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(54) **Title**: FASTING MIMICKING DIET (FMD) AND GLUCOSE LOWERING DRUGS PROTECT NORMAL CELLS AND GENERATE CANCER SENSITIZING CONDITIONS IN RESPONSE TO STANDARD AND HIGH GLUCOSE CONDITIONS INDUCED BY RAPAMYCIN AND DEXAMETHASONE

(57) **Abstract**:

FASTING MIMICKING DIET (FMD) AND GLUCOSE LOWERING DRUGS PROTECT NORMAL CELLS AND GENERATE CANCER SENSITIZING CONDITIONS IN RESPONSE TO STANDARD AND HIGH GLUCOSE CONDITIONS INDUCED BY RAPAMYCIN AND DEXAMETHASONE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. provisional application Serial No. 62/148,451 filed April 16, 2015, the disclosure of which is hereby incorporated in its entirety by reference herein.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] The invention was made with Government support under Contract No. 1PO1AG034906 awarded by the National Institutes of Health. The Government has certain rights to the invention.

TECHNICAL FIELD

[0003] In at least one aspect, the present invention relates to methods to protect normal tissues from increased toxicity/sensitization to chemotherapy drugs induced by the raised circulating glucose levels upon administration of rapamycin and the palliative drug dexamethasone.

BACKGROUND

[0004] Cancer management and treatment has significantly improved over the past century. However, the standard of care is still predominantly uses chemotherapy, radiotherapy or their combination. Both modes of treatment are associated with a multitude of side effects ranging from discomfort to development of secondary cancers and organ toxicity especially heart and liver. To increase efficacy of cancer treatment and to help with management of symptoms, other drugs such as dexamethasone are often used in combination with chemo- and radio-therapy. Dexamethasone (Dexa), commonly combined with chemotherapy, is often used as a

palliative drug that has also been shown to be effective in treating multiple myeloma, leukemia, and lymphoma. However, treatment with Dexa can be causative of a number of side effects including, fluid retention, weight gain, heartburn, insomnia and elevated levels of blood glucose.

[0005] It has previously been shown that Short-Term Starvation (STS) is an effective practice to ease the discomfort associated with cancer treatment while increasing the efficacy of such treatments. Moreover, it has been demonstrated that STS regimens are an effective method in protecting normal cells and tissues during chemotherapy (Differential Stress Resistance or DSR) (Raffaghello *et.al., PNAS.* 2008; PMID: 18378900, and Lee *et.al. Cancer Research.* 2010; PMID: 20145127).

[0006] It has been previously shown that fasting can sensitize cancer cells but not normal cells to chemotherapy, a phenomena referred to as Differential Stress Sensitization (DSS), which efficacy has been attributed to the reduction in circulating glucose and IGF-1 levels (**Figure 2**) (Lee *et.al. Sci Transl Med.* 2012; PMID: 22323820). However, a 10- to 14-day re-feeding period between fasting cycles is needed to recover the body weight loss.

[0007] 5' AMP-activated protein kinase (AMPK) is an enzyme up-regulated during STS/FMD regimen and which plays a role in cellular energy homeostasis and has been associated with lifespan extension. AMPK is also considered a metabolic tumor suppressor (Luo *et.al. Future Oncol.* 2010; PMID: 20222801). Metformin, is an AMPK activator that leads to the reduction of circulating glucose (**Figure 1E**) and has potential for the treatment/prevention of cancer.

[0008] The central players that regulate metabolism in all living cells do so by modulating normal-cell growth in part by regulating serine/threonine protein kinases, which has led to the modified standard of care that included administration of kinases inhibitors such as rapamycin (Rapa) in combination with chemotherapy. Kinases and other signal transduction inhibitors can delay cancer growth and are widely used but, like dexamethasone, can also cause major side effects to normal cells.

[0009] Accordingly, there is a need for treatment protocols that (i) mitigate the side effects associated with the adjunct drugs used in chemotherapy, and (ii) can maintain reduced

glucose levels during the "re-feeding" period between STS cycles or to substitute STS/FMD and sensitize cancer cells.

SUMMARY

[0010] The present invention solves one or more problems of the prior art by providing, in at least one embodiment, a method for treating hyperglycemia or reducing glycemia in a subject undergoing chemotherapy or other cancer therapy. The method includes a step of identifying a subject undergoing chemotherapy and being administered a hyperglycemia-inducing agent. Short-term starvation, a fasting mimicking diet, or insulin are administered for a first time period to the subject to prevent or reverse hyperglycemia and sensitization to chemotherapy associated with increased glucose levels.

[0011] Various embodiments of the invention, alleviate or treat symptoms of chemotherapy which can be worsened by the complementary administration of rapamycin and the steroid medication dexamethasone. It is shown below (**Figure 1B-C)** that the administration of dexamethasone and rapamycin (**Figure 1B, D)** for the treatment of chemotherapy-associated side can cause sensitization of animals to chemotherapy. As shown below (**Figure 1B-D**), the administration of insulin to reduce circulating glucose levels in control mice, as well as in animals undergoing Rapa and Dexa treatment, can reverse the toxicity of doxorubicin and of other chemotherapy drugs. Because of the wide use of rapamycin and dexamethasone for the treatment of certain tumors in humans, these results have important implications for the safety of patients and efficacy of those therapies.

[0012] Because of its effects in reducing circulating glucose levels (**Figure 1E**) and up-regulating AMPK, which we have shown to inactivate PKA signaling, Metformin has the potential to be used as a STS-mimicking drug to (i) reverse the hyperglycemia-associated cytotoxic effects of chemotherapy and, when administered during the re-feeding period by both acting on glucose levels and PKA, to (ii) potentiate/prolong the effect of STS in reducing the tumor-progression, again by acting on both glucose and AMPK-PKA signaling. Thus, metformin can promote both differential stress resistance and differential stress sensitization by both reducing glucose levels and acting on PKA signaling as described in Raffaghello *et.al., PNAS.* 2008; PMID: 18378900 and Lee *et.al. Sci Transl Med.* 2012; PMID: 22323820.

[0013] In another embodiment, a method of replacing or enhancing effects of a fasting mimicking diet (FMD) on cancer cell sensitization is provided. The method includes a step of identifying a subject receiving chemotherapy or another cancer therapy. Metformin is then administered to the subject by administering to the subject.

[0014] In another embodiment, a method of promoting differential stress is provided. The method includes a step of identifying a subject with one or more of breast cancer, ovarian cancer, colorectal cancer, melanoma, prostate cancer, cervical cancer, epidermoid carcinoma, neuroblastoma, or any additional cancer type. Metformin is administered to the subject to reduce glucose levels and promote differential stress sensitization to specifically kill cancer but not normal cells.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] **FIGURE 1A, 1B, 1C, 1D and 1E**. Increase in circulating glucose levels mediates the sensitization of the host to chemotherapy. Administration of rapamycin (Rapa) or dexamathasone (Dexa) (**A**) caused an increase in glucose levels that was significantly reduced by insulin and, even more, by STS (**B**). Asterisks in **B** indicate the significance of each group compared to the ad lib (AL) group. The significance of each group compared to its internal control is indicated with daggers (i.e. the daggers on Rapa + ins and Dexa + ins indicate the significance compared to AL + ins). For the stress resistance experiment shown in **C** and **D** we followed the schedule shown in **A**. Rapamycin and Dexamethasone where administrated ip for 14 days prior DXR injection (day 0). Following the administration of 24 mg/kg of DXR, the animals where monitored for signs of distress and the survival was recorded (**C** and **D**). STS, ad lib, and ad lib + ins groups reported in **C** and **D** were shared groups and have the same values in both graphs. (**E**) Blood glucose levels in mice injected ip with metformin- 50 mg/kg (saline for control mice). One-way ANOVA test was performed and differences with p-value<0.05 were considered significant (p-value<0.05, 0.01 and 0.001 are indicated as *, *, and ***, respectively).

[0016] **FIGURE 2.** Effect of glucose restriction on DXR sensitivity of 9 different mouse and human cancer cell lines. Control groups were cultured in DMEM supplemented with 2.0 g/L glucose, while the glucose restriction groups were cultured in DMEM supplemented with 0.5 g/L glucose. Survival was determined by MTT reduction. The cancer cell line tested were: 4T1

(mouse breast cancer), B16 (mouse melanoma), GL26 (mouse glioma), C42B (human prostate cancer), MCF-7 (human breast cancer), HeLa (human cervical cancer), A431 (human epidermoid carcinoma), ACN (human neuroblastoma), and MZ2-MEL (human melanoma).

[0017] **TABLE 1.** Micronutrient content provided by the Fasting Mimicking Diet (FMD)

DETAILED DESCRIPTION

[0018] Reference will now be made in detail to presently preferred compositions, embodiments and methods of the present invention which constitute the best modes of practicing the invention presently known to the inventors. The Figures are not necessarily to scale. However, it is to be understood that the disclosed embodiments are merely exemplary of the invention that may be embodied in various and alternative forms. Therefore, specific details disclosed herein are not to be interpreted as limiting, but merely as a representative basis for any aspect of the invention and/or as a representative basis for teaching one skilled in the art to variously employ the present invention.

[0019] Except in the examples, or where otherwise expressly indicated, all numerical quantities in this description indicating amounts of material or conditions of reaction and/or use are to be understood as modified by the word "about" in describing the broadest scope of the invention. Practice within the numerical limits stated is generally preferred. Also, unless expressly stated to the contrary: percent, “parts of,” and ratio values are by weight; the description of a group or class of materials as suitable or preferred for a given purpose in connection with the invention implies that mixtures of any two or more of the members of the group or class are equally suitable or preferred; description of constituents in chemical terms refers to the constituents at the time of addition to any combination specified in the description and does not necessarily preclude chemical interactions among the constituents of a mixture once mixed; the first definition of an acronym or other abbreviation applies to all subsequent uses herein of the same abbreviation and applies *mutatis mutandis* to normal grammatical variations of the initially defined abbreviation; and, unless expressly stated to the contrary, measurement of a property is determined by the same technique as previously or later referenced for the same property.

[0020] It is also to be understood that this invention is not limited to the specific embodiments and methods described below, as specific components and/or conditions may, of course, vary. Furthermore, the terminology used herein is used only for the purpose of describing particular embodiments of the present invention and is not intended to be limiting in any way.

[0021] It must also be noted that, as used in the specification and the appended claims, the singular form "a," "an," and "the" comprise plural referents unless the context clearly indicates otherwise. For example, reference to a component in the singular is intended to comprise a plurality of components.

[0022] Throughout this application, where publications are referenced, the disclosures of these publications in their entireties are hereby incorporated by reference into this application to more fully describe the state of the art to which this invention pertains.

[0023] Abbreviations:

[0024] "AL" mean *ad lib*.

[0025] "FMD" means fasting mimicking diet.

[0026] "STS" means short-term starvation.

[0027] "DSR" means differential stress resistance.

[0028] "DSS" means differential stress seneitization.

[0029] "DXR" means doxorubicin.

[0030] "Rapa" means rapamycin.

[0031] "Dexa" means dexamethasone.

[0032] "ins" means insulin.

[0033] "AMPK" means 5' AMP-activated protein kinase.

[0034] "ip" means intraperitoneal.

[0035] The terms "kilocalorie" (kcal) and "Calorie" refer to the food calorie.

[0036] The term "calorie" refers to the so-called small calorie.

[0037] The term "subject" refers to a human or animal, including all mammals such as primates (particularly higher primates), sheep, dog, rodents (e.g., mouse or rat), guinea pig, goat, pig, cat, rabbit, and cow.

[0038] The term "fasting mimicking diet" means a diet that provides the subject with a calorie restricted diets formulated in a way to generate changes in glucose, ketone bodies, IGF-1 and IGFBP1 similar to those caused by fasting but able to provide high nourishment and minimize hunger.

[0039] In an embodiment, methods for treating hyperglycemia in a subject undergoing chemotherapy are provided. The method includes a step of identifying a subject undergoing chemotherapy and being administered a hyperglycemia-inducing agent. Short-term starvation, a fasting mimicking diet (FMD) or insulin are administered for a first time period to the subject to prevent or reverse hyperglycemia and sensitization to chemotherapy associated with increased glucose levels. In the context of the present embodiment, preventing hyperglycemia or sensitization means reducing the probability that these side effect will occur. In general, the FMD diet provides less than about 1000 kilocalories per day, while STS provides no calories when administered. In a refinement, the hyperglycemia-inducing agent is a kinase inhibitor or a corticosteroid. Specific examples of hyperglycemia-inducing agent include, rapamycin, steroid medications including dexamethasone, and the like, and combinations thereof. In a refinement, short-term starvation or a fasting mimicking diet is repeated a plurality of times at predetermined intervals. For example, short-term starvation or a fasting mimicking diet can be repeated at intervals from two weeks to 2 months. Typically, he subject is administered a normal diet i.e., re-feeding period) in between these repetitions. In this context, a normal diet is a diet of sufficient caloric intake to maintain the patient weight. In a refinement, the normal caloric intake provides the subject with 1500 to 2500 kcal or 1800 to 2300 kcal, or 1800 to 2000 kcal.

[0040] Examples of STS protocols are found in U.S. Pat. Appl. Nos. 12/430,058 and 13/488,590; the entire disclosures of which are hereby incorporated by reference. In a variation,

the STS diet provides a hypo-caloric 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. In a refinement, the STS/FMD diet may be administered to the subject for 3-10 consecutive days prior to when the subject is exposed to chemotherapy. The diet may also be administered to the subject for 24 hours following the exposure. Preferably, the diet may be administered to the subject for both 3-10 consecutive days prior to when the subject is exposed to chemotherapy and 24 hours following the exposure.

[0041] In another variation, the STS diet provides 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 subject 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 subject is exposed to chemotherapy, 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 subject's exposure to chemotherapy. The diet may also be administered to the subject for 24 hours following the exposure. Preferably, the diet may be administered to the subject for both 3-10 consecutive days prior to the subject's exposure to chemotherapy and 24 hours following the exposure.

[0042] In another variation, the STS/FMD protocol involves fasting mimicking diets. For example, the subject suffering from cancer may be fasted for 48-140 hours prior to one round of chemotherapy or 4-56 hours following the chemotherapy. Preferably, the subject suffering from cancer is given a FMD for 48-140 hours prior to one round of chemotherapy and 4-56 hours following the chemotherapy.

[0043] Examples of FMD diets are found in U.S. Pat. Appl. Nos. 14060494 and 14178953 and WIPO Pub. No. WO2011/050302 and WIPO Pub. No. WO2011/050302; the

entire disclosures of which are hereby incorporated by reference. Typically, in the FMD protocol a subject's diet is substituted for a predetermined number of days (i.e. 5 days). During this period, subjects consume plenty of water. For healthy subjects of normal weight (Body Mass Index or BMI between 18.5-25), the diet is consumed once a month (5 days on the diet and 25-26 days on their normal diet) for the first 3 months and every 3 months thereafter (5 days every 3 months). The weight of the subject is measured and the subject must regain at least 95% of the weight lost during the diet before the next cycle is begun. Subjects with BMI of less than 18.5 should not undertake the FMD unless recommended and supervised by a physician. The same regimen (once every month for 3 months followed by once every 3 months thereafter) can be adopted for the treatment, or in support of the treatment, of all of the conditions presented in the patent applications. U.S. Pat. Appl. No. 14178953 provides a low protein version of the FMD diet.

[0044] In one variation, the FMD set forth in U.S. Pat. Appl. Nos. 12/430,058 is used in the methods set forth above. This diet includes nutrition facts relative to calories, macronutrients and micronutrients. Calories are consumed according to the user's body weight. Total calorie consumption is 4.5-7 calorie per pound (or 10-16 calorie per kilogram) for day 1 and 3-5 calorie per pound (or 7-11 calorie per kilogram) for day 2 to 5. Figures 12-14 provides listings of the nutrients for day one through day five. In addition to the macronutrients, the diet should contain less than 30 g of sugar on day 1 and less than 20 g of sugar on days 2-5. The diet should contain less than 28 g of proteins on day 1 and less than 18 g of proteins on days 2-5. The diet should contain between 20 and 30 grams of monounsaturated fats on day 1 and 10-15 grams of monounsaturated fats on days 2-5. The diet should contain between 6 and 10 grams of polyunsaturated fats on day 1 and 3-5 grams of polyunsaturated fats on days 2-5. The diet should contain less than 12 g of saturated fats on day 1 and less than 6 grams of saturated fats on days 2-5. Typically, the fats on all days are derived from a combination of the following: Almonds, Macadamia Nuts, Pecans, Coconut, Coconut oil, Olive Oil and Flaxseed. In a refinement, the FMD diet includes over 50% of the recommended daily value of dietary fiber on all days. In the further refinement, the amount of dietary fiber is greater than 15 grams per day on all five days. The diet should contain 12-25 grams of glycerol per day on days 2-5. In a refinement, glycerol is provided at 0.1 grams per pound body weight/day.

[0045] In a refinement, the FMD includes the following micronutrients (at least 95% non-animal based): over 5,000 IU of vitamin A per day (days 1-5); 60-240 mg of vitamin C per day (days 1-5); 400-800 mg of Calcium per day (days 1-5); 7.2-14.4 mg of Iron per day (days 1-5); 200-400 mg of Magnesium per day (days 1-5); 1-2 mg of copper per day (days 1-5); 1-2 mg of Manganese per day (days 1-5); 3.5-7 mcg of Selenium per day (days 1-5); 2-4 mg of Vitamin B1 per day (days 1-5); 2-4 mg of Vitamin B2 per day (days 1-5); 20-30 mg of Vitamin B3 per day (days 1-5); 1-1.5 mg of Vitamin B5 per day (days 1-5); 2-4 mg of Vitamin B6 per day (days 1-5); 240-480 mcg of Vitamin B9 per day (days 1-5); 600-1000 IU of Vitamin D per day (days 1-5); 14-30 mg of Vitamin E per day (days 1-5); over 80 mcg of Vitamin K per day (days 1-5); 16-25 mcg Vitamin B12 are provided during the entire 5-day period; 600 mg of Docosahexaenoic acid (DHA, algae-derived) are provided during the entire 5-day period. The FMD diet provides high micronutrient content mostly (i.e., greater than 50 percent by weight) from natural sources including: Kale, Cashews, Yellow Bell Pepper, Onion, Lemon Juice, Yeast, Turmeric. Mushroom, Carrot, Olive Oil, Beet Juice, Spinach, Tomato, Collard, Nettle, Thyme, Salt, Pepper, Vitamin B12 (Cyanocobalamin), Beets, Butternut Squash, Collard, Tomato, Oregano, Tomato Juice, Orange Juice, Celery, Romaine Lettuce, Spinach, Cumin, Orange Rind, Citric Acid, Nutmeg, Cloves, and combinations thereof. Table 1 provides an example of additional micronutrient supplementation that can be provided in the FMD diet:

[0046] Table 1. Micronutrient Supplementation

	Supplement	**Formula**	**Amount**	**Amount Range**	**Unit**
Vit A			1250 IU	900-1600	IU
Vit C	Ascorbic Acid	$C_6H_8O_6$	*15.0000*	10-20	mg
Ca	Calcium Carbonate	$CaCO_3$	*80.0000*	60-100	mg
Fe	Ferrous Fumarate	$C_4H_2FeO_4$	*4.5000*	3-6	mg
Vit D3	Cholecalciferol	$C_{27}H_{44}O$	*0.0025*	0.001-0.005	mg
Vit E	dl-Alpha Tocopheryl Acetate	$C_{29}H_{50}O_2$	*5.0000*	3-7	mg
Vit K	Phytonadione		*0.0200*	0.1-0.04	mg

Vit B1	Thiamine Mononitrate	$C_{12}H_{17}N_5O_4S$	*0.3750*	0.15-0.5	mg
Vit B2	Riboflavin E101	$C_{17}H_{20}N_4O_6$	*0.4250*	0.2-0.6	mg
Vit B3	Niacinamide	$C_6H_6N_2O$	*5.0000*	3-7	mg
Vit B5	Calcium Pantothenate	$C_{18}H_{32}CaN_2O_{10}$	*2.5000*	1.5-4.0	mg
Vit B6	Pyridoxine Hydrochloride	$C_8H_{11}NO_3 \cdot HCl$	*0.5000*	0.3-0.7	mg
Vit B7	Biotin	$C_{10}H_{16}N_2O_3S$	*0.0150*	0.01-0.02	mg
Vit B9	Folic Acid	$C_{19}H_{19}N_7O_6$	*0.1000*	0.07-0.14	mg
Vit B12	Cyanocobalamin	$C_{63}H_{88}CoN_{14}O_{14}P$	*0.0015*	0.001-0.002	mg
Cr	Chromium Picolinate	Cr(C6H4NO2)3	*0.0174*	0.014-0.022	mg
Cu	Cupric Sulfate	CuSO4	*0.2500*	0.18-0.32	mg
I	Potassium Iodide	KI	*0.0375*	0.03-0.045	mg
Mg	Magnesium Oxide	MgO	*26.0000*	20-32	mg
Mn	Manganese Sulfate	$MnSO_4$	*0.5000*	0.3-0.7	mg
Mo	Sodium Molybdate	Na_2MoO_4	*0.0188*	0.014-0.023	mg
Se	Sodium Selenate	Na_2O_4Se	*0.0175*	0.014-0.023	mg
Zn	Zinc Oxide	**Zn**O	*3.7500*	3-5	mg

[0047] In another embodiment, a diet package for implemented the method forth above is provided. The diet package includes a first set of rations for a first diet to be administered for a first time period to a subject, the first diet providing from 4.5 to 7 kilocalories per pound of subject for a first day and 3 to 5 kilocalories per pound of subject per day for a second to fifth day of the first diet. The diet package includes rations that provide less than 30 g of sugar on the first day; less than 20 g of sugar on the second to fifth days; less than 28 g of proteins on the first day; less than 18 g of proteins on days the second to fifth days; 20 to 30 grams of monounsaturated fats on the first day; 10 to15 grams of monounsaturated fats on the second to fifth days; between 6 and 10 grams of polyunsaturated fats on the first day; 3 to 5 grams of

polyunsaturated fats on the second to fifth days; less than 12 g of saturated fats on the first day; less than 6 grams of saturated fats on the second to fifth days; and 12 to 25 grams of glycerol per day on the second to fifth days. In a refinement, the diet package further includes sufficient rations to provide the micronutrients set forth above. In a further refinement, the diet package provides instructions providing details of the methods set forth above.

[0048] In refinement of the embodiments set forth above, a 5-day supply of diet includes: soups/broths, soft drinks, nut bars and supplements. The diet is administered as follows: 1) on the first day a 1000-1200 kcal diet with high micronutrient nourishment as set forth above is provided; 2) for the next 4 days a daily diet of 650- 800 kcal plus a drink containing a glucose substitution carbon source providing between 60-120 kcal are provided.

[0049] In another refinement of the embodiments set forth above, a 6-day low-protein diet protocol includes: soups/broths, soft drinks, nut bars, and supplements. The diet is administered as follows: 1) on the first day a 1000-1200 kcal diet plus with high micronutrient nourishment is provided; 2) for the next 3 days a daily diet of less than 200 kcal plus a drink containing a glucose substitution carbon source providing between 60 and 120 kcal. This substitution carbon source does not interfere with the effect of fasting on stem cell activation; 3) on the 5th day the subject consumes a normal diet; and 4) on day 6 an additional replenishment foods consisting of a high fat source of 300 kcal and a micronutrient nourishment mix on day 6 replenishment foods consisting of a high fat source of 300 kcal and a micronutrient nourishment mix are provided in addition to normal diet.

[0050] In still another refinement, a diet protocol includes: 6-day supply of low-protein diet includes: soups/broths, soft drinks, nut bars, and supplements. 1) on the first day a 1000-1200 kcal diet with high micronutrient nourishment is provided; 2) for the next 3 days a daily diet of 600 to 800 kcal which contains less than 10 grams of protein and less than 200 kcal from sugars; 3) on the 5th day the subject receives a normal diet; and 4) on day 6 an additional replenishment foods consisting of a high fat source of 300 kcal and a micronutrient nourishment mix on day 6 replenishment foods consisting of a high fat source of 300 kcal and a micronutrient nourishment mix are provided in addition to normal diet.

[0051] Although the FMD diet encompasses virtually any source of fat, sources high in unsaturated fat, including monounsaturated and polyunsaturated fat sources, are particularly useful (e.g., omega-3/6 essential fatty acids). Suitable examples of monounsaturated food sources include, but are not limited to, peanut butter, olives, nuts (e.g., almonds, pecans, pistachios, cashews), avocado, seeds (e.g., sesame), oils (e.g., olive, sesame, peanut, canola), etc. Suitable examples of polyunsaturated food sources include, but are not limited to, walnuts, seeds (e.g., pumpkin, sunflower), flaxseed, fish (e.g., salmon, tuna, mackerel), oils (e.g., safflower, soybean, corn). The first diet also includes a component selected from the group consisting of vegetable extracts, minerals, omega-3/6 essential fatty acids, and combinations thereof. In one refinement, such a vegetable extract provides the equivalent of 5 recommended daily servings of vegetables. Suitable sources for the vegetable extract include, but are not limited to, bokchoy, kale, lettuce, asparagus, carrot, butternut squash, alfalfa, green peas, tomato, cabbage, cauliflower, beets. Suitable sources for the omega-3/6 essential fatty acids include fish such as salmon, tuna, mackerel, bluefish, swordfish, and the like.

[0052] In another variation, a method based on the administration of Metformin (*N,N*-Dimethylimidodicarbonimidic diamide) to mimic the effects of fasting to reverse the hyperglycemia-associated cytotoxic effects of chemotherapy and/or to potentiate/prolong the effect of STS in reducing the tumor-progression when administered during the re-feeding period is provided. The method includes a step of identifying a subject undergoing chemotherapy and having hyperglycemia and/or being administered a hyperglycemia-inducing agent as set forth above. Metformin is administered to the subject to reverse the cytotoxic effects. Metformin is administered in a dosage range from 1 to 2.5 mg/day depending on the response of the patient to the drug. The Metformin can be administered for 1 day, 1 to 5 days, 1 to 10 days, or 1 to 14 days or more depending on the subject's response. The In another variation, a method for treating hyperglycemia or the negative effects of normo-glycemia in a subject undergoing chemotherapy or another cancer therapy is provided. The method includes a step of identifying a subject undergoing chemotherapy and being administered a hyperglycemia-inducing agent. Short-term starvation or a fasting mimicking diet, or insulin is administered for a first time period to the subject to prevent or reduce glucose levels and sensitize cancer cells to chemotherapy or other cancer therapy. The details of this variation regarding the administration of short-term starvation or a fasting mimicking diet are the same as those set forth above. During a re-feeding period, a

normal diet is administered to the subject in between administration of the short-term starvation or a fasting mimicking diet also as set forth above. Metformin is administered to the subject during this re-feeding period. Metformin is administered in a dosage range from 1 to 2.5 mg/day depending on the response of the patient to the drug. The Metformin can be administered for 1 day, 1 to 5 days, 1 to 10 days, or 1 to 14 days or more depending on the subject's response. The steps of administration of short-term starvation or a fasting mimicking diet and administering the re-feeding period with Metformin administration is repeated is repeated a plurality of times at predetermined intervals. As set forth above, in a refinement, these steps are repeated at intervals from two weeks to 2 months.

[0053] In another embodiment, a method of replacing or enhancing the effect of the FMD on cancer cell sensitization is provided. The method includes a step of identifying a subject receiving chemotherapy or another cancer therapy. Metformin is then administered to the subject by administering to the subject. In a refinement, Metformin is administered in a dosage range from 1 to 2.5 mg/day depending on the response of the patient to the drug. The Metformin can be administered for 1 day, 1 to 5 days, 1 to 10 days, 1 to 14 days or 1 to 60 days or more depending on the subject's response.

[0054] In another embodiment, a method of promoting differential stress is provided. The method includes a step of identifying a subject with one or more of breast cancer, ovarian cancer, colorectal cancer, melanoma, prostate cancer, cervical cancer, epidermoid carcinoma, neuroblastoma, or any additional cancer type. Metformin is administered to the subject to reduce glucose levels and promote differential stress sensitization to specifically kill cancer but not normal cells. Metformin is administered in a dosage range from 1 to 2.5 mg/day depending on the response of the patient to the drug. The Metformin can be administered for 1 day, 1 to 5 days, 1 to 10 days, 1 to 14 days, or 1 to 60 days or more depending on the subject's response.

[0055] 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.

[0056] The present invention has been tested in *in vitro* and *in vivo* murine models. STS and FMD have also been tested in different clinical trials which have shown the safety and feasibility of the two dietary interventions. FMD diet has shown to be as effective as STS in evoking DSR.

[0057] Methods: Rapamycin or Dexamethasone were daily administrated intraperitoneally (ip) for a period of 14 days prior the beginning of Short term starvation (STS) or fasting mimicking diet (FMD). The end of the dietary intervention coincided with the administration of doxorubicin by intravenous injection. Mice in the insulin (ins) groups also received insulin injection every 12h for the 48h preceding doxorubicin administration. The animals were then being observed for sign of pain or distress for the following days and the survival was recorded (**Figure 1A, C, and D**).

[0058] Metformin (50 mg/kg) was diluted in saline and administrated by intraperitoneal (*i.p.*) injection. Circulating glucose levels were monitored following metformin administration (**Figure 1E**).

[0059] Diet (mouse): Mice were maintained on irradiated TD.7912 rodent chow (Harlan Teklad). In brief, this diet contains 3.T5kcal/g of digestible energy with calories supplied by protein, carbohydrate and fat in a percent ratio of 25: 58: 17. Food was provided ad lib. On average, mice in the control group consumed 14.9 kcal/day (or 3.9 g/day), Our experimental FMD diet is based on a nutritional screen that identified ingredients allowing high nourishment during periods of low calorie consumption (Brandhorst, Wei et al., 2013). Prior to supplying the FMD diet, animals were transferred into fresh cages to avoid feeding on residual chow and coprophagy. The FMD diet consists of two different components designated as day 1 diet and day 2-4 diet that were fed in this order, respectively. The day 1 diet contains 1.88 kcal/g and was designed to adapt the mouse to a period of low caloric intake during the subsequent feeding days. The day 2-4 diet is identical on all feeding days and contains 0.36 kcal/g. The day 1 and days 2-4 diets were fed as the average intake (~4 g) of the *ad lib* fed control group every two weeks. Due to the different caloric densities of the supplied FMD diet, mice in this cohort had a ~50% reduction in consumed calories on day 1 and consumed 9.7% of the control cohort on days 2 to 4. Mice consumed all the supplied food on each day of the FMD regimen and showed no signs of

food aversion. After the end of the day 2-4 diet, we supplied TD.7912 chow *ad lib* for 10 days before starting another FMD cycle.

[0060] Diet (Human): The FMD will substitute the normal diet of a cancer patient for a period of 5 to 21 days with a 17 day maximum for most patients (see below) with frequency to be determined based on the frequency and efficacy of other treatments, with more frequent use needed when other treatments are not effective in cancer treatment. The ability of the patient to regain weight before the next cycle is initiated must also be considered, with patients with more severe symptoms able to regain weight receiving the diet as frequently as the other treatments are given and patients who are not regaining weight or are unable to undergo the full dietary period being placed on the FMD only after they return to the normal weight (weight before treatment is initiated but also BMI above 18). The FMD consists of ingredients which are Generally Regarded As Safe (RGAS). Calories are consumed according to the subject's body weight. For day 1, total calorie consumption is 4.5-7 calorie per pound (or 10-16 calorie per kilogram). The diet should be at least 90% plant based. The day 1 diet should contain less than 30 g of sugars, less than 28 g of plant based proteins, 20-30 grams of plant based monounsaturated fats, 6-10 g of plant based polyunsaturated fats and 2-12 g of plant based saturated fats. For days 2-21, total calorie consumption is 3-5 calorie per pound (or 7-11 calorie per kilogram). The days 2-21 diet should contain less than 20 g of sugars, less than 18 g of plant based proteins, 10-15 g of plant based monounsaturated fats, 3-5 g of plant based polyunsaturated fats and 1-6 grams of plant based saturated fats, 10-30 grams of glycerol diluted in 1 liter of water/day, based on body weight (10 grams for a 100 pound person, 20 grams for a 200 pound person and 30 grams for a 300 pound person). Diet should also be high nourishment containing approximately 50% of the RDA (daily) for vitamins, minerals + essential fatty acids. The minimum length will be 5 or 6 days and the maximum length 21 days (based on safety data and standard of care practice at fasting clinics).

[0061] *In vitro* dose response of cancer cell lines to DXR: glucose restriction was applied to cells 24 hours before and 24 hours during DXR treatment. Control groups were cultured in DMEM supplemented with 2.0 g/L glucose while the glucose restriction groups were cultured in DMEM supplemented with 0.5 g/L glucose. Survival was determined by MTT reduction.

[0062] Stress resistance - 12 weeks old female C57BL/6 mice were divided in the following experimental groups; *ad lib* (*ad libitum* feeding), STS/FMD, DXR, STS/FMD + DXR. In order to observe the response to every treatment in presence or not of rapamycin and dexamethasone, each group was present as triplicate where one of the sets underwent rapamycin treatment and one underwent dexamethasone treatment. The administration of rapamycin was performed for a period of l4 days at the end of which a high dose of doxorubicin was administrated iv (24 mg/kg/mouse). The administration of dexamethasone was performed for a period of l4 days at the end of which a high dose of doxorubicin was administrated iv (24 mg/kg/mouse). The animals belonging to the STS + DXR groups were fed a very low calorie and no protein FMD for 48h prior the injection of doxorubicin. Following doxorubicin injection the animals were monitored every day and the survival was recorded (**Figure 1A, B, and C**). Mice in the insulin (ins) groups also received insulin injection every 12h for the 48h preceding doxorubicin administration.

[0063] It is observed that the administration of the kinase inhibitor rapamycin, corticosteroid drugs such as dexamethasone and of other hyperglycemia-inducing drugs during chemotherapy sensitizes mice to the drug leading to an increased mortality (**Figure 1C and 1D**). In addition, the sensitization of the animals is positively associated with an increase of circulating blood glucose (**Figure 1B**). However, when the glucose levels are reduced by either STS, FMD, or the administration of insulin, this sensitizing effect is completely or partially reversed, respectively (**Figure 1B-D**). The experiments show that administration of insulin, STS, or FMD in combination with rapamycin and dexamethasone: a) offer a powerful tool to reduce the hyperglycemic state induced by rapamycin and dexamethasone (**Figure 1B**) and b) to reverse the toxic effects associated with the hyperglycemia induced by the two drugs (**Figure 1C and 1D**).

[0064] While exemplary embodiments are described above, it is not intended that these embodiments describe all possible forms of the invention. Rather, the words used in the specification are words of description rather than limitation, and it is understood that various changes may be made without departing from the spirit and scope of the invention. Additionally, the features of various implementing embodiments may be combined to form further embodiments of the invention.

WHAT IS CLAIMED IS:

1. A method for treating a hyperglycemia in a subject undergoing chemotherapy, the method comprising:

a) identifying a subject undergoing chemotherapy and having hyperglycemia and/or being administered a hyperglycemia-inducing agent; and

b) administering short-term starvation (STS), a fasting mimicking diet (FMD) or insulin to the subject for a first time period to prevent or reverse hyperglycemia and sensitization to chemotherapy associated with increased glucose levels.

2. The method of claim 1 wherein the hyperglycemia-inducing agent is selected form the group consisting of rapamycin, steroid medications including dexamethasone, and combinations thereof.

3. The method of claim 1 wherein short term starvation is administered for 48-140 hours prior to a round of chemotherapy and/or 4-56 hours following a round of chemotherapy.

4. The method of claim 1 wherein the FMD is administered for 48-140 hours prior to a round of chemotherapy and/or 4-56 hours following a round of chemotherapy.

5. The method of claim 1 wherein step b) is repeated a plurality of times at predetermined intervals.

6. The method of claim 5 wherein step b) is repeated at intervals from two weeks to 2 months.

7. The method of claim 6 wherein the subject is administered a normal diet in between repetition of step b).

8. The method of claim 1 wherein the first time period is from 3 to 10 days.

9. The method of claim 1 wherein the hyperglycemia-inducing agent is rapamycin.

10. The method of claim 1 wherein the hyperglycemia-inducing agent is a steroid medication.

11. The method of claim 1 wherein the hyperglycemia-inducing agent is dexamethasone.

12. A method for treating a hyperglycemia in a subject undergoing chemotherapy, the method comprising:

a) identifying a subject undergoing chemotherapy and having hyperglycemia and/or being administered a hyperglycemia-inducing agent; and

b) administering Metformin to subject to mimic effects of fasting.

13. The method of claim 12 wherein the hyperglycemia-inducing agent is selected form the group consisting of rapamycin, steroid medications including dexamethasone, and combinations thereof.

14. The method of claim 12 wherein the Metformin is administered at a dose of 1 to 2.5 mg/day.

15. A method for treating a hyperglycemia in a subject undergoing chemotherapy or other cancer therapy, the method comprising:

a) identifying a subject undergoing chemotherapy and having hyperglycemia and/or being administered a hyperglycemia-inducing agent;

b) administering short-term starvation (STS), a fasting mimicking diet (FMD) or insulin to the subject for a first time period to prevent or reverse hyperglycemia and sensitization to chemotherapy associated with increased glucose levels; and

c) administering a normal diet to the subject after step b).

16. The method of claim 15 wherein the hyperglycemia-inducing agent is selected form the group consisting of rapamycin, steroid medications including dexamethasone, and combinations thereof.

17. The method of claim 15 wherein steps b) and c) is repeated a plurality of times at predetermined intervals.

18. The method of claim 17 wherein steps b) and c) is repeated at intervals from two weeks to 2 months.

19. The method of claim 15 wherein the first time period is from 3 to 10 days.

20. A method of replacing or enhancing an effect of a fasting mimicking diet on cancer cell sensitization, by administering metformin to patients receiving chemotherapy or another cancer therapy.

21. A method where metformin is administered in combination with breast cancer, ovarian cancer, colorectal cancer, melanoma, prostate cancer, cervical cancer, epidermoid carcinoma, neuroblastoma, or any additional cancer type in order to reduce glucose levels and promote differential stress sensitization to specifically kill cancer but not normal cells.

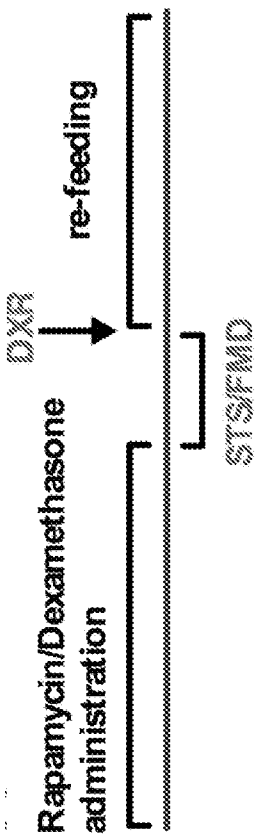

Fig. 1A

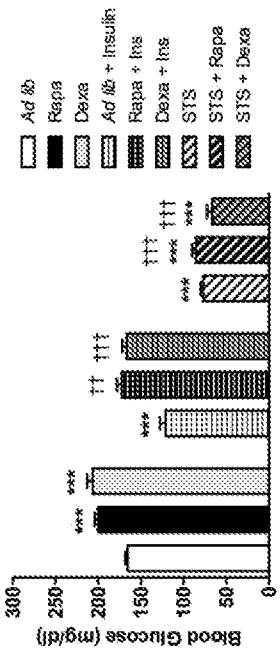

Fig. 1B

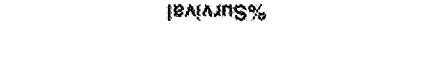

Fig. 1C

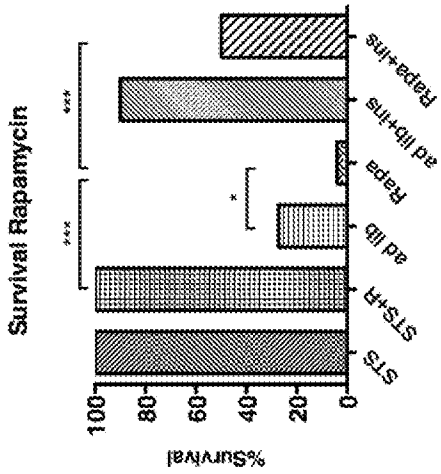

Fig. 1D

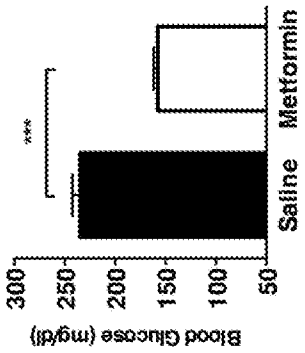

Fig. 1E

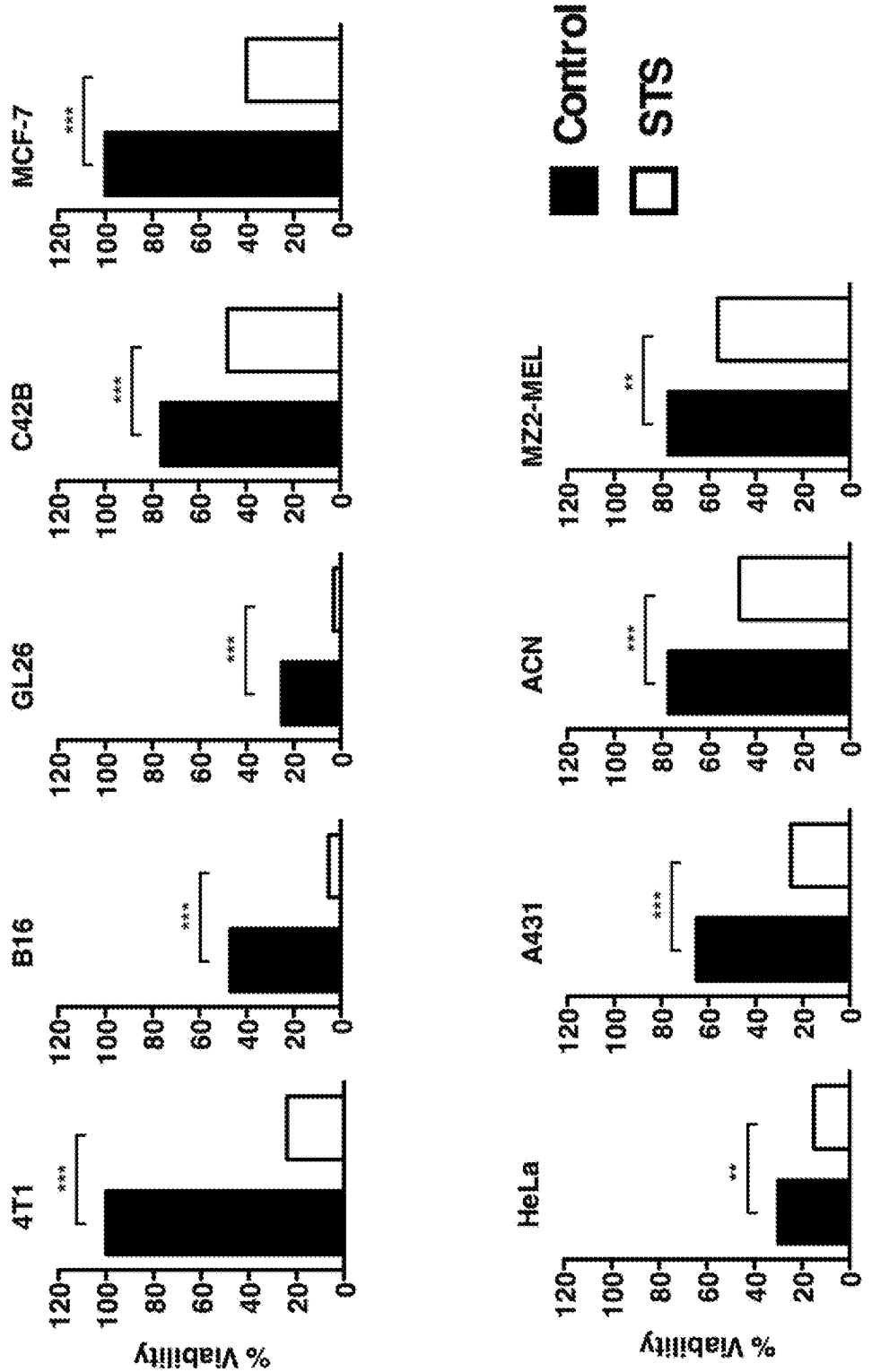
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Fig. 2

PATENT COOPERATION TREATY

PCT

DECLARATION OF NON-ESTABLISHMENT OF INTERNATIONAL SEARCH REPORT

(PCT Article 17(2)(a), Rules 13*ter*.1(c) and (d) and 39)

Applicant's or agent's file reference	**IMPORTANT DECLARATION**	Date of mailing (*day/month/year*)
USC0140PCT		23 September 2016 (23.09.2016)
International application No.	International filing date (*day/month/year*)	(Earliest) Priority date (*day/month/year*)
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International Patent Classification (IPC) or both national classification and IPC

A61K 31/436(2006.01)i, A61K 31/573(2006.01)i, A61P 35/00(2006.01)i

Applicant

UNIVERSITY OF SOUTHERN CALIFORNIA

This International Searching Authority hereby declares, according to Article 17(2)(a), that **no international search report will be established** on the international application for the reasons indicated below.

1. ☒ The subject matter of the international application relates to:
   - a. ☐ scientific theories
   - b. ☐ mathematical theories
   - c. ☐ plant varieties
   - d. ☐ animal varieties
   - e. ☐ essentially biological processes for the production of plants and animals, other than microbiological processes and the products of such processes
   - f. ☐ schemes, rules or methods of doing business
   - g. ☐ schemes, rules or methods of performing purely mental acts
   - h. ☐ schemes, rules or methods of playing games
   - i. ☒ methods for treatment of the human body by surgery or therapy
   - j. ☐ methods for treatment of the animal body by surgery or therapy
   - k. ☐ diagnostic methods practised on the human or animal body
   - l. ☐ mere presentation of information
   - m. ☐ computer programs for which this International Searching Authority is not equipped to search prior art
2. ☐ The failure of the following parts of the international application to comply with prescribed requirements prevents a meaningful search from being carried out:
   ☐ the description ☐ the claims ☐ the drawings
3. ☐ A meaningful search could not be carried out without the sequence listing; the applicant did not, within the prescribed time limit:
   - ☐ furnish a sequence listing in the form of an Annex C/ST.25 text file, and such listing was not available to the International Searching Authority in a form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.
   - ☐ furnish a sequence listing on paper or in the form of an image file complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.
   - ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13ter.1(a) or (b).
4. Further coments:

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权利要求书1页 说明书10页 附图3页

(54)发明名称

响应于雷帕霉素和地塞米松诱导的标准和高葡萄糖条件禁食模仿膳食(FMD)和降葡萄糖药物保护正常细胞并产生癌症敏感条件

(57)摘要

用于治疗正在经历化疗的受试者的高血糖症和高血糖症依赖性副作用的方法包括鉴别正在经历化疗和给予高血糖症诱导剂的受试者的步骤。在第一时间段将短期饥饿或禁食模仿膳食给予受试者以预防高血糖症以及对与葡萄糖水平提高相关的化疗的敏感。在STS/FMD周期之间给予STS-模拟药物二甲双胍来保持再喂食期间的STS样/FMD样条件。

CN 107613979 A

1.一种用于治疗正在经历化疗的受试者的高血糖症的方法,所述方法包括:

a)鉴别正在经历化疗和患有高血糖症和/或给予高血糖症诱导剂的受试者;以及

b)在第一时间段将短期饥饿(STS)、禁食模仿膳食(FMD)或胰岛素给予受试者以预防或逆转高血糖症以及对与葡萄糖水平提高相关的化疗的敏感。

2.权利要求1所述的方法,其中,所述高血糖症诱导剂选自由雷帕霉素、包括地塞米松的类固醇药物及其组合组成的组。

3.权利要求1所述的方法,其中,在一轮化疗之前给予短期饥饿48～140小时和/或在一轮化疗之后给予短期饥饿4～56小时。

4.权利要求1所述的方法,其中,在一轮化疗之前给予FMD 48～140小时和/或在一轮化疗之后给予FMD 4～56小时。

5.权利要求1所述的方法,其中,以预定的间隔多次重复步骤b)。

6.权利要求5所述的方法,其中,以2周至2个月的间隔重复步骤b)。

7.权利要求6所述的方法,其中,在步骤b)的重复之间给予受试者正常膳食。

8.权利要求1所述的方法,其中,所述第一时间段为3～10天。

9.权利要求1所述的方法,其中,所述高血糖症诱导剂为雷帕霉素。

10.权利要求1所述的方法,其中,所述高血糖症诱导剂为类固醇药物。

11.权利要求1所述的方法,其中,所述高血糖症诱导剂为地塞米松。

12.一种用于治疗正在经历化疗的受试者的高血糖症的方法,所述方法包括:

a)鉴别正在经历化疗和患有高血糖症和/或给予高血糖诱导剂的受试者;以及

b)给予受试者二甲双胍以模仿禁食的效果。

13.权利要求12所述的方法,其中,所述高血糖症诱导剂选自由雷帕霉素、包括地塞米松的类固醇药物及其组合组成的组。

14.权利要求12所述的方法,其中,以1～2.5mg/日的剂量给予二甲双胍。

15.一种用于治疗正在经历化疗或其他癌症治疗的受试者的高血糖症的方法,所述方法包括:

a)鉴别正在经历化疗和患有高血糖症和/或给予高血糖症诱导剂的受试者;

b)在第一时间段给予受试者短期饥饿(STS)、禁食模仿膳食(FMD)或胰岛素以预防或逆转高血糖症以及对与葡萄糖水平提高相关的化疗的敏感;以及

c)在步骤b)之后给予受试者正常膳食。

16.权利要求15所述的方法,其中,所述高血糖症诱导剂选自由雷帕霉素、包括地塞米松的类固醇药物及其组合组成的组。

17.权利要求15所述的方法,其中,以预定的间隔多次重复步骤b)和c)。

18.权利要求17所述的方法,其中,以2周至2个月的间隔重复步骤b)和c)。

19.权利要求15所述的方法,其中,所述第一时间段为3～10天。

20.一种替代或增强禁食模仿膳食对癌细胞敏感的作用的方法,通过将二甲双胍给予接受化疗或另一种癌症疗法的患者来进行。

21.一种用于降低葡萄糖水平并促进差异胁迫敏感以特异地杀伤癌细胞而不杀伤正常细胞的方法,其中将给予二甲双胍与乳腺癌、卵巢癌、结肠直肠癌、黑色素瘤、前列腺癌、宫颈癌、表皮样癌、神经母细胞瘤或任何其他癌症类型相结合。

响应于雷帕霉素和地塞米松诱导的标准和高葡萄糖条件禁食模仿膳食(FMD)和降葡萄糖药物保护正常细胞并产生癌症敏感条件

[0001] 相关申请的交叉引用

[0002] 本申请要求2015年4月16日提交的美国临时申请系列号62/148,451的权益,其公开内容据此通过引用全文并入本文中。

[0003] 关于联邦资助的研究或开发的声明

[0004] 根据由国立卫生研究院批出的合约号1PO1AG034906,利用政府支持做出本发明。政府对本发明享有一定权利。

技术领域

[0005] 在至少一方面,本发明涉及在施用雷帕霉素和缓解药地塞米松时,保护正常细胞免受由升高的循环葡萄糖水平诱导的对化疗药物的提高的毒性/敏感的方法。

背景技术

[0006] 在过去的一个世纪,癌症管理和治疗已经显著改善。然而,治疗的标准仍然主要使用化疗、放疗或其组合。这两种治疗方式都伴随着许多副作用,从不适到继发性癌症的发展和器官毒性,特别是心脏和肝脏毒性。为了增加癌症治疗的疗效并帮助管理症状,其他药物如地塞米松经常与化疗和放疗联合使用。通常与化疗联合的地塞米松(Dexa)经常用作缓解药,该缓解药还显示出对治疗多发性骨髓瘤、白血病和淋巴瘤有效。然而,用Dexa治疗可能会引起许多副作用,包括液体潴留、体重增加、胃灼热、失眠和血糖水平升高。

[0007] 之前已经显示短期饥饿(STS)是缓解与癌症治疗相关的不适,同时提高该治疗的效果的有效实践。此外,已经证明STS方案是在化疗期间保护正常细胞和组织的有效方法(差异胁迫抗性(Differential Stress Resistance),或DSR)(Raffaghello等人,PNAS.2008;PMID:18378900和Lee等人.癌症研究(Cancer Research).2010;PMID:20145127)

[0008] 之前已经显示禁食可以使癌细胞对化疗敏感而不使正常细胞对化疗敏感,禁食是一种称为差异胁迫敏感(DSS)的现象,其功效归因于循环葡萄糖和IGF-1水平的降低(图2)(Lee等人.Sci TranslMed.2012;PMID:22323820)。然而,在禁食周期之间需要10至14天的再喂食期来恢复体重的损失。

[0009] 5'AMP活化蛋白激酶(AMPK)是在STS/FMD方案期间上调的酶,其在细胞能量内稳态中起作用,并且与寿命延长有关。AMPK也被认为是代谢性肿瘤抑制因子(Luo等人.Future Oncol.2010;PMID:20222801)。二甲双胍是引起循环葡萄糖降低的AMPK激活剂(图1E),并具有治疗/预防癌症的潜能。

[0010] 所有活细胞中调节代谢的中心成员通过调节丝氨酸/苏氨酸蛋白激酶而部分地调节正常细胞生长来调节代谢,这导致了包括施用激酶抑制剂如雷帕霉素(Rapa)联合化疗的治疗标准的改变。激酶和其他信号转导抑制剂可以延缓癌症生长并被广泛使用,但像地塞

米松一样也可能导致对正常细胞的重大副作用。

[0011] 因此,需要如下的治疗方案:(i)减轻与化疗中使用的辅助药物相关的副作用,以及(ii)可以在STS周期之间的“再喂食”期间维持降低的葡萄糖水平,或替代STS/FMD并使癌细胞敏感。

发明内容

[0012] 本发明通过如下方式解决了现有技术的一个或多个问题,在至少一种实施方式中提供用于治疗经历化疗或其他癌症治疗的受试者的高血糖症或降低血糖的方法。该方法包括鉴别正在经历化疗和给予高血糖诱导剂的受试者的步骤。在第一时间段将短期饥饿、禁食模仿膳食或胰岛素给予受试者以预防或逆转高血糖症以及对与葡萄糖水平提高相关的化疗的敏感。

[0013] 本发明的各种实施方式减轻或治疗化疗的症状,所述症状可以通过互补给予雷帕霉素和类固醇药物地塞米松而恶化。以下显示了(图1B至图1C),给予地塞米松和雷帕霉素(图1B、图1D)来治疗化疗相关的副作用可以导致动物对化疗敏感。如下显示的(图1B至图1D),给予胰岛素以降低对照小鼠以及经历Rapa和Dexa治疗的动物的循环葡萄糖水平,可以逆转阿霉素和其他化疗药物的毒性。因为雷帕霉素和地塞米松广泛用于治疗人类某些肿瘤,这些结果对患者的安全性和那些疗法的效果具有重要的意义。

[0014] 因为二甲双胍在降低循环葡萄糖水平(图1E)和上调我们已经显示使PKA信号传导失活的AMPK中的作用,二甲双胍具有用作STS模仿药物的潜能,以便(i)当在再喂食期期间给予时,通过作用于葡萄糖水平和PKA,逆转化疗的与高血糖症相关的细胞毒性作用,以及(ii)同样通过作用于葡萄糖和AMPK-PKA信号传导,加强/延长STS在减少肿瘤进展中的作用。因此,二甲双胍可以通过降低葡萄糖水平和作用于PKA信号传导两者来促进差异胁迫抗性和差异胁迫敏感,如Raffaghello等人,PNAS.2008;PMID:18378900以及Lee等人.Sci TranslMed.2012;PMID:22323820中所述。

[0015] 在另一种实施方式中,提供了替代或增强禁食模仿膳食(FMD)对癌细胞敏感的作用的方法。该方法包括鉴别接受化疗或另一种癌症治疗的受试者的步骤。然后通过给予受试者将二甲双胍给予受试者。

[0016] 在另一种实施方式中,提供了促进差异胁迫的方法。该方法包括鉴别患有乳腺癌、卵巢癌、结肠直肠癌、黑色素瘤、前列腺癌、宫颈癌、表皮样癌、神经母细胞瘤或任何其他癌症类型中的一种或多种的受试者。将二甲双胍给予受试者来降低葡萄糖水平并促进差异胁迫敏感以特异地杀伤癌细胞而不杀伤正常细胞。

附图说明

[0017] 图1A、1B、1C、1D和1E。循环葡萄糖水平的增加介导宿主对化疗的敏感。给予雷帕霉素(Rapa)或地塞米松(Dexa)(A)导致显著地被胰岛素降低的葡萄糖水平的增加,甚至更多地被STS降低(B)的葡萄糖水平的增加。B中的星号表明各组与随意(AL)组相比的显著性。各组与其内部对照相比的显著性用短剑表示(即Rapa+ins和Dexa+ins上的短剑是指与AL+ins相比的显著性)。对于C和D中所示的胁迫抗性实验,我们按照A中所示的计划进行。在DXR注射之前(第0天)腹膜内注射给予雷帕霉素和地塞米松14天。在给予24mg/kg DXR之后,记录

监测死亡迹象和存活的动物(C和D)。C和D中报告的STS组、随意组和随意+ins组是共享组，并且在两幅图中具有相同值。(E)腹膜内注射50mg/kg二甲双胍的小鼠的血糖水平(对照小鼠注射盐水)。进行单因素ANOVA检验，p值<0.05的差异被认为是显著的(p值<0.05、0.01和0.001分别以*、*和***表示)

[0018] 图2.葡萄糖限制对9种不同的小鼠和人类癌细胞系的DXR敏感性的作用。在补充了2.0g/L葡萄糖的DMEM中培养对照组，而在补充了0.5g/L葡萄糖的DMEM中培养葡萄糖限制组。通过MTT还原来确定存活率。测试的癌细胞系为：4T1(小鼠乳腺癌)、B16(小鼠黑色素瘤)、GL26(小鼠神经胶质瘤)、C42B(人前列腺癌)、MCF-7(人乳腺癌)、HeLa(人宫颈癌)、A431(人表皮样癌)、ACN(人神经母细胞瘤)和MZ2-MEL(人黑色素瘤)。

[0019] 表1.禁食模仿膳食(FMD)提供的微量营养素含量

具体实施方式

[0020] 现在将详细参考本发明目前优选的组成、实施方式和方法，它们组成了实践发明人目前已知的本发明的最佳方式。附图不一定是按比例的。然而，应该理解的是，所公开的实施方式仅仅是可以以各种且可选形式呈现的本发明的示例。因此，本文公开的特定细节不应解释为限制，而仅仅是本发明任意方面的代表性基础和/或教导本领域技术人员多方面利用本发明的代表性基础。

[0021] 除了在这些实例中，或另外明确表示之外，本描述中所有表示物质的量或反应条件和/或用途的数量应理解为由描述本发明最宽范围的词语“约”来修饰。所述数值限制内的实践通常是优选的。而且，除非明确相反说明：百分比、“份数”和比值是以重量计的；对出于与本发明有关的给定目的适合或优选的一组或一类物质的描述意味着所述组或类的成员中的任意两个或更多个的混合物是同等适合或优选的；化学术语中对组分的描述是指在添加至描述中指定的任意组合时的组分，一旦混合并不必然排除混合物的组分之间的化学相互作用；首字母缩写或其他缩写的首次定义适用于本文所有随后相同缩写的使用，并适用于对最初定义的缩写的正常语法变化的必要的修改；以及，除非明确相反说明，性质的测量是通过与对同一性质前后参考的相同技术来确定的。

[0022] 还应理解的是，本发明不限于以下描述的具体实施方式和方法，因为特定组分和/或条件当然可以变化。而且，本文使用的术语仅出于描述本发明的特定实施方式的目的使用，而不是意图以任意方式限制。

[0023] 还必须注意的是，如在说明书和所附权利要求书中所使用的，单数形式“一个/一种(“a”)”、“一个/一种(“an”)”和“所述(“the”)”包括复数指代物，除非上下文另有明确说明。例如，提及单数组分旨在包括多种组分。

[0024] 贯穿本申请，当引用出版物时，这些出版物的公开内容据此通过引用全文并入本申请中以更充分地描述本发明所属的技术状态。

[0025] 缩写：

[0026] “AL”意思是随意。

[0027] “FMD”是指禁食模仿膳食。

[0028] “STS”是指短期饥饿。

[0029] “DSR”是指差异胁迫抗性。

[0030] “DSS”是指差异胁迫敏感。

[0031] “DXR”是指阿霉素。

[0032] “Rapa”是指雷帕霉素。

[0033] “Dexa”是指地塞米松。

[0034] “ins”是指胰岛素。

[0035] “AMPK”是指5’AMP活化蛋白激酶。

[0036] “ip”是指腹膜内。

[0037] 术语“千卡”(kcal)和“大卡”指的是食物卡路里。

[0038] 术语“卡”指的是所谓的小卡路里。

[0039] 术语“受试者”是指人类或动物,包括所有哺乳动物,例如灵长类动物(特别是较高级的灵长类动物)、绵羊、狗、啮齿动物(例如小鼠或大鼠)、豚鼠、山羊、猪、猫、兔子和牛。

[0040] 术语“禁食模仿膳食”是指这样的膳食,即向受试者提供以类似于通过禁食所引起的产生葡萄糖、酮体、IGF-1和IGFBP1变化但能够提供高营养和使饥饿最小化的方式来配制的限制卡路里的膳食。

[0041] 在一种实施方式中,提供了用于治疗经历化疗的受试者的高血糖症的方法。该方法包括鉴别正在经历化疗并给予高血糖症诱导剂的受试者。在第一时间段将短期饥饿、禁食模仿膳食(FMD)或胰岛素给予受试者以预防或逆转高血糖症以及对与葡萄糖水平提高相关的化疗的敏感。在本实施方式的上下文中,预防高血糖症或敏感是指减少这些副作用发生的可能性。一般来说,FMD膳食每日提供少于约1000千卡,而STS在给予时不提供卡路里。在改进中,高血糖症诱导剂是激酶抑制剂或皮质类固醇。高血糖症诱导剂的具体实例包括雷帕霉素、类固醇药物(包括地塞米松等)及其组合。在改进中,短期饥饿或禁食模仿膳食以预定的间隔重复多次。例如,短期饥饿或禁食模仿膳食可以以两周到两个月的间隔来重复。典型地,在这些重复之间向受试者给予正常膳食(即再喂食期)。在该上下文中,正常饮食是足以维持患者体重的热量摄入的饮食。在改进中,正常的热量摄入向受试者提供1500至2500kcal或1800至2300kcal或1800至2000kcal。

[0042] STS方案的实例见于第12/430,058号和第13/488,590号美国专利申请中;其全部公开内容在此通过引用并入。在变形中,STS膳食提供低卡路里或无卡路里膳食。该膳食包含能够为人受试者提供营养同时提供不超过813～957kcal(例如,不超过700、500、300或100kcal,或0kcal)的总能量以及不超过30～60g(例如,不超过20、10或5g,或0g)蛋白质的膳食物质。如果膳食材料中存在碳水化合物,那么不超过一半的能量在碳水化合物中。在改进中,在受试者暴露于化疗之前,STS/FMD膳食可以连续给予受试者3～10天。也可以在暴露之后,将该膳食给予受试者24小时。优选地,该膳食可以在受试者暴露于化疗之前连续给予受试者3～10天,并且在暴露之后给予24小时。

[0043] 在另一种变形中,STS膳食提供营养同时每日每kg受试者体重提供不超过11kcal(例如,不超过8、5或2kcal,或0kcal)能量,以及每日每kg动物或人体重提供不超过0.4g(例如,不超过0.3、0.2或0.1g,或0g)蛋白质。如果膳食中存在碳水化合物,那么不超过一半的能量在碳水化合物中。在一些实施方式中,该膳食能够提供每日不超过700kcal(例如600、400或200kcal,或0kcal)总能量。当受试者暴露于化疗时,动物或人的正常细胞受到保护,而异常细胞如癌细胞不受保护。在受试者暴露于化疗之前,STS/FMD膳食可以连续给予人或

动物3～10天。也可以在暴露之后，将该膳食给予受试者24小时。优选地，该膳食可以在受试者暴露于化疗之前连续给予受试者3～10天，并且在暴露之后给予24小时。

[0044] 在另一种变形中，STS/FMD方案涉及禁食模仿膳食。例如，患有癌症的受试者可以在一轮化疗之前禁食48～140小时或在化疗后禁食4～56小时。优选地，在一轮化疗之前向患有癌症的受试者提供FMD 48～140小时，并且在化疗后提供FMD 4～56小时。

[0045] FMD膳食的实例见于第14060494号和第14178953号美国专利申请以及第WO2011/050302号WIPO公开和第WO2011/050302号WIPO公开中；其全部公开内容通过引用在此并入。典型地，在FMD方案中，受试者的饮食被取代预定的天数(即5天)。在该时期，受试者消耗大量的水。为了健康的受试者具有正常体重(体重指数或BMI在18.5～25)，该膳食在前3个月每个月消耗一次(消耗该膳食5天，消耗正常膳食25～26天)，之后每3个月消耗一次(每3个月5天)。在消耗膳食期间，在开始下一轮之前，测量受试者的体重，受试者必须恢复体重损失的至少95％。BMI小于18.5的受试者不应该进行FMD，除非医师推荐和监督。可以采用相同的方案(3个月中每个月一次，之后每3个月一次)用于治疗或支持治疗，在专利申请中提供了全部条件。第14178953号美国专利申请提供了低蛋白形式的FMD膳食。

[0046] 在一种变形中，第12/430,058号美国专利申请中提出的FMD用于上述的方法中。该膳食包括涉及卡路里、大量营养素和微量营养素的营养标示。根据使用者的体重消耗卡路里。总卡路里消耗量为第1天每磅4.5～7卡(或每千克10～16卡)，第2至5天每磅3～5卡(或每千克7～11卡)。图12至图14提供了从第1天至第5天的营养素的清单。除了大量营养素以外，膳食应该在第1天含有少于30g糖，在第2至5天含有少于20g糖。膳食应该在第1天含有少于28g蛋白质，在第2至5天含有小于18g蛋白质。膳食应该在第1天含有20～30g单不饱和脂肪，在第2至5天含有10～15g单不饱和脂肪。膳食应该在第1天含有6～10g多不饱和脂肪，在第2至5天含有3～5g多不饱和脂肪。膳食应该在第1天含有少于12g饱和脂肪，在第2至5天含有少于6g饱和脂肪。典型地，在全部的几天中的脂肪来自以下的组合：杏仁、澳洲坚果、山核桃、椰子、椰子油、橄榄油和亚麻籽。在改进中，在全部的几天中，FMD膳食包括超过50％的每日推荐值的膳食纤维。在进一步的改进中，在全部5天中，膳食纤维的量每日大于15g。在第2至5天，膳食应该每日含有12～15g甘油。在改进中，以每日每磅体重0.1g提供甘油。

[0047] 在改进中，FMD包括以下微量营养素(至少95％非动物基微量营养素)：每日超过5,000IU的维生素A(第1至5天)；每日60～240mg维生素C(第1至5天)；每日400～800mg钙(第1至5天)；每日7.2～14.4mg铁(第1至5天)；每日200～400mg镁(第1至5天)；每日1～2mg铜(第1至5天)；每日1～2mg锰(第1至5天)；每日3.5～7mg硒(第1至5天)；每日2～4mg维生素B1(第1至5天)；每日2～4mg维生素B2(第1至5天)；每日20～30mg维生素B3(第1至5天)；每日1～1.5mg维生素B5(第1至5天)；每日2～4mg维生素B6(第1至5天)；每日240～480mg维生素B9(第1至5天)；每日600～1000IU维生素D(第1至5天)；每日14～30mg维生素E(第1至5天)；每日超过80mg维生素K(第1至5天)；在整个5天期间提供16～25mg维生素B12；在整个5天期间提供600mg二十二碳六烯酸(DHA，来自藻类)。FMD膳食主要从天然来源提供高微量营养素含量(即大于50wt％)：所述天然来源包括：无头甘蓝、腰果、黄椒、洋葱、柠檬汁、酵母、姜黄、蘑菇、胡萝卜、橄榄油、甜菜汁、菠菜、番茄、羽衣甘蓝、荨麻、百里香、盐、胡椒、维生素B12(氰钴胺)、甜菜、胡桃南瓜、羽衣甘蓝、番茄、牛至、番茄汁、橙汁、芹菜、直立莴苣、菠菜、小茴香、橙皮、柠檬酸、肉豆蔻、丁香以及其组合。表1提供了FMD膳食中可以提供的另外的微量营养素

补充物的实例：

[0048] 表1.微量营养素补充物

[0049]

	补充物	分子式	量	量范围	单位
维生素A			1250 IU	900-1600	IU
维生素C	抗坏血酸	$C_6H_8O_6$	*15.0000*	10-20	mg
Ca	碳酸钙	$CaCO_3$	*80.0000*	60-100	mg
Fe	富马酸亚铁	$C_4H_2FeO_4$	*4.5000*	3-6	mg
维生素D3	胆钙化醇	$C_{27}H_{44}O$	*0.0025*	0.001-0.005	mg
维生素E	dl-α-生育酚乙酸酯	$C_{29}H_{50}O_2$	*5.0000*	3-7	mg
维生素K	植物甲萘醌		*0.0200*	0.1-0.04	mg

[0050]

维生素B1	硝酸硫胺	$C_{12}H_{17}N_5O_4S$	*0.3750*	0.15-0.5	mg
维生素B2	核黄素B101	$C_{17}H_{20}N_4O_6$	*0.4250*	0.2-0.6	mg
维生素B3	烟酰胺	$C_6H_6N_2O$	*5.0000*	3-7	mg
维生素B5	泛酸钙	$C_{18}H_{32}CaN_2O_{10}$	*2.5000*	1.5-4.0	mg
维生素B6	盐酸吡哆醇	$C_8H_{11}NO_3 \cdot HCl$	*0.5000*	0.3-0.7	mg
维生素B7	生物素	$C_{10}H_{16}N_2O_3S$	*0.0150*	0.01-0.02	mg
维生素B9	叶酸	$C_{19}H_{19}N_7O_6$	*0.1000*	0.07-0.14	mg
维生素B12	氰钴胺	$C_{63}H_{88}CoN_{14}O_{14}P$	*0.0015*	0.001-0.002	mg
Cr	吡啶甲酸铬	Cr(C6H4NO2)3	*0.0174*	0.014-0.022	mg
Cu	硫酸铜	CuSO4	*0.2500*	0.18-0.32	mg
I	碘化钾	KI	*0.0375*	0.03-0.045	mg
Mg	氧化镁	MgO	*26.0000*	20-32	mg
Mn	硫酸锰	$MnSO_4$	*0.5000*	0.3-0.7	mg
Mo	钼酸钠	Na_2MoO_4	*0.0188*	0.014-0.023	mg
Se	硒酸钠	Na_2O_4Se	*0.0175*	0.014-0.023	mg
Zn	氧化锌	ZnO	*3.7500*	3-5	mg

[0051] 在另一种实施方式中，提供了一种用于实施上述方法的膳食包。该膳食包包括用于在第一时间段给予受试者的第一膳食的第一套配给，该第一膳食在第1天为每磅受试者提供4.5～7千卡，在第2至5天每日为每磅受试者提供3～5千卡。该膳食包包括如下的配给：在第1天提供少于30g糖；在第2至5天提供少于20g糖；在第1天提供少于28g蛋白质；在第2至5天提供少于18g蛋白质；在第1天提供20～30g单不饱和脂肪；在第2至5天提供10～15g单不饱和脂肪；在第1天提供6～10g多不饱和脂肪；在第2至5天提供3～5g多不饱和脂肪；在第1天提供少于12g饱和脂肪；在第2至5天提供少于6g饱和脂肪；以及在第2至5天每日提供12～25g甘油。在改进中，膳食包进一步包括足以提供上述微量营养素的配给。在进一步改进中，膳食包提供了提供上述方法的细节的说明。

[0052] 在上述实施方式的改进中，膳食的5天供应包括：汤/肉汤、软饮料、坚果棒和补充物。如下给予膳食：1)在第1天，提供1000～1200kcal膳食与上述的高微量营养素营养品；2)在接下来的4天，提供650～800kcal的每日膳食加上提供60～120kcal的含有葡萄糖替代碳源的饮料。

[0053] 在上述实施方式的另一种改进中，6天低蛋白膳食方案包括：汤/肉汤、软饮料、坚果棒和补充物。如下给予膳食：1)在第1天，提供1000～1200kcal膳食与高微量营养素营养品；2)在接下来的3天，提供少于200kcal的每日膳食加上提供60～120kcal的含有葡萄糖替代碳源的饮料。该替代碳源不影响禁食对干细胞活化的影响；3)在第5天，受试者消耗正常膳食；以及4)在第6天，除了正常膳食之外，提供由300kcal的高脂肪源和微量营养素营养品混合物组成的另外的补充食品。

[0054] 在又另一种改进中，膳食方案包括：6天低蛋白膳食供给，包括：汤/肉汤、软饮料、坚果棒和补充物。1)在第1天，提供1000～1200kcal膳食与高微量营养素营养品；2)在接下来的3天，提供600～800kcal的每日膳食，其中含有少于10g蛋白质和来自糖的热量少于200kcal；3)在第5天，受试者消耗正常膳食；以及4)在第6天，除了正常膳食之外，提供由300kcal的高脂肪源和微量营养素营养品混合物组成的另外的补充食品。

[0055] 虽然FMD膳食几乎包含任何脂肪来源，包括单不饱和脂肪来源和多不饱和脂肪来源的不饱和脂肪高的来源是尤其有用的(例如，ω-3/6必需脂肪酸)。单不饱和食物来源的合适的实例包括但不限于花生酱、橄榄、坚果(如杏仁、山核桃、开心果、腰果)、鳄梨、种子(如芝麻)、油(如橄榄、芝麻、花生、卡诺拉)等。多不饱和食物来源的合适的实例包括但不限于核桃、种子(例如南瓜、向日葵)、亚麻籽、鱼(例如鲑鱼、金枪鱼、鲭鱼)、油(例如红花、大豆、玉米)。第一膳食还包括选自由植物提取物、矿物质、ω-3/6必需脂肪酸及其组合组成的组的组分。在一种改进中，这样的蔬菜提取物提供了5份每日推荐的蔬菜的等价物。蔬菜提取物的合适的来源包括但不限于上海青、无头甘蓝、莴苣、芦笋、胡萝卜、胡桃南瓜、苜蓿、青豆、西红柿、卷心菜、花椰菜、甜菜。ω-3/6必需脂肪酸的合适的来源包括鱼，如鲑鱼、金枪鱼、鲭鱼、跳鱼(bluefish)、剑鱼等

[0056] 在另一种变形中，提供了基于在再喂食期间给予时，给予二甲双胍(N,N-二甲基亚氨基二碳亚胺二酰胺)以模仿禁食效果来逆转化疗的与高血糖症相关的细胞毒性作用和/或加强/延长STS在降低肿瘤进展中的作用的方法。该方法包括如上述的鉴别正在经历化疗和患有高血糖症和/或给予高血糖症诱导剂的受试者的步骤。将二甲双胍给予受试者以逆转细胞毒性作用。根据患者对药物的响应以1～2.5mg/日的剂量范围给予二甲双胍。可以根据受试者的响应给予二甲双胍1天、1至5天、1至10天或1至14天或更多。在另一种变形中，提供了用于治疗正在经历化疗或其他癌症治疗的受试者的高血糖症或血糖正常的负面作用的方法。该方法包括鉴别正在经历化疗和施用了高血糖诱导剂的受试者的步骤。在第一时间段将短期饥饿或禁食模仿膳食或胰岛素给予受试者以预防或降低葡萄糖水平以及使癌细胞对化疗或其他癌症治疗敏感。关于给予短期饥饿或禁食模仿膳食的该变形的细节与上述的那些相同。在再喂食期间，也在给予如上所述的短期饥饿或禁食模仿膳食之间将正常膳食给予受试者。在该再喂食期间将二甲双胍给予受试者。根据患者对药物的响应以1～2.5mg/日的剂量范围给予二甲双胍。可以根据受试者的响应给予二甲双胍1天、1至5天、1至10天或1至14天或更多。以预定的间隔多次重复给予短期饥饿或禁食模仿膳食和在二甲双胍给予期间给予再喂食的步骤。如上所述，在改进中，以2周至2个月的间隔重复这些步骤。

[0057] 在另一种实施方式中，提供了替代或增强FMD对癌细胞敏感的作用的方法。该方法包括鉴别正在接受化疗或另一种癌症治疗的受试者的步骤。然后通过给予受试者向受试者给予二甲双胍。在改进中，根据患者对药物的响应以1～2.5mg/日的剂量范围给予二甲双

胍。可以根据受试者的响应给予二甲双胍1天、1至5天、1至10天、1至14天或1至60天或更多。

[0058] 在另一种实施方式中，提供了促进差异胁迫的方法。该方法包括鉴别患有乳腺癌、卵巢癌、结肠直肠癌、黑色素瘤、前列腺癌、宫颈癌、表皮样癌、神经母细胞瘤或任何其他癌症类型中的一种或多种的受试者的步骤。将二甲双胍给予受试者以降低葡萄糖水平并促进差异胁迫敏感来特异地杀伤癌细胞而不杀伤正常细胞。根据患者对药物的响应以1～2.5mg/日的剂量范围给予二甲双胍。可以根据受试者的响应给予二甲双胍1天、1至5天、1至10天、1至14天或1至60天或更多。

[0059] 以下实例旨在说明而非限制本发明的范围。虽然这些实施例是可利用的那些的典型，可以可选地利用本领域技术人员已知的其它程序。事实上，基于本文的教导，在不需要过度实验的情况下，本领域普通技术人员可容易地设想并产生进一步的实施方式。

[0060] 已经在体外和体内鼠模型中测试了本发明。STS和FMD也已经在不同的临床试验中测试，其显示了两种膳食干预的安全性和可行性。FMD膳食已显示在唤起DSR上与STS一样有效。

[0061] 方法：在短期饥饿(STS)或禁食模仿膳食(FMD)开始之前，每日腹膜内(ip)给予雷帕霉素或地塞米松14天的时间。在膳食干预结束时，正好通过静脉内注射给予阿霉素。胰岛素(ins)组中的小鼠在给予阿霉素之前还每12小时接受胰岛素注射共48小时。然后在之后的几天观察动物的疼痛或死亡迹象，记录存活率(图1A、图1C和图1D)。

[0062] 用盐水稀释二甲双胍(50mg/kg)并通过腹膜内(i.p.)注射给药。在给予二甲双胍后监测循环葡萄糖水平(图1E)。

[0063] 膳食(小鼠)：以辐照TD.7912啮齿类食物(Harlan Teklad)供养小鼠。简言之，此膳食包含3.T5千卡/克的具有由蛋白质、碳水化合物和脂肪以25:58:17的百分率提供的卡路里的易消化能量。随意提供食物。对照组中的小鼠平均消耗14.9kcal/日(或3.9g/日)，我们的实验FMD膳食基于营养筛查，该营养筛查在低卡路里消耗期间鉴定允许高营养品的成分(Brandhorst,Wei等人,2013)。在供应FMD膳食之前，将动物转移至新笼子中以避免食用残留食物和食粪。FMD膳食由指定为第1天膳食和第2至4天膳食的两种不同组分组成，所述两种不同组分分别以此顺序喂食。第1天膳食包含1.88kcal/g并且设计为使小鼠在随后的喂食时间期间适应低卡路里摄入的时期。第2-4天膳食在所有的喂食时间是相同的并包含0.36kcal/g。每两周，按照随意喂食对照组的平均摄入(～4克)喂食第1天和第2-4天膳食。由于供应的FMD膳食的不同卡路里密度，在此队列中的小鼠在第1天消耗的卡路里减少～50％，并且在第2至4天消耗对照队列的9.7％。小鼠在FMD方案的每一天消耗掉所有的供应食物并且没有显示出厌食迹象。在第2至4天膳食结束后，在开始另一个FMD周期之前，我们随意供应TD.7912食物，持续10天。

[0064] 膳食(人)：FMD会替代癌症患者的正常膳食5至21天的时间，对大多数患者来说17天是最大值(见下)，其中频率基于其他治疗的频率和疗效来确定，当癌症治疗中其他治疗不起作用时需要更频繁地使用。在启动下一轮之前患者恢复体重的能力也必须考虑，其中能够恢复体重的具有更严重症状的患者，与提供的其他治疗一样频繁地接受膳食，没有恢复体重或不能经历完整膳食时期的患者仅在他们恢复正常体重后(启动治疗之前的体重，而且BMI高于18)接受FMD。FMD由公认安全(RGAS)的成分组成。根据受试者的体重消耗卡路里。第1天，总卡路里消耗量为每磅4.5～7卡(或每千克10～16卡)。膳食应该是至少90％植

物基的膳食。第1天膳食应该含有少于30g糖，少于28g植物基蛋白，20～30g植物基单不饱和脂肪，6～10g植物基多不饱和脂肪，以及2～12g植物基饱和脂肪。在第2至21天，总卡路里消耗为每磅3～5卡(或每千克7～11卡)。第2至21天膳食应该含有少于20g糖，少于18g植物基蛋白，10～15g植物基单不饱和脂肪，3～5g植物基多不饱和脂肪，1～6g植物基饱和脂肪，根据体重稀释于1L水/日(100磅的人10g，200磅的人20g，300磅的人30g)中的10～30g甘油。膳食还应该是含有约50％RDA(每日)的维生素、矿物质加必需脂肪酸的高营养物。最小长度将为5或6天，最大长度将为21天(基于在禁食门诊的治疗实践的安全性数据和标准)

[0065] 癌细胞系对DXR的体外剂量响应：在DXR治疗前24小时和DXR治疗期间的24小时，将葡萄糖限制施用于细胞。在补充了2.0g/L葡萄糖的DMEM中培养对照组，而在补充了0.5g/L葡萄糖的DMEM中培养葡萄糖限制组。通过MTT还原来确定存活率。

[0066] 胁迫抗性-将12周龄雌性C57BL/6小鼠分到以下实验组中：随意(随意喂养)、STS/FMD、DXR、STS/FMD+DXR。为了在存在或不存在雷帕霉素和地塞米松的情况下观察对每种处理的响应，每组以一次三份提供，其中一组经历雷帕霉素处理，一组经历地塞米松处理。在14天的时间内给予雷帕霉素，结束时静脉内给予高剂量的阿霉素(每只小鼠24mg/kg)。在14天的时间内给予地塞米松，结束时静脉内给予高剂量的阿霉素(每只小鼠24mg/kg)。在注射阿霉素之前，喂养属于STS+DXR组的动物非常低的卡路里并且无蛋白的FMD 48小时。在注射阿霉素之后，每天监测动物，并记录存活率。(图1A、图1B和图1C)。胰岛素(ins)组中的小鼠在给予阿霉素之前还每12小时接受胰岛素注射，共48小时。

[0067] 观察到，给予激酶抑制剂雷帕霉素，皮质类固醇药物如地塞米松以及化疗期间的其他高血糖症诱导药物使小鼠对药物敏感而导致死亡率增加(图1C和图1D)。另外，动物的敏感与循环血糖的增加呈正相关(图1B)。然而，当分别通过STS，FMD或给予胰岛素降低葡萄糖水平时，这种致敏作用完全或部分逆转(图1B至图1D)。实验显示了与雷帕霉素和地塞米松组合给予胰岛素、STS或FMD：a)提供了减少由雷帕霉素和地塞米松诱导的高血糖症状态的强效工具(图1B)，以及b)逆转了与由这两种药物诱导的高血糖症相关的毒性作用(图1C和图1D)。

[0068] 虽然上面描述了示例性实施方式，但是并不意味着这些实施方式描述了本发明的所有可能的形式。相反，说明书中使用的词语为描述性的词语而非限制性的词语，并且应该理解的是在不脱离本发明的精神和范围的情况下可以进行各种变化。另外，各种实施的实施方式的特征可以结合起来形成本发明的另外的实施方式。

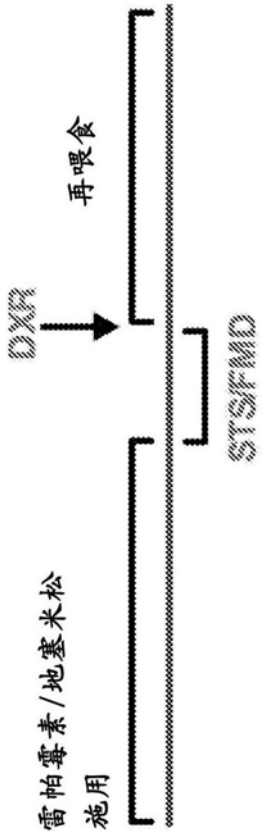
再喂食
DXR
STS/FMD
雷帕霉素/地塞米松
施用

图1A

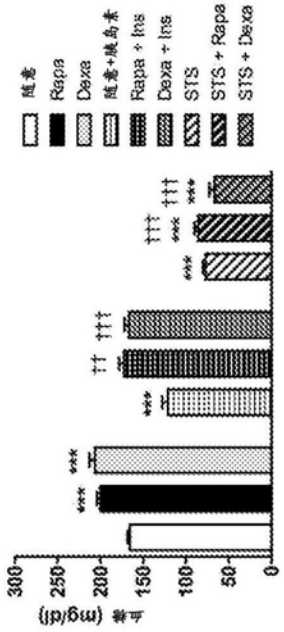
随意
Rapa
Dexa
随意+胰岛素
Rapa + Ins
Dexa + Ins
STS
STS + Rapa
STS + Dexa
血糖 (mg/dl)
300
250
200
150
100
50
0

图1B

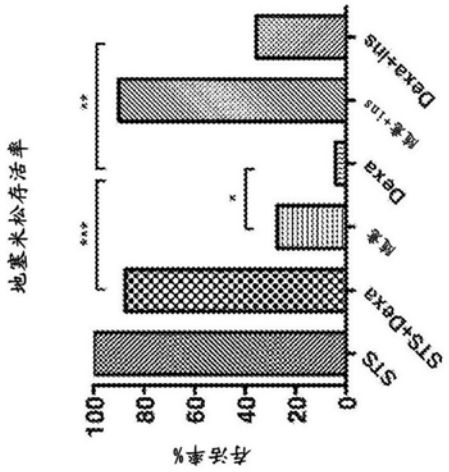
地塞米松存活率
存活率%
100
80
60
40
20
0
STS
STS+Dexa
Dexa
胰素
胰素+Ins
Dexa+Ins
*
**
***

图1C

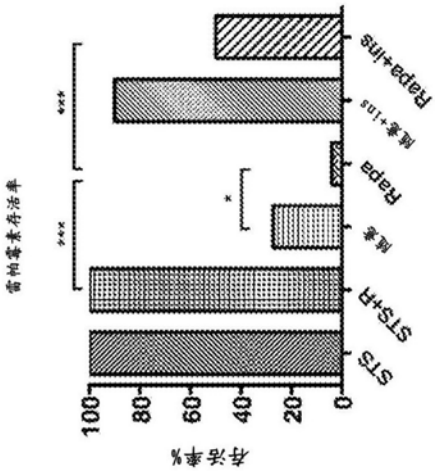
雷帕霉素存活率
存活率%
100
80
60
40
20
0
STS
STS+R
Rapa
胰素
胰素+Ins
Rapa+Ins
*
***
***

图1E

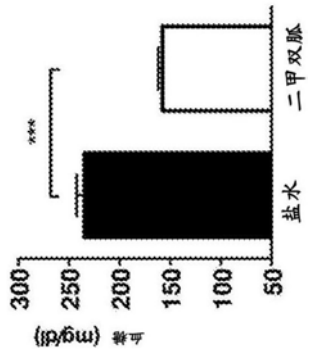
血糖 (mg/dl)
300
250
200
150
100
50
盐水
二甲双胍
***

图1E

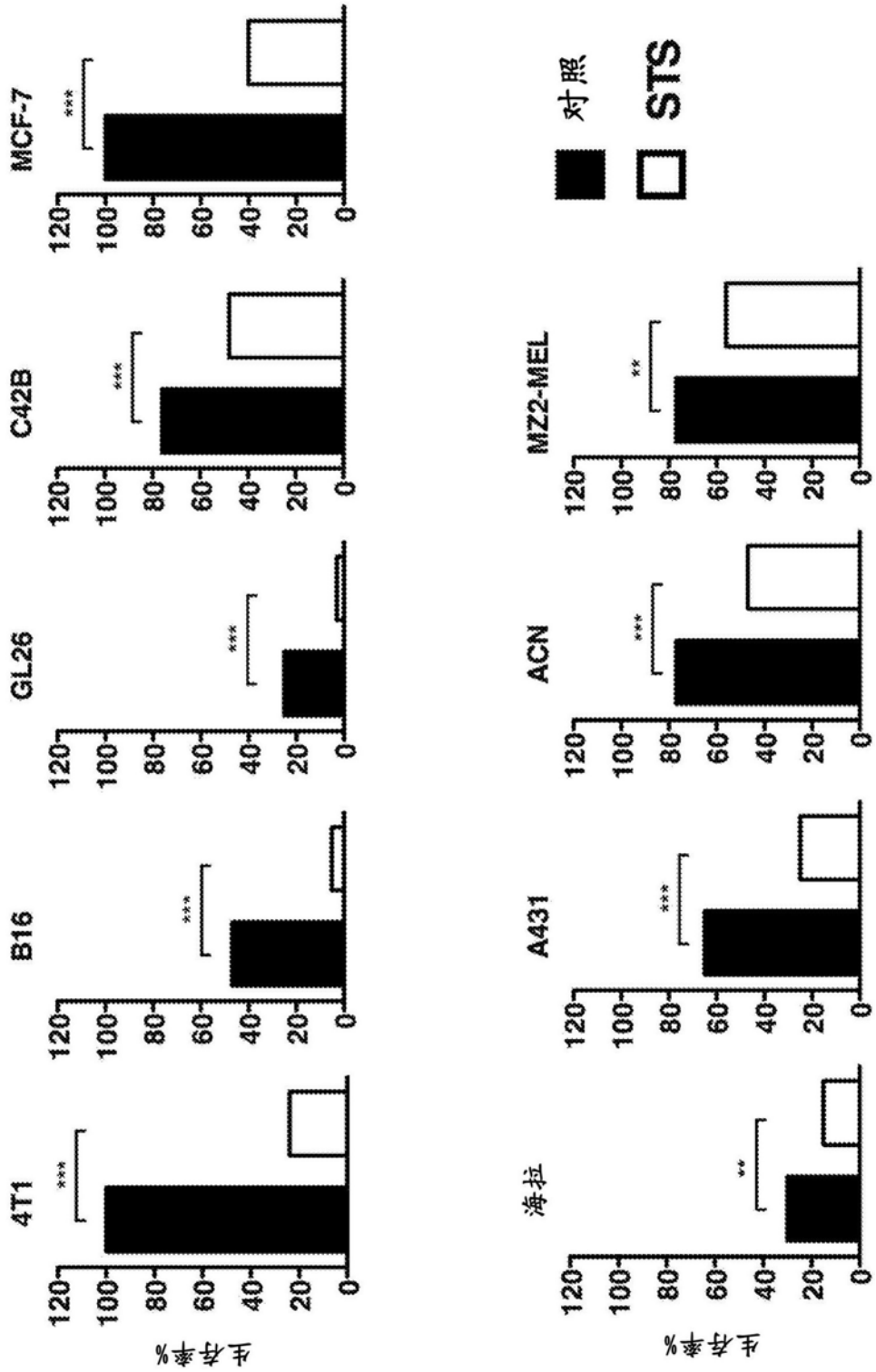
对照
STS
4T1
B16
GL26
C42B
MCF-7
海拉
A431
ACN
MZ2-MEL
生存率%
0
20
40
60
80
100
120
***
**

图2

Abstract

A method for treating a hyperglycemia and hyperglycemia-dependent side effects in a subject undergoing chemotherapy includes a step of identifying a subject undergoing chemotherapy and being administered a hyperglycemia-inducing agent. Short-term starvation or a fasting mimicking diet is administered for a first time period to the subject to prevent hyperglycemia and sensitization to chemotherapy associated with increased glucose levels. The STS-mimicking drug Metformin is administrated between STS/FMD cycles to maintain STS-/FMD-like conditions during the re-feeding period.