Caloric Restriction in Primates and Relevance to Humans

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ABSTRACT: Dietary caloric restriction (CR) is the *only* intervention conclusively and reproducibly shown to slow aging and maintain health and vitality in mammals. Although this paradigm has been known for over 60 years, its precise biological mechanisms and applicability to humans remain unknown. We began addressing the latter question in 1987 with the first controlled study of CR in primates (rhesus and squirrel monkeys, which are evolutionarily much closer to humans than the rodents most frequently employed in CR studies). To date, our results strongly suggest that the same beneficial "antiaging" and/or "antidisease" effects observed in CR rodents also occur in primates. These include lower plasma insulin levels and greater sensitivity; lower body temperatures; reduced cholesterol, triglycerides, blood pressure, and arterial stiffness; elevated HDL; and slower age-related decline in circulating levels of DHEAS. Collectively, these biomarkers suggest that CR primates will be less likely to incur diabetes, cardiovascular problems, and other age-related diseases and may in fact be aging more slowly than fully fed counterparts.

Despite these very encouraging results, it is unlikely that most humans would be willing to maintain a 30% reduced diet for the bulk of their adult life span, even if it meant more healthy years. For this reason, we have begun to explore CR mimetics, agents that might elicit the same beneficial effects as CR, *without* the necessity of dieting. Our initial studies have focused on 2-deoxyglucose (2DG), a sugar analogue with a limited metabolism that actually reduces glucose/energy flux without decreasing food intake in rats. In a six-month pilot study, 2DG lowered plasma insulin and body temperature in a manner analagous to that of CR. Thus, metabolic effects that mediate the CR mechanism can be attained pharmacologically. Doses were titrated to eliminate toxicity; a long-term longevity study is now under way. In addition, data from other laboratories suggest that at least some of the same physiological/metabolic end points that are associated with the beneficial effects of underfeeding may be obtained from other potential CR mimetic agents, some naturally occurring in food products. Much work remains to be done, but taken together, our successful results with CR in primates and 2DG administration to rats suggest that it may indeed be possible to obtain the health- and longevity-promoting effects of the former intervention without actually decreasing food intake.

KEYWORDS: Caloric Restriction; Primates; Humans

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INTRODUCTION

It has been known for over six decades that reduced caloric intake is the only conclusive and reproducible intervention shown to retard aging and extend healthy life span in mammals.[1] Dietary caloric restriction (CR) maintains physiological and behavioral functions and delays and reduces the severity of many age-related diseases in a variety of animal models.[2] These range from invertebrates to primates; data from the latter are recently beginning to suggest strong parallels between beneficial CR effects in experimental rodents and monkeys.[3]

The first large-scale controlled study of CR in primates was initiated by us at the National Institute on Aging in 1987.[4] Major objectives included determination of whether this intervention could indeed slow aging and maintain health and vitality in rhesus and squirrel monkeys in a manner analogous to its well-documented effects in rodents and, by inference, might be applicable to humans. Results from this and similar projects, subsequently begun at other institutions, bode well for the possibility that certain indices of aging and age-associated diseases may indeed be diminished by CR in humans as well.[5] These include a slower age-related decline in circulating levels of the steroid hormone dehydroepiandrosterone sulfate (DHEAS); lower plasma concentrations of glucose, insulin, cholesterol, and triglycerides; lower systolic and diastolic blood pressure as well as arterial stiffness; and increases in insulin sensitvity and levels of HDL2b.[3,5] Taken together, these findings suggest that CR primates will be less likely to contract diabetes, cardiovascular disease, cancer, and other pathologies of aging than fully fed counterparts. Thus, opportunities for an entire new area of dietary therapeutic intervention might eventually be available.

In addition, CR monkeys have lower body temperatures and initially reduced metabolic rates, symptomatic of a fundamental shift from a growth and reproductive strategy to a life-maintenance mode.[6] These physiological adaptations are also analogous to those elicited by CR in rodents, whose protective mechanisms have been enhanced, and median and maximal life spans extended.[1,2] Such alterations are undoubtedly linked with the "antidisease" effects as well and suggest possible biological mechanisms by which beneficial effects might be exerted.

Unfortunately, it is very doubtful that most humans would be willing to adopt a CR lifestyle, even with the promise of an extended, healthy life span. Difficulties with compliance and problems associated with so-called yo-yo and fad dieting are leading examples of practical arguments against CR for people. Moreover, enjoyment of food is a positive, life quality–enriching experience for most of us and renders an "appetite suppressant" strategy equally unpalatable. Thus, we have chosen to explore potential "CR mimetics" as a means of achieving the beneficial effects of restriction *without* dieting or suppressing appetite.

PHYSIOLOGICAL AND METABOLIC HALLMARKS OF CR IN RODENTS AND PRIMATES

TABLE 1 compares some of the most robust physiological and metabolic indices of the CR state in rodents and primates. Although lower plasma insulin concentrations and body temperature are probably the most reproducible of these, all are use-

TABLE 1. Summary of findings from the NIA Study of Calorie Restriction in Nonhuman Primates

Finding	Reference	Agrees with rodent data
▼ Body weight	Lane *et al.*, J. Nutr. **125:** 1600	yes
▼ Fat and lean mass	Lane *et al.*, Am. J. Physiol. **31:** E941	yes
▼ Trunk:leg fat radio	DeAngelis *et al.*, Gerontologist (Oct. 98)**	NR
▼ Time to sexual maturity	Roth *et al.*, Endocrine J. **1:** 227	yes
▼ Time to skeletal maturity	Lane *et al.*, J. Nutr. **125:** 1600	yes
▼ Fasting glucose/insulin	Lane *et al.*, Am. J. Physiol. **31:** E942	yes
▲ Insulin sensitivity	DeAngelis *et al.*, Gerontologist (Oct. 98)**	NR
▼ Metabolic rate (short-term)	Lane *et al.*, Proc. Natl. Acad. Sci. USA **93:** 4159	yes
◆ Metabolic rate (long-term)	Lane *et al.*, J. Gerontol. Biol. Sci. 50A: B295	yes
▼ Body temperature	Lane *et al.*, Proc. Natl. Acad. Sci. USA **93:** 4159	yes
◆ or ▼ Locomotion	Weed *et al.*, Physiol. Behav **62** (1): 97	yes
▼ Serum triglycerides	Verdery *et al.,* Am. J. Physiol. **36:** E 714	yes
▲ Serum HDL 2B	Verdery *et al.,* Am. J. Physiol. **36:** E 714	yes
▼ IGF-1/growth hormone	Cocchi *et al.*, Neuroends. Lett **17:** 181	yes
▼ IL-6	Lane *et al.,* J. Nutr. **125:** 1600	yes
◆ Testosterone	Unpublished	NR
◆ Estradiol, LH, FSH, prog	Handy *et al.*, Gerontologist (Oct. 98)**	NR
◆ Wound closure rate	Roth *et al.*, Gerontol. Biol. Sci. **52A:** B98	yes
◆ Fibroblast clonal proliferation	Pendergrass *et al.*, J. Cell. Physiol. (in press)	?
◆ β-Gal senescent cells	Pendergrass *et al.*, J. Cell. Physiol. (in press)	?
▼ Rate of decline in DHEAS	Lane *et al.*, J. Clin . Endocrinol. Metb. **82** (7): 2093	?
▼ Lymphocyte number	Weindruch *et al.*, Aging **9**(4): 304	yes
◆ Lymphocyte calcium response	Grossman *et al.*, J. Cell Physiol. **162:** 298	no

NOTE: ▼ decreased; ▲ increased; ◆ no effect or change; NR, not reported; ** abstract.

ful and can serve as a basis for evaluating the short-term effects of potential CR mimetics. Obviously, it is too early to ascertain whether life span is extended by CR in primates, although preliminary morbidity and mortality data exhibit very promising trends in this direction.[5] Similarly, while longevity experiments with CR mimetics in rodents are quite feasible, much faster suggestive evidence as to efficacy may be obtained from shorter-term physiological assessments.

We have, therefore, sought to establish a battery of such measures in order to screen candidate CR mimetics. Our strategy is to combine and compare data from our own studies as well as the existing literature to determine what agents might be the most promising. In light of the critical relationship between glucoregulation, energy metabolism, and the beneficial effects of CR in both rodents and primates (TABLE 1), we have chosen to explore compounds that exert their initial biological effects at these levels.

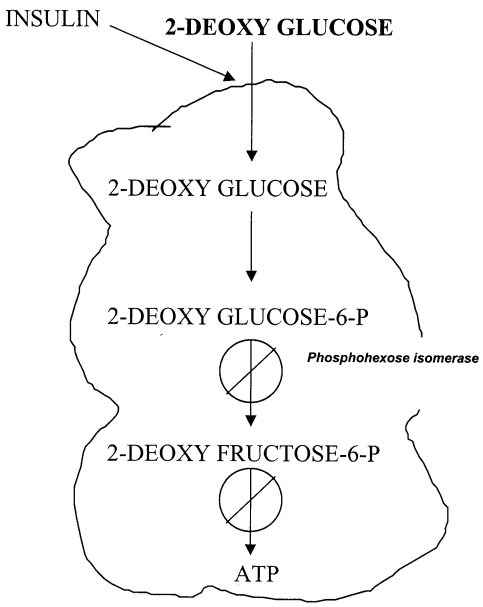

FIGURE 1. Effects of 2-deoxyglucose on cellular glucose metabolism. 2-Deoxyglucose enters the cell and is phosphorylated but is not metabolized further. 2-Deoxyglucose phosphate inhibits further metabolism of native glucose molecules.

2-DEOXYGLUCOSE—THE FIRST CANDIDATE

A substantial body of data on the physiological and metabolic effects of the glucose analogue 2-deoxyglucose (2DG) has been compiled over the last two decades. This sugar has been employed for a variety of purposes, ranging from tracing native glucose molecule transport and processing to actually blocking energy metabolism. FIGURE 1 shows a schematic of the effects of 2DG on glucose metabolism. Its primary effect is to serve as an extremely strong competitor of the enzyme phosphohexose isomerase, which converts glucose-6-phosphate to fructose-6-phosphate. As a consequence, the former phosphate builds up and is eventually excreted, while metabolism of native glucose is greatly reduced. Because of this latter effect, 2DG is quite toxic at higher concentrations.

Despite this potential toxicity, however, 2DG at lower dosages and/or in very short-term experiments has been shown to elicit some of the very same physiological and metabolic effects as CR. These include lowering body temperature[7] and reducing tumor formation[8] in rats, and elevating circulating glucocorticoid hormone concentrations in humans.[9] It was, therefore, decided to examine the longer-term effects of various concentrations of 2DG administered in chow on several metabolic hallmarks of CR in rats. TABLE 2 illustrates the design of this experiment.[10]

TABLE 2. Experimental design: 2-deoxy glucose treatment

Group	*n*	Treatment
Ad lib	20	Ad lib
Pair-fed	20	Pair-fed to lowest intake
0.2%	20	0.2% 2-dg in diet
0.4%	20	0.4% 2-dg in diet
0.6%	20	0.6% 2-dg in diet

EFFECTS OF SIX-MONTH 2DG ADMINISTRATION TO FISCHER RATS

The highest dose of 2DG did, indeed, prove to be toxic, as four rats in that group died in the first five weeks of the study.[10] Unfortunately, the cause of death could not be determined, although unidentifiable vacuolar inclusions were noted in some 2DG-treated animals. Since the 0.6% dose appeared to define the lower limits of the toxicity range, it was decided to administer this concentration of 2DG only every other week, leaving this experimental group untreated on alternate weeks. This strategy proved to be successful, as no further deaths occurred.

Food intake was substantively affected by 2DG only in the first month or two, as the animals adapted to the taste of the chow (mostly at the highest dose, reflecting also the toxicity discussed above).[10] Consumption by most animals was essentially identical to that of the *ad libitum* control group during the remainder of the study. Thus, the objective of maintaining nonrestricted dietary intake was indeed achieved. FIGURE 2 shows the corresponding effect of 2DG on body weight. In general, rats at the two highest concentrations exhibited slight reductions in weight over the entire six months of the experiment, in the 5–10% range.[10] Since food consumption was not significantly altered, these decreases are consistent with a CR mimetic action by the sugar analogue.

Having satisfied requirements for lack of toxicity, maintenance of food intake, and weight control, it became important to assess some metabolic indices in the experimental rats. FIGURE 3 depicts body temperature over the course of the study. Although a fair amount of variability is apparent over the relatively narrow range of physiological temperature, animals in the 0.4% and 0.6% groups did indeed exhibit reductions during most of the experiment.[10] Thus, one important metabolic index of the CR state was achieved by 2DG administration.

A consistent, but statistically nonsignificant, trend toward lower plasma glucose levels relative to controls (on the order of 5%) was also observed in the 2DG groups at 13 and 24 weeks.[10] These results are illustrated in FIGURE 4. Although reductions in blood sugar are often observed in CR rodents and primates, a much more robust metabolic hallmark is decreased insulin concentration (TABLE 1).[3,5] FIGURE 4 also shows the latter, which is reduced by about 30%, comparable to most CR studies, in the 0.4% group of rats.[10]

Taken together, the above findings demonstrated for the first time that key physiological effects of CR could indeed be achieved by long-term administration of low concentrations of 2DG to rats, *without* significantly affecting food intake. Of course, these results beg the obvious question of whether these same experimental animals

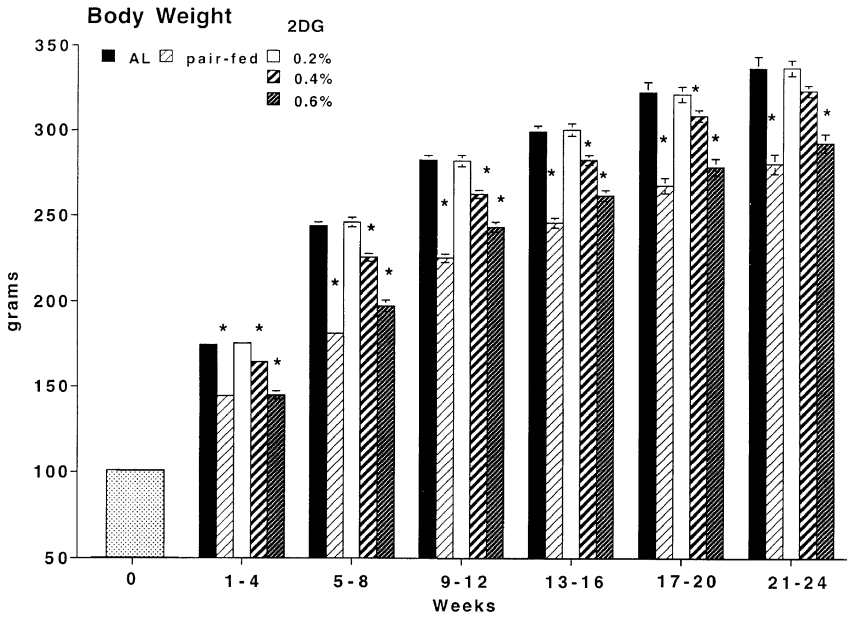

FIGURE 2. Effect of 2-deoxyglucose on body weight in Fischer-344 rats. Values are means ± standard errors. Details can be obtained from Reference 10.

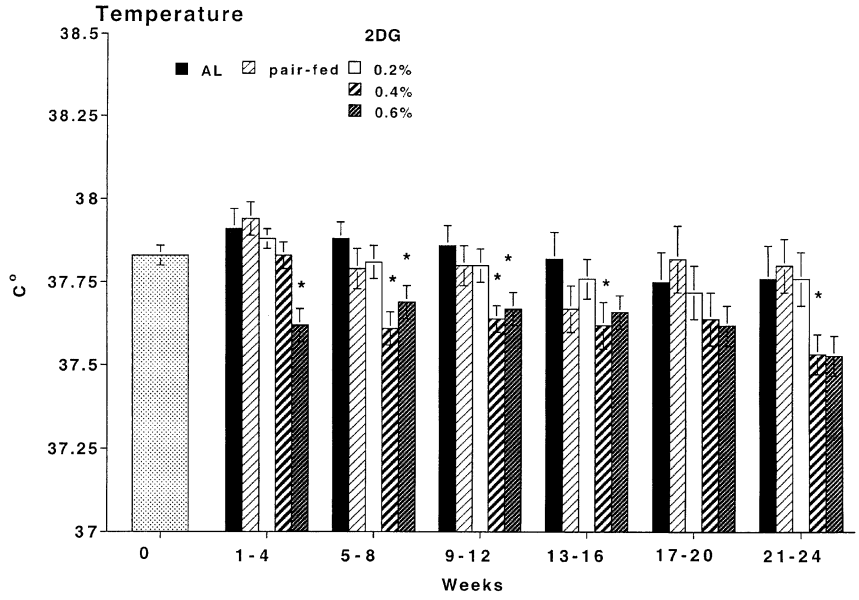

FIGURE 3. Effect of 2-deoxyglucose on temperature in Fischer-344 rats. Experimental design and data presentation are as for FIGURE 3, and details are in Reference 10.

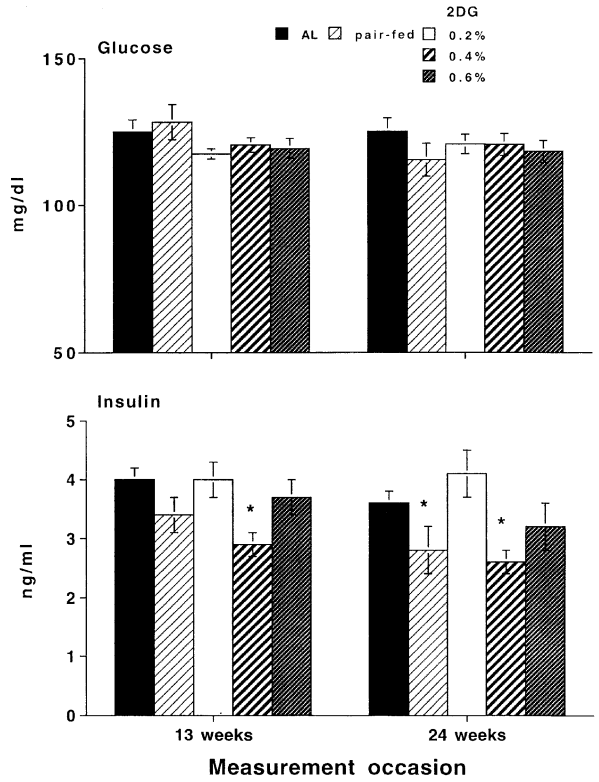

FIGURE 4. Effects of 2-deoxyglucose on plasma glucose and insulin levels in Fischer-344 rats. Details are as for FIGURES 2 and 3.

will actually age at a slower rate, incur the diseases of aging later and to a lesser extent, and live longer than *ad libitum*–fed control counterparts. The latter experiment is currently under way, having been initiated in December of 1999 with 140 additional rats and employing 2DG dosages of 0.3% and 0.4%. Various physiological/metabolic as well as extensive pathological assessments will be performed. It will take another two to three years to determine whether the sugar analogue can indeed be employed as a true CR mimetic.

OTHER POTENTIAL CR MIMETIC STRATEGIES

If the 2DG-treated rats in the ongoing longevity study do actually outlive their controls, an entire new research area will be opened, with 2DG as the "lead" compound. Of course, a wider range between the toxic and the beneficial effects of potential CR mimetic agents would be highly desirable, if not essential, if this strategy is ever to be applied to humans. Consequently, whether or not the 2DG animals ex-

TABLE 3. Inhibitory effects of gymnemoside b and gymnemic acids on the increase in serum glucose of oral glucose-loaded rats

	Increase in serum glucose (mg/dL) one half hour after oral glucose
Control	77.4 ± 7.6
Gymnemic bacid	61.2 ± 7.6
Control	62.9 ± 4.3
Gymnemic acid III	46.3 ± 6.9
Control	65.5 ± 4.7
Gymnemic acid V	41.9 ± 6.1
Control	66.9 ± 2.3
Gymnemic acid VII	51.5 ± 5.2

NOTE: Adapted from Yoshikawa *et al.*, 1997, Clin. Pharm. Bull. **45:** 1671.

hibit increased health, vitality, and longevity, other such compounds must be identified and evaluated.

In fact, a fairly large literature (both scientific and folk/popular) exists in the area of medicinals that might elicit some of the same beneficial effects as CR. An exhaustive treatise is well beyond the scope of the present review, but a few illustrative examples can provide some perspective with regard to the possibilities.

Gymnema Sylvestre and Antidiabetics

The leaves of the Asian plant *Gymnema sylvestre*, have long been employed as a folk remedy for diabetes and its related symptoms.[11] Called "gur-mar" or "gurmara," which means "sugar destroyer" in Hindi, *Gymnema* has played an active role in Ayurvedic medicine for nearly three millenia.[12] More recently, it has been marketed as a health food/herbal medicine in Japan, where it is purported to be effective against both diabetes and obesity.[11] Although the plant and its extracts appear to exert multiple biological effects, including reducing the perception of sweetness,[13] the end point most relevant to CR mimicry is probably a reduction in blood sugar concentrations, especially following an oral glucose load.[11]

Matsuda and his colleagues have identified a number of active ingredients, falling into a broad class of triterpines called gymnemosides as well as some gymnemic acids, that elicit this particular response.[11] TABLE 3 is an adaptation of their results from the most effective of these agents, showing reductions of approximately 20 to 30% in serum glucose concentrations 30 minutes after oral administration of 100 mg per kg of body weight. The effect appears to be exerted at the level of glucose adsorption through the intestine, far removed from that of 2DG, which inhibits an enzyme early in the process of glucose metabolism. Nevertheless, lowering of serum glucose concentrations would qualify as a CR mimetic effect. Of course, a more reproducible end point would be a reduction in circulating insulin levels, but unfortunately this effect was not examined.[11]

These results do suggest, however, that certain other antidiabetic agents might mimic CR by lowering insulin concentrations and/or sensitizing target cells and tissues to its effects. A number of such agents are currently available, but again for present purposes a few examples will suffice to demonstrate possible research opportunities. Two classes of compounds with specific effects on both lipid and carbohydrate metabolism are at present prominent in this category. These are the biguanides and the thiazolidinediones. At least one study has already reported that a member of the former group, phenformin, extends both mean and maximal life span in mice.[14] Unfortunately, however, the mouse strain employed, C3H/Sn, is cancer prone, with a mean life span of only 450 days. Furthermore, food intake was not determined, so it is uncertain how much of the effect might be attributable to appetite suppression. Whether similar potential antiaging effects can be achieved with longer-lived rodent strains remains to be determined.

Another biguanide, metformin, is more commonly employed clinically. Although these agents have been available for nearly 30 years, their exact mechanism(s) of action is still uncertain. Their biological effects include lowering blood lipids, reducing gluconeogenesis (the opposite of CR), and decreasing glucose adsorption through the intestine (like the gymnemosides).[15] Their most consistent effect, however, seems to be the facilitation of glucose entry into cells,[15,16] which results in increased insulin sensitivity (like CR). The thiazolidinedione that has received the most recent attention is troglitizone. Its precise effects are even less well understood than those of the biguanides. However, it does appear to regulate certain genes involved in both lipid and carbohydrate metabolism and, like the biguanides, increases glucose entry into cells.[17] Thus, antidiabetics may indeed offer at least some of the beneficial effects of CR, probably without the same degree of restriction, but to what extent the effects are due to appetite suppression remains to be determined.

Garcinia Cambogia

One other Ayurvedic folk medicine with potential CR mimetic effects on fat metabolism is the Brindall berry, *Garcinia cambogia.*[18] This fruit has traditionally been used as a preservative for fish and condiments in Indian cooking; extracts are used as food supplements throughout the world. *Garcinia* has been alleged to exert weight control by increasing lipolysis, decreasing lipogenesis, and appetite suppression, although the latter does not appear to be an absolute requirement for the other two effects. At the biochemical level, the principal active ingredient appears to be hydroxycitrate, which inhibits isocitrate lyase.[18] This enzyme plays a key role in lipogensis, although it is not clear how its inhibition facilitates lipoloysis as well. In any case, the aggregate effects of *Garcinia* on lipid metabolism bode well for the possibility that this aspect of actual CR can indeed be mimicked by pharmacological or nutraceutical means.

SUMMARY AND CONCLUSIONS

Data from an ongoing 13-year study in monkeys strongly suggest that CR can exert the same beneficial antiaging, antidisease effects in primates as it does in rodents and lower animals. Although conclusive longevity results will not be available for a

number of years, preliminary morbidity and mortality data are consistent with the possibility of both an extended life span and health span for CR primates, ultimately including humans. Unfortunately, long-term 30% restriction of food intake is beyond the capability of most people, even with the promise of such benefits.

For this reason, a more practical strategy is that of CR mimetics, to achieve the positive effects of restriction without actually reducing caloric consumption. The first candidate CR mimetic is 2-deoxyglucose, or 2DG. A six-month pilot experiment, in which it was fed to rats, demonstrated that very low dosages could be administered without significant toxicity or reduction in dietary intake yet still elicit some of the critical physiological/metabolic end points of CR. These included lower circulating insulin concentrations and lower body temperatures. A longevity study is currently under way.

Regardless of the results of the latter investigation, which is expected to take two to three years, it will be extremely useful to screen other potential CR mimetics, with wider "effective to toxic dose ranges" than 2DG. The fact that certain other pharmacological agents, as well as naturally occurring food/nutraceutical products, can achieve at least some of the same metabolic effects as CR also bodes well for the feasibility of this approach. It is expected that a number of exciting results from studies in this new area will occur in the very near future, which, it is hoped, will ultimately lead to the possibility of "eating one's cake and having it, too."

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