Could Carbohydrate -Concentrated Diets Mimic Calorie Restriction in Slowing the Aging Process?

Mark F. McCarty, NutriGuard Research, 1051 Hermes Ave., Encinitas, CA 92024

Abstract

Although calorie restriction and alternate-day fasting have potential for slowing the aging process while decreasing risk for vascular disease, diabetes, cancer and dementia, a "carbohydrate-concentrated" diet strategy, entailing long daily periods of carbohydrate abstinence, may prove to be a much more practical strategy for achieving these aims. The life extension and healthy aging benefits of calorie restriction appear to be mediated primarily by sustained reductions in plasma levels of insulin, free IGF-I, and glucose, which in turn induce down-regulation of Akt and mTORC1, and up-regulation of Sirt1, AMPK, and the FOXO transcription factors. The health protective consequences of these effects include up-regulation of apoptosis – helping to rid the body of pre-cancerous cells – and autophagy, which aids control of oxidative stress while clearing cells of structurally damaged proteins and membranes. Increased FOXO activity boosts expression of many antioxidant enzymes and aids DNA repair; increased Sirt1 activity has an anti-inflammatory impact. Calorie restriction also promotes central neuroprotection by increasing brain expression of neural growth factors, Sirt1, and heat shock proteins. The benefits evoked by calorie restriction are not crucially dependent on calorie deficit per se, since alternate day fasting has likewise been shown to increase maximal lifespan and promote neuroprotection – even if animals maintain their previous calorie intakes by eating ravenously on the fed days. Hence, repeated prolonged episodes of diminished insulin, free IGF-I, and glucose appear sufficient to achieve the aging retardant and health benefits associated with calorie restriction. Modified alternate-day fasting – allowing modest calorie intake during the "fasting" days – has shown clinical utility for ameliorating inflammatory disorders and promoting weight loss, but is probably too rigorous to achieve widespread popularity. Carbohydrate-concentrated (CC) diets – in which most of the day's carbohydrate intake is segregated to one main meal, and optional accessory low-carb meals are allowed – appear to have considerable utility for weight control, and their prolonged periods of relatively low insulin, free IGF-I, and glucose may evoke to some degree the manifold health and pro-longevity benefits seen in calorically-restricted or alternate-day-fasted rodents. Moreover, adherence to such regimens appears to be quite feasible, as suggested by the widespread popularity of the CC diet program promulgated by Drs. Rachael and Richard Heller as a weight control strategy. CC diets composed of plant foods are likely to have the greatest positive impact on health and longevity, as they should reduce plasma IGF-I levels, while also promoting muscle insulin sensitivity and thereby down-regulating insulin secretion.

Calorie Restriction Slows the Aging Process

It is well known that, in rodents, sustained calorie restriction – allowing rodents to eat only 60-80% of the calories they would eat if given free access to food – can extend not only their average lifespan, but also their *maximum* lifespan by as much as 50%. (Maximum lifespan refers to the average lifespan achieved by the 10% of a population that survives longest – though this term is also sometimes used to refer to the longest life that a member of a given species has achieved.) This increase in maximum lifespan is literally associated with a delay in the aging process in many respects; in other words, the onset of many functional decrements and cosmetic changes characteristically seen with aging is significantly delayed. In particular, the onset of cancer tends to be delayed; calorically-restricted rodents might or might not experience the same lifelong incidence of cancer as rodent who are not restricted, but at any given age, fewer of the calorically restricted animals would have developed cancer. And there is a comparable delay in the onset of other common fatal non-infectious disorders in rodents, such as kidney failure.

Moreover, calorie restriction is quite protective to the brain, in part because it increases the expression of certain neural growth factors, antioxidant enzymes, and heat shock proteins that improve the tolerance of neurons for oxidative stress and a number of types of toxic insult, and that can promote repair of the damaged brain. Remarkably, calorie restriction boosts brain levels of neural precursor cells that can generate new neurons, thereby promoting learning and brain repair. And calorie restriction has shown protective effects in rodent models of Alzheimer's, Parkinson's, and Huntington's diseases, as well as of stroke.[1]

A meta-analysis of rodent calorie restriction studies by Merry concludes that the increase in mean and maximal lifespan achieved by calorie restriction is linearly proportional to both the degree of calorie restriction, and the proportion of total lifespan that calorie restriction is implemented.[2] If these findings are also pertinent to humans, the implication is that even a modest degree of calorie restriction implemented for a significant part of one's lifetime might have a worthwhile impact on rate of aging and life expectancy.

Pertinent Experience in Primates – Okinawans and Macaques

Speaking in favor of this possibility is a remarkable natural experiment on the island of Okinawa. Prior to the 1970s, people on Okinawa, on average, were consuming about 11% fewer calories daily than would be considered appropriate for their modest BMIs of about 21.[3] This reflects the fact that their dietary staple, sweet potato, is rather calorically dilute. Also, their agriculture was somewhat inefficient and prone to episodic crop failures; this gave rise to an ethic that food should be conserved, and that people should not completely fill their stomach at meals. This may help to explain why the mean and maximal lifespan of elderly Okinawans today is about 5 years longer than that of Americans. Indeed, Okinawans are known for having the highest proportion of centenarians in the world. Moreover, a reliable biomarker of aging, serum levels

of the hormone DHEA (which declines steadily during aging), is significantly higher in aging Okinawans than in Americans of comparable age; thus, not only do they live longer, but their rate of aging truly appears to be slowed. And this despite that fact that they haven't been calorically restricted since the 1970s!

The possibility that calorie restriction can indeed slow aging in primates appears to be supported by ongoing studies at the Wisconsin National Primate Research Center, where the impact of lifelong 30% calorie restriction is being evaluated in rhesus macaque monkeys. The data accumulated to-date show that calorie restriction prevents or postpones the onset of insulin resistance syndrome and its complications, and retards the onset of cancer and other age-related disorders that can be fatal.[4] Perhaps surprisingly (as calorie restriction does not promote anabolic activity), it also retards the onset of sarcopenia – the progressive loss of muscle mass typically seen with aging.[5] Calorie restriction has not influenced mortality from causes unrelated to aging - complications of anesthesia, injury, endometriosis, gastric bloat. (By analogy, you would not expect calorie restriction to protect humans from car wrecks or appendicitis.) But at any given age, the monkeys who were calorie restricted were only *one-third* as likely as unrestricted monkeys to have died from age-related causes – cancer, diabetes, and cardiovascular diseases; this reduction in risk is highly statistically significant. Particularly notable was the impact of calorie restriction on glucose metabolism – at the time of the last report, 16 of 38 control monkeys had developed diabetes or pre-diabetes, whereas none of the restricted monkeys had this problem. The restricted monkeys naturally were much leaner, the volume of gray matter in certain regions of the brain was better preserved, and they had a more youthful appearance when they reached the average lifespan of the species (about 27 years).[4, 6] These studies must progress further before conclusions can be reached regarding average or maximal lifespan.

Scientists are still striving to understand why calorie restriction increases maximum longevity and delays aging. A reduction in signaling by the key growth factors insulin and IGF-I appears to be of key importance in this regard. But increased activity of certain enzymes which detect reduced intracellular energy availability, notable sirtuin-1 (sirt1) and possibly also AMP-activated kinase (AMPK), may play an adjuvant role in this regard.

A Key Role for Down-Regulation of Insulin/IGF-I Signaling

Reduced calorie availability extends maximal lifespan not only in rodents, but also lower organisms such as yeast, worms, and fruit flies; in each case, reduced signaling by hormones that are functionally and structurally homologous to the insulin and IGF-I produced in mammals appears to be a key mediator of this life-extending effect.[7] With respect to rodents, calorie restriction decreases diurnal insulin levels, since less food is ingested to evoke insulin release and hepatic glycogen stores are less ample; moreover, circulating levels of IGF-I are also reduced, and this IGF-I is less bioactive owing the an increase in hepatic IGFBP-1 secretion that results from the lower insulin levels.[8] A very key role for decreased IGF-I activity in lifespan

extension is suggested by the fact that, even when given free access to food, mice that have been genetically bioengineered to produce less IGF-I (owing to a deficit of growth hormone production, or genetic resistance to the activity of growth hormone), or whose receptors for IGF-I are less functional, tend to achieve an increased maximal lifespan.[7] (Nonetheless, concurrent calorie restriction boosts the longevity in these mice still further, indicating that decreased IGF-I activity is not the sole mediator of the pro-longevity effect of calorie restriction.[9]) In regard to humans, a recent study has found that human centenarians, as contrasted to younger people, are more prone to carry altered forms of the gene for the IGF-I receptor which render the receptor less responsive to IGF-I or that associate with lower IGF-I levels; this suggests that reduced IGF-I signaling in humans may also be associated with increased potential to achieve extreme old age.[10, 11]

In their target tissues, insulin and IGF-I activate the mammalian target of rapamycin complex 1 (mTORC1). Recent rodent studies shown that drugs which inhibit this enzyme complex – namely the immunosuppressive drug rapamycin and the antidiabetic medication metformin – can extend maximal lifespan in some strains of rodents.[12-16] Since rapamycin doesn't alter levels of insulin or of IGF-I (metformin may modestly reduce their levels), it can be concluded that suppression of mTORC1 activity is one of the key mediators of the favorable effects of calorie restriction on aging.

mTORC1 is a key regulator of an intracellular process known as macroautophagy.[17] This process sweeps cellular proteins and organelles, including mitochondria, into lysosomes, where they are digested to yield their chemical constituents; these can then be reutilized in biosyntheses. Although rampant macroautophagy can precipitate cell death, a more gradual but persistent macroautophagy, when coupled appropriately with increased biosynthesis of new proteins and organelles, can optimize cellular function by clearing away structurally damaged proteins and organelles and replacing them with "shiny new" ones. mTORC1 activity suppresses this protective process.[17] For unknown reasons, aging tends to decrease cellular capacity for macroautophagy, whereas long-term calorie restriction tends to slow or prevent this age-related loss of capacity. (It is not surprising that acute calorie restriction activates macroautophagy – as it opposes mTORC1 activity; but why chronic caloric restriction opposes the age-related decline in *capacity* for macroautophagy is much less clear.) There is considerable speculation that the ability of calorie restriction – or other measures which chronically decrease IGF-I or mTORC1 signaling – to activate macroautophagy and, in the long run, to prevent an age-related decline in this process, is one of the key mediators of the pro-longevity impact of these strategies.[18-21] Indeed, it is intuitively appealing that measures which help to keep our cellular proteins and membranes structurally and functionally pristine could have an "anti-aging" effect. Thus, Cuervo refers to calorie restriction as "the ultimate cleansing diet".[21]

The delay in cancer incidence associated with calorie restriction may be largely attributable to the fact that insulin/IGF-I activity exerts a pro-proliferative impact on many tissues prone to give

rise to cancer – thereby increasing the rate at which mutations resulting from inaccurate DNA replication accumulate in stem cells – while at the same time this activity tends to suppress protective apoptosis in pre-cancerous initiated cells.[22] This latter effect stems from activation of the kinase Akt as well as mTORC1, each of which work in various complementary ways to oppose apoptosis.[23, 24] Remarkably, Dunn and collegues have shown that in cancer-prone mice (heterozygous for p53 deficiency and treated with a carcinogen that initiates bladder cancer), the ability of dietary restriction to slow bladder cancer progression is completely abrogated in mice receiving continuous infusions of IGF-I.[25] With respect to humans, relatively low plasma levels of IGF-I and/or insulin (the latter can increase IGF-I bioactivity by suppressing hepatic secretion of IGFBP-1) have been associated with decreased risk for a number of types of cancer.[26-30] Moreover, there is recent evidence that Laron dwarves – in whom homozygosity for a dysfunctional growth hormone receptor leads to markedly lower plasma levels of IGF-I – may be at exceptionally low risk for cancer.[31, 32]

Cellular Energy Deficit Activates Sirt1 and AMPK

There is however good reason to believe that factors beyond insulin/IGF-I down-regulation contribute to the aging-retardant impact of calorie restriction. In particular, activation of the enzyme sirtuin-1 (sirt1) likely plays a role in this regard. Sirt1, found primarily in the nucleus of cells, can remove acetyl groups from gene-regulatory proteins that bind to DNA; by removing these acetyl groups, sirt1 changes the function of these regulatory proteins in a way that modulates the range of proteins which the cell synthesizes. Sirt1 tends to be activated when cellular fuels such as glucose or fatty acids are in short supply and insulin/IGF-I are relatively low (as occurs during fasting or calorie restriction); the resulting increase in sirt1's activity exerts a range of effects that suppress inflammation, increase the biogenesis and the fat-burning capacity of mitochondria, help to prevent cell death by suicide in long-lived cells (such as neurons and cardiac fibers), boost the expression of antioxidant enzymes, aid DNA repair, stimulate macroautophagy, and help prevent autoimmune disorders.[33-45]

Yeast, worms, and flies make enzymes functionally homologous to sirtuin-1in mammals, and strategies (aside from calorie restriction) which boost the activity of these enzymes in these lower organisms tend to increase their maximal lifespans.[46-48] Hence, it is suspected that an increase in sirt1 activity may be another of the mediators of the life-extending impact of calorie restriction in rodents; indeed, one recent study found that, in genetically modified mice that lack sirt1 activity, calorie restriction fails to increase maximal lifespan.[49] In rodents, high daily doses of the phytochemical resveratrol can activate sirt1 in many tissues, and considerable interest has been generated by a study showing that resveratrol can diminish weight gain and increase lifespan in mice fed high-calorie diets.[50] Unfortunately, no impact on maximal lifespan was noted in mice fed a more healthful diet – suggesting that sirt1 may not be sufficient to slow aging if insulin and IGF-I activities remain rather high; nonetheless, resveratrol had a number of

favorable functional effects on aging mice, suggestive of healthy aging.[51] (Unfortunately, owing to unfavorable pharmacokinetics in humans, its utility for humans in very much in doubt.)

Another key enzyme activated by low-energy conditions in cells (and hence by calorie restriction) is AMP-activated kinase (AMPK). This remarkable enzyme has the ability to increase the activity of sirt1 while simultaneously decreasing the activity of the mTORC1 complex (as you will recall, a key mediator of the effects of insulin and IGF-I).[52-55] As we have noted, the antidiabetic drug metformin, whose clinical benefits reflect activation of AMPK, has in fact been found to increase the lifespan of some but not all strains of rodents. In human diabetics, metformin therapy has been associated with decreased risk for cancer, heart attack, and stroke – and, unlike most other diabetic medications, tends to promote weight loss.[56-60] Thus, some researchers think that metformin may have true "anti-aging" potential.[13, 15]

It may not be accidental that all of the key "anti-aging" mediators we have discussed – sirt1, AMPK, and decreased activity of insulin/IGF-I – tend to activate the macroautophagy process that prevents cellular accumulation of damaged proteins and membranes.

FOXO Factor Activation Promotes Longevity

Other regulatory proteins influenced by the activities of insulin/IGF-I, AMPK, and sirt1 are the closely related transcription factors FOXO1 and FOXO3a. These function to increase the synthesis of enzymes which have crucial antioxidant functions – such as catalase and the mitochondrial form of superoxide dismutase – and others which promote efficient DNA repair.[61-65] FOXO activity therefore plays a key role in antioxidant defense and in cancer prevention.

Insulin and IGF-I signaling suppress FOXO activity by promoting the exclusion of FOXO factors from the nucleus and stimulating their degradation. Conversely, both AMPK and sirt1 act on nuclear FOXO factors to boost their activity.[66-69] For these reasons, the growth factor reduction and the cellular fuel shortage associated with calorie restriction tend to promote FOXO activity, much like they promote autophagy. The FOXO factors are structurally and functionally homologous to a protein made by worms, known as DAF-16, that is a crucial mediator of the lifespan-extending impact of calorie restriction in these organisms; calorie restriction activates DAF-16 in worms, and genetically-altered worms which can't make DAF-16 get no benefit from calorie restriction.[70, 71] Analogously, in mice that have a genetically diminished capacity to make FOXO1, calorie restriction, while still extending lifespan, has minimal impact on cancer risk.[72]

Several variant forms of the FOXO3a gene occur in human populations; these variations potentially could influence the amount of FOXO3a protein made, and/or the structure and function of the protein. Studies in several different populations are showing that certain specific forms of this gene are more common in people who have achieved a ripe old age.[73-76] Very likely, these forms of the gene are more highly active.

AMPK and Sirt1 also collaborate to promote the nuclear translocation and activation of PPAR-gamma coactivator-1alpha (PGC-1alpha), which plays a key role in the induction of mitochondrial biogenesis.[77, 78] One of its effects in this regard is to act as a coactivator for FOXO3a in promoting transcription of genes that code for mitochondrial antioxidant enzymes.[64] It undoubtedly is no accident that enzymes which activate macroautophagy (and hence promote lysis of aging mitochondria) concurrently induce production of new mitochondria.

Slowing Aging - Calorie Restriction Per Se May Not be Necessary

Unfortunately, the calorie restriction literature is of questionable practical significance for humans, as few humans, on a chronic basis, are likely to subject themselves to significant daily calorie restriction by obsessively counting calories (except as a temporary measure for treating obesity). Temporary calorie restriction in the obese is reasonably feasible, since burning fat derived from excess fat stores can compensate for the deficit of food calories supplied, but once excess fat is lost, daily calorie restriction becomes much less pleasant; typically, it is associated with gnawing hunger, anxiety and tiredness. (It is telling that calorically-restricted monkeys virtually attack the food that is presented to them, and calorically-restricted rodents consume almost immediately their daily food allotments.) Some rather heroic and stoic members of the Calorie Restriction Society are succeeding in moderately restricting their calorie intakes on a chronic basis, but don't look for this lifestyle strategy to become popular any time soon.

It is therefore of considerable interest that alternate day fasting – fasting animals every other day, and letting them consume as much as they want on alternate days - has also been shown to boost maximal longevity in rodents.[79-81] Remarkably, this benefit is seen even in strains of mice that eat twice or nearly twice as many calories on their feeding days as they would consume per day if given free access to food.[82] In other words, alternate day fasting seems to boost longevity even if there is no net decrement in calorie intake. Presumably, this implies that the metabolic benefits achieved during intermittent fasts of 36 hours or so – associated with notably decreased levels of insulin, free IGF-I, and glucose, and likely also up-regulation of sirt1 and AMPK activity - are not overridden by shorter intermittent episodes in which insulin, free IGF-I, and glucose are considerably increased.

Mattson has suggested that the moderate stress evoked by intermittent fasting can be viewed as a type of "hormesis" that amplifies expression of protective cellular factors that in turn work to delay the aging process. Mattson's group has also shown that alternate-day fasting has potent neuroprotective effects on the central nervous system – protecting the brain from oxidants, chemical toxins, or temporary deficits of blood flow – that are at least as great as those achieved with daily calorie restriction.[83]

Anti-inflammatory Benefits of Modified Alternate-Day Fasting

Unfortunately, clinical evaluations of strict alternate day fasting in non-obese subjects have concluded that hunger and weakness on the fasting days make it unlikely that most people would stick with such a regimen in the long term.[84] Johnson and Laub have therefore proposed a "modified alternate-day fasting" regimen, in which 20-50% of ad libitum calorie intake is allowed on "fasting" days; in their clinical experience, this can achieve far higher compliance that strict alternate-day fasting. [85-87] They indicate that they have monitored over 500 people who have attempted this regimen, and note that a wide range of clinical disorders appear to be benefited by this strategy, including conditions such as insulin resistance, cardiac arrhythmias, autoimmune diseases (such as rheumatoid arthritis), osteoarthritis, and periodontal disease. In a more formal 8-week clinical study enrolling overweight people with asthma, they found that asthma symptoms improved markedly during this period, while initial body weight fell by about 8% and risk factors related to insulin resistance syndrome improved concurrently.[86] They also draw attention to an intriguing Spanish clinical study, published in 1957, in which half the residents of a retirement home were subjected to alternate-day calorie restriction – receiving 900 calories on restricted days, and 2300 calories on fed days – and the other half of the residents, fed ad-libitum, served as controls. Over a three year period, the residents subjected to alternate-day restriction experienced about half as many deaths and days of hospitalization as the non-restricted control group.[85]

The marked anti-inflammatory effects seen in the asthma study, and also noted in patients with various autoimmune disorders undertaking an alternate day modified fasting regimen, is particularly striking, and may prove to have clinical utility for managing such disorders. Johnson and Laub note that activation of sirt1 suppresses the pro-inflammatory activity of the gene-regulatory protein complex NF-kappaB.[35, 36] Excessive activation of NF-kappaB is at the root of many inflammatory disorders – and other scientists have shown that sirt1 mRNA is increased in the skeletal muscle fibers of people who are engaged in strict alternate day fasting.[88] Moreover, exposure of human liver cancer cells to blood serum obtained from people who have been doing alternate day fasting increases the level of sirt1 protein in these cells by 20%.[89] The anti-inflammatory clinical benefits observed by Johnson and Laub may therefore reflect (at least in part) an increase of sirt1 activity in the immune cells of people who are doing an alternate day modified fasting regimen.

For years, doctors have known that the pain of rheumatoid arthritis remits substantially when patients fast for several days; unfortunately, the pain tends to come back soon after they start eating again.[90, 91] The observations of Johnson and Laub may mean that the anti-inflammatory benefits achieved by strict fasting can be maintained on a sustained basis in people who do a modified alternate day fasting regimen. The day of substantial calorie consumption permitted on the Johnson regimen does not seem to offset the benefits of sirt1 activation achieved during the modified fast, since control of inflammation was nearly as good on fed days as on fasted days;

this might reflect the fact that sirt1, and some of the protective proteins whose synthesis it promotes, have long "lifespans" that extend well into the fed day.

Carbohydrate-Concentrated Diets May Represent a Practical Alternative

The modified alternate-day fasting strategy may prove to have very worthwhile practical merit for patients with chronic inflammatory conditions which respond well to it – particularly if they are also overweight. Patients who are already lean, or people who aren't afflicted with such conditions, may be more apt to find this strategy too rigorous for long-term compliance.

It is however quite conceivable that shorter intermittent fasts – such as those achievable by consuming only one large meal a day – could also provide some of the aging-retardant and neuroprotective benefits evoked by alternate-day fasting or calorie restriction. Indeed, Mattson has urged that one-meal-per-day regimens should be assessed clinically.[92] And Herring has proposed that consuming all one's daily calories in a single 5 hour period – which he calls a "fast-5 diet" can be reasonably practical.[93] Anecdotally, he reports that this strategy has enabled him to achieve and maintain a quite significant weight loss, and that he and many others – who likewise report worthwhile weight loss – have been able to comply with this regimen in the long term.

Jeremy J. Stone has recently introduced the concept of "carbohydrate-concentrated (CC) diets" as a generalization of the one-meal-per-day approach.[94] By definition, a CC diet is one in which the great preponderance of the day's carbohydrate – and all of the high-glycemic-index carbohydrate – is consumed within a single daily main meal. One or two accessory meals quite low in carbohydrates are also permitted, but are not required. This strategy is very similar to a diet program promulgated previously by Drs. Rachael and Richard Heller (differing primarily in that the Hellers additionally require that the main meal be consumed in a single hour). The Hellers referred to their program as "The Carbohydrate Addicts Diet" and popularized it in several best-selling books.[95-97] They reported anecdotally that this strategy was quite helpful for achieving weight loss and weight maintenance in most compliant subjects, and was also associated with notable improvements in cardiovascular risk factors. The great popularity of the Hellers' books – despite the fact that they were ignored by the mainstream medical community – may have reflected not only the efficacy of this approach for weight control, but also the fact that their regimen was reasonably easy to comply with, inasmuch as people were allowed to (but not required to) eat up to 3 meals daily.

Very recently, a controlled trial by Israeli researchers has demonstrated that a carbohydrate-concentrated approach can amplify weight loss in the context of concurrent calorie restriction.[98] Overweight policemen were randomized into two groups; all volunteers were asked to consume 1300-1500 kcal daily, and both groups ate the same types of food, but a further requirement for one of the groups was that virtually all of the day's carbohydrates be consumed at dinner. At the

end of the 18 week study, average weight loss had been significantly greater in the CC group (11.6 kg) than in the control group (9.1 kg). The CC group also achieved greater improvements in risk factors such as fasting glucose, insulin resistance, LDL and HDL cholesterol, C-reactive protein, and IL-6. Moreover, the increase in adiponectin level (averaged over a 12-hour period) was dramatically higher in the CC group (44%) than in the controls (14%); this difference was disproportionate to the relative losses of body fat, and possibly reflects a specific impact of CC dieting. Numerical self-rating of hunger enabled the researchers to conclude that hunger was better controlled throughout the day on the CC regimen, likely accounting for the greater weight loss achieved by that group. They speculated that this improvement in hunger control resulted from a shift in the diurnal pattern of leptin secretion, such that higher levels of leptin were seen during daytime hours. (Alternatively, increased hepatic fat oxidation during the lengthy low-carb periods may have played a role – as discussed below.) Although this Israeli study entailed conscious calorie restriction, it seems likely that the greater weight loss associated with a CC regimen would also be seen during CC dieting without conscious restriction – as reported by the Hellers.

Stone's original contribution is to propose that the CC diet approach may be of benefit not only from the standpoint of healthful weight control, but that it might also evoke, to at least a modest extent, many of the metabolic benefits associated with alternate-day fasting or calorie restriction, owing to the fairly long consecutive hours of relatively low insulin, free IGF-I, and glucose entailed by such a regimen. And he has also drawn attention to the fact that calorie-restricted animals are effectively practicing CC dieting, as they tend to consume their entire day's food allotment within 1-2 hours of receiving it. Masoro also noted this, and went on to show that, in the context of severe (40%) calorie reduction, giving mice two meals or one meal daily had no impact on their mean or maximal longevity.[99] Nonetheless, this does not prove that CC dieting per se, with food intake in ad-libitum amounts, might not have a favorable impact on rodent (or human) longevity or healthy aging. To test this, one could train mice to consume all of their day's food (or carbohydrate) within a (say) two hour period every day; mice likely would soon learn to do this if deprived of their food after two hours every day. The health, leanness, and longevity of such mice could then be compared to that of mice given free access to food all day.

CC dieting generalizes the one-meal-a-day approach in that it allows (but does not require) accessory meals or snacks that are quite low in carbohydrates. The insulin secretion provoked by these meals will hence be a function of their protein content. But protein, calorie per calorie, tends to boost insulin secretion less than most carbohydrates. Moreover, protein ingestion also causes pancreatic release of glucagon, a hormone which opposes the impact of insulin on the liver, and boosts the capacity of the liver to burn fat. Efficient hepatic fat oxidation tends to control hunger; it does so by sending signals to the brain via the vagus nerve, and also by boosting the availability of fuel for the brain via increased hepatic production of ketones bodies and increased liver production of glucose via gluconeogenesis.[100, 101] This probably explains

why, when people have been eating low-carb "Atkins" style diets for days or weeks at a time, their adaptive loss of hunger is not quenched by ingesting low-carb protein-rich foods – despite a boost in insulin secretion – whereas a carb-rich meal can bring their appetite roaring back. Another key effect of glucagon is that it tends to increase the liver's production of IGFBP-1, opposing the impact of insulin in that regard.[102, 103] An increase in IGFBP-1 opposes the bioactivity of IGF-I, and is one of the key reasons why the low insulin environment associated with calorie restriction tends to prevent or postpone cancer and slow the aging process.

So while CC diets that include accessory meals won't keep insulin levels as low as they would be during a strict 20-hour fast, the compensatory impact of the accessory meals on glucagon secretion will tend to aid hunger control, and also may mitigate the impact of modest insulin rises on cancer risk and aging. Hence, the aging-retardant benefit of CC diets with accessory meals may be little different from that achieved by one-meal-per-day strategies, and the intervals between main meals might reasonably be referred to as "quasi-fasting".

It is also quite conceivable that a CC diet strategy, if adopted as a continuing lifestyle, could sustain a modest but meaningful degree of "spontaneous" calorie restriction – particularly if the accessory meals are relatively low in fat as suggested by the Hellers, or are omitted entirely. In overweight people actively losing fat during the first few months of such a diet, the calorie restriction may be rather substantial, as the burning of stored fat compensates for dietary calories not consumed. However, even after weight equilibrates, CC dieters may be consuming fewer calories than they did previously. This may reflect the appetite control mechanisms evoked by quasi-fasting metabolism during most of the day (as documented in the Israeli study), which would help to restrain calorie consumption during the accessory meals; indeed, in the Hellers' experience, many subjects on CC diets chose to skip one or both of the accessory meals. Furthermore, during the main meal, it can be uncomfortable and unnatural to consume too great a number of calories in a short space of time.

Critics could justly argue that the degree of the reductions in insulin, free IGF-I, and glucose achieved during the 36-hour fasts entailed by alternate-day fasting are likely to be more substantial, and the duration of these reductions longer, than on CC diets. While such objections are accurate, it has been established that alternate-day fasting is not a practical alternative for most humans – whereas CC dieting appears to be substantially more practical. The real question is whether the less lengthy intervals of decreased insulin, free IGF-I and glucose associated with CC dieting will be sufficient to evoke in some measure the protective metabolic effects seen with calorie restriction. To begin to answer this question, long-term clinical trials (preferably of 6-12 months duration) evaluating the impact of ad-libitum CC dieting on parameters such as daily calorie intake, body composition, urinary C-peptide (to quantify diurnal insulin secretion), vascular risk factors, markers for oxidative stress or inflammation, psychological function, proliferation rate in epithelial tissues (perhaps keratinocytes),[104] and the activity of sirt1, AMPK, and antioxidant enzymes in leukocytes, would be desirable. (It is crucial to emphasize that such

studies must allow ad libitum calorie intake – studies which require a person's entire typical daily calorie consumption to be consumed within one meal[105] will not replicate the long-term impact of CC dieting guided by appetite, and clearly will abrogate the weight-loss benefits of this strategy.)

Moreover, even if rodent studies reveal that CC dieting is not likely to impact maximal longevity to a meaningful degree, the possibility that worthwhile effects on cancer risk and healthful aging might be achieved will still merit consideration. At the very least, CC diets may often help to reverse metabolic syndrome by promoting weight control – no mean benefit in itself.

Lessons from Ramadan

During the month of Ramadan, Moslems eat a pre-dawn breakfast and then abstain from food or drink until sundown, at which time they often enjoy a prolonged feast. While this is clearly different from CC dieting (as breakfast will usually contain carbohydrate), Ramadan fasting does entail 12 consecutive hours without food each day. A number of medical studies have evaluated impacts of Ramadan fasting on risk factors. Although the results of these studies are not uniform, there is a trend (significant in some studies) toward weight loss and fat loss during the month. Rather substantial increases in HDL cholesterol and apo-A1 are also typically reported, insulin sensitivity tends to rise, and marked reductions of serum IL-6 and C-reactive protein, suggestive of improved adipocyte function, have also been noted.[106-110] These latter effects appear to be disproportionate to the usually modest weight loss achieved. It will be of interest to determine whether analogous effects are evoked by CC dieting.

Amplifying the Benefits with Plant-Based Food Choices

Recently, longevity researchers at Washington University were surprised to discover that long-term calorie restriction does not notably lower serum IGF-I levels in humans – even though such restriction lowers IGF-I by about 40% in rodents.[111] (This likely reflects the fact that, whereas calorie restriction down-regulates growth hormone secretion in rodents, it fails to do so in humans.[112, 113]) In light of the central role which IGF-I bioactivity is believed to play in the regulation of aging, this must have come as a bit of a shock to practitioners of calorie restriction! Fortunately, it is likely that calorie restriction lowers IGF-I *bioactivity* in humans, since the low insulin levels associated with it up-regulate the IGF-I antagonist IGFBP-1. Nonetheless, it seems likely that calorie restriction per se will have a lesser impact on IGF-I bioactivity in humans than it does it rodents.

The silver lining of the Washington University research is the discovery that serum IGF-I levels are indeed lower – by about 30% - in people who habitually consume whole-food vegan diets of moderate protein content, even when they are making no effort to restrict calories.[111] Decreased IGF-I levels in those consuming vegan or quasi-vegan diets have been reported previously, and arguably may reflect a down-regulatory impact of moderate essential amino acid restriction on

hepatic IGF-I production.[114-116] This phenomenon may help to explain the relative rarity of "Western" cancers in societies whose traditional diets are quasi-vegan.[117] It also should be noted that, at the time that Okinawans were engaged in calorie restriction, their food choices were incredibly low in animal products – only about 3% of total calories![3] The Okinawans are the only clear example we have at present of an increase in maximal lifespan achieved by humans; whether their quasi-vegan traditional diet was key to this remains unknown.

The possible benefit of moderate essential amino acid restriction brings to mind the interesting phenomenon that dietary methionine restriction – giving rodents ad libitum access to food that is relatively low in methionine (20% of the content of control diets) achieves an increase in mean and maximal longevity about half as great as that achieved by substantial calorie restriction.[118, 119] More recent studies show that decreasing dietary methionione content by 40-80% is associated with a reduction in mitochondrial complex I superoxide generation, and that this phenomenon appears to be primarily responsible for the decrease in mitochondrial superoxide production observed during calorie restriction.[120, 121] It has been suggested that, inasmuch as most plant proteins are relatively low in methionine relative to animal proteins, a vegan diet of modest protein content might be capable of evoking, at least to a modest degree, the life extension and antioxidant benefit achieved by methionine restriction in rodents.[122, 123]

Vegan diets – and Mediterranean food choices as well – tend to have a favorable effect on muscle insulin sensitivity owing to the low saturate-unsaturate ratio of their dietary fat.[124] This of course would be expected to down-regulate diurnal insulin secretion – an effect which would presumably complement the impact of CC diets on diurnal insulin. Conversely, saturated fat not only tends to increase LDL cholesterol, but it is more likely than unsaturates (especially monounsaturates) to provoke inflammation in the arterial endothelium, liver, or other tissues.[125, 126] These findings are probably pertinent to a recent epidemiological analysis, employing data from the prospective Nurses' Health Study and the Health Professionals' Follow-Up Study, which found that those who followed low-carbohydrate diets rich in animal products tended to have increased mortality compared to controls, whereas those following low-carbohydrate diets composed primarily of plant products had decreased mortality compared to controls.[127]

These considerations appear to imply that CC diets featuring moderate-protein vegan food choices are likely to achieve the greatest reductions in diurnal insulin and in free IGF-I, and hence may have the greatest potential to achieve some of the metabolic benefits associated with calorie restriction or alternate-day fasting. The accessory meals with such a regimen could feature green leafy vegetables and other low-carb vegetables, vinegar, oils, nuts, and soy products such as tofu or edamame; or one or both accessory meal could be skipped altogether. The present author has been following such a regimen, at least several days a week, for about a year, so he can attest that it is not impossible.

With respect to the fat employed with CC dieting, almonds, which provide primarily oleic acid, may have special utility for minimizing diurnal insulin secretion. Jenkins and colleagues have reported that isocaloric substitution of 36 g of almonds for a muffin in a day's diet is associated with a reduction of diurnal insulin secretion (assessed by urinary C-peptide output) by about a third; the degree of benefit observed is disproportionate to that expected from withdrawing the muffin carbohydrates, and presumably reflects a special property of almonds.[128]

It should also be noted that, even though dietary fat provokes little insulin secretion and does not directly raise blood glucose, an excess of free fatty acids (and possibly ketone bodies) in fat people overconsuming fatty foods is likely to abrogate the up-regulatory effect of low glucose on sirt1 and AMPK activities. Moreover, an excess of ectopic fat in skeletal muscle – particularly saturated fat – can have an adverse impact on insulin sensitivity and hence raise diurnal insulin secretion. Notably, feeding rodents a low-carb, high-fat diet does not extent their lifespans. Exercising moderation in the fat content of accessory meals (and the main meal) is therefore advisable if CC diets are to have an optimal impact on weight control and health. Indeed, the Hellers emphasize this.

A quick comment on exercise is also warranted. Aerobic training done during fasting metabolism – or the quasi-fasting metabolism that prevails most of the day during CC dieting - should have the most worthwhile impact on loss of body fat and improvement of insulin sensitivity, and hence should aid the minimization of diurnal insulin associated with CC dieting.[129-131] Moderate-intensity aerobic training may also lower serum IGF-I slightly.[132]

Metformin or Berberine as Adjuvants for CC Diets

It seems unlikely that 20 hours or so of carbohydrate avoidance will decrease glucose levels and glycogen stores as greatly as they are during the fasting period of alternate-day fasting; indeed, that may be why CC diets are less stressful and hence more practical. As a result, CC diets may not activate AMPK and Sirt1 – which effectively function as cellular calorie sensors – as thoroughly as alternate-day fasting does. However, activation of AMPK has emerged as the key mechanism of action of the widely employed diabetes drug metformin;[133, 134] moreover, AMPK has recently been shown to boost Sirt1 activity by increasing expression of nicotinamide phosphoribosyltransferase, the enzyme which generates Sirt1's obligate substrate NAD+.[135, 136] In addition, metformin's well known ability to down-regulate gluconeogenesis and hepatic glucose output could be expected to further diminish serum glucose and insulin levels. Hence, employing metformin as an adjuvant to CC diets would likely potentiate the ability of such diets to suppress signaling via insulin/IGF-I and mTORC1, while boosting AMPK, Sirt1, and Foxo activities – better mimicking the key metabolic effects of calorie restriction thought to mediate its favorable impact on longevity.

Unlike some diabetes drugs, metformin cannot provoke severe hypoglycemia, and hence can be used safely by non-diabetics. Moreover, a growing number of epidemiological studies and the UK Prospective Diabetes Study conclude that metformin-treated diabetics are less likely than diabetics treated with other agents (establishing a comparable degree of glycemic control) to experience heart attack, stroke, and many types of cancer; indeed, their total mortality is decreased.[137-140] In pre-diabetics, metformin therapy helps to prevent or postpone the onset of diabetes.[141] The possibility that metformin could be employed as a calorie restriction mimetic has been raised by a number of researchers, and lifelong treatment with metformin has increased average and sometimes maximal lifespan in some though not all strains of rodents.[142-146] Since metformin is quite safe in recommended doses, and is well tolerated aside from some GI upset that afflicts a minority of patients and tends to become less significant with time, this agent may have practical potential as an adjuvant to CC dieting and other diet strategies intended to retard the aging process.

Alternatively, the herb-derived compound berberine, long employed in China as a diabetes therapy, can also activate AMPK, and hence may have pro-longevity potential comparable to that of metformin.[147-149] Berberine has the adjunctive advantage that it up-regulates hepatic expression of the LDL receptor by a post-transcriptional mechanism that is complementary to the impact of statins.[150, 151]

Because of its ability to boost longevity and Sirt1 activity in "lower" organisms, resveratrol has also attracted attention as a potential calorie restriction mimetic. However, there is now evidence that resveratrol-mediated activation of Sirt1 is downstream from, and wholly dependent on, AMPK activation.[152-154] Furthermore, resveratrol's stimulatory impact on AMPK, reflecting partial inhibition of mitochondrial ATP synthase, requires low micromolar concentrations which are difficult to achieve or sustain in humans with feasible oral doses.[155-158] Moreover, unlike metformin or berberine, resveratrol has never been shown to aid glycemic control in diabetics – an expected consequence of AMPK activation. These considerations suggest that resveratrol has considerably less practical potential for extension of human lifespan than do either metformin or berberine.

Remarkably, recent studies by Hardie and colleagues establish that the majority of agents know to activate AMPK/Sirt1 – including metformin, berberine, and resveratrol - do so by compromising the efficiency of the mitochondrial respiratory chain or the mitochondrial ATP synthase, and thereby raising the cellular AMP/ATP ratio; 2-deoxyglucose achieves a comparable effect by inhibiting glycolysis.[155] Hence, these agents create a "fuel shortage" signal comparable to that evoked by calorie deficit.

A Caution – IGF-I Activity Promotes Vascular Health

Insulin and IGF-I activation endothelial nitric oxide synthase (eNOS) via the PI3K/Akt signaling pathway; they also boost endothelial expression of eNOS mRNA and protein.[159-162] There is ample evidence that efficient eNOS activity works in various ways to counter atherogenesis and decrease risk for myocardial infarction and stroke.[163] Delafontaine and colleagues have shown that, in atherosclerosis-prone ApoE-deficient mice, a modest genetically-determined decrease in serum IGF-I exacerbates atherogenesis; conversely, continual infusion of IGF-I inhibits atherogenesis in apo-E deficiency, while boosting aortic eNOS expression.[162, 164] These authors also demonstrate that IGF-I counteracts the pro-oxidative impact of oxidized LDL on endothelial cells – an effect independent of eNOS activity.

Some but not all epidemiological studies have associated low-normal IGF-I levels with increased risk for ischemic heart disease or stroke.[165] It is hard to say whether low IGF-I is actually the mediator of this increased risk, since metabolic syndrome tends to down-regulate IGF-I levels (its tendency to boost IGF-I bioactivity results in a feedback reduction of hepatic IGF-I biosynthesis), and the aging process per se leads to a decrease in serum IGF-I. Intriguingly, whereas quasi-vegan Third World societies have typically enjoyed low risk for coronary disease (presumably because their LDL levels tend to be quite low), some – especially those which heavily salt their food and hence are prone to hypertension – are at high risk for stroke, and it has been suggested that low serum IGF-I may play a role in this phenomenon.[166] (Low LDL cholesterol may also contribute to increased risk for hemorrhagic stroke in these populations.) Low serum IGF-I may also have a negative impact on prognosis following stroke;[167] in part, this may reflect the fact that IGF-I supports the function of endothelial progenitor cells by stimulating eNOS.[162, 168]

These phenomena do not come into play in rodent calorie restriction studies because most strains of rodents are not prone to die from coronary disease or stroke. They may be much more pertinent to humans. How ironic that the same signaling pathway that aids vascular health, also increases cancer risk and blocks protective autophagy!

The vascular health of people who achieve worthwhile weight loss from CC diets should benefit from a favorable impact on metabolic syndrome; this likely will outweigh any countervailing effect of decreased insulin/IGF-I on vascular eNOS activity. Low insulin aids blood pressure control by down-regulating sympathetic activity and renal sodium retention, and any anti-inflammatory effect of CC diets should also benefit vascular health.[169] Nevertheless, in CC dieters who are at vascular risk owing to an adverse lipoprotein profile and/or hypertension, adjunctive measures to boost vascular NO bioactivity - thereby compensating for the impact of low insulin/IGF-I - may be warranted. Strategies that may be useful in this regard include aerobic exercise training; agents, such as spirulina or high-dose folate, which inhibit endothelial oxidative stress; polyphenols (such as quercetin or cocoa flavanols) which provoke increased

eNOS activity; metformin or berberine and possibly vinegar (which stimulate eNOS via AMPK); supplemental citrulline; and a high intake of dietary nitrate.[170-177] (Obviously, optimization of the lipoprotein profile and of blood pressure should also be indicated in this situation.)

Summing Up

It is commonly thought that the aging-retardant benefits observed in rodent calorie restriction studies are not likely to be feasibly achievable by humans – because the self-denial required would be too rigorous and would need to be implemented for most of the lifespan, or because rodents might be inherently more responsive to this strategy. But the meta-analysis of rodent studies conducted by Merry concludes that the increase in life expectancy achieved should be proportional both to the degree of restriction and the proportion of the lifespan this restriction is implemented – implying that some worthwhile benefit should be achievable even if modest restriction is implemented for less than half the lifespan. Moreover, the five-year increase in maximum lifespan observed in elderly Okinawans – who were only moderately restricted for about half their lives - in conjunction with the encouraging results now being observed in ongoing calorie restriction studies with rhesus monkeys, suggest that primates, like rodents, are indeed reasonably responsive to calorie restriction. And the discovery that alternate-day fasting has longevity and hormesis benefits analogous to those of calorie restriction, even if weekly calorie intake does not decline notably, suggests that diet strategies more practical than daily underfeeding may be capable of achieving comparable benefits for health and longevity. Indeed, the marked anti-inflammatory benefits of modified alternate-day fasting in humans provide some support for this view.

In this regard, the CC diet strategy discussed here is of particular interest in light of its demonstrable practicality. The experience of the Hellers and of Herring shows that a great many people are capable of adopting this lifestyle on a continuing basis, often with positive consequences for body weight and risk factors. And recent Israeli research confirms that carbohydrate concentration does indeed tend to promote appropriate weight loss.

At minimum, CC diets have considerable potential for promoting leanness and thereby reducing risk for metabolic syndrome and the panoply of distressing health consequences – hypertension, heart attack, stroke, congestive heart failure, non-alcoholic steatohepatitis, dementia, many types of cancer, and possibly even osteoarthritis – that flow from it. This is doubly true for CC diets that are plant-based, as vegan diets per se promote leanness and insulin sensitivity. However, a key advantage of the CC diet concept is that it is ecumenical – people do not need to give up their traditional food choices to adopt it, merely to change the times at which they eat those foods. Hence, the CC approach has the potential for considerable popular appeal.

But there is also a reasonable prospect that CC diets will provide protection from age-linked degenerative diseases beyond that conferred by leanness per se, while also slowing the aging

process. It is clear from rodent studies that repeated extended episodes in which insulin, free IGF-I, and glucose levels are lowered can replicate the health-protective and aging-retardant benefits associated with daily calorie restriction. It is quite conceivable that the low-insulin episodes associated with CC diets may be long enough and intense enough to evoke these benefits in humans to a meaningful degree. Moreover, long-term compliance with such diets might well be associated with a modest reduction in daily calorie intake (beyond that predictable from decreased body mass) even after weight equilibration has occurred - thereby achieving a measure of genuine calorie restriction without necessitating rigorous "calorie counting". Particularly in light of the evident practical potential of CC diets for promoting leanness, this strategy merits formal clinical evaluation in long-term studies; some of these studies should examine its impact on biomarkers that are typically modulated by calorie restriction or alternate-day fasting, while also estimating calorie intake after weight plateaus.

Acknowledgement

I'd like to thank my friend and colleague Dr. Jeremy J. Stone for sharing with me his innovative insights regarding CC diets, and for helpful suggestions on the manuscript. We have worked together to develop a new website devoted to the CC diet concept: http://catalyticlongevity.org/.

References

(1) Mattson MP. Neuroprotective signaling and the aging brain: take away my food and let me run. *Brain Res* 2000 December 15;886(1-2):47-53.

(2) Merry BJ. Molecular mechanisms linking calorie restriction and longevity. *Int J Biochem Cell Biol* 2002 November;34(11):1340-54.

(3) Willcox BJ, Willcox DC, Todoriki H, Fujiyoshi A, Yano K, He Q, Curb JD, Suzuki M. Caloric restriction, the traditional Okinawan diet, and healthy aging: the diet of the world's longest-lived people and its potential impact on morbidity and life span. *Ann N Y Acad Sci* 2007 October;1114:434-55.

(4) Colman RJ, Anderson RM, Johnson SC, Kastman EK, Kosmatka KJ, Beasley TM, Allison DB, Cruzen C, Simmons HA, Kemnitz JW, Weindruch R. Caloric restriction delays disease onset and mortality in rhesus monkeys. *Science* 2009 July 10;325(5937):201-4.

(5) Colman RJ, Beasley TM, Allison DB, Weindruch R. Attenuation of sarcopenia by dietary restriction in rhesus monkeys. *J Gerontol A Biol Sci Med Sci* 2008 June;63(6):556-9.

(6) Bendlin BB, Canu E, Willette A, Kastman EK, McLaren DG, Kosmatka KJ, Xu G, Field AS, Colman RJ, Coe CL, Weindruch RH, Alexander AL, Johnson SC. Effects of aging and calorie restriction on white matter in rhesus macaques. *Neurobiol Aging* 2010 June 10.

(7) Bartke A. Minireview: role of the growth hormone/insulin-like growth factor system in mammalian aging. *Endocrinology* 2005 September;146(9):3718-23.

(8) McCarty MF. Up-regulation of IGF binding protein-1 as an anticarcinogenic strategy: relevance to caloric restriction, exercise, and insulin sensitivity. *Med Hypotheses* 1997 April;48(4):297-308.

(9) Al-Regaiey KA, Masternak MM, Bonkowski M, Sun L, Bartke A. Long-lived growth hormone receptor knockout mice: interaction of reduced insulin-like growth factor i/insulin signaling and caloric restriction. *Endocrinology* 2005 February;146(2):851-60.

(10) Bonafe M, Barbieri M, Marchegiani F, Olivieri F, Ragno E, Giampieri C, Mugianesi E, Centurelli M, Franceschi C, Paolisso G. Polymorphic variants of insulin-like growth factor I (IGF-I) receptor and phosphoinositide 3-kinase genes affect IGF-I plasma levels and human longevity: cues for an evolutionarily conserved mechanism of life span control. *J Clin Endocrinol Metab* 2003 July;88(7):3299-304.

(11) Suh Y, Atzmon G, Cho MO, Hwang D, Liu B, Leahy DJ, Barzilai N, Cohen P. Functionally significant insulin-like growth factor I receptor mutations in centenarians. *Proc Natl Acad Sci U S A* 2008 March 4;105(9):3438-42.

(12) Harrison DE, Strong R, Sharp ZD, Nelson JF, Astle CM, Flurkey K, Nadon NL, Wilkinson JE, Frenkel K, Carter CS, Pahor M, Javors MA, Fernandez E, Miller RA. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature* 2009 July 16;460(7253):392-5.

(13) Anisimov VN, Egormin PA, Bershtein LM, Zabezhinskii MA, Piskunova TS, Popovich IG, Semenchenko AV. Metformin decelerates aging and development of mammary tumors in HER-2/neu transgenic mice. *Bull Exp Biol Med* 2005 June;139(6):721-3.

(14) Anisimov VN, Berstein LM, Egormin PA, Piskunova TS, Popovich IG, Zabezhinski MA, Tyndyk ML, Yurova MV, Kovalenko IG, Poroshina TE, Semenchenko AV. Metformin slows down aging and extends life span of female SHR mice. *Cell Cycle* 2008 September 1;7(17):2769-73.

(15) McCarty MF. Chronic activation of AMP-activated kinase as a strategy for slowing aging. *Med Hypotheses* 2004;63(2):334-9.

(16) Blagosklonny MV. Calorie restriction: decelerating mTOR-driven aging from cells to organisms (including humans). *Cell Cycle* 2010 February 15;9(4):683-8.

(17) Pattingre S, Espert L, Biard-Piechaczyk M, Codogno P. Regulation of macroautophagy by mTOR and Beclin 1 complexes. *Biochimie* 2008 February;90(2):313-23.

(18) Bergamini E, Cavallini G, Cecchi L, Donati A, Dolfi C, Gori Z, Innocenti B, Maccheroni M, Marino M, Masini M, Paradiso C, Pollera M, Trentalance A. A proposed mechanism of the antiaging action of diet restriction. *Aging (Milano)* 1998 April;10(2):174-5.

(19) Cuervo AM, Bergamini E, Brunk UT, Droge W, Ffrench M, Terman A. Autophagy and aging: the importance of maintaining "clean" cells. *Autophagy* 2005 October;1(3):131-40.

(20) Bergamini E, Cavallini G, Donati A, Gori Z. The role of autophagy in aging: its essential part in the anti-aging mechanism of caloric restriction. *Ann N Y Acad Sci* 2007 October;1114:69-78.

(21) Cuervo AM. Calorie restriction and aging: the ultimate "cleansing diet". *J Gerontol A Biol Sci Med Sci* 2008 June;63(6):547-9.

(22) Baserga R, Peruzzi F, Reiss K. The IGF-1 receptor in cancer biology. *Int J Cancer* 2003 December 20;107(6):873-7.

(23) Song G, Ouyang G, Bao S. The activation of Akt/PKB signaling pathway and cell survival. *J Cell Mol Med* 2005 January;9(1):59-71.

(24) Graff JR, Konicek BW, Carter JH, Marcusson EG. Targeting the eukaryotic translation initiation factor 4E for cancer therapy. *Cancer Res* 2008 February 1;68(3):631-4.

(25) Kari FW, Dunn SE, French JE, Barrett JC. Roles for insulin-like growth factor-1 in mediating the anti-carcinogenic effects of caloric restriction. *J Nutr Health Aging* 1999;3(2):92-101.

(26) Chaves J, Saif MW. IGF system in cancer: from bench to clinic. *Anticancer Drugs* 2011 March;22(3):206-12.

(27) Gallagher EJ, LeRoith D. The proliferating role of insulin and insulin-like growth factors in cancer. *Trends Endocrinol Metab* 2010 October;21(10):610-8.

(28) Rowlands MA, Gunnell D, Harris R, Vatten LJ, Holly JM, Martin RM. Circulating insulin-like growth factor peptides and prostate cancer risk: a systematic review and meta-analysis. *Int J Cancer* 2009 May 15;124(10):2416-29.

(29) Giovannucci E. Metabolic syndrome, hyperinsulinemia, and colon cancer: a review. *Am J Clin Nutr* 2007 September;86(3):s836-s842.

(30) Lann D, LeRoith D. The role of endocrine insulin-like growth factor-I and insulin in breast cancer. *J Mammary Gland Biol Neoplasia* 2008 December;13(4):371-9.

(31) Guevara-Aguirre J, Balasubramanian P, Guevara-Aguirre M, Wei M, Madia F, Cheng CW, Hwang D, Martin-Montalvo A, Saavedra J, Ingles S, de CR, Cohen P, Longo VD. Growth hormone receptor deficiency is associated with a major reduction in pro-aging signaling, cancer, and diabetes in humans. *Sci Transl Med* 2011 February 16;3(70):70ra13.

(32) Steuerman R, Shevah O, Laron Z. Congenital IGF1 deficiency tends to confer protection against post-natal development of malignancies. *Eur J Endocrinol* 2011 April;164(4):485-9.

(33) Cohen HY, Miller C, Bitterman KJ, Wall NR, Hekking B, Kessler B, Howitz KT, Gorospe M, de CR, Sinclair DA. Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. *Science* 2004 July 16;305(5682):390-2.

(34) Takayama K, Ishida K, Matsushita T, Fujita N, Hayashi S, Sasaki K, Tei K, Kubo S, Matsumoto T, Fujioka H, Kurosaka M, Kuroda R. SIRT1 regulation of apoptosis of human chondrocytes. *Arthritis Rheum* 2009 September;60(9):2731-40.

(35) Yeung F, Hoberg JE, Ramsey CS, Keller MD, Jones DR, Frye RA, Mayo MW. Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. *EMBO J* 2004 June 16;23(12):2369-80.

(36) Chen J, Zhou Y, Mueller-Steiner S, Chen LF, Kwon H, Yi S, Mucke L, Gan L. SIRT1 protects against microglia-dependent amyloid-beta toxicity through inhibiting NF-kappaB signaling. *J Biol Chem* 2005 December 2;280(48):40364-74.

(37) Tanno M, Kuno A, Yano T, Miura T, Hisahara S, Ishikawa S, Shimamoto K, Horio Y. Induction of manganese superoxide dismutase by nuclear translocation and activation of SIRT1 promotes cell survival in chronic heart failure. *J Biol Chem* 2010 March 12;285(11):8375-82.

(38) Olmos Y, Valle I, Borniquel S, Tierrez A, Soria E, Lamas S, Monsalve M. Mutual dependence of Foxo3a and PGC-1alpha in the induction of oxidative stress genes. *J Biol Chem* 2009 May 22;284(21):14476-84.

(39) Yuan Z, Zhang X, Sengupta N, Lane WS, Seto E. SIRT1 regulates the function of the Nijmegen breakage syndrome protein. *Mol Cell* 2007 July 6;27(1):149-62.

(40) Sequeira J, Boily G, Bazinet S, Saliba S, He X, Jardine K, Kennedy C, Staines W, Rousseaux C, Mueller R, McBurney MW. sirt1-null mice develop an autoimmune-like condition. *Exp Cell Res* 2008 October 1;314(16):3069-74.

(41) Zhang J, Lee SM, Shannon S, Gao B, Chen W, Chen A, Divekar R, McBurney MW, Braley-Mullen H, Zaghouani H, Fang D. The type III histone deacetylase Sirt1 is essential for maintenance of T cell tolerance in mice. *J Clin Invest* 2009 October;119(10):3048-58.

(42) Gerhart-Hines Z, Rodgers JT, Bare O, Lerin C, Kim SH, Mostoslavsky R, Alt FW, Wu Z, Puigserver P. Metabolic control of muscle mitochondrial function and fatty acid oxidation through SIRT1/PGC-1alpha. *EMBO J* 2007 April 4;26(7):1913-23.

(43) Canto C, Gerhart-Hines Z, Feige JN, Lagouge M, Noriega L, Milne JC, Elliott PJ, Puigserver P, Auwerx J. AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity. *Nature* 2009 April 23;458(7241):1056-60.

(44) Morselli E, Maiuri MC, Markaki M, Megalou E, Pasparaki A, Palikaras K, Criollo A, Galluzzi L, Malik SA, Vitale I, Michaud M, Madeo F, Tavernarakis N, Kroemer G. The life span-prolonging effect of sirtuin-1 is mediated by autophagy. *Autophagy* 2010 January;6(1):186-8.

(45) Lee IH, Cao L, Mostoslavsky R, Lombard DB, Liu J, Bruns NE, Tsokos M, Alt FW, Finkel T. A role for the NAD-dependent deacetylase Sirt1 in the regulation of autophagy. *Proc Natl Acad Sci U S A* 2008 March 4;105(9):3374-9.

(46) Guarente L, Picard F. Calorie restriction--the SIR2 connection. *Cell* 2005 February 25;120(4):473-82.

(47) Ghosh HS. The anti-aging, metabolism potential of SIRT1. *Curr Opin Investig Drugs* 2008 October;9(10):1095-102.

(48) Canto C, Auwerx J. Caloric restriction, SIRT1 and longevity. *Trends Endocrinol Metab* 2009 September;20(7):325-31.

(49) Boily G, Seifert EL, Bevilacqua L, He XH, Sabourin G, Estey C, Moffat C, Crawford S, Saliba S, Jardine K, Xuan J, Evans M, Harper ME, McBurney MW. SirT1 regulates energy metabolism and response to caloric restriction in mice. *PLoS ONE* 2008;3(3):e1759.

(50) Baur JA, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A, Prabhu VV, Allard JS, Lopez-Lluch G, Lewis K, Pistell PJ, Poosala S, Becker KG, Boss O, Gwinn D, Wang M, Ramaswamy S, Fishbein KW, Spencer RG, Lakatta EG, Le CD, Shaw RJ, Navas P, Puigserver P, Ingram DK, de CR, Sinclair DA. Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* 2006 November 16;444(7117):337-42.

(51) Pearson KJ, Baur JA, Lewis KN, Peshkin L, Price NL, Labinskyy N, Swindell WR, Kamara D, Minor RK, Perez E, Jamieson HA, Zhang Y, Dunn SR, Sharma K, Pleshko N, Woollett LA, Csiszar A, Ikeno Y, Le CD, Elliott PJ, Becker KG, Navas P, Ingram DK, Wolf NS, Ungvari Z, Sinclair DA, de CR. Resveratrol delays age-related deterioration and mimics transcriptional aspects of dietary restriction without extending life span. *Cell Metab* 2008 August;8(2):157-68.

(52) Fulco M, Cen Y, Zhao P, Hoffman EP, McBurney MW, Sauve AA, Sartorelli V. Glucose restriction inhibits skeletal myoblast differentiation by activating SIRT1 through AMPK-mediated regulation of Nampt. *Dev Cell* 2008 May;14(5):661-73.

(53) Costford SR, Bajpeyi S, Pasarica M, Albarado DC, Thomas SC, Xie H, Church TS, Jubrias SA, Conley KE, Smith SR. Skeletal muscle NAMPT is induced by exercise in humans. *Am J Physiol Endocrinol Metab* 2010 January;298(1):E117-E126.

(54) Shaw RJ. LKB1 and AMP-activated protein kinase control of mTOR signalling and growth. *Acta Physiol (Oxf)* 2009 May;196(1):65-80.

(55) Hoyer-Hansen M, Jaattela M. AMP-activated protein kinase: a universal regulator of autophagy? *Autophagy* 2007 July;3(4):381-3.

(56) Libby G, Donnelly LA, Donnan PT, Alessi DR, Morris AD, Evans JM. New users of metformin are at low risk of incident cancer: a cohort study among people with type 2 diabetes. *Diabetes Care* 2009 September;32(9):1620-5.

(57) Landman GW, Kleefstra N, van Hateren KJ, Groenier KH, Gans RO, Bilo HJ. Metformin associated with lower cancer mortality in type 2 diabetes: ZODIAC-16. *Diabetes Care* 2010 February;33(2):322-6.

(58) Papanas N, Maltezos E, Mikhailidis DP. Metformin and cancer: licence to heal? *Expert Opin Investig Drugs* 2010 June 18.

(59) Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. *Lancet* 1998 September 12;352(9131):854-65.

(60) Hermansen K, Mortensen LS. Bodyweight changes associated with antihyperglycaemic agents in type 2 diabetes mellitus. *Drug Saf* 2007;30(12):1127-42.

(61) Kops GJ, Dansen TB, Polderman PE, Saarloos I, Wirtz KW, Coffer PJ, Huang TT, Bos JL, Medema RH, Burgering BM. Forkhead transcription factor FOXO3a protects quiescent cells from oxidative stress. *Nature* 2002 September 19;419(6904):316-21.

(62) Li M, Chiu JF, Gagne J, Fukagawa NK. Age-related differences in insulin-like growth factor-1 receptor signaling regulates Akt/FOXO3a and ERK/Fos pathways in vascular smooth muscle cells. *J Cell Physiol* 2008 November;217(2):377-87.

(63) Tan WQ, Wang K, Lv DY, Li PF. Foxo3a inhibits cardiomyocyte hypertrophy through transactivating catalase. *J Biol Chem* 2008 October 31;283(44):29730-9.

(64) Olmos Y, Valle I, Borniquel S, Tierrez A, Soria E, Lamas S, Monsalve M. Mutual dependence of Foxo3a and PGC-1alpha in the induction of oxidative stress genes. *J Biol Chem* 2009 May 22;284(21):14476-84.

(65) Furukawa-Hibi Y, Kobayashi Y, Chen C, Motoyama N. FOXO transcription factors in cell-cycle regulation and the response to oxidative stress. *Antioxid Redox Signal* 2005 May;7(5-6):752-60.

(66) Greer EL, Banko MR, Brunet A. AMP-activated protein kinase and FoxO transcription factors in dietary restriction-induced longevity. *Ann N Y Acad Sci* 2009 July;1170:688-92.

(67) Colombo SL, Moncada S. AMPKalpha1 regulates the antioxidant status of vascular endothelial cells. *Biochem J* 2009 July 15;421(2):163-9.

(68) Kobayashi Y, Furukawa-Hibi Y, Chen C, Horio Y, Isobe K, Ikeda K, Motoyama N. SIRT1 is critical regulator of FOXO-mediated transcription in response to oxidative stress. *Int J Mol Med* 2005 August;16(2):237-43.

(69) An BS, Tavera-Mendoza LE, Dimitrov V, Wang X, Calderon MR, Wang HJ, White JH. Stimulation of Sirt1-regulated FoxO protein function by the ligand-bound vitamin D receptor. *Mol Cell Biol* 2010 October;30(20):4890-900.

(70) Morris BJ. A forkhead in the road to longevity: the molecular basis of lifespan becomes clearer. *J Hypertens* 2005 July;23(7):1285-309.

(71) Murphy CT. The search for DAF-16/FOXO transcriptional targets: approaches and discoveries. *Exp Gerontol* 2006 October;41(10):910-21.

(72) Yamaza H, Komatsu T, Wakita S, Kijogi C, Park S, Hayashi H, Chiba T, Mori R, Furuyama T, Mori N, Shimokawa I. FoxO1 is involved in the antineoplastic effect of calorie restriction. *Aging Cell* 2010 June;9(3):372-82.

(73) Willcox BJ, Donlon TA, He Q, Chen R, Grove JS, Yano K, Masaki KH, Willcox DC, Rodriguez B, Curb JD. FOXO3A genotype is strongly associated with human longevity. *Proc Natl Acad Sci U S A* 2008 September 16;105(37):13987-92.

(74) Flachsbart F, Caliebe A, Kleindorp R, Blanche H, von Eller-Eberstein H, Nikolaus S, Schreiber S, Nebel A. Association of FOXO3A variation with human longevity confirmed in German centenarians. *Proc Natl Acad Sci U S A* 2009 February 24;106(8):2700-5.

(75) Anselmi CV, Malovini A, Roncarati R, Novelli V, Villa F, Condorelli G, Bellazzi R, Puca AA. Association of the FOXO3A locus with extreme longevity in a southern Italian centenarian study. *Rejuvenation Res* 2009 April;12(2):95-104.

(76) Li Y, Wang WJ, Cao H, Lu J, Wu C, Hu FY, Guo J, Zhao L, Yang F, Zhang YX, Li W, Zheng GY, Cui H, Chen X, Zhu Z, He H, Dong B, Mo X, Zeng Y, Tian XL. Genetic association of FOXO1A and FOXO3A with longevity trait in Han Chinese populations. *Hum Mol Genet* 2009 December 15;18(24):4897-904.

(77) Canto C, Auwerx J. PGC-1alpha, SIRT1 and AMPK, an energy sensing network that controls energy expenditure. *Curr Opin Lipidol* 2009 April;20(2):98-105.

(78) Canto C, Jiang LQ, Deshmukh AS, Mataki C, Coste A, Lagouge M, Zierath JR, Auwerx J. Interdependence of AMPK and SIRT1 for metabolic adaptation to fasting and exercise in skeletal muscle. *Cell Metab* 2010 March 3;11(3):213-9.

(79) Goodrick CL, Ingram DK, Reynolds MA, Freeman JR, Cider NL. Differential effects of intermittent feeding and voluntary exercise on body weight and lifespan in adult rats. *J Gerontol* 1983 January;38(1):36-45.

(80) Goodrick CL, Ingram DK, Reynolds MA, Freeman JR, Cider NL. Effects of intermittent feeding upon growth and life span in rats. *Gerontology* 1982;28(4):233-41.

(81) Goodrick CL, Ingram DK, Reynolds MA, Freeman JR, Cider N. Effects of intermittent feeding upon body weight and lifespan in inbred mice: interaction of genotype and age. *Mech Ageing Dev* 1990 July;55(1):69-87.

(82) Anson RM, Guo Z, de CR, Iyun T, Rios M, Hagepanos A, Ingram DK, Lane MA, Mattson MP. Intermittent fasting dissociates beneficial effects of dietary restriction on glucose metabolism and neuronal resistance to injury from calorie intake. *Proc Natl Acad Sci U S A* 2003 May 13;100(10):6216-20.

(83) Mattson MP, Duan W, Guo Z. Meal size and frequency affect neuronal plasticity and vulnerability to disease: cellular and molecular mechanisms. *J Neurochem* 2003 February;84(3):417-31.

(84) Heilbronn LK, Smith SR, Martin CK, Anton SD, Ravussin E. Alternate-day fasting in nonobese subjects: effects on body weight, body composition, and energy metabolism. *Am J Clin Nutr* 2005 January;81(1):69-73.

(85) Johnson JB, Laub DR, John S. The effect on health of alternate day calorie restriction: eating less and more than needed on alternate days prolongs life. *Med Hypotheses* 2006;67(2):209-11.

(86) Johnson JB, Summer W, Cutler RG, Martin B, Hyun DH, Dixit VD, Pearson M, Nassar M, Telljohann R, Maudsley S, Carlson O, John S, Laub DR, Mattson MP. Alternate day calorie

restriction improves clinical findings and reduces markers of oxidative stress and inflammation in overweight adults with moderate asthma. *Free Radic Biol Med* 2007 March 1;42(5):665-74.

(87) Johnson JB, Laub DR. *The alternate day diet*. New York: Perigee; 2008.

(88) Heilbronn LK, Civitarese AE, Bogacka I, Smith SR, Hulver M, Ravussin E. Glucose tolerance and skeletal muscle gene expression in response to alternate day fasting. *Obes Res* 2005 March;13(3):574-81.

(89) Allard JS, Heilbronn LK, Smith C, Hunt ND, Ingram DK, Ravussin E, de CR. In vitro cellular adaptations of indicators of longevity in response to treatment with serum collected from humans on calorie restricted diets. *PLoS ONE* 2008;3(9):e3211.

(90) Hafstrom I, Ringertz B, Gyllenhammar H, Palmblad J, Harms-Ringdahl M. Effects of fasting on disease activity, neutrophil function, fatty acid composition, and leukotriene biosynthesis in patients with rheumatoid arthritis. *Arthritis Rheum* 1988 May;31(5):585-92.

(91) Palmblad J, Hafstrom I, Ringertz B. Antirheumatic effects of fasting. *Rheum Dis Clin North Am* 1991 May;17(2):351-62.

(92) Mattson MP. The need for controlled studies of the effects of meal frequency on health. *Lancet* 2005 June 4;365(9475):1978-80.

(93) Herring BW. *The Fast-5 Diet and the Fast-5 Lifestyle*. Jacksonville, FLA: Fast-5 LLC; 2005.

(94) Stone JJ. Concentrate your carbs! 2011.
Ref Type: Unpublished Work

(95) Heller RF, Heller RF. *The Carbohydrate Addict's Diet*. New York: Signet; 1993.

(96) Heller RF, Heller RF, Vagnini FJ. *The Carbohydrate Addict's Healthy Heart Program*. New York: Ballantine; 1999.

(97) Heller RF, Heller RF. Hyperinsulinemic obesity and carbohydrate addiction: the missing link is the carbohydrate frequency factor. *Med Hypotheses* 1994 May;42(5):307-12.

(98) Sofer S, Eliraz A, Kaplan S, Voet H, Fink G, Kima T, Madar Z. Greater Weight Loss and Hormonal Changes After 6 Months Diet With Carbohydrates Eaten Mostly at Dinner. *Obesity (Silver Spring)* 2011 April 7.

(99) Masoro EJ, Shimokawa I, Higami Y, McMahan CA, Yu BP. Temporal pattern of food intake not a factor in the retardation of aging processes by dietary restriction. *J Gerontol A Biol Sci Med Sci* 1995 January;50A(1):B48-B53.

(100) Scharrer E. Control of food intake by fatty acid oxidation and ketogenesis. *Nutrition* 1999 September;15(9):704-14.

(101) Friedman MI. Obesity and the hepatic control of feeding behavior. *Drug News Perspect* 2007 November;20(9):573-8.

(102) Suwanichkul A, DePaolis LA, Lee PD, Powell DR. Identification of a promoter element which participates in cAMP-stimulated expression of human insulin-like growth factor-binding protein-1. *J Biol Chem* 1993 May 5;268(13):9730-6.

(103) Hilding A, Moller C, Hall KE. Glucagon and GLP-1 stimulate IGFBP-1 secretion in Hep G2 cells without effect on IGFBP-1 mRNA. *Growth Horm IGF Res* 2002 February;12(1):60-8.

(104) Bruss MD, Thompson AC, Aggarwal I, Khambatta CF, Hellerstein MK. The effects of physiological adaptations to calorie restriction on global cell proliferation rates. *Am J Physiol Endocrinol Metab* 2011 April;300(4):E735-E745.

(105) Stote KS, Baer DJ, Spears K, Paul DR, Harris GK, Rumpler WV, Strycula P, Najjar SS, Ferrucci L, Ingram DK, Longo DL, Mattson MP. A controlled trial of reduced meal frequency without caloric restriction in healthy, normal-weight, middle-aged adults. *Am J Clin Nutr* 2007 April;85(4):981-8.

(106) Aksungar FB, Topkaya AE, Akyildiz M. Interleukin-6, C-reactive protein and biochemical parameters during prolonged intermittent fasting. *Ann Nutr Metab* 2007;51(1):88-95.

(107) Maislos M, Khamaysi N, Assali A, bou-Rabiah Y, Zvili I, Shany S. Marked increase in plasma high-density-lipoprotein cholesterol after prolonged fasting during Ramadan. *Am J Clin Nutr* 1993 May;57(5):640-2.

(108) Maislos M, bou-Rabiah Y, Zuili I, Iordash S, Shany S. Gorging and plasma HDL-cholesterol--the Ramadan model. *Eur J Clin Nutr* 1998 February;52(2):127-30.

(109) Shariatpanahi ZV, Shariatpanahi MV, Shahbazi S, Hossaini A, Abadi A. Effect of Ramadan fasting on some indices of insulin resistance and components of the metabolic syndrome in healthy male adults. *Br J Nutr* 2008 July;100(1):147-51.

(110) Adlouni A, Ghalim N, Saile R, Hda N, Parra HJ, Benslimane A. Beneficial effect on serum apo AI, apo B and Lp AI levels of Ramadan fasting. *Clin Chim Acta* 1998 March 23;271(2):179-89.

(111) Fontana L, Weiss EP, Villareal DT, Klein S, Holloszy JO. Long-term effects of calorie or protein restriction on serum IGF-1 and IGFBP-3 concentration in humans. *Aging Cell* 2008 October;7(5):681-7.

(112) Lopez-Varela S, Chacon F, Cano P, Arce A, Esquifino AI. Differential responses of circulating prolactin, GH, and ACTH levels and distribution and activity of submaxillary lymph node lymphocytes to calorie restriction in male Lewis and Wistar rats. *Neuroimmunomodulation* 2004;11(4):247-54.

(113) Redman LM, Veldhuis JD, Rood J, Smith SR, Williamson D, Ravussin E. The effect of caloric restriction interventions on growth hormone secretion in nonobese men and women. *Aging Cell* 2010 February;9(1):32-9.

(114) Allen NE, Appleby PN, Davey GK, Kaaks R, Rinaldi S, Key TJ. The associations of diet with serum insulin-like growth factor I and its main binding proteins in 292 women meat-eaters, vegetarians, and vegans. *Cancer Epidemiol Biomarkers Prev* 2002 November;11(11):1441-8.

(115) Ngo TH, Barnard RJ, Tymchuk CN, Cohen P, Aronson WJ. Effect of diet and exercise on serum insulin, IGF-I, and IGFBP-1 levels and growth of LNCaP cells in vitro (United States). *Cancer Causes Control* 2002 December;13(10):929-35.

(116) McCarty MF. Hepatic monitoring of essential amino acid availability may regulate IGF-I activity, thermogenesis, and fatty acid oxidation/synthesis. *Med Hypotheses* 2001 February;56(2):220-4.

(117) McCarty MF. Insulin and IGF-I as determinants of low "Western" cancer rates in the rural third world. *Int J Epidemiol* 2004 August;33(4):908-10.

(118) Orentreich N, Matias JR, DeFelice A, Zimmerman JA. Low methionine ingestion by rats extends life span. *J Nutr* 1993 February;123(2):269-74.

(119) Richie JP, Jr., Leutzinger Y, Parthasarathy S, Malloy V, Orentreich N, Zimmerman JA. Methionine restriction increases blood glutathione and longevity in F344 rats. *FASEB J* 1994 December;8(15):1302-7.

(120) Sanz A, Caro P, Ayala V, Portero-Otin M, Pamplona R, Barja G. Methionine restriction decreases mitochondrial oxygen radical generation and leak as well as oxidative damage to mitochondrial DNA and proteins. *FASEB J* 2006 June;20(8):1064-73.

(121) Caro P, Gomez J, Sanchez I, Naudi A, Ayala V, Lopez-Torres M, Pamplona R, Barja G. Forty percent methionine restriction decreases mitochondrial oxygen radical production and leak at complex I during forward electron flow and lowers oxidative damage to proteins and mitochondrial DNA in rat kidney and brain mitochondria. *Rejuvenation Res* 2009 December;12(6):421-34.

(122) Lopez-Torres M, Barja G. Lowered methionine ingestion as responsible for the decrease in rodent mitochondrial oxidative stress in protein and dietary restriction possible implications for humans. *Biochim Biophys Acta* 2008 November;1780(11):1337-47.

(123) McCarty MF, Barroso-Aranda J, Contreras F. The low-methionine content of vegan diets may make methionine restriction feasible as a life extension strategy. *Med Hypotheses* 2009 February;72(2):125-8.

(124) McCarty MF. Dietary saturate/unsaturate ratio as a determinant of adiposity. *Med Hypotheses* 2010 July;75(1):14-6.

(125) Krogmann A, Staiger K, Haas C, Gommer N, Peter A, Heni M, Machicao F, Haring HU, Staiger H. Inflammatory response of human coronary artery endothelial cells to saturated long-chain fatty acids. *Microvasc Res* 2011 January;81(1):52-9.

(126) Gentile CL, Pagliassotti MJ. The role of fatty acids in the development and progression of nonalcoholic fatty liver disease. *J Nutr Biochem* 2008 September;19(9):567-76.

(127) Fung TT, van Dam RM, Hankinson SE, Stampfer M, Willett WC, Hu FB. Low-carbohydrate diets and all-cause and cause-specific mortality: two cohort studies. *Ann Intern Med* 2010 September 7;153(5):289-98.

(128) Jenkins DJ, Kendall CW, Marchie A, Josse AR, Nguyen TH, Faulkner DA, Lapsley KG, Singer W. Effect of almonds on insulin secretion and insulin resistance in nondiabetic hyperlipidemic subjects: a randomized controlled crossover trial. *Metabolism* 2008 July;57(7):882-7.

(129) Bahadori B, McCarty MF, Barroso-Aranda J, Gustin JC, Contreras F. A "mini-fast with exercise" protocol for fat loss. *Med Hypotheses* 2009 October;73(4):619-22.

(130) Derave W, Mertens A, Muls E, Pardaens K, Hespel P. Effects of post-absorptive and postprandial exercise on glucoregulation in metabolic syndrome. *Obesity (Silver Spring)* 2007 March;15(3):704-11.

(131) Van PK, Szlufcik K, Nielens H, Pelgrim K, Deldicque L, Hesselink M, Van Veldhoven PP, Hespel P. Training in the fasted state improves glucose tolerance during fat-rich diet. *J Physiol* 2010 November 1;588(Pt 21):4289-302.

(132) Nishida Y, Matsubara T, Tobina T, Shindo M, Tokuyama K, Tanaka K, Tanaka H. Effect of low-intensity aerobic exercise on insulin-like growth factor-I and insulin-like growth factor-binding proteins in healthy men. *Int J Endocrinol* 2010;2010.

(133) Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, Wu M, Ventre J, Doebber T, Fujii N, Musi N, Hirshman MF, Goodyear LJ, Moller DE. Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest* 2001 October;108(8):1167-74.

(134) Musi N, Hirshman MF, Nygren J, Svanfeldt M, Bavenholm P, Rooyackers O, Zhou G, Williamson JM, Ljunqvist O, Efendic S, Moller DE, Thorell A, Goodyear LJ. Metformin increases AMP-activated protein kinase activity in skeletal muscle of subjects with type 2 diabetes. *Diabetes* 2002 July;51(7):2074-81.

(135) Fulco M, Cen Y, Zhao P, Hoffman EP, McBurney MW, Sauve AA, Sartorelli V. Glucose restriction inhibits skeletal myoblast differentiation by activating SIRT1 through AMPK-mediated regulation of Nampt. *Dev Cell* 2008 May;14(5):661-73.

(136) Wang P, Xu TY, Guan YF, Tian WW, Viollet B, Rui YC, Zhai QW, Su DF, Miao CY. Nicotinamide phosphoribosyltransferase protects against ischemic stroke through SIRT1-dependent adenosine monophosphate-activated kinase pathway. *Ann Neurol* 2011 February;69(2):360-74.

(137) Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. *Lancet* 1998 September 12;352(9131):854-65.

(138) Ruderman NB, Cacicedo JM, Itani S, Yagihashi N, Saha AK, Ye JM, Chen K, Zou M, Carling D, Boden G, Cohen RA, Keaney J, Kraegen EW, Ido Y. Malonyl-CoA and AMP-activated protein kinase (AMPK): possible links between insulin resistance in muscle and early endothelial cell damage in diabetes. *Biochem Soc Trans* 2003 February;31(Pt 1):202-6.

(139) Decensi A, Puntoni M, Goodwin P, Cazzaniga M, Gennari A, Bonanni B, Gandini S. Metformin and cancer risk in diabetic patients: a systematic review and meta-analysis. *Cancer Prev Res (Phila)* 2010 November;3(11):1451-61.

(140) Roussel R, Travert F, Pasquet B, Wilson PW, Smith SC, Jr., Goto S, Ravaud P, Marre M, Porath A, Bhatt DL, Steg PG. Metformin use and mortality among patients with diabetes and atherothrombosis. *Arch Intern Med* 2010 November 22;170(21):1892-9.

(141) Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 2002 February 7;346(6):393-403.

(142) Anisimov VN. Metformin for aging and cancer prevention. *Aging (Albany NY)* 2010 November;2(11):760-74.

(143) Anisimov VN, Berstein LM, Egormin PA, Piskunova TS, Popovich IG, Zabezhinski MA, Tyndyk ML, Yurova MV, Kovalenko IG, Poroshina TE, Semenchenko AV. Metformin slows down aging and extends life span of female SHR mice. *Cell Cycle* 2008 September 1;7(17):2769-73.

(144) Anisimov VN, Berstein LM, Egormin PA, Piskunova TS, Popovich IG, Zabezhinski MA, Kovalenko IG, Poroshina TE, Semenchenko AV, Provinciali M, Re F, Franceschi C. Effect of metformin on life span and on the development of spontaneous mammary tumors in HER-2/neu transgenic mice. *Exp Gerontol* 2005 August;40(8-9):685-93.

(145) Spindler SR, Mote PL. Screening candidate longevity therapeutics using gene-expression arrays. *Gerontology* 2007;53(5):306-21.

(146) McCarty MF. Chronic activation of AMP-activated kinase as a strategy for slowing aging. *Med Hypotheses* 2004;63(2):334-9.

(147) Lee YS, Kim WS, Kim KH, Yoon MJ, Cho HJ, Shen Y, Ye JM, Lee CH, Oh WK, Kim CT, Hohnen-Behrens C, Gosby A, Kraegen EW, James DE, Kim JB. Berberine, a natural plant product, activates AMP-activated protein kinase with beneficial metabolic effects in diabetic and insulin-resistant states. *Diabetes* 2006 August;55(8):2256-64.

(148) Turner N, Li JY, Gosby A, To SW, Cheng Z, Miyoshi H, Taketo MM, Cooney GJ, Kraegen EW, James DE, Hu LH, Li J, Ye JM. Berberine and its more biologically available derivative, dihydroberberine, inhibit mitochondrial respiratory complex I: a mechanism for the action of berberine to activate AMP-activated protein kinase and improve insulin action. *Diabetes* 2008 May;57(5):1414-8.

(149) Yin J, Xing H, Ye J. Efficacy of berberine in patients with type 2 diabetes mellitus. *Metabolism* 2008 May;57(5):712-7.

(150) Kong W, Wei J, Abidi P, Lin M, Inaba S, Li C, Wang Y, Wang Z, Si S, Pan H, Wang S, Wu J, Wang Y, Li Z, Liu J, Jiang JD. Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins. *Nat Med* 2004 December;10(12):1344-51.

(151) Kong WJ, Wei J, Zuo ZY, Wang YM, Song DQ, You XF, Zhao LX, Pan HN, Jiang JD. Combination of simvastatin with berberine improves the lipid-lowering efficacy. *Metabolism* 2008 August;57(8):1029-37.

(152) Canto C, Auwerx J. Calorie Restriction: Is AMPK a Key Sensor and Effector? *Physiology (Bethesda)* 2011 August;26(4):214-24.

(153) Um JH, Park SJ, Kang H, Yang S, Foretz M, McBurney MW, Kim MK, Viollet B, Chung JH. AMP-activated protein kinase-deficient mice are resistant to the metabolic effects of resveratrol. *Diabetes* 2010 March;59(3):554-63.

(154) Canto C, Jiang LQ, Deshmukh AS, Mataki C, Coste A, Lagouge M, Zierath JR, Auwerx J. Interdependence of AMPK and SIRT1 for metabolic adaptation to fasting and exercise in skeletal muscle. *Cell Metab* 2010 March 3;11(3):213-9.

(155) Hawley SA, Ross FA, Chevtzoff C, Green KA, Evans A, Fogarty S, Towler MC, Brown LJ, Ogunbayo OA, Evans AM, Hardie DG. Use of cells expressing gamma subunit variants to identify diverse mechanisms of AMPK activation. *Cell Metab* 2010 June 9;11(6):554-65.

(156) Zheng J, Ramirez VD. Inhibition of mitochondrial proton F0F1-ATPase/ATP synthase by polyphenolic phytochemicals. *Br J Pharmacol* 2000 July;130(5):1115-23.

(157) Walle T, Hsieh F, DeLegge MH, Oatis JE, Jr., Walle UK. High absorption but very low bioavailability of oral resveratrol in humans. *Drug Metab Dispos* 2004 December;32(12):1377-82.

(158) Boocock DJ, Faust GE, Patel KR, Schinas AM, Brown VA, Ducharme MP, Booth TD, Crowell JA, Perloff M, Gescher AJ, Steward WP, Brenner DE. Phase I dose escalation pharmacokinetic study in healthy volunteers of resveratrol, a potential cancer chemopreventive agent. *Cancer Epidemiol Biomarkers Prev* 2007 June;16(6):1246-52.

(159) Michell BJ, Griffiths JE, Mitchelhill KI, Rodriguez-Crespo I, Tiganis T, Bozinovski S, de Montellano PR, Kemp BE, Pearson RB. The Akt kinase signals directly to endothelial nitric oxide synthase. *Curr Biol* 1999 July 29;9(15):845-8.

(160) Salt IP, Morrow VA, Brandie FM, Connell JM, Petrie JR. High glucose inhibits insulin-stimulated nitric oxide production without reducing endothelial nitric-oxide synthase Ser1177 phosphorylation in human aortic endothelial cells. *J Biol Chem* 2003 May 23;278(21):18791-7.

(161) Fisslthaler B, Benzing T, Busse R, Fleming I. Insulin enhances the expression of the endothelial nitric oxide synthase in native endothelial cells: a dual role for Akt and AP-1. *Nitric Oxide* 2003 June;8(4):253-61.

(162) Sukhanov S, Higashi Y, Shai SY, Vaughn C, Mohler J, Li Y, Song YH, Titterington J, Delafontaine P. IGF-1 reduces inflammatory responses, suppresses oxidative stress, and decreases atherosclerosis progression in ApoE-deficient mice. *Arterioscler Thromb Vasc Biol* 2007 December;27(12):2684-90.

(163) Yetik-Anacak G, Catravas JD. Nitric oxide and the endothelium: history and impact on cardiovascular disease. *Vascul Pharmacol* 2006 November;45(5):268-76.

(164) Shai SY, Sukhanov S, Higashi Y, Vaughn C, Rosen CJ, Delafontaine P. Low circulating insulin-like growth factor I increases atherosclerosis in ApoE-deficient mice. *Am J Physiol Heart Circ Physiol* 2011 May;300(5):H1898-H1906.

(165) Colao A. The GH-IGF-I axis and the cardiovascular system: clinical implications. *Clin Endocrinol (Oxf)* 2008 September;69(3):347-58.

(166) McCarty MF. IGF-I activity may be a key determinant of stroke risk--a cautionary lesson for vegans. *Med Hypotheses* 2003 September;61(3):323-34.

(167) De SA, Brouns R, Uyttenboogaart M, De RS, Moens M, Wilczak N, Luijckx GJ, De KJ. Insulin-Like Growth Factor I Serum Levels Influence Ischemic Stroke Outcome. *Stroke* 2011 June 23.

(168) Cittadini A, Monti MG, Castiello MC, D'Arco E, Galasso G, Sorriento D, Saldamarco L, De PA, Napoli R, Iaccarino G, Sacca L. Insulin-like growth factor-1 protects from vascular stenosis and accelerates re-endothelialization in a rat model of carotid artery injury. *J Thromb Haemost* 2009 November;7(11):1920-8.

(169) Reaven GM, Hoffman BB. A role for insulin in the aetiology and course of hypertension? *Lancet* 1987 August 22;2(8556):435-7.

(170) Gielen S, Sandri M, Erbs S, Adams V. Exercise-induced Modulation of Endothelial Nitric Oxide Production. *Curr Pharm Biotechnol* 2011 January 11.

(171) McCarty MF, Barroso-Aranda J, Contreras F. Potential complementarity of high-flavanol cocoa powder and spirulina for health protection. *Med Hypotheses* 2010 February;74(2):370-3.

(172) Loke WM, Hodgson JM, Proudfoot JM, McKinley AJ, Puddey IB, Croft KD. Pure dietary flavonoids quercetin and (-)-epicatechin augment nitric oxide products and reduce endothelin-1 acutely in healthy men. *Am J Clin Nutr* 2008 October;88(4):1018-25.

(173) Davis BJ, Xie Z, Viollet B, Zou MH. Activation of the AMP-activated kinase by antidiabetes drug metformin stimulates nitric oxide synthesis in vivo by promoting the association of heat shock protein 90 and endothelial nitric oxide synthase. *Diabetes* 2006 February;55(2):496-505.

(174) Sakakibara S, Murakami R, Takahashi M, Fushimi T, Murohara T, Kishi M, Kajimoto Y, Kitakaze M, Kaga T. Vinegar intake enhances flow-mediated vasodilatation via upregulation of endothelial nitric oxide synthase activity. *Biosci Biotechnol Biochem* 2010;74(5):1055-61.

(175) Schwedhelm E, Maas R, Freese R, Jung D, Lukacs Z, Jambrecina A, Spickler W, Schulze F, Boger RH. Pharmacokinetic and pharmacodynamic properties of oral L-citrulline and L-arginine: impact on nitric oxide metabolism. *Br J Clin Pharmacol* 2008 January;65(1):51-9.

(176) Lundberg JO, Carlstrom M, Larsen FJ, Weitzberg E. Roles of dietary inorganic nitrate in cardiovascular health and disease. *Cardiovasc Res* 2011 February 15;89(3):525-32.

(177) McCarty MF, Barroso-Aranda J, Contreras F. High-dose folate and dietary purines promote scavenging of peroxynitrite-derived radicals--clinical potential in inflammatory disorders. *Med Hypotheses* 2009 November;73(5):824-34.