wild type cultures were diluted and plated onto SC-Trp plates (no carbon source) or onto plates supplemented with glucose (Glc, 2%), ethanol (EtOH, 0.8%) or glycerol (Gly, 3%) as carbon source. Every two days, tryptophan (or with additional glucose) was added to the plates. Colony formation was monitored 2 days after the addition of tryptophan. Data represent mean and SEM, n=3.

[0056] FIG. 9G. Effect of glycerol on stress resistance and life span. Chronological survival of wild type (DBY746) and msn2Δ msn4Δ gis1Δ mutants grown in normal (SC+2% glucose), calorie-restricted (SC+1% glucose), or glucose/glycerol (SC+1%+1%) medium. Data represent mean and SEM of 4 cultures analyzed.

[0057] FIG. 9H. Effect of glycerol on stress resistance and life span. Day 3 wild type cells grown in SDC medium were washed three times with water and incubated in water (CR/

extreme starvation) with or without glycerol (0.1% or 1%). Plot shows a representative experiment (mean of duplicates) repeated three times with similar results.

[0058] FIG. 9I. Effect of glycerol on stress resistance and life span. Yeast grown in SDC was sampled (1 ml) at indicated time points. [1,2,3-³H] Glycerol (ARC, Inc) was added to the aliquot and incubated at 30° C. with shaking for 24 hours. Cells were then washed three times with water. The cellular [³H]-content was determined by scintillation counting (Wallac 1410, Pharmacia) and normalized to cell number (viability by CFU). Data represent mean and SEM of 4 cultures analyzed.

[0059] FIG. 10A. Dietary substitution of sugar with glycerol protects mice from paraquat toxicity. Two groups of five mice each were ad libitum fed with either the control or glycerol diet for six days. Food intake per 100 g body weight was slightly higher in the group fed with the glycerol diet.

[0060] FIG. 10B. Dietary substitution of sugar with glycerol protects mice from paraquat toxicity. Blood glucose levels were measured prior to paraquat injection (6 days after the initiation of diet. *p=0.05, unpaired t-test, two tailed.)

[0061] FIG. 10C. Dietary substitution of sugar with glycerol protects mice from paraquat toxicity. Survival curves after intraperitoneal injection of 50 mg/kg paraquat (7.5 mg/ml in PBS). (D) Body weight of mice after the paraquat treatment.

[0062] FIG. 11A. Laboratory values of blood cell counts for case 1. (A) Neutrophils.

[0063] FIG. 11B. Laboratory values of blood cell counts for case 1. Lymphocytes.

[0064] FIG. 11C. Laboratory values of blood cell counts for case 1. White blood cells, WBC.

[0065] FIG. 11D. Laboratory values of blood cell counts for case 1. Red blood cells, RBC.

[0066] FIG. 11E. Laboratory values of blood cell counts for case 1. Platelets.

[0067] FIG. 11F. Laboratory values of blood cell counts for case 1. Haemoglobin, Hgb.

[0068] FIG. 11G. Laboratory values of blood cell counts for case 1. Haematocrit, Hct.

[0069] FIG. 11H. Laboratory values of blood cell counts for case 1. Body weight.

[0070] FIG. 12. Self-reported side-effects after chemotherapy for case 1.

[0071] FIG. 13. Self-reported side-effects after chemotherapy for case 2.

[0072] FIG. 14A. Laboratory values of blood cell counts for case 3. Neutrophils.

[0073] FIG. 14B. Laboratory values of blood cell counts for case 3. Lymphocytes.

[0074] FIG. 14C. Laboratory values of blood cell counts for case 3. White blood cells, WBC.

[0075] FIG. 14D. Laboratory values of blood cell counts for case 3. Red blood cells, RBC.

[0076] FIG. 14E. Laboratory values of blood cell counts for case 3. Platelets.

[0077] FIG. 14F. Laboratory values of blood cell counts for case 3. Haemoglobin, Hgb.

[0078] FIG. 14G. Laboratory values of blood cell counts for case 3. Haematocrit, Hct.

[0079] FIG. 14H. Laboratory values of blood cell counts for case 3. Prostate specific antigen (PSA) level.

[0080] FIG. 15. Self-reported side-effects after chemotherapy for case 3.

[0081] FIG. 16A. Laboratory values of blood cell counts for case 4. Neutrophils.

[0082] FIG. 16B. Laboratory values of blood cell counts for case 4. Lymphocytes.

[0083] FIG. 16C. Laboratory values of blood cell counts for case 4. White blood cells, WBC.

[0084] FIG. 16D. Laboratory values of blood cell counts for case 4. Red blood cells, RBC.

[0085] FIG. 16E. Laboratory values of blood cell counts for case 4. Platelets.

[0086] FIG. 16F. Laboratory values of blood cell counts for case 4. Haemoglobin, Hgb.

[0087] FIG. 16G. Laboratory values of blood cell counts for case 4. Haematocrit, Hct.

[0088] FIG. 17. Self-reported side-effects after chemotherapy for case 4.

[0089] FIG. 18A. Laboratory values of blood cell counts for case 5. Neutrophils.

[0090] FIG. 18B. Laboratory values of blood cell counts for case 5. Lymphocytes.

[0091] FIG. 18C. Laboratory values of blood cell counts for case 5. White blood cells, WBC.

[0092] FIG. 18D. Laboratory values of blood cell counts for case 5. Red blood cells, RBC.

[0093] FIG. 18E. Laboratory values of blood cell counts for case 5. Platelets.

[0094] FIG. 18F. Laboratory values of blood cell counts for case 5. Haemoglobin, Hgb.

[0095] FIG. 18G. Laboratory values of blood cell counts for case 5. Haematocrit, Hct.

[0096] FIG. 18H. Laboratory values of blood cell counts for case 5. Prostate specific antigen (PSA) level.

[0097] FIG. 19A. Laboratory values of blood cell counts for case 6. Neutrophils.

[0098] FIG. 19B. Laboratory values of blood cell counts for case 6. Lymphocytes.

[0099] FIG. 19C. Laboratory values of blood cell counts for case 6. White blood cells, WBC.

[0100] FIG. 19D. Laboratory values of blood cell counts for case 6. Red blood cells, RBC.

[0101] FIG. 19E. Laboratory values of blood cell counts for case 6. Platelets.

[0102] FIG. 19F. Laboratory values of blood cell counts for case 6. Haemoglobin, Hgb.

[0103] FIG. 19G. Laboratory values of blood cell counts for case 6. Haematocrit, Hct.

[0104] FIG. 20A. Laboratory values of blood cell counts for case 9. Neutrophils.

[0105] FIG. 20B. Laboratory values of blood cell counts for case 9. Lymphocytes.

[0106] FIG. 20C. Laboratory values of blood cell counts for case 9. White blood cells, WBC.

[0107] FIG. 20D. Laboratory values of blood cell counts for case 9. Red blood cells, RBC.

[0108] FIG. 20E. Laboratory values of blood cell counts for case 9. Platelets.

[0109] FIG. 20. Laboratory values of blood cell counts for case 9. Haemoglobin, Hgb.

[0110] FIG. 20G. Laboratory values of blood cell counts for case 9. Haematocrit, Hct.

[0111] FIG. 21. Self-reported side-effects after chemotherapy for case 10.

[0112] FIG. 22A. Self-reported side-effects after chemotherapy with or without fasting. Data represent average of CTC rating post all cycles reported by all the patients in this study.

[0113] FIG. 22B. Self-reported side-effects after chemotherapy with or without fasting. Data represent average of CTC rating from matching fasting and non-fasting cycles.

[0114] FIG. 23A. The effect of 72 hour fasting on weight. 30-week old CD-1 mice were fasted for 72 hours and sacrificed. Blood was collected via cardiac puncture under deep anesthesia, and blood glucose was measured immediately. Plasma was analyzed for GH and IGF-levels (Cohen). GH is a pulsatile hormone and therefore requires a large sample size to obtain significant results. All P values were calculated by Student's t-test except for IGFBP-1 which was done by the Mann-Whitney U test.

[0115] FIG. 23B. The effect of 72 hour fasting on glucose levels. 30-week old CD-1 mice were fasted for 72 hours and sacrificed. Blood was collected via cardiac puncture under deep anesthesia, and blood glucose was measured immediately. Plasma was analyzed for GH and IGF-levels (Cohen). GH is a pulsatile hormone and therefore requires a large sample size to obtain significant results. All P values were calculated by Student's t-test except for IGFBP-1 which was done by the Mann-Whitney U test.

[0116] FIG. 23C. The effect of 72 hour fasting on GH/IGF-I axis. 30-week old CD-1 mice were fasted for 72 hours and sacrificed. Blood was collected via cardiac puncture under deep anesthesia, and blood glucose was measured immediately. Plasma was analyzed for GH and IGF-levels (Cohen). GH is a pulsatile hormone and therefore requires a large sample size to obtain significant results. All P values were calculated by Student's t-test except for IGFBP-1 which was done by the Mann-Whitney U test.

[0117] FIG. 23D. The effect of 72 hour fasting on GH/IGF-I axis. 30-week old CD-1 mice were fasted for 72 hours and sacrificed. Blood was collected via cardiac puncture under deep anesthesia, and blood glucose was measured immediately. Plasma was analyzed for GH and IGF-levels (Cohen). GH is a pulsatile hormone and therefore requires a large sample size to obtain significant results. All P values were calculated by Student's t-test except for IGFBP-1 which was done by the Mann-Whitney U test.

[0118] FIG. 23E. The effect of 72 hour fasting on GH/IGF-I axis. 30-week old CD-1 mice were fasted for 72 hours and sacrificed. Blood was collected via cardiac puncture under deep anesthesia, and blood glucose was measured immediately. Plasma was analyzed for GH and IGF-levels (Cohen). GH is a pulsatile hormone and therefore requires a large sample size to obtain significant results. All P values were calculated by Student's t-test except for IGFBP-1 which was done by the Mann-Whitney U test.

[0119] FIG. 23F. The effect of 72 hour fasting on GH/IGF-I axis. 30-week old CD-1 mice were fasted for 72 hours and sacrificed. Blood was collected via cardiac puncture under deep anesthesia, and blood glucose was measured immediately. Plasma was analyzed for GH and IGF-levels (Cohen). GH is a pulsatile hormone and therefore requires a large sample size to obtain significant results. All P values were calculated by Student's t-test except for IGFBP-1 which was done by the Mann-Whitney U test.

[0120] FIG. 24. The conserved regulatory pathways of stress resistance in response to starvation/calorie restriction. In yeast, nutrient-sensing pathways controlled by Sch9, Tor,

and Ras converge on the protein kinase Rim15. In turn, the stress response transcription factors Msn2, Msn4, and Gis1 transactivate stress response genes and enhance cellular protection, which leads to life span extension. In mice and humans, a short-term starvation leads to a significant reduction in circulating IGF-I levels. The partially conserved IGF-I signaling pathways negatively regulate the FoxO family transcription factors through Akt. Ras and Tor also function downstream of IGF-I, although their roles in the regulation of stress resistance and aging are poorly understood. Mice deficient in type 5 adenylyl cyclase (AC) are stress resistant, analogous to the adenylyl cyclase deficient yeast. Notably, oncogenic mutations that cause the hyperactivation of IGF-I, Akt, Ras, Tor and PKA are among the most common in human cancers [20].

[0121] FIG. 25A. *in vitro* DSR to CP treatments by reducing IGF-I. Primary rat glial cells and rat glioma cell lines (C6, 9L, and A10-85) cell lines were tested. Cells were pre-incubated in DMEM/F12 with 1% serum and neutralizing anti-IGF-IR monoclonal antibody alpha-IR3 (1 ug/ml) for 24 hours. Cytotoxicity (LDH assay) was determined following CP treatment (15 mg/ml; n=12).

[0122] FIG. 25B. *in vitro* DSR to CP treatments by reducing IGF-I. Primary rat glial cells and rat glioma cell lines (C6, 9L, and A10-85) cell lines were tested. Cells were pre-incubated in medium with either 1% (STS) or 10% FBS for 24 hours. Cytotoxicity (LDH assay) was determined following CP treatment (15 mg/ml; n=12).

[0123] FIG. 25C. *in vitro* DSR to CP treatments by reducing IGF-I. Primary rat glial cells and rat glioma cell lines (C6, 9L, and A10-85) cell lines were tested. Cells were pre-incubated in medium with 1% serum with or without rhIGF-I (100 ng/ml) for 48 hours. Cytotoxicity (LDH assay) was determined following CP treatment (12 mg/ml; n=21). ***P<0.0001 by Student's t test.

[0124] FIG. 26A. R⁺ and R⁻ cells were grown to confluence and treated with DXR (0-500 μ M) in DMEM/F12 supplemented with 10% FBS for 24 hours. Viability was determined by the relative degree of MTT reduction compared to untreated; mean $\pm$ SD. *P<0.05, **P<0.01, ***P<0.001 by Student's t test; R⁺ vs. R⁻ cells at same DXR concentration.

[0125] FIG. 26B. R⁺ and R⁻ cells were grown to confluence and treated with DXR (0-500 μ M) in DMEM/F12 supplemented with 10% FBS for 48 hours. Viability was determined by the relative degree of MTT reduction compared to untreated; mean $\pm$ SD. *P<0.05, **P<0.01, ***P<0.001 by Student's t test; R⁺ vs. R⁻ cells at same DXR concentration.

[0126] FIG. 26C. Comet assay for experiments of FIGS. 26A and 26B.

[0127] FIG. 26D. Cells overexpressing IGF-IR or with IGF-IR deficiency (R⁺ and R⁻) were treated with 50 μ M DXR for 1 hour. Significant DNA damages were observed in the DXR treated R⁺ cells, while R⁻ cells were protected from DXR induced DNA damage. **P<0.01, ****P<0.0001 by Student's t test; R⁺ control vs. R⁺ DXR; R⁻ control vs. R⁻ DXR; R⁺ DXR vs. R⁻ DXR. Similar results were obtained from two independent experiments. Representative experiment is shown.

[0128] FIG. 27A. The effect of Sch9-/Ras2-deficiencies on DSR against DXR in *S. cerevisiae*. Wild type (DBY746), sch9 Δ , sch9 Δ , ras2 Δ , RAS2^{val19}, and sch9 Δ RAS2^{val19} strains were inoculated at OD₆₀₀=0.1, grown separately in glucose media, and treated with DXR (200 μ M) 24 hours after initial inoculation. Viability was measured as colony forming units

(CFU) onto appropriate selective media. Data from 3 independent experiments are shown as mean $\pm$ SE. *P<0.05 by Student's t test, sch9 Δ ras2 Δ vs. sch9 Δ RAS2^{val19}.

[0129] FIG. 27B. The effect of Sch9-/Ras2-deficiencies on DSR against DXR in *S. cerevisiae*. Mutation frequency over time, measured as Can^r mutants/10⁶ cells. Strains shown are wild type (WT), cells lacking Sch9 and/or Ras2, and cells overexpressing constitutively active Ras2^{val19}. Data represent the mean $\pm$ SEM (n=3-5 experiments). Cells were treated with DXR (200 μ M) on day 1. Mutation frequency of wild type untreated cells was reported as control. *P<0.05 by Student's t test, sch9 Δ ras2 Δ vs. sch9 Δ RAS2^{val19}.

[0130] FIG. 28A. Stress resistance testing in LID mice with various high-dose chemotherapeutic drugs. LID and control mice received (A) a single injection of 100 mg/kg etoposide (Eto, P=0.064).

[0131] FIG. 28B. Stress resistance testing in LID mice with various high-dose chemotherapeutic drugs. LID and control mice received a single injection of 500 mg/kg CP (P=0.001).

[0132] FIG. 28C. Stress resistance testing in LID mice with various high-dose chemotherapeutic drugs. LID and control mice received (C) a single injection of 400 mg/kg 5-fluorouracil (5-FU, P=0.148).

[0133] FIG. 28D. Stress resistance testing in LID mice with various high-dose chemotherapeutic drugs. LID and control mice received two injections of doxorubicin (DXR). The first injection of 20 mg/kg was given on day zero, and the second injection of 28 mg/kg was given on day 22 (P=0.022). Toxicity evaluated by percent survival is shown. P values by Peto's log rank test.

[0134] FIG. 29A. Differential stress resistance (DSR) against 2 cycles of high-dose DXR in melanoma bearing LID mice. (A) Timeline of experimental procedures.

[0135] FIG. 29B. Differential stress resistance (DSR) against 2 cycles of high-dose DXR in melanoma bearing LID mice. Bioluminescence imaging of B16Fluc melanoma bearing LID mice and control mice treated with 2 cycles of high-dose DXR. Five mice were randomly selected and followed throughout the experiment to monitor tumor progression or regression.

[0136] FIG. 29C. Differential stress resistance (DSR) against 2 cycles of high-dose DXR in melanoma bearing LID mice. Survival rate comparison between B16Fluc melanoma bearing LID and control mice treated with 2 cycles of high-dose DXR (P<0.05).

[0137] FIG. 29D. Differential stress resistance (DSR) against 2 cycles of high-dose DXR in melanoma bearing LID mice. The data in FIG. 29C represent all deaths resulting from both metastasis and DXR toxicity. Therefore, the data was analyzed to represent only DXR toxicity related deaths.

[0138] FIG. 29E. Differential stress resistance (DSR) against 2 cycles of high-dose DXR in melanoma bearing LID mice. Weight of LID and control mice.

[0139] FIG. 29F. Differential stress resistance (DSR) against 2 cycles of high-dose DXR in melanoma bearing LID mice. DXR induced cardiomyopathy in control and LID mice. Heart failure is a major outcome of acute DXR toxicity [76]. Histological slides of the heart from DXR treated control mice showed loss of myofibrils and infiltration of immune cells, whereas DXR dependent cardiac myopathy was not observed in LID mice. Hematoxylin and eosin staining. Representative slide shown. Bar, 100 μ m.

[0140] FIG. 30. A model for differential stress resistance (DSR) in response to short-term starvation (STS) and reduced

IGF-I. Normal cells respond to starvation or the absence of growth signals by undergoing cell cycle arrest and shifting energy to maintenance. Since one of the hallmarks of cancer cells is the ability to grow or remain in a growth mode regardless of external regulatory signals (including IGF-IR, Ras, and Akt), cancer cells are predicted to fail to or only partially enter into a protective maintenance mode in response to starvation and low IGF-I.

[0141] FIG. 31. DIFFERENTIAL STRESS RESISTANCE BY STARVATION. In normal cells, downstream effectors of the IGF-I and other growth factor pathways, including the Akt, Ras and other proto-oncogenes, are down-regulated in response to the reduction in growth factors caused by starvation. This down-regulation blocks/reduces growth and promotes protection to chemotherapy. By contrast, oncogenic mutations render tumor cells less responsive to STS due to their independence from growth signals. Therefore, cancer cells fail to or only partially respond to starvation conditions and continue to promote growth instead of protection against oxidative stress and high dose chemotherapy.

[0142] FIG. 32A. Differential Stress Resistance in Starved Mammalian Cells. Primary rat glial cells were tested. (* $p < 0.05$, ** $p < 0.01$).

[0143] FIG. 32B. Differential Stress Resistance in Starved Mammalian Cells. Rat glioma cell lines (C6) were tested. (* $p < 0.05$, ** $p < 0.01$).

[0144] FIG. 32C. Differential Stress Resistance in Starved Mammalian Cells. Rat glioma cell lines (A10-85) were tested. (* $p < 0.05$, ** $p < 0.01$).

[0145] FIG. 32D. Differential Stress Resistance in Starved Mammalian Cells. Rat glioma cell lines (RG2) were tested. (* $p < 0.05$, ** $p < 0.01$).

[0146] FIG. 32E. Differential Stress Resistance in Starved Mammalian Cells. Human glioma (LN229) cell lines were tested. (* $p < 0.05$, ** $p < 0.01$).

[0147] FIG. 32F. Differential Stress Resistance in Starved Mammalian Cells. Human neuroblastoma (SH-SY5Y) cell lines were tested. (* $p < 0.05$, ** $p < 0.01$).

[0148] FIG. 33A. Short-term starvation (STS) Protects Mice From Chemo-toxicity. Mice from 3 different genetic backgrounds (A: A/J B: CD-1 C: Nude) were starved for 48-60 hours and challenged with high-dose etoposide (100-110 mg/kg). (** $p < 0.01$, *** $p < 0.05$).

[0149] FIG. 33B. Short-term starvation (STS) Protects Mice From Chemo-toxicity. Mice from 3 different genetic backgrounds (A: A/J B: CD-1 C: Nude) were starved for 48-60 hours and challenged with high-dose etoposide (100-110 mg/kg). (** $p < 0.01$, *** $p < 0.05$).

[0150] FIG. 33C. Short-term starvation (STS) Protects Mice From Chemo-toxicity. Mice from 3 different genetic backgrounds (A: A/J B: CD-1 C: Nude) were starved for 48-60 hours and challenged with high-dose etoposide (100-110 mg/kg). (** $p < 0.01$, *** $p < 0.05$).

[0151] FIG. 33D. Survival for the experiments of FIGS. 33A-C.

[0152] FIG. 33E. Short-term starvation (STS) Protects Mice From Chemo-toxicity. 8-week old CD-1 female mice were starved for 48 hours prior to and 24 h following administration of 12 mg/kg of cisplatin. ($p < 0.05$)

[0153] FIG. 33F. Short-term starvation (STS) Protects Mice From Chemo-toxicity. (F) 15-week old A/J female mice were starved for 48 hours and challenged with 16 mg/kg of doxorubicin. ($p < 0.05$)

[0154] FIG. 34A. Differential Stress Resistance in Starved Mice with Neuroblastoma. NXS2 (neuroblastoma)-bearing mice were starved for 48 hours (STS) prior to chemotherapy with high-dose etoposide (80 mg/kg).

[0155] FIG. 34B. Differential Stress Resistance in Starved Mice with Neuroblastoma. Experimental procedures. (C) STS may sensitize NXS2 cells against doxorubicin and cisplatin.

[0156] FIG. 35. Differential Stress Resistance in Starved Mice with B16 Melanoma Cells. STS sensitizes B16 melanoma cells against DXR: Mice starved 48 hour prior to chemotherapy showed a greater tumor response which was further quantified using bioluminescence technology.

DETAILED DESCRIPTION OF THE INVENTION

[0157] The present invention is based, at least in part, upon the unexpected discovery that dietary compositions comprising reduced level of methionine, tryptophan, all amino acids, or protein, dietary compositions comprising glycerol as a substitute for monosaccharides, disaccharides, and polysaccharides, and hypocaloric or calorie free diets with reduced level of energy, carbohydrates, or protein, as well as fasting, can be used to protect subjects against chemotherapy, radiotherapy, oxidative stress, or aging.

[0158] More specifically, one dietary composition of the invention contains 0-0.2% (e.g., 0.02%, 0.05%, 0.1%, or 0.15%) by weight L-methionine and at least 0.05% (e.g., 0.1%, 0.5%, 1%, or 2%) by weight of each of L-tryptophan, L-isoleucine, L-leucine, L-lysine, L-phenylalanine, L-threonine, and L-valine, but no protein. In some embodiments, the composition also contains one or more amino acids selected from the group consisting of L-alanine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, L-glycine, L-proline, L-serine, L-tyrosine, L-arginine, and L-histidine, e.g., each in the amount of at least 0.05% (e.g., 0.1%, 0.5%, 1%, or 2%) by weight. In some embodiments, the composition contains a normal amount of each of L-tryptophan, L-isoleucine, L-leucine, L-lysine, L-phenylalanine, L-threonine, L-valine, L-alanine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, L-glycine, L-proline, L-serine, L-tyrosine, L-arginine, and L-histidine.

[0159] A second dietary composition of the invention contains 0-0.2% (e.g., 0.02%, 0.05%, 0.1%, or 0.15%) by weight L-tryptophan and at least 0.05% (e.g., 0.1%, 0.5%, 1%, or 2%) by weight of each of L-methionine, L-isoleucine, L-leucine, L-lysine, L-phenylalanine, L-threonine, L-valine, but no protein. In some embodiments, the composition also contains one or more amino acids selected from the group consisting of L-alanine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, L-glycine, L-proline, L-serine, L-tyrosine, L-arginine, and L-histidine, e.g., each in the amount of at least 0.05% (e.g., 0.1%, 0.5%, 1%, or 2%) by weight. In some embodiments, the composition contains a normal amount of each of L-methionine, L-isoleucine, L-leucine, L-lysine, L-phenylalanine, L-threonine, L-valine, L-alanine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, L-glycine, L-proline, L-serine, L-tyrosine, L-arginine, and L-histidine.

[0160] A third dietary composition of the invention contains L-methionine, L-tryptophan, L-isoleucine, L-leucine, L-lysine, L-phenylalanine, L-threonine, L-valine, L-alanine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, L-glycine, L-proline, L-serine, L-tyrosine,

L-arginine, and L-histidine, each in the amount of 0-0.2% (e.g., 0.02%, 0.05%, 0.1%, or 0.15%) by weight, but no protein.

[0161] A fourth dietary composition of the invention contains glycerol as a substitute for monosaccharides (e.g., glucose), disaccharides, and polysaccharides.

[0162] A dietary composition of the invention can be used to protect an animal or human against chemotherapy, radiotherapy, oxidative stress, or aging. More specifically, an animal or human may be fed with a dietary composition of the invention. When the animal or human is exposed to chemotherapy, radiotherapy, or oxidative stress, normal cells, but not abnormal cells such as cancer cells, in the animal or human are protected. For example, the composition may be administered to the animal or human for 3-10 consecutive days prior to the animal or human is exposed to chemotherapy, radiotherapy, or oxidative stress. The composition may also be administered to the animal or human for 24 hours following the exposure. Preferably, the composition may be administered to the animal or human for both 3-10 consecutive days prior to the animal or human is exposed to chemotherapy, radiotherapy, or oxidative stress and 24 hours following the exposure. For protection of an animal or human against aging, the composition may be administered every third meal or every 3-10 days.

[0163] Examples of chemotherapy include, but are not limited to, etoposide, doxorubicin, cisplatin, 5-FU, gemcitabine, cyclophosphamide, docetaxel, cyclophosphamide, carboplatin, GMZ, and paclitaxel. These drugs may be used individually or in combination.

[0164] The invention also provides a hypocaloric or calorie free diet. The diet contains dietary materials capable of providing nutrition to a human subject while providing no more than 813-957 kcal (e.g., no more than 700, 500, 300, or 100 kcal, or 0 kcal) total energy, and no more than 30-36 g (e.g., no more than 20, 10, or 5 g, or 0 g) protein. If carbohydrates are present in the dietary materials, no more than half of the energy is in the carbohydrates.

[0165] A diet of the invention can be administered to an animal or human (e.g., once or in 3 portions a day) for protection against chemotherapy, radiotherapy, oxidative stress, or aging. For example, the diet may be administered to the animal or human for 3-10 consecutive days prior to the animal or human is exposed to chemotherapy, radiotherapy, or oxidative stress. The diet may also be administered to the animal or human for 24 hours following the exposure. Preferably, the diet may be administered to the animal or human for both 3-10 consecutive days prior to the animal or human is exposed to chemotherapy, radiotherapy, or oxidative stress and 24 hours following the exposure. For protection of an animal or human against aging, the diet may be administered every third meal or every 3-10 days.

[0166] The invention further provides a method of protecting an animal or human against chemotherapy, radiotherapy, oxidative stress, or aging by administering to an animal or human a diet capable of providing nutrition while providing no more than 11 kcal (e.g., no more than 8, 5, or 2 kcal, or 0 kcal) energy per kg body weight of the animal or human per day and no more than 0.4 g (e.g., 0.3, 0.2, or 0.1 g or 0 g) protein per kg body weight of the animal or human per day. If carbohydrates are present in the diet, no more than half of the energy is in the carbohydrates. In some embodiments, the diet is capable of providing no more than 700 kcal (e.g., 600, 400, or 200 kcal or 0 kcal) total energy per day. When the animal

or human is exposed to chemotherapy, radiotherapy, or oxidative stress, normal cells, but not abnormal cells such as cancer cells, in the animal or human are protected. For example, the diet may be administered to the animal or human for 3-10 consecutive days prior to the animal or human is exposed to chemotherapy, radiotherapy, or oxidative stress. The diet may also be administered to the animal or human for 24 hours following the exposure. Preferably, the diet may be administered to the animal or human for both 3-10 consecutive days prior to the animal or human is exposed to chemotherapy, radiotherapy, or oxidative stress and 24 hours following the exposure. For protection of an animal or human against aging, the diet may be administered every third meal or every 3-10 days.

[0167] In addition, the invention provides a method of protecting an animal or human against chemotherapy by fasting an animal or human suffering from cancer prior to or following chemotherapy. For example, an animal or human suffering from cancer may be fasted for 48-140 hours prior to one round of chemotherapy or 4-56 hours following the chemotherapy. Preferably, an animal or human suffering from cancer is fasted for 48-140 hours prior to one round of chemotherapy and 4-56 hours following the chemotherapy. When the animal or human is exposed to chemotherapy, normal cells, but not cancer cells, in the animal or human are protected. In some embodiments, the animal or human is fasted for no more than 180 hours prior to and following one round of chemotherapy.

[0168] It was observed in animals that fasting 48-60 hours pre-chemo +/-24 hours post chemo protects mice and sensitizes cancer cells against chemotherapy. Further, as shown below, in cancer patients, fasting or a very low calorie diet protected patients but not cancer cells against chemotherapy. The very low calorie/fasting diet also appeared to sensitize cancer cells to chemo. It was also observed in animal studies that fasting sensitized various cancers to several types of chemotherapy. In addition, in animal studies, fasting caused a 75% reduction in IGF-I and a 75-90% reduction in IGF-I was sufficient to protect animals but to sensitize cancer cells against chemotherapy. Moreover, human clinical trials showed that 5-day fasting and/or a low calorie/low protein/low sugar diet caused a 75% or higher reduction in IGF-I (Thissen et al. (1994) *Endocrine Review* 15 (1):80-101). Therefore, the very low calorie/low sugars but also very low protein diet will protect animals and human against chemotherapy and sensitize many types of cancer cells against chemotherapy.

[0169] The following examples are intended to illustrate, but not to limit, the scope of the invention. While such examples are typical of those that might be used, other procedures known to those skilled in the art may alternatively be utilized. Indeed, those of ordinary skill in the art can readily envision and produce further embodiments, based on the teachings herein, without undue experimentation.

Example I

[0170] The strategies to treat cancer have focused largely on increasing the toxicity to tumor cells. The inventor has departed from the classic tumor-centric drug development focused on tumor killing and put focus on increasing the protection of normal cells. Recently, the inventor reported that a short-term starvation (STS; 40-60 hours) can enhance host resistance to chemotherapy while concomitantly enhancing tumor sensitivity to chemotherapy-induced apoptosis.

tosis (Differential Stress Resistance, DSR) (1). The foundation of STS comes from the work of Dr. Longo in the aging field where growth-factor suppression and calorie restriction (CR) increase lifespan and stress resistance in various organisms. However, although a STS is a powerful method to differentially protect the host, it could have limited application in clinical settings. Therefore, the inventor investigated alternative pharmaceutical interventions that could also enhance host resistance against chemotherapy. During the search, the inventor determined 3 promising preparations that provided increased protection to the host against chemotherapy drugs. The pharmaceutical preparations that were effective in enhancing resistance against chemotherapy were 1) a methionine restricted amino acid mix (LMA1), 2) a tryptophan restricted amino acid mix (LTA1), and 3) glycerol (G1). LMA1 is effective only if the diet lacks other sources of methionine and LTA1 is effective only if the diet lacks other sources of tryptophan. Finally, G1 is effective in combination with a glucose-restricted diet. Interestingly, despite the fact that the diets were isocaloric and the food intake was similar, LMA1/LTA1 treated animals showed a lower weight profile. This suggests that LMA1/LTA1 allow the animals to shift the energy towards 'maintenance' rather than 'growth/reproduction', and therefore increases resistance against chemotherapy toxicity.

LMA1 Mix

[0171] Methionine restriction has been shown to increase lifespan and stress resistance in laboratory rodents (2, 3). Therefore, the effect of a low methionine amino acid mix (LMA1) in the absence of proteins in the diet in protection against chemotherapy toxicity in laboratory rodents was investigated. 5 days prior to chemotherapy, eight mice were given the LMA1 mix in combination with a protein-free diet (Harlan, TD. 07789). Methionine levels in the LMA1 mix were 20% of that of the control diet (TD. 07788). Following the 5-day LMA1 diet, mice were intravenously injected with a high-dose of doxorubicin (DXR, a widely used chemotherapy drug). To determine the degree of toxicity, mice were monitored daily for weight loss and abnormal behavior. Body weight and food intake was recorded daily. LMA1 treated mice recovered from the weight loss more quickly compared to the control group (FIG. 1). Furthermore, LMA1-treated mice showed significantly higher survival rate compared to the control mice following high-dose chemotherapy (63% vs. 13% respectively) (FIG. 1).

LTA1 Mix

[0172] As with methionine restriction, a diet with low levels of tryptophan has also been shown to increase lifespan and decrease some age-related disease including cancer (4-7). Based on the fact that there is a strong correlation between longevity and stress resistance, the inventors believed that treatment of mice with a low tryptophan amino acid mix in the absence of other sources of tryptophan could also provide increased stress resistance in addition to lifespan extension. 10 days prior to chemotherapy, eight mice were treated with the LTA1 mix in combination with a diet lacking protein (Harlan, TD. 07790). Tryptophan levels in the LTA1 mix was 20% of that of the control diet (TD. 07788). Toxicity was determined as done with the LMA1 mix experiments. The LTA1 mix improved weight management after chemotherapy, causing a quicker recovering of the weight loss com-

pared to controls (FIG. 2). Also, mice treated with the LTA1 mix had a 4-fold higher survival rate compared to the controls (50% vs 12.5%) (FIG. 2).

G1 Mix

[0173] Calorie restriction enhances stress resistance and extends life span in model organism ranging from yeast to mammals (Longo, 2003) (8, 9). In view of our recent results with starvation showing effects in the protection against multiple chemotherapy and the beneficial effects of carbon source substitution with glycerol in life span and stress resistance in yeast, the effect of feeding mice with glycerol on protection against toxins was studied. Two groups of five mice each were fed ad libitum for six days with two isocaloric diets, the control diet (Teklad 8604 chow supplemented with 40% starch/sucrose/maltose dextrin) or the G1 diet containing glycerol (supplemented with 40% glycerol). Although the mice on the glycerol diet ate slightly more than those on the control diet, they showed an 18% reduction in blood glucose level by day 6 (FIG. 3). Both groups of mice were then given a single dose of 50 mg/kg paraquat intraperitoneally and put back on a normal diet (8604 chow). Paraquat is known to cause S-phase arrest of liver and lung cells (10) and lead to death (11). All mice in the control group were dead by day 3, whereas three out of five glycerol-fed mice fully protected from the paraquat toxicity (FIG. 3C, $p < 0.05$) and regained normal body weight five days after paraquat treatment (FIG. 3D). These results indicate that dietary carbon source substitution with glycerol enhances oxidative stress resistance in vivo and has the potential to mimic calorie restriction in higher eukaryotes.

Materials and Methods

LMA1 and LTA1

[0174] LMA1 and LTA1 are based on purified synthetic amino acid mixes (1) and were custom manufactured for us by Harlan Tekal in a 1/2" pellet form. All groups including Control (TD. 07788), LMA1 (TD. 07790), and LTA1 (TD. 07789) received isocaloric diets (3.9 Kcal/g).

[0175] LMA1 mix: CD-1 mice, weighing 25-30 g, were prefed for 5 days prior to chemotherapy with purified synthetic amino acids mixes containing either normal (0.86%) or low (0.17%) levels of methionine.

[0176] LTA1 mix: CD-1 mice, weighing 25-30 g, were prefed for 5 days prior to chemotherapy with purified synthetic amino acids mixes containing either normal (0.86%) or low (0.17%) levels of tryptophan.

TABLE I

Composition of control diet	
Formula	g/Kg
L-Alanine	3.5
L-Arginine HCl	12.1
L-Asparagine	6.0
L-Aspartic Acid	3.5
L-Cystine	3.5
L-Glutamic Acid	40.0
Glycine	23.3
L-Histidine HCl, monohydrate	4.5
L-Isoleucine	8.2
L-Leucine	11.1
L-Lysine HCl	18.0

TABLE I-continued

Composition of control diet	
Formula	g/Kg
L-Methionine	8.6
L-Phenylalanine	7.5
L-Proline	3.5
L-Serine	3.5
L-Threonine	8.2
L-Tryptophan	1.8
L-Tyrosine	5.0
Sucrose	344.53
Corn Starch	150.0
Maltodextrin	150.0
Soybean Oil	80.0
Cellulose	30.0
Mineral Mix, AIN-93M-MX (94049)	35.0
Calcium Phosphate, monobasic, monohydrate	8.2
Vitamin Mix, AIN-93-VX (94047)	19.5
Choline Bitartrate	2.75
TBHQ, antioxidant	0.02

Doxorubicin Studies in Mice

[0177] Following treatments with LMA1 or LTA1, mice were intravenously injected with 24-26 mg/kg doxorubicin (Bedford Laboratories) with 30 gauge insulin syringes (Becton, Dickinson and Company). Doxorubicin was dissolved in purified water and diluted in saline to a final concentration of 5 mg/ml. All doxorubicin injections were followed by a saline/heparin wash to minimize endothelial cell damage. To determine toxicity and efficacy, mice were monitored routinely for weight loss and general behavior. Body weight was recorded once daily throughout the experiment. Mice found moribund were sacrificed by CO₂ narcosis and necropsy was performed. Since cardiotoxicity is the major cause of death from acute doxorubicin toxicity, we prepared histological slides to examine the degree of damage at the tissue level.

Glycerol Diet in Mice

[0178] A/J mice, weighing 18-24 g, were given a 40% glycerol diet (w/w) for 6 days. The diet composed of 60% pellet (Harlan Teklad, Diet 8604) and 40% glycerol (Bio-Serv, NJ) by weight. Briefly, pellets were finely ground using a food processor and mixed with USP grade glycerol. The density of glycerol (1.26 g/ml) was taken into account when mixing with pellet powder. Food was dried for 4 days. Since glycerol is hygroscopic, it absorbed atmospheric moisture and increased the pellet weight 3% during the first 3 days of the drying process and maintained stable weight thereafter. Blood glucose levels were measured on day 6. The tail vein was minimally punctured using a sterile 31 gauge needle and briefly bled. Blood glucose levels were determined with a Precision Xtra blood glucose monitoring system (Abbott Laboratories). Since glycerol is metabolized primarily in the liver and kidneys, these organs were collected at the time of necropsy for histological examination.

Paraquat Studies in Mice

[0179] Following the 6 days of glycerol diet, mice were injected with paraquat (Sigma). Paraquat was prepared in phosphate buffered saline (PBS) at 7.5 mg/ml and injected at 50 mg/kg intraperitoneally using a 31 gauge syringe (Becton, Dickinson and Co). Immediately following paraquat administration, animals were returned to the normal diet (Harlan

Teklad, Diet 8604). Mice were monitored every 2 hours for 4 days and body weight was recorded once daily throughout the experiment. Body weight measures were divided into 2 phases—glycerol diet phase and post-paraquat phase—and analyzed. Mice were sacrificed when they showed signs of stress or pain and also determined to have no chance of recovery by highly trained and experienced researchers. Since the lung is the major target organ, sacrificed mice were necropsied and the lung was collected for histological examination. Briefly, the lung slices were fixed in 4% formaldehyde, paraffin embedded and sectioned to 4 μ m thickness, and H&E stained.

REFERENCES

- [0180]** 1. Raffaghello L, Lee C, Safdie F M, Wei M, Madia F, Bianchi G, & Longo V D (2008) *Proc Natl Acad Sci USA*.
- [0181]** 2. Miller R A, Buehner G, Chang Y, Harper J M, Sigler R, & Smith-Wheelock M (2005) *Aging Cell* 4, 119-125.
- [0182]** 3. Orentreich N, Matias J R, DeFelice A, & Zimmerman J A (1993) *J Nutr* 123, 269-274.
- [0183]** 4. Ooka H, Segall P E, & Timiras P S (1988) *Mech Ageing Dev* 43, 79-98.
- [0184]** 5. Segall P E & Timiras P S (1976) *Mech Ageing Dev* 5, 109-124.
- [0185]** 6. Timiras P S, Hudson D B, & Segall P E (1984) *Neurobiol Aging* 5, 235-242.
- [0186]** 7. Anisimov V N (2001) *Exp Gerontol* 36, 1101-1136.
- [0187]** 8. Masoro E J (2005) *Mech Ageing Dev* 126, 913-922.
- [0188]** 9. Kennedy B K, Steffen K K, & Kaeberlein M (2007) *Cell Mol Life Sci* 64, 1323-1328.
- [0189]** 10. Matsubara M, Yamagami K, Kitazawa Y, Kawamoto K, & Tanaka T (1996) *Arch Toxicol* 70, 514-518.
- [0190]** 11. Migliaccio E, Giorgio M, Mele S, Pelicci G, Reboldi P, Pandolfi P P, Lanfranccone L, & Pelicci P G (1999) *Nature* 402, 309-313.
- [0191]** 12. Rogers Q R & Harper A E (1965) *J Nutr* 87, 267-273.

Example II

Sch9-Regulated Carbon Source Substitution is as Effective as Calorie Restriction in Life Span Extension Summary

[0192] The effect of calorie restriction (CR) on life span extension, demonstrated in organisms ranging from yeast to mice, may involve the down-regulation of pathways including Tor, Akt, and Ras. Here we present genetic and gene expression data suggesting that yeast Sch9 (a homolog of both mammalian kinases Akt and S6K) is a central component of a network that controls a common set of genes implicated in a metabolic switch from the TCA cycle and respiration to glycolysis and glycerol biosynthesis. During chronological survival, mutants lacking SCH9 depleted extracellular ethanol, reduced stored lipids but synthesized and released glycerol. Deletion of the glycerol biosynthesis genes GPD1, GPD2 or RHR2, among the most up-regulated in long-lived sch9 Δ , tor1 Δ , and ras2 Δ mutants, was sufficient to reverse chronological life span extension and stress resistance in sch9 Δ mutants. Replacement of glucose or ethanol

with glycerol as carbon source caused a longevity extension comparable to that caused by calorie restriction or starvation. Replacement of glucose-based carbohydrates with glycerol in the mouse diet reduced glucose level and enhanced resistance to oxidative stress. These results suggest that "carbon source substitution" (CS) represents a new strategy to delay aging and protect cells against damage.

Introduction

[0193] Mutations that decrease the activities of the Akt/PKB, Tor, and Ras pathways extend the lifespan of several model organisms, suggesting that the underlying mechanisms of longevity regulation are conserved in many eukaryotic organisms (Kenyon, 2001; Longo and Finch, 2003). Akt/PKB is a highly conserved serine-threonine kinase shown to function in the Daf-2 longevity pathway of *Caenorhabditis elegans* (Paradis et al., 1999). Homologous longevity modulating pathways were also identified in *Drosophila* and mice (Kenyon, 2001). In yeast, Sch9, which shares high sequence identity with the mammalian kinases Akt/PKB and S6K, is part of a nutrient-sensing pathway whose downregulation extends the chronological lifespan (CLS, the survival time of population of non-dividing yeast) by up to 3-fold (Fabrizio et al., 2001). The Ras G-proteins are also evolutionary conserved and implicated in cell division in response to glucose/growth factors. The deletion of RAS2 doubles the CLS of yeast (Fabrizio et al., 2003). In mammals, a role for Ras in longevity control has not been established conclusively but, together with Akt, Ras is one of the major mediators of IGF-I signaling, which has been shown to promote aging (Holzenberger, 2004; Longo, 2004). Another conserved nutrient-responsive pathway, regulating cell growth and cell-cycle progression, involves the protein kinase target of rapamycin, TOR, which has been associated with life span regulation in *C. elegans* and *Drosophila*. Knockdown of LET-363/CeTOR, starting at the first day of the adult life, more than doubled the life span of worm (Vellai et al., 2003). Similarly, a reduced activity of Daf-15, the worm ortholog of the mammalian mTOR-interacting protein raptor, promotes life span extension (Jia et al., 2004). In flies, overexpression of dominant-negative dTOR or TOR-inhibitory dTsc1/2 proteins also leads to longevity extension (Kapahi et al., 2004). Moreover, knockdown of CeTOR does not further extend the life span of worms subject to dietary restriction (DR) and inhibition of TOR protects flies from the deleterious effects of rich food, suggesting the beneficial effect of DR is, at least in part, mediated by TOR (Hansen et al., 2007; Kapahi et al., 2004).

[0194] Two TOR orthologs, TOR1 and TOR2, have been identified in yeast. Both Tor1 and Tor2 mediate growth-related signaling in a rapamycin-sensitive manner, whereas Tor2 has an additional rapamycin-insensitive function in controlling the cell-cycle-dependent organization of actin cytoskeleton (Loewith et al., 2002). Reduction of the TOR pathway activity results in an extension of yeast replicative life span (RLS), the number of daughter cells generated by individual mother cells (Kennedy et al., 1994; Mortimer and Johnston, 1959), comparable to that obtained when Sch9 is inactivated (Kaeberlein et al., 2005a; Kaeberlein and Kennedy, 2005). Furthermore, a high throughput assay to measure the CLS of individual yeast deletion mutants identified several long-lived strains carrying deletions of genes implicated in the Tor pathway (Powers et al., 2006). Addi-

tional evidence supporting an inverse correlation between Tor1 activity and CLS has recently been provided (Bonawitz et al., 2007).

[0195] The aging-regulatory function of both yeast Tor1 and Sch9 mediates the calorie restriction (CR)-dependent RLS extension. The down-regulation of either pathway mimics the effect of lowering the glucose content of the medium, and no further extension of RLS is observed when the sch9 Δ or the tor1 Δ mutants are calorie restricted (Kaeberlein et al., 2005b). Ethanol produced during fermentative growth is used as carbon source during diauxic shift and post-diauxic phase, when the yeast cells switch from rapid growth to slow budding and eventually ceasing proliferation (Gray et al., 2004; Lillie and Pringle, 1980). Switching yeast grown in glucose/ethanol medium to water models an extreme CR/starvation condition for non-dividing cells. This severe form of CR doubles chronological survival of wild type yeast (Fabrizio and Longo, 2003). In contrast to RLS, CR-induced increase of CLS is only partially mediated by Sch9 (Fabrizio et al., 2005; Wei et al., 2008).

[0196] Despite the extensive body of work demonstrating a link between nutrient-sensing pathways and life span regulation in different organisms, the key mechanisms responsible for delaying the aging process are still elusive. The direct correlation between life span extension and the ability to withstand different stress challenges, which has been observed in different model organisms, indicates that the activation of cellular protection represents an important survival strategy (Longo and Fabrizio, 2002). Our previous studies suggest that superoxide plays an important role in aging and age-dependent mortality, but protection against superoxide only accounts for a small portion of the potent effect of mutations in SCH9 and RAS2 on life span (Fabrizio et al., 2003). The connection between calorie restriction and the Sch9, Tor, and Ras2 pathways as well as the mechanisms of CR-dependent effects on life span are poorly understood. Here we present evidence that changes in the expression of a set of genes controlled by Sch9 but also Tor and Ras lead to a metabolic switch to glycerol production, which causes enhanced cellular protection and life span extension. Replacement of glucose or ethanol with glycerol as carbon source is as effective as calorie restriction in promoting cellular protection and life span extension. Dietary substitution of sugars with glycerol also protected mice against oxidative stress, suggesting that carbon source substitution (CSS) has the potential to trigger some of the protective effects of calorie restriction or starvation in higher eukaryotes.

Results

Genetic Interactions Between SCH9, and RAS2 and TOR1

[0197] Using a genetic approach, we examined the relationship between Sch9, Tor1, and Ras2 in regulating cellular protection against stress and life span. The effects on life span and stress resistance caused by deficiency in Tor1 activity are less robust than those observed in the strains lacking Sch9 or Ras2. We did not observe any significant difference in mean lifespan or stress resistance between sch9 Δ and the tor1 Δ sch9 Δ double knockout strains (FIGS. 4A and 4G). By contrast, the deletion of TOR1 in a mutant carrying a transposon insertion in the promoter region of SCH9, which only reduces SCH9 expression (Fabrizio et al., 2001), caused a further increase of resistance to heat and to the superoxide-generating agent menadione, but not to H₂O₂ (FIG. 4B), suggesting

that the lack of TOR1 contributes to the further inactivation of the Sch9 pathway. This result is in agreement with the recent study showing that Sch9 is a direct target of rapamycin-sensitive Tor complex 1 (TORC1) (Urban et al., 2007). In fact, reducing the TORC1 activity either by deleting TCO89, which encodes a TORC1 component, or by rapamycin treatment increased cell resistance to heat and H₂O₂. Since Sch9 activity is associated with an age-dependent increase of mutation frequency (Fabrizio et al., 2005), we examined the interaction between Sch9 and Tor1 in the regulation of genomic instability during chronological aging. Whereas the *tor1Δ* mutant was slightly less susceptible than wild type cells to genomic instability (measured as age-dependent frequency of mutations of the CAN1 gene) between day 1 and 7, there was no significant change in the mutation frequency of the double *tor1Δ sch9Δ* mutant compared to that of the *sch9Δ* mutant (FIG. 4E). Overexpression of TOR1 only slightly reduced the stress resistance phenotype of *sch9Δ*. However, resistance to stress and life span extension of *tor1Δ* was abolished by overexpressing SCH9 (FIG. 5F). Taken together, these data are in agreement with a shared signaling pathway between Tor and Sch9 in life span regulation and suggest an upstream role of Tor1 in Sch9 signaling (FIG. 4H).

[0198] Both Tor and Ras/cAMP-PKA signalings are known to regulate stress-responsive (STRE) genes (Zurita-Martinez and Cardenas, 2005). Elevating Ras activity by ectopically expressing constitutively active Ras2 (*ras2^{Val19}*) reversed the life span extension and the stress resistance of *tor1Δ* mutants (FIG. 4F). Conversely, deletion of RAS2 has an additive effect to *tor1Δ* with respect to stress resistance but not life span (FIGS. 4C and 4G), suggesting an overlapping in longevity modulation by Tor1 and Ras2.

[0199] We have previously shown that longevity regulations controlled by Tor1, Sch9, and Ras2 converge on the protein kinase Rim15 (Wei et al., 2008). Rim15 positively regulates stress response transcription factors (TFs) Msn2/4 and Gis1, which activate genes involved in cellular protection. Interestingly, enhancement of stress resistance and life span extension associated with Ras2 deficiency requires both the STRE-binding TFs Msn2/4 and PDS-binding Gis1, whereas the *sch9Δ*-mediated longevity regulation mainly depends on the latter (Fabrizio et al., 2001; Wei et al., 2008). These results indicate that the common downstream effectors are differentially modulated by the Sch9 and Ras2. In fact, the *ras2Δ sch9Δ* double knockout cells exhibited higher stress

resistance than either of the single deletion mutants (FIG. 4D). It also showed a 5-fold increase in mean life span compared to wild type cells (FIG. 5G). The triple *sch9Δ ras2Δ tor1Δ* deletion mutant, however, did not show any further increase of life span or stress resistance (FIGS. 4D and 4G). These results depict a life-span regulatory network composed of parallel but partially connected signaling pathways controlled by Tor/Sch9 and Ras (FIG. 4H).

Gene Expression Profiles of Long-Lived Mutants

[0200] To identify the mediators of life span extension downstream of the Tor/Sch9 and Ras pathways, we carried out DNA microarray analyses for all three major long-lived mutants: *sch9Δ*, *tor1Δ*, and *ras2Δ*. Total RNA was extracted from 2.5 day-old cultures of long-lived mutants and wild type cells. This age was selected to avoid both the noise that may arise from a small fraction of cells that are still dividing at younger ages (day 1-2) and the general decrease in metabolism and consequently in gene expression that normally occurs at older ages (day 4-5) (Fabrizio and Longo, 2003). The cRNA obtained from total RNA was hybridized to gene chips that allow the detection of 5841 of the 5845 genes present in *S. cerevisiae*. Three independent populations of each genotype were analyzed. A total of 800 genes showed a greater than 2-fold change in expression relative to wild type cells. Among these, 63 genes were consistently up-regulated more than 2-fold in all three mutants, and 25 genes were consistently down-regulated (FIG. 5A). The mRNA levels of seven of the most up-regulated and one most down-regulated genes in both the *tor1Δ* and *sch9Δ* mutants were confirmed by quantitative RT-PCR and/or Northern blot. Based on the pairwise comparison of the long-lived mutants, the up- and down-regulation of genes in these long-lived mutants are significantly overlapping, suggesting that the Ras, Tor, and Sch9-centered longevity regulatory network controls a common set of down-stream genes (Table 1). To identify features common to the three long-lived mutants we performed a gene ontology (GO) analysis of the microarray data by Wilcoxon rank test. Although the data point to common changes in all 3 long-lived mutants, the GO category analysis indicated a divergence in expression pattern between *ras2Δ* and the other two mutants, which is in agreement with our genetic analysis of two parallel signaling pathways controlled by Sch9 and Ras2, and is consistent with the role of Sch9 and Tor in the same life span regulatory pathway (Table 1 and FIG. 4H) (Wei, 2008).

TABLE 1

Gene ontology (GO) analysis of expression profiles of long-lived mutants									
GO*	GO ID	Gene #	Annotation	sch9Δ		tor1Δ		ras2Δ	
				p	q	p	q	p	q
Positively affected TIGO categories									
C	GO:0005842	83	cytosolic large ribosomal subunit	0.00E+00	0.00E+00	0.00E+00	0.00E+00	1.64E-12	2.37E-10
C	GO:0005843	63	cytosolic small ribosomal subunit	0.00E+00	0.00E+00	0.00E+00	0.00E+00	7.49E-09	6.49E-07
P	GO:0016125	37	sterol metabolism	5.65E-03	6.20E-02	7.50E-03	7.56E-02	7.51E-05	2.32E-03
P	GO:0046365	33	monosaccharide catabolism	1.32E-03	2.01E-02	2.94E-05	1.02E-03	8.81E-06	3.81E-04

TABLE 1-continued

Gene ontology (GO) analysis of expression profiles of long-lived mutants									
GO*	GO ID	Gene #	Annotation	sch9Δ		tor1Δ		ras2Δ	
				p	q	p	q	p	q
Negatively affected TIGO categories									
C	GO:0005762	43	mitochondrial large ribosomal subunit	1.56E-19	3.32E-17	1.13E-20	4.29E-18	1.34E-20	4.29E-18
C	GO:0005763	34	mitochondrial small ribosomal subunit	6.94E-13	4.93E-11	3.17E-13	2.54E-11	4.83E-14	4.41E-12
C	GO:0016591	74	DNA-directed RNA polymerase II, holoenzyme	1.61E-05	2.29E-04	9.05E-05	8.65E-04	4.97E-10	2.27E-08
C	GO:0000502	46	proteasome complex	3.92E-04	2.56E-03	4.51E-43	1.72E-02	1.35E-08	4.79E-07
C	GO:0005743	158	mitochondrial inner membrane	2.64E-16	2.82E-14	3.56E-17	5.70E-15	3.14E-09	1.34E-07
F	GO:0008080	37	N-acetyltransferase activity	6.89E-03	2.32E-02	6.43E-03	2.25E-02	3.16E-04	2.20E-03
P	GO:0016570	59	histone modification	1.56E-03	7.85E-03	2.16E-04	1.64E-03	7.30E-06	1.14E-04
P	GO:0006365	67	35S primary transcript processing	1.93E-03	9.16E-03	3.84E-06	7.23E-05	4.05E-03	1.59E-02
P	GO:0007005	95	mitochondrion organization and biogenesis	6.62E-05	7.02E-04	1.32E-04	1.07E-03	4.51E-06	8.02E-05
P	GO:0016044	31	membrane organization and biogenesis	2.09E-03	9.85E-03	1.38E-03	7.18E-03	9.74E-03	3.06E-02
P	GO:0006626	47	protein-mitochondrial targeting	8.33E-06	1.27E-04	1.46E-06	3.11E-05	4.04E-04	2.59E-03
P	GO:0009060	82	aerobic respiration	2.66E-08	8.96E-07	4.73E-09	1.78E-07	1.32E-06	3.01E-05
P	GO:0006119	46	oxidative phosphorylation	7.03E-07	2.04E-05	9.01E-07	2.31E-05	1.57E-04	1.24E-03
P	GO:0006118	31	electron transport	1.22E-04	1.03E-03	1.01E-04	9.28E-04	4.29E-03	1.65E-02

*C, Cellular component;
F, molecular function; and
P, biological process

Gene ontology (GO) analysis of expression profiles of long-lived mutants. Significantly up- or down-regulated categories were shown ($p < 0.01$). q-value was also calculated to correct the multi-testing error.

Metabolic Changes Associated with Longevity-Extension

[0201] Gene expression profile comparison between long-lived mutants and wild type cells reveals a consistent down-regulation of the genes encoding mitochondrial proteins, including those that function in the TCA cycle, oxidative phosphorylation, mitochondrial ribosomal proteins, as well as proteins targeted to mitochondria. The expression of glycolytic/fermentative genes, but not of gluconeogenic genes, was instead up-regulated. Interestingly, several genes coding for high-affinity glucose transporters or putative glucose transporters, known to be inhibited by high glucose concentrations (Ozcan and Johnston, 1999), were up-regulated indicating that the long-lived mutants may have entered a starvation-like mode in which glucose uptake is maximized. Considering that the extracellular glucose was exhausted in mutants as well as wild type cells by day 1-2, the major substrate available for fermentation by day 2.5 is probably

glycogen, which is normally accumulated by yeast in the late phases of exponential growth (Werner-Washburne et al., 1993).

[0202] Genes involved in stationary phase survival, sporulation, meiosis, and stress response (FMP45, GRE1, IME1, RPI1, SPS100, and TAH1) were among the most upregulated genes in all three long-lived mutants. To test their contribution to life span extension and stress resistance in long-lived mutants, we originated a set of double mutants carrying the deletion of SCH9, RAS2 or TOR1 in combination with that of one of the most up-regulated genes. Whereas the deletion of either FMP45 or YDL218W slightly reduced the mean life span of the sch9Δ mutants (FIG. 5B), they have no effect on ras2Δ mutants. The deletion of IME1 or RPI1 did not affect either the stress resistance or the life span extension caused by the lack of Sch9 (FIG. 5B). Deletion of YLR012C, the most down-regulated gene, did not affect significantly the life span or the stress resistance of the cell.

[0203] Several genes coding for proteins that function in the ergosterol biosynthesis were up-regulated in the long-lived mutants. Ergosterol is the predominant sterol in yeast and is structurally closely related to cholesterol. Besides

being a structural component of the cellular membrane, ergosterol affects phospholipid synthesis, lipid rafts formation, signal transduction, as well as aerobic energy metabolism (Parks et al., 1995). The deletion of either HMG1 or ERG28 caused a significant decrease in both heat and oxidative stress resistance in the *sch9Δ* mutants. However, the deletion of ERG5, the most up-regulated ergosterol biosynthesis gene in our microarray analysis, did not reverse longevity extension or reduced stress resistance associated with the *sch9Δ* mutants. Notably, the ergosterol biosynthetic genes that were upregulated in all three long-lived mutants are those involved in converting squalene to ergosterol, which require molecular oxygen and often involve oxidation of NADPH to NADP⁺. The upregulation may reflect a hypoxic environment during the post-diauxic phase survival of these long-lived mutants and suggests a link between redox state of the cell and survival. Taken together, these results indicate that the deletion of many single genes among the most up-regulated in long-lived mutants has little effect on life span.

Increased Expression of Glycerol Biosynthetic Genes in Long-Lived Mutants

[0204] In addition to the lower expression of TCA cycle and respiratory genes and higher expression of glycolytic/fermentative genes, we also observed an up-regulation of the genes implicated in the metabolism of glycerol, a byproduct of the overflow metabolism when there is enhanced glycolytic flux and limited respiration capacity (FIGS. 6A and 6B). Significant up-regulation of genes involved in glycerol metabolism (21 genes) was observed in *sch9Δ* and *ras2Δ* mutants (p-value of 0.0058 and 0.0142, Wilcoxon rank test, one-sided, respectively). In yeast, glycerol is produced from either triacylglycerol or dihydroxy-acetone-phosphate (DHAP), a glycolysis intermediate (FIG. 6A). Whereas the genes encoding the lipases responsible for the hydrolysis of triacylglycerol were slightly up-regulated, GPD1 and GPD2, encoding the key enzymes required for glycerol production from DHAP, showed higher levels of expression in all the long-lived mutants (FIGS. 6B and 6C), suggesting that part of the glucose utilized by these mutants is redirected towards glycerol biosynthesis.

[0205] In fact, high level of intracellular glycerol was observed in the *sch9Δ* mutants compared to that in wild type cells at day 3 (FIG. 7A). In wild type cells the level of extracellular glycerol reached a peak at day 2 but was mostly depleted by day 3. In the *sch9Δ* culture, however, a much elevated level of glycerol was measured in the medium up to day 9 (FIG. 7B). By contrast, ethanol produced during the exponential growth, and most likely in the post-diauxic phase as well, was depleted early in *sch9Δ* mutants but not in wild type cells (FIGS. 7C and 7D) (Fabrizio et al., 2005), suggesting a metabolic switch from biosynthesis and release of ethanol in wild type cells to that of glycerol in *sch9Δ* mutants. Glycerol accumulation could be accompanied by the depletion of other carbon sources as well. Nile red staining of the lipid body indicated that the levels of triacylglycerol and other neutral lipids in *sch9Δ* mutants were consistently lower compared to that in wild type cells across all ages (FIG. 7E), which is in agreement with a modest but consistent increase of mRNA levels of lipolytic enzymes converting lipids to glycerol. Accumulation of extracellular glycerol also occurred for *tor1Δ* and *ras2Δ* mutants, but was lower than that observed for *sch9Δ* mutants.

Glycerol Biosynthesis Genes are Required for Life Span Extension in *Sch9Δ*

[0206] To further examine the role of glycerol biosynthesis in life span regulation, we generated strains lacking Rhr2, the yeast DL-glycerol-3-phosphatase, in the *sch9Δ* background. The *rhr2Δ sch9Δ* double mutant failed to accumulate glycerol extracellularly (FIG. 8A). Deletion of RHR2 abolished the life span extension as well as the resistance to heat and oxidative stresses associated with the lack of SCH9 in the DBY746 genetic background (FIGS. 8B and 8C). Utilizing the yeast KO collection (BY4741 genetic background), we deleted SCH9 in strains lacking key glycerol biosynthetic genes. Deficiency in either of the NAD-dependent glycerol 3-phosphate dehydrogenase genes, GPD1 or GPD2, did not cause a significant life span change in wild type BY4741 cells. However, the deletion of either GPD1 or GPD2, led to the reversion of the longevity extension associated with *Sch9* deficiency (FIG. 8D). Similarly, the deletion of RHR2 abolished the life span extension in the *sch9Δ* mutant (FIG. 8D). By contrast, lack of Hor2, a redundant isoenzyme of DL-glycerol-3-phosphatase, did not affect the life span of the *sch9Δ* mutant. The difference between these two isoenzymes may be explained by the fact that Rhr2 is the predominant isoenzyme in the cell (Norbeck et al., 1996). In agreement with the major role of Rhr2, the mRNA level of YIG1, coding for an inhibitor of Rhr2 (Granath et al., 2005), was down-regulated in all long-lived mutants (FIG. 6B). Notably, the life span of *rhr2Δ* mutants in the BY4741 genetic background was similar to that of wild type cells although some *rhr2Δ* cultures showed regrowth/gasping (Fabrizio et al., 2004).

[0207] Cells lacking both Rhr2 and Hor2 have been shown to be hypersensitive to the superoxide anion generator, paraquat, suggesting a role for glycerol biosynthesis in cellular protection beyond osmotic stress (Pahlman et al., 2001). We tested the role of glycerol biosynthetic genes in the stress resistance of *sch9Δ* mutants. Hypersensitivity to heat and peroxide-induced oxidative stress was observed in the RHR2-null strain, but not in *gpd1Δ*, *gpd2Δ*, or *hor2Δ* mutants in the BY4741 background (FIG. 8E). Furthermore, cells lack Yig1, the Rhr2 inhibitor, were slightly more resistant to stress compared to wild type cells (FIG. 8E). The stress resistance phenotype of *sch9Δ* mutants was partially reversed by deletion of GPD1, GPD2, or RHR2 (FIG. 8E). There appears to be redundancy in glycerol-mediated response to stress such that deficiency of one enzyme can be compensated by activation of others in the glycerol biosynthesis pathway. Deletion of SCH9 greatly enhanced stress resistance to heat and H₂O₂ of *rhr2Δ* mutant, possibly due to the upregulation of the Hor2 level. Since glycerol phosphatases (Rhr2 and Hor2) are not the rate-limiting enzymes for glycerol production (Pahlman et al., 2001), upregulations of Gpd1 and Gpd2 may also contribute to the rescue of the *rhr2Δ* stress sensitive phenotype in cells lacking SCH9. A similar redundancy exists between Gpd1 and Gpd2. Although little or no effect was seen in either of the single deletion mutants, *gpd1/2Δ* double knockout strain is hypersensitive to heat and hydrogen peroxide treatment. The triple *sch9Δ gpd1Δ gpd2Δ* mutant showed severe growth defects and low saturation density in the liquid culture, which prevented us from utilizing this mutant for epistatic studies. Taken together, these results underscore the importance of glycerol biosynthesis in promoting cellular protection and life span extension in the SCH9 deficient mutants.

Mechanisms of Glycerol-Dependent Life Span Extension

[0208] Glycerol can protect against stress in part because of its function as a chemical chaperone (Meng et al., 2001; Deocaris, 2006; Wojda, 2003). To test the role of glycerol in protecting against heat-induced protein misfolding, we examined the activity loss and recovery of a heat sensitive bacterial luciferase (Parsell et al., 1994) in wild type and *sch9Δ* cells. Whereas exposing wild type cells to heat stress (55° C. for 1 hour) led to a ~80% reduction of luciferase activity, only a 20-40% loss of activity was observed in *sch9Δ* mutants (FIG. 9A), which is consistent with the enhanced stress resistance phenotype of *sch9Δ* (FIG. 4). However, pretreatment of wild type cells with low concentration of glycerol had no protective effect on the heat-induced loss and the recovery of luciferase activity (FIG. 9B), indicating the heat resistance phenotype of *sch9Δ* does not depend on extracellular glycerol. Similar results were obtained in the BY4741 genetic background.

[0209] Intracellular accumulation of glycerol also contributes to protection against osmotic stress (Albertyn et al., 1994; Wojda et al., 2003). Addition of 0.1% of glycerol to the medium slightly enhanced the resistance to osmotic stress of wild type yeast (FIG. 9C). When exposed to high concentration of NaCl, the *sch9Δ* and *ras2Δ* mutants exhibited enhanced resistance to hyperosmolarity compared to the *tor1Δ* mutant, which in turn was better protected than wild type cells (FIG. 9D), suggesting that increased resistance against hyperosmolarity may be part of the general stress response shared by all long-lived mutants. These data are also consistent with the reports that high osmolarity growth conditions extend both RLS and CLS in yeast (Kaeberlein et al., 2002; Murakami et al., 2008). With regard to life span, however, extracellular supplementation of glycerol (0.1% and 1%) to the wild type yeast culture at day 3, when the glycerol level is high in the long-lived *sch9Δ* mutants (FIG. 7B), did not show any beneficial effect (FIG. 9E).

Glycerol Provides a Carbon Source without Affecting the Anti-Aging Effect of Calorie-Restriction

[0210] Ethanol, as a carbon source, elicits pro-aging signaling and promotes cell death. Removing ethanol either by evaporation or by switching yeast cells from expired medium to water, which represents a condition of extreme calorie restriction/starvation, extends yeast chronological life span (Fabrizio et al., 2005). The metabolic switch to ethanol utilization and glycerol biosynthesis removes the detrimental effect of pro-aging carbon sources (glucose and ethanol) and creates an environment that mimics calorie restriction in the *sch9Δ* mutant culture (FIG. 7D). To elucidate the role of different carbon sources on yeast survival, we used an in situ assay to monitor cell survival on plate, which allowed us: a) to study the effect of different carbon sources in the presence of all the other nutrients, b) to control the exact amount of carbon source to which the cells are exposed over the whole experiment, similarly to the experimental conditions used for the RLS studies of calorie restriction.

[0211] One day old tryptophan auxotrophic cells were plated on SC plates lacking tryptophan (SC-Trp). Every two days, tryptophan was added to one of the set of plates generated on the same day to allow growth and monitor survival. We monitored colony formation to determine the viability of the cells. The survival curve of approximately 200 wild type DBY746 cells plated onto SC plates supplemented with 2% glucose is reminiscent of that in the standard liquid medium paradigm (FIG. 9F). Removal of carbon source from the

SC-Trp plates caused a 70% increase in mean life span, which was partially reversed by the presence of low concentration of ethanol (FIG. 9F) in agreement of our earlier findings (Fabrizio et al., 2005). Substitution of glucose with high level of glycerol (3%) did not trigger the pro-aging signaling as seen with glucose or ethanol (FIG. 9F). Thus, the metabolic switch to glycerol biosynthesis in the long-lived *sch9Δ* mutants may represent a genetically induced “carbon source substitution” that can be as effective as that of calorie restriction.

Life Span Extension after the Switch to Glycerol Medium Depends on CR-Transcription Factors

[0212] Calorie restriction-induced cellular protection and life span extension in yeast depends on the protein kinase Rim15 and its downstream stress response transcription factors Msn2/4 and Gis1, all of which are negatively regulated by Sch9, Tor, and Ras (Wei et al., 2008). When yeast were grown in isocaloric medium containing either glucose (2%) or glucose/glycerol (1% each), a 1.5-fold increase in mean life span was observed in yeast cultured in glucose/glycerol medium (FIG. 9G). This pro-longevity effect of the glucose/glycerol diet was mostly dependent, as is that of calorie restriction, on the stress response transcriptional factors (FIG. 9G).

Glycerol is Taken Up by *Sch9Δ* Mutants.

[0213] The metabolic switch in the *sch9Δ* mutants not only removes the pro-aging/death signaling from glucose/ethanol or other carbon sources but also produces a carbon source for long-term survival. We switched wild type cells from the ethanol-containing medium to water containing 0.1% glycerol. A small extension of life span was observed in addition to that of extreme calorie restriction (FIG. 9H), suggesting that glycerol may provide nutritional support or additional protection under the starvation condition. In fact, we show that yeast cells actively uptake the exogenous [1,2,3-³H] glycerol during the post-diauxic phase, entered by *S. cerevisiae* after most of the extracellular glucose is depleted (FIG. 9I). The utilization of glycerol is also supported by our microarray analysis, which shows that the genes involved in the catabolic metabolism of glycerol are up-regulated under the extreme calorie restriction/starvation (water) condition in wild type cells.

Substitution of Glycerol as Dietary Carbon Source Enhances Stress Resistance in Mice

[0214] Calorie restriction enhances stress resistance and extends life span in model organism ranging from yeast to mammals (Longo, 2003; Kennedy et al., 2007; Masoro, 2005). In view of the beneficial effects of carbon source substitution with glycerol in life span and stress resistance in yeast, we studied the effect of CSC in mice. Two groups of five mice each were fed ad libitum for six days with two isocaloric diets, the control diet (Teklad 8604 chow supplemented with 40% starch/sucrose/maltose dextrin) or the glycerol diet (supplemented with 40% glycerol). Although the mice on the glycerol diet ate slightly more than those on the control diet, they showed an 18% reduction in blood glucose level by day 6 (FIGS. 10A and 10B). Both groups of mice were then given a single dose of 50 mg/kg paraquat intraperitoneally and put back on normal diet (8604 chow). Paraquat is known to cause S-phase arrest of liver and lung cells (Matsubara et al., 1996) and lead to death (Migliaccio et al., 1999). All mice in the control group were dead by day 3, whereas three out of five glycerol-fed mice fully protected

from the paraquat toxicity (FIG. 10C, $p < 0.05$) and regained normal body weight five days after paraquat treatment (FIG. 10D). These results indicate that dietary carbon source substitution with glycerol enhances oxidative stress resistance in vivo and has the potential to mimic calorie restriction in higher eukaryotes.

DISCUSSION

[0215] Model organisms such as yeast, worms, and flies have been instrumental in the discovery of life span regulatory pathways that have a common evolutionary origin. Among these, the insulin/IGF-I-like pathways control longevity in organisms as phylogenetically distant as yeast and mice. Akt, Tor, and Ras function in the mammalian IGF-I signaling pathway and have been implicated in life span regulation in different model organisms (Kennedy et al., 2007; Longo and Finch, 2003). In this study, we show that longevity regulatory pathways control the shift from respiration to glycolysis and glycerol biosynthesis. This metabolic switch, which leads to the removal of pro-aging carbon sources and glycerol accumulation, creates an environment in the *sch9Δ* culture that mimics calorie restriction without removing the carbon source.

[0216] The genetic and genomic data revealed two parallel longevity signaling pathways controlled by Sch9 and Ras, in agreement with our previous work (Fabrizio et al., 2001). The beneficial effects of reduced activities of both pathways is additive (FIGS. 4D and 4G), and the *sch9Δ ras2Δ* double mutant is one of the longest lived genetic mutants (Partridge and Gems, 2002). In agreement with the genetic data, the gene expression profile of the day 2.5-old *ras2Δ* mutant shows that approximately 67% of the genes differentially expressed are not significantly changed in the other two mutants (FIG. 5A). Our genetic analysis of the interactions between the Tor pathway and the other two life-span regulatory pathways indicates a stronger overlap between the Tor1 and Sch9 pathways in the regulation of stress resistance, longevity, and age-dependent genomic instability. It also suggests that TORC1 functions upstream of Sch9 in the regulation of these readouts in agreement with what has been proposed by others (Jorgensen et al., 2004) and with the demonstration of the direct phosphorylation of Sch9 by TORC1 (Urban et al., 2007). Our microarray analysis indicates similarities but also differences between the set of genes controlled by Tor and Ras. On the one hand, TOR1 deletion further increased the heat-shock resistance of *ras2Δ* mutants, and on the other hand no additional life span extension was observed. Furthermore, the overexpression of constitutively active Ras2 abolished CLS extension associated with deficiency of TOR1, suggesting an overlapping of the two pathways and possibly an upstream role of TORC1.

[0217] Despite the higher degree of differential expression profile observed in *ras2Δ* mutants, there are remarkable similarities in the expression pattern of genes involved in key metabolic pathways in all three long-lived mutants. The genome-wide association (transcription factor binding motif enrichment test) and the genetic analyses indicate that longevity modulation by the Tor/Sch9 and Ras signaling depends on the protein kinase Rim15 and its downstream stress response transcription factors, Msn2/4 and Gis1 (Cheng et al., 2007; Wei et al., 2008). The most striking result is that genes involved in glycolysis/fermentation are consistently upregulated, while mitochondrial related genes are down-regulated, in all three long-lived mutants, suggesting a cellular state that

favors glycolysis and diminished mitochondrial functions including TCA cycle and oxidative phosphorylation. Part of our results may appear to contradict recent results showing that respiration is upregulated in the *tor1Δ* mutant (Bonawitz et al., 2007). This discrepancy may be explained by the difference in the time point of observation. Bonawitz and colleagues measured higher respiration rates in exponentially growing or day 1 *tor1Δ* cultures relative to wild type yeast. By day 2 this difference was no longer observed (Bonawitz et al., 2007). The role of respiration in replicative life span regulation is still unclear. On the one hand, increased respiration has been shown to mediate the beneficial effect of CR (0.5% glucose) (Lin et al., 2002); on the other hand, growth on lower glucose-containing medium (0.05% glucose) can extend the replicative life span of respiratory-deficient yeast (Kaeberlein et al., 2005a). Moreover, the studies from Jazwinski's group indicated that respiration does not directly affect replicative longevity (Kirchman et al., 1999). The different effect of respiration on life span may also be contributed to the experimental systems used for life span studies. The replicative life span analysis is mostly carried out on the solid rich YPD medium, where cells are constantly exposed to glucose and other nutrients. The energy required for growth is mainly derived from fermentation. In contrast, our chronological longevity studies are performed by monitoring population survival in a non-dividing phase in which fermentation is minimized (Fabrizio and Longo, 2003).

[0218] The gene expression profiles of long-lived mutants showed induction of the expression of key genes required for glycerol biosynthesis. High levels of extracellular and intracellular glycerol were detected in the *sch9Δ* culture and triglyceride catabolism appeared to contribute to glycerol generation (FIG. 7). This shift towards the production of glycerol represents a fundamental metabolic change in the physiology of the long-lived mutants. Interestingly, mutants lacking Sir2, another gene implicated in CR-dependent and—independent life span regulation (Kaeberlein et al., 2005a; Kaeberlein et al., 1999; Lin et al., 2000), also deplete the pro-aging carbon source ethanol (Fabrizio et al., 2005). Expression profile analysis of the *sir2Δ* mutant, like the *sch9Δ* mutant, shows upregulation of glycerol biosynthetic genes, suggesting a role of glycerol biosynthesis in the Sir2-dependent life span regulation (Fabrizio et al., 2005).

[0219] Genetic analysis performed by deleting genes required for glycerol biosynthesis in the *sch9Δ* mutant indicates that glycerol production is required for life span regulation and stress resistance (FIG. 8). Increased glycerol biosynthesis may contribute to life span regulation through several distinct mechanisms. First, cells lacking Sch9 utilize glucose and ethanol and accumulate glycerol, a non-pro-aging carbon source, which effectively leads to a "self-imposed" CSS. CR, achieved by lowering glucose in growth medium or removing ethanol extends the yeast CLS (Fabrizio et al., 2005; Smith et al., 2007; Wei et al., 2008). Conversely, addition of low concentration of ethanol reverses life span extension induced by CR or deletion of SCH9 (Fabrizio et al., 2005). Here we show that cells lacking Sch9 deplete pro-aging carbon sources and activate glycerol biosynthesis. In addition to acting as a "phantom carbon source" that does not promote aging as glucose or ethanol, glycerol caused a minor but further enhancement of survival of cells under starvation conditions, suggesting that it provides nutritional support, which was confirmed by its uptake by non-dividing cells (FIGS. 9H and 9I). Second, production and accumulation of

glycerol may contribute- to cellular protection since glycerol enhances resistance to osmotic stress and functions as molecular chaperone stabilizing/renaturing the newly synthesized or heat-inactivated proteins. Third, glycerol production may affect aging through the modulation of the redox balance of the cell, since its production contributes to the maintenance of the cell.

[text missing or illegible when filed]

1. A dietary composition comprising:

0-0.2% (by weight) L-methionine;

L-tryptophan, L-isoleucine, L-leucine, L-lysine, L-phenylalanine, L-threonine, and L-valine in the amount of at least 0.05% (by weight) each; and

no protein.

2. The composition of claim 1, further comprising one or more amino acids selected from the group consisting of L-alanine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, L-glycine, L-proline, L-serine, L-tyrosine, L-arginine, and L-histidine.

3. A method of protecting an animal or human against chemotherapy, comprising administering the composition of claim 1 to an animal or human, thereby protecting the animal or human against chemotherapy.

4. The method of claim 3, further comprising exposing the animal or human to the chemotherapy.

5. The method of claim 4, wherein the composition is administered to the animal or human for 3-10 consecutive days prior to the exposing step, 24 hours following the exposing step, or a combination thereof.

6. A dietary composition comprising:

0-0.2% (by weight) L-tryptophan;

L-methionine, L-isoleucine, L-leucine, L-lysine, L-phenylalanine, L-threonine, L-valine in the amount of at least 0.05% (by weight) each; and

no protein.

7. The composition of claim 6, further comprising one or more amino acids selected from the group consisting of L-alanine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, L-glycine, L-proline, L-serine, L-tyrosine, L-arginine, and L-histidine.

8. A method of protecting an animal or human against chemotherapy, comprising administering the composition of claim 6 to an animal or human, thereby protecting the animal or human against chemotherapy.

9. The method of claim 8, further comprising exposing the animal or human to the chemotherapy.

10. The method of claim 9, wherein the composition is administered to the animal or human for 3-10 consecutive days prior to the exposing step, 24 hours following the exposing step, or a combination thereof.

11. A dietary composition comprising:

L-methionine, L-tryptophan, L-isoleucine, L-leucine, L-lysine, L-phenylalanine, L-threonine, L-valine, L-alanine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, L-glycine, L-proline,

L-serine, L-tyrosine, L-arginine; and L-histidine in the amount of 0-0.2% (by weight) each; and

no protein.

12. A method of protecting an animal or human against chemotherapy, radiotherapy, oxidative stress, or aging, comprising administering the composition of claim 11 to an animal or human, thereby protecting the animal or human against chemotherapy, radiotherapy, oxidative stress, or aging.

13. The method of claim 12, further comprising exposing the animal or human to the chemotherapy, radiotherapy, or oxidative stress.

14. The method of claim 13, wherein the composition is administered to the animal or human for 3-10 consecutive days prior to the exposing step, 24 hours following the exposing step, or a combination thereof.

15. The method of claim 12, wherein the composition is administered every third meal or every 3-10 days to protect the animal or human against aging.

16. A dietary composition comprising glycerol as a substitute for monosaccharides, disaccharides, and polysaccharides.

17. A method of protecting an animal or human against chemotherapy or oxidative stress, comprising administering the composition of claim 16 to an animal or human, thereby protecting the animal or human against chemotherapy or oxidative stress.

18. The method of claim 17, further comprising exposing the animal or human to the chemotherapy or oxidative stress.

19. The method of claim 18, wherein the composition is administered to the animal or human for 3-10 consecutive days prior to the exposing step, 24 hours following the exposing step, or a combination thereof.

20. A hypocaloric or calorie free diet comprising: dietary materials capable of providing nutrition to a human subject while providing no more than 813-957 kcal total energy, no more than half of which is in carbohydrates if the carbohydrates are present in the dietary materials, wherein the dietary materials include no more than 30-36 g protein.

21. The diet of claim 20, wherein the dietary materials are capable of providing no more than 700 kcal total energy.

22-26. (canceled)

27. A method of protecting an animal or human against chemotherapy, comprising:

fasting an animal or human suffering from cancer for 48-140 hours prior to one round of chemotherapy, 4-56 hours following the chemotherapy, or a combination thereof; and

exposing the animal or human to the chemotherapy.

28. The method of claim 27, wherein the animal or human is fasted for no more than 180 hours prior to and following one round of chemotherapy.

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