Sugar substitutes: their energy values, bulk characteristics, and potential health benefits[1,2]

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ABSTRACT Restriction of dietary energy extends life and reduces incidences of disease in animals. These benefits would likely extend to humans. However, diet restriction in animals imposes reductions of 30–50% in food intake, which is probably unacceptable to humans. Low-energy sweeteners used in beverages offer minor reductions in energy intake. However, they lack the bulk required for baked goods and other sugar-rich foods. Full-bulk sweeteners providing about one-half the energy of sugar are under development for such uses. Laxation limits their acceptable dose. Even within such limitations, they can help achieve the health benefits for humans indicated by diet restriction. D-Tagatose, a new candidate sweetener, is nearly as sweet as sucrose and has the bulk of sucrose, yet provides zero available energy. We discuss its potential contribution to human diet restriction along with its specific effect in delaying the aging effects of glycosylation. *Am J Clin Nutr* 1995;62(suppl):1161S-8S.

KEY WORDS Diet restriction, low-energy bulk sweeteners, D-tagatose

ENERGY INTAKE AND HEALTH

Dieting to improve health started with simple correlations between obesity and disease. Momentum has been added by more detailed epidemiologic and dietary studies on longevity and disease. We now have the knowledge to influence our lives to a greater extent than imaginable only a few decades ago. Moreover, it is becoming evident that rigorous control of energy intake provides significantly increased life span and marked postponements and reductions in incidences of many serious illnesses in mammals; these benefits probably extend to humans. Major physiologic benefits of chronic energy-intake restriction, also called dietary restriction, are reported from the cellular to the primate level.

BENEFITS OF DIETARY RESTRICTION

A review article on diet restriction in weaned and adult rodents reports that the numbers of epididymal and perirenal fat cells are reduced by energy restriction (1). Restriction of energy intake delays death caused by neoplasms. However, this effect was not achieved through the restriction of dietary fat or protein components of the diet without overall energy restriction. The review summarizes that "restriction of dietary energy maintains most physiologic systems in a youthful state and retards a broad spectrum of disease processes" (1).

A subsequent review cites works establishing that diet restriction markedly extends the maximum life span, retards the aging process itself as shown by an increase in mortality rate doubling time, maintains consistently lower plasma glucose and plasma insulin concentrations, and retards or prevents "almost all age-associated disease processes" (2).

Chronic restriction of energy intake was found to inhibit autoimmune disease in several strains of autoimmune-prone mice, to correct a variety of cell population abnormalities, to correct interleukin 2 production, and to prolong life (3). Diet restriction was shown to decrease the incidence of neoplastic and non-neoplastic lesions in rats and mice, including preputial, mammary, pituitary, skin, pancreas, clitoral, thyroid, liver, and bladder lesions (4). Moreover, the incidences were less in diet-restricted animals challenged with carcinogens than in nonrestricted controls. When p53-knockout mice (genetically predisposed to spontaneous tumors) were put on restricted diets, tumorigenesis was delayed (SD Hurstin, SN Perkins, JM Phan, unpublished observations, 1994).

Sprague-Dawley rats after 2 y of diet restriction had the same brain weight as controls fed ad libitum, but less body fat and lower heart, lung, kidney, adrenal, thyroid, and pituitary weights (5). Diet restriction was correlated with decreased incidence and severity of degenerative or proliferative lesions in those organs. Numerous strains of mice genetically prone to various diseases of aging developed less renal disease, cardiac disease, vascular disease, and malignancies when maintained on restricted diets (RA Good, unpublished observations, 1994). Diet restriction also reduced heightened susceptibility to infection that was related to age. Restriction of as little as 10% was found to inhibit tumorigenesis in rats (6). In a 2-y tumor onset study in Sprague-Dawley rats, diet restriction increased survival time in both males and females by a factor of more than two (5).

Additional age-associated pathologic lesions cited as being ameliorated in rats or mice by diet restriction include the following: nephropathy, cardiomyopathy, gastric ulcer, osteodystrophy, cataract, lymphoproliferative disease, lung tumors, leukemia, and lymphoma (7). The following physiologic

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processes were also maintained in a youthful state: parathyroid hormone concentration, plasma calcitonin concentration, response of adipocytes to lipolytic hormones, dopamine receptors in corpus striatum, ability to learn a maze, development of vascular smooth muscle tension, gamma crystallin concentration in lens, female reproductive function, collagen cross-linking, hepatic protein biosynthesis, hepatic proteolysis, DNA repair in splenocytes, α-2u-globulin expression, blood glutathione concentration, and hepatic cytochrome oxidase content. In vivo studies of cell turnover and in vitro studies (8) of clonal proliferation concluded that diet restriction slows the loss of in vitro replicative potential otherwise found with increasing animal age (8).

It has been proposed that diet restriction extends the life span by increasing the intrinsic sensitivity of damaged or diseased target cells to apoptotic cell death (9). In a diet restriction study of mice, a twofold increase in apoptotic bodies was observed in livers of mice fed restricted diets compared with those of controls fed ad libitum (SJ James-Gaylor, L Muskhelishvilli, WG Sheldon, et al, unpublished observations, 1994). At 18 and 24 mo of age, male mice fed ad libitum had a 29% incidence of hepatic tumors, whereas there were none in the diet-restricted mice.

Diet restriction also decreased the expression of endogenous and exogenous retroviruses in mice and decreased all variables of Friend virus disease (10). Diet-restricted rats have also been shown to have enhanced homeostatic capacity compared with rats of similar age fed ad libitum (JL Pipkin, WG Hinson, LE Lyn-cook, et al, unpublished observations, 1994). An overall general increase in homeostasis was found in aged rats after prolonged diet restriction.

Pancreas cells perpetuated in cell culture from diet-restricted rats continued to show the effects of lower tumorigenesis that diet restriction had established in the animals in life (BS Hass, D He, RW Hart, et al, unpublished observations, 1994). Additionally, diet restriction had a positive effect on cognitive and sensorimotor performance of mice tested for trainability and reaction times (MJ Forster, H Lal, unpublished observations, 1994).

Age retardation effects of diet restriction have been found at the level of the cell membrane (11). Various indexes of cell membrane composition and function were monitored in diet-restricted female Wistar rats. It was found "that diet energy restriction preserves the normal physicochemical properties of cell membranes, protecting them from age-dependent alterations" (11). The life span of the diet-restricted animals was significantly extended. Diet-restricted rats in four feeding studies had a mortality rate doubling time that averaged 197 d compared with an average of 102 d for controls fed ad libitum (12).

POSSIBLE APPLICABILITY OF DIET RESTRICTION TO HUMANS

The National Institute on Aging started a study of diet restriction in rhesus and squirrel monkeys in 1987 (13). Because these primates have life spans in the 40-y range, age-induced changes in biochemistry and anatomy are tracked for early evidence of any effect of diet restriction. About 4.5 y into the study, significant differences in body weights developed between the test and control rhesus monkeys with a similar trend in the squirrel monkeys (14). Higher concentrations of serum alkaline phosphatase were found in the diet-restricted monkeys. Alkaline phosphatase normally declines with age in the subject species. All animals were in excellent health as determined by physical examinations, hematology, and blood chemistry.

An early, if not the first, scientific study of the applicability of diet restriction to humans was performed in middle-aged, nonobese men (EJM Velthuis-te Wierik et al, unpublished observations, 1994). Subjects restricted to 80% of their habitual energy intake for 10 wk lost an average of 7.4 ± 2.6 kg, mostly fat. The subjects' blood pressures decreased and their lipid profiles improved. There were no adverse effects on mental performance, feelings of hunger, satiety, or mood. A slight decrease in physical performance of the test subjects was noted, but was not correlated with weight loss.

If diet restriction should prove effective for humans, would-be benefactors will face a difficult choice: the immediate pleasure of liberal indulgence in food or privation for the promise of better health and increased longevity. In most of the animal studies reported, intakes were restricted to 50–70% of normal diets. Although human dose-response data may show effects with lesser degrees of diet restriction (EJM Velthuis-te Wierik et al, unpublished observations, 1994), it seems likely that significant effects will require significant diet restriction. If so, it will take extraordinary willpower to secure the increasingly apparent benefits of diet restriction.

The use of high-intensity sweeteners, such as aspartame and saccharin, in beverages can effect a minor reduction in daily energy intake. However, the major opportunities for energy control lie in replacing sugar in bulk foods such as baked goods, ice cream, frozen desserts, processed foods, and condiments. In these food products, the bulk of sugar is intrinsically important to the product. The use of high-intensity sweeteners requires incorporation of a low-energy bulking agent into the product. This approach has not resulted in many satisfactory products. The formulations attempted have suffered from decreased palatability or from physical limitations, such as decomposition on heating. Those formulations surviving the heat of baking do not undergo the browning effect important to baked goods.

FULL-BULK SWEETENERS

Zero- or low-energy, full-bulk, alternative carbohydrate sweeteners may play a useful role as one factor in helping to make severely energy-restricted diets bearable. Several such sweeteners have been developed to fill the major market unaddressed by high-intensity sweeteners. Such bulking agents typically have an energy content of approximately one-half of sucrose's 16.5 kJ/g. Principal among these are "polyols," sugar alcohols with a generally sweet taste, but lower intensity than sucrose and having an undesirable cooling effect. Characteristics of some presently available full-bulk, alternative carbohydrate sweeteners are shown in **Table 1**.

A problem limiting the role of these full-bulk sweeteners in human diet restriction is their inducement of laxation. Although the Food and Drug Administration (FDA) does not consider laxation a toxic effect, it does require that labels of products containing such sweeteners provide cautionary information including serving size limitations. Tolerances deter-

TABLE 1
Comparative evaluation of alternative carbohydrate sweeteners and bulking agents[1]

	Sorbitol[2]	Mannitol[3]	Xylitol[4]	Palatinit[5]	Lactitol[6]	Polydextrose[7]	Neosugar[8]	Maltitol[9]	Erythritol[10]
Raw materials	Glucose	Fructose	Birchwood, almond shells, straw	Sucrose	Lactose	Glucose, sorbitol, citric acid	Sucrose	Starch	Glucose
Chemical processing steps	1	1	2	2	1	1	1	3	1
Sweetness (× sucrose)	0.6	0.5	0.7–1.0	0.45–0.65	0.3–0.4	0	0.3–0.6	0.9	<0.5–0.75
Browning on baking	No	No	No	No	No	No	No	No	No
Available energy (% of sucrose)	75	50	100	50–75	50	25–50	25–50	50–90	≈10
Laxative effect	High	Very high	High	Medium	High	Medium	Medium	Low	Low-medium
Cariogenicity	Low	Low	Anti	None	None	None	None	None	None
Deliquescence (% RH)[11]	65–70	>70	65–70	85–90	85–90	55–60	80–85	90–95	90–95
pH stability	2–6	2–6	2–6	2–6	2–6	2–6	>6	2–6	2–6
Taste characteristics	Sweet, cooling	Sweet, cooling	Sweet, cooling	Sweet, slightly cooling	Sweet, cooling	Slightly bitter	Sweet, clean	Sweet, slightly cooling	Sweet, cooling
Insulin requirement	None	None	Low	Low	None	None	Varies	High	None

[1] Information compiled by Biospherics Incorporated, Beltsville, MD.
[2] Manufactured by ADM, Decatur, IL; Roquette, France; Lonza, Switzerland; and others.
[3] Manufactured by Roquette and Getec, Brazil.
[4] Manufactured by Ajinomoto, Japan; Finnsugar, Finland; and others.
[5] Manufactured by Palatinit Sussungsmittel, Germany.
[6] Manufactured by CCA-Biochem, Netherlands.
[7] Manufactured by Pfizer, Groton, CT.
[8] Manufactured by Meiji Seika, Japan; Coors, Golden, CO; and Beghin-Say, France.
[9] Manufactured by Mitsubishi, Japan; and Roquette.
[10] Manufactured by Nikken Chemical, Japan, and Pfizer.
[11] Relative humidity.

mined for humans not previously having consumed the respective sweeteners are presented in **Table 2**. If these products, which provide approximately one-half the energy of sucrose (Table 1), are used at their laxation limits of ≈20–60 g/meal, they can make diet restriction more acceptable. For example, a 60-g serving of cake would typically contain ≈20 g sucrose that would provide 330 kJ. A one-for-one substitution of 20 g palatinit would thus halve the available energy. The cake would be only about one-half as sweet as a cake made with sucrose (Table 1), but a total of 495 kJ might be saved per day if a piece of cake, or a similar product, made with palatinit rather than sucrose was incorporated as part of each of three meals. This represents a reduction of 5.4% for females and 4.1% for males based on the recommended average adult daily energy intake (9205 kJ for females and 12 134 kJ for males) (24).

TABLE 2
Laxation values for selected carbohydrates

Compound	Mean LTV[1]	Reference
	g/meal	
Sorbitol (n = 21)	23	(15)
Mannitol	10–20 (estimate)	(16)
Xylitol	20 (estimate)	(17)
Palatinit (n = 10)	29	(18)
Lactitol	24–50	(19)
Polydextrose (n = 21)	30	(20)
Maltitol (n = 10)	60	(21, 22)
Erythritol	40 (estimate)	(23)
HSH (Hystar)	30–60 (estimate)	W Raleigh, personal communication, 1990

[1] Laxative threshold values for unadapted, adult subjects dosed one time as part of a normal meal.

L-Glucose and the L-sugars

For nearly two decades, our laboratory has sought to develop alternative carbohydrate sweetener and bulking agents. Our early work was with the L-sugars (25, 26). We thought that their enantiomeric structure would make them resistant to the body's digestive enzymes and that, as a result, they would provide no available energy. When we unexpectedly found them to be sweet, they were experimentally incorporated into several food products, many of which met with favorable evaluations. L-Glucose, in particular, seemed promising.

Methods to determine available energy, developed at the Agricultural and Food Research Council Institute of Food Research, were applied to L-glucose, L-gulose, and L-fructose (27, 28). Sucrose and cellulose served as controls. The results of this calculation of energy values are shown in **Table 3**. Whereas L-glucose contains the same, ≈16 kJ/g, as do the other carbohydrates when measured in a bomb calorimeter, it is distinguished by having a slightly negative value for available energy. Thus, a little more energy is consumed in metabolizing the small amount of energy available from L-glucose than is liberated. As seen in **Table 4**, essentially all of the L-glucose

TABLE 3
Energy values determined for selected carbohydrates[1]

Supplement	Gross energy (bomb calorimetry)	Food energy	Proportion gross energy used
	kJ/g	kJ/g	
Sucrose	16.5	16.3	0.99
Cellulose	17.3	0.1	0.01
L-Gulose	15.7	10.1	0.64
L-Fructose	15.7	6.2	0.40
L-Glucose	16.0	−0.8	−0.04
D-Tagatose	15.6	−0.5	−0.03

[1] From G Livesey, unpublished observations, 1987 (sucrose, cellulose, L-gulose, L-fructose, and L-glucose) and 1989 (D-tagatose).

passes through the urine after intravenous administration in rats. After oral administration, L-glucose produces slightly more metabolic carbon dioxide than when administered intravenously. That small increase is probably produced by some microorganisms in the intestine that can metabolize the compound. However, Table 4 shows that even those microorganisms have difficulty adapting to L-glucose over time. This is in contrast with the adaptation seen for oral administration of the other L-sugars also shown in Table 4. The only significant difference in partitioning of the carbon-14 among the various compartments measured for L-glucose is that the intravenously dosed adapted rat discharged less of the label in its feces than did its unadapted cohort. That this result was not caused by microbial adaptation in the intestine is shown by the lack of any increase in exhaled label by the adapted animals.

There may be some adaptation to L-glucose by the rat as opposed to adaptation by intestinal microflora, as revealed by the high percentage of carbon-14 recovered in the breath of rats dosed orally with the other L-sugars. Microbial metabolism would produce degradation products that could cross the intestinal membrane and be available for metabolism by the animal. Because microbial metabolism generally takes place in the lower intestine of rats, much of any such degradation products might exit with the feces, which would partly account for the reduced energy values determined for the other L-sugars. However, if the adaptation measurement for L-glucose does represent metabolism, the net result is the denial of energy, or an actual negative net flux of available energy to the ingesting animal. This is further supported by the slightly negative available energy value obtained after the longer, 56-d adaptation period that produced the L-glucose animal data in Table 3. Although the same phenomenon of decreased fecal partition is shown for orally administered L-fructose in Table 4, Table 3 shows that L-fructose yields ≈40% of the available energy (6.2 kJ/g) in sucrose.

L-Glucose shows promise as a sugar substitute from an energy standpoint but has several drawbacks. Although it has a clean, sweet taste, the sugar is only ≈0.6 times as sweet as sucrose. This amount of sweetness may be suitable for some food products and may be compensated for in others by addition of a small amount of a high-intensity sweetener. However, rat experiments showed that laxation would limit doses in the same manner as for the compounds shown in Table 1. This would still allow significant use, as discussed for palatinit. Most difficult to overcome, however, has been the high cost of production. Although progress has been made, no method has yet been shown to produce L-glucose, or the other L-sugars, at market-competitive costs.

D-TAGATOSE, A NONFATTENING, FULL-BULK SWEETENER

We wanted to study L-tagatose, one of the L-sugar monosaccharides within our patent claims (29), for use as an edible sweetener; however, we could not foresee an economic method for its manufacture. Instead, we chose to examine D-tagatose because its structure is similar to that of D-fructose, but with an inversion about one optically active center (**Figure 1**). Our studies with the L-sugars indicated that this single inversion might render the D-tagatose molecule low in available energy.

The primary attribute for evaluation of a sweetener is taste. D-Tagatose proved to have an excellent, sucrose-like taste, with no cooling effect, aftertaste, or potentiation of off-flavors. Standard taste-panel tests on 10% aqueous solutions of D-tagatose showed it to be 92% as sweet as sucrose. However, initial experimental results did not support our low-energy theory. Use of [^{14}C]D-tagatose showed it to be largely metabolized by both the oral and intravenous routes of administration, as revealed in Table 4.

We were surprised, therefore, when subsequent rat feeding studies showed weight- and fat-sparing effects of D-tagatose. Sprague-Dawley rats in groups of six each were fed a commercial stock diet (Certified Rodent Chow #5002; Ralston Purina, Richmond, IN) plus 15% (wt:wt) D-tagatose for an adaptation period of 14 d. One group of rats was then continued on the same diet and another group was maintained on the same diet except sucrose was substituted on an equal weight basis for the D-tagatose. Rats were pair-fed on the basis of the average daily food consumption of the D-tagatose group. Abrupt changes in diet generally result in temporary weight loss. The group switched to sucrose showed such a loss for a period of ≈7 d, after which weight gain commenced. At day 47 in the study, the two groups were at essentially the same weight, ≈735 g/animal. With this equivalent weight as the starting point for comparison, **Figure 2** presents curves generated by daily weight measurements of each group. At the end of the 60-d comparison, the average weight for the D-tagatose-fed animals was slightly > 760 g compared with the corresponding average weight of 819 g for the sucrose-fed animals, showing > 7% less weight gain for the D-tagatose-fed rats.

D-TAGATOSE SUPPLIES NO AVAILABLE ENERGY

On the basis of the 60-d test period, the sucrose-fed rats gained an average of 86 g each, more than three times the average 28 g gained by the rats fed D-tagatose. Analyses of the rat carcasses showed that this excess weight of the sucrose-fed group was composed of ≈90% fat. No difference in protein content was found between the sucrose-fed and D-tagatose-fed rats. Because it has been shown that there is no reduction in metabolic rate per unit of body mass in diet-restricted animals (1), it seems that D-tagatose does not liberate net available energy beyond that consumed in metabolizing it. This inefficient energy cycle effectively denies the animal food energy from D-tagatose and is consistent with findings that the

TABLE 4
Metabolism of ^{14}C-rare sugars by unadapted and adapted Sprague-Dawley rats[1]

	Percentage ^{14}C recovered						
	Breath	Urine	Feces	Skin	Carcass	Cage wash	Total
				%			
L-Glucose[2]							
Oral							
Unadapted	9.3 ± 3.3	72.6 ± 4.0	15.5 ± 15.6	1.7 ± 1.8	1.0 ± 0.0	0.6 ± 0.6	100.7
Adapted	6.5 ± 3.7	60.8 ± 21.6	20.8 ± 10.2	0.3 ± 0.1	1.7 ± 0.8	1.8 ± 0.2	91.9
IV							
Unadapted	1.0 ± 0.0	107.4 ± 5.0	18.1 ± 20.4	0.1 ± 0.0	0.4 ± 0.1	0.7 ± 0.9	127.7
Adapted	0.6 ± 0.2	91.1 ± 16.1	3.3 ± 3.2	0.1 ± 0.0	0.3 ± 0.1	0.5 ± 0.2	95.8
L-Gulose[2]							
Oral							
Unadapted	34.2 ± 1.8	18.6 ± 4.4	37.1 ± 0.9	1.0 ± 0.2	3.6 ± 0.1	0.7 ± 0.0	95.2
Adapted	61.2 ± 8.0	10.1 ± 2.0	12.5 ± 5.4	2.0 ± 0.3	7.8 ± 1.1	0.1 ± 0.0	93.7
IV							
Unadapted	3.3 ± 0.0	97.8 ± 0.2	1.2 ± 1.0	0.3 ± 0.1	0.9 ± 0.3	0.2 ± 0.1	103.7
Adapted	4.1 ± 0.7	106.4 ± 21	1.9 ± 1.6	0.3 ± 0.1	1.1 ± 0.3	0.2 ± 0.1	114.0
L-Fructose[2]							
Oral							
Unadapted	36.1 ± 1.6	21.1 ± 4.6	44.3 ± 10.5	1.2 ± 0.1	8.0 ± 11.4	1.2 ± 0.5	111.9
Adapted	50.0 ± 5.3	14.6 ± 4.2	13.1 ± 3.0	2.5 ± 0.7	8.4 ± 1.4	0.1 ± 0.1	88.7
IV							
Unadapted	1.6 ± 0.2	102.1 ± 6.2	3.7 ± 2.2	0.2 ± 0.0	0.9 ± 0.3	0.2 ± 0.1	108.7
Adapted	2.2 ± 0.0	96.3 ± 5.9	4.3 ± 1.8	0.2 ± 0.0	1.0 ± 0.4	1.6 ± 2.3	105.6
D-Tagatose[3]							
Oral							
Unadapted	49.4 ± 9.8	5.8 ± 0.6	28.7 ± 10.0	—	9.4 ± 1.0	—	93.3
Adapted	67.9 ± 2.7	5.2 ± 0.5	11.4 ± 1.5	—	10.8 ± 2.1	—	95.3
IV							
Unadapted	36.6 ± 2.2	42.6 ± 5.6	4.8 ± 4.9	—	8.6 ± 0.4	—	92.6

[1] $\bar{x}$ ± SE. Single doses of ^{14}C-rare sugars were administered orally or intravenously (IV) after 28 d ad libitum feeding of a commercial stock diet (unadapted) or a diet containing 10% (wt:wt) rare sugar (adapted); water was consumed ad libitum. Rats were maintained on diets while monitored in individual metabolism chambers for 72 h after dosing, and killed immediately for assays.
[2] From studies for Biospherics Inc by an industrial toxicology laboratory inspected by the Food and Drug Administration.
[3] From studies conducted by Biospherics Inc.

"decrease in (available) energy intake per rat does underlie the antiaging action of dietary restriction" (1). Energy value studies similar to those reported above for the L-sugars were also performed for D-tagatose at the Agricultural and Food Research Council Institute. D-Tagatose had no available energy value, actually indicating a slight negative value as seen in Table 3. Accordingly, we patented its use and decided to pursue it as a full-bulk sweetener (30).

SAFETY AND TOLERANCE OF D-TAGATOSE

We next investigated any possible toxicity of D-tagatose. Product safety tests required by the FDA in petitions for approval of food additives were initiated at independent industrial toxicology laboratories. Tests for acute toxicity, mutagenicity (Ames assay), chromosomal aberrations in Chinese hamster ovary cells, and mouse lymphoma forward mutation have now been completed in addition to subchronic feeding studies. Rat teratology studies are also complete. No toxic effects of agatose have been found. In vitro tests with *Actinomyces viscosus*, *Streptococcus mutans*, and *Streptococcus sanguis* have shown D-tagatose to be virtually noncariogenic.

In the rat feeding studies, up to 20% (wt:wt) D-tagatose was supplied in the diet. The variables monitored included laxation. **Table 5** shows no stool-softening effect on naive rats at the 5% level. Laxation was seen at higher doses, but with virtually complete accommodation and normal stool occurring within 3 d. Even at the 20% level, apparent adaptation to D-tagatose quickly overcame laxation.

Laxation is essentially an osmotic effect. The large numbers of small molecules introduced into the intestine, being non- or slowly metabolizable, draw water from the body into the intestine. This effect is heightened if the small molecules are not actively transported across the membrane of the small intestine. A good model for the human small intestine for investigating this effect is the pig small intestine. Accordingly, a pig absorption study was performed in 1989 for Raffinerie Tirlemontoise (Tiense, Belgium) at the State Centre of Animal Nutrition, State University of Ghent in Belgium (unpublished). One pig was fed 5% D-tagatose in its normal diet of barley, extracted soybean meal, and vitamin and mineral supplements, but was restricted to 85% of its normal quantity of feed. In addition, 1.4% by wt of bacteriostatics (neomycin and bacitracin) was included in addition to 1.0% by wt Celite (Manville Corpora-

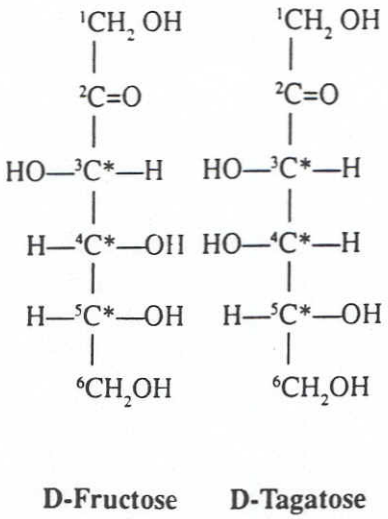

FIGURE 1. Comparison of D-fructose and D-tagatose molecular structures. Asterisk designates chiral carbon.

tion, Denver, CO) as an inert marker. Samples of digested material were removed hourly from the cannulated ileum in the course of an 8-h test period.

Analyses of the pig's digestive contents showed that 78 ± 8% of the D-tagatose was absorbed from the small intestine. This was in contrast with the 5 ± 7% absorption found in the same test for lactitol. This would seem to indicate a significantly lesser laxation effect for D-tagatose. Nonetheless, tests of both compounds produced soft, unformed stools throughout the 8-h period. The experimenters state that laxation caused by the antibiotics might have masked the beneficial effect indicated by the higher absorption rate found for D-tagatose.

Human laxation tests similar to those reported for other carbohydrate sweetener and bulking agents in Table 2 were performed with D-tagatose on a total of seven unadapted subjects. The mean laxative threshold value was 40 g/meal in single-meal tests, approximately one-third greater than that for palatinit used in the diet restriction calculation above. Although this value is not the highest shown for compounds in Table 2, D-tagatose, as seen in Table 3, is the only molecule other than L-glucose that provides no, or slightly negative, available energy. Whether humans would adapt to D-tagatose to overcome laxation, as do mice and rats, remains to be tested.

D-TAGATOSE'S POSSIBLE ROLE IN HELPING ACHIEVE DIET RESTRICTION

If naive or adapted tolerance permits the substitution of D-tagatose for conventional carbohydrates at 40 g/meal for three meals daily, available energy intake would decrease by 1980 kJ/d (three meals × 40 g × 16.5 kJ/g). Based on recommended daily energy intakes (24), the substitution would constitute 21.5% and 16.3% reductions in available energy for adult females and adult males, respectively. If laxation does not interfere or if it is accommodated, these considerable reductions could be accomplished with no sacrifice in bulk consumed. This would be more palatable than outright restriction of food intake. In ad libitum feeding studies in rats adapted to D-tagatose, the sugar did not engender an offsetting increased intake of food to compensate for the displaced energy, in contrast with control rats provided cellulose in their diets (Biospherics, unpublished report, 1989). Total diet intake did not differ significantly between the experimental and control rats. Studies should be undertaken to determine whether humans react similarly. To the extent that they do, the case for the use of D-tagatose in helping achieve diet restriction would be enhanced. Dietary goals for the United States call for 58% of daily energy intake to be in the form of carbohydrate (31).

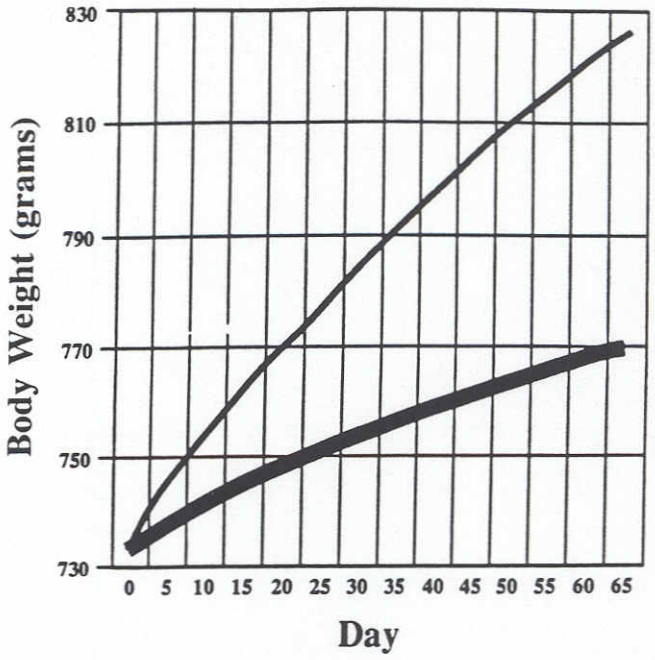

FIGURE 2. Body weight in groups of six Sprague-Dawley rats (23-wk-old males) fed diets containing sucrose (thin line) or D-tagatose (thick line). Rats were housed individually, acclimated for 7 d with standard rat feed (ad libitum), adapted for 14 d with 15% by wt D-tagatose in rat feed (ad libitum), and further adapted to each group's respective test sugar for 26 d. The group fed the sucrose diet received the group-average amount of diet consumed by the D-tagatose-fed group. The test period began when both groups achieved the same mean body weight.

Various mechanisms proposed by which diet restriction operates to extend life and reduce disease include the following: effects on neural and endocrine regulatory systems, lowering of plasma glucose, increasing rates of glucose utilization (but not increasing overall metabolic rates) per unit of metabolic mass, lowering plasma insulin concentrations, reducing production of reactive oxygen molecules, increasing concentrations of protective enzymes, decreasing lipid peroxidation, and increasing removal of such products (1). The overall effect is to produce "a change in metabolic characteristics that enables carbohydrate fuel and oxygen to be utilized in a less harmful fashion" (1). A review of mechanisms includes cell membrane composition and function, effects on the Na-K ATPase-dependent pump, effects on other ion active transport systems, and changes in phospholipid metabolism (32). Another paper reports that diet restriction affects most, if not all, cells in living organisms and alters the expression of many genes at the level of transcription (33).

TABLE 5
Soft stool observations during subchronic oral toxicity study in rats fed D-tagatose[1]

Group (percent by wt of D-tagatose)	Day in life						
	0	1	2	3	4	5	6–95[2]
Males							
0%	0	0	0	0	0	0	0
5%	0	0	0	0	0	0	0
10%	0	1	1	0	0	0	1
15%	0	17	17	18	0	0	1
20%	0	20	19	18	1	0	0
Females							
0%	0	0	0	0	0	0	0
5%	0	0	0	0	0	0	0
10%	0	3	4	2	0	0	0
15%	0	17	16	15	0	0	0
20%	0	20	20	20	0	0	5

$n = 20$.
[1] Each animal was housed individually and given ad libitum access to water and a commercial diet including indicated percentage of D-tagatose. No anogenital staining was observed in any of the groups. Studies performed by an industrial toxicology laboratory inspected by the Food and Drug Administration.
[2] Number of observations of soft stools out of possible 2020 observations for each dose group.

POTENTIAL ANTI-AGING EFFECTS OF D-TAGATOSE

Without specific consideration of diet restriction, studies of glycosylation of protein have identified it as one of the major factors responsible for aging, including aging in humans (34, 35). This cross-linking of protein molecules by glucose in both muscle and brain tissues accretes with time and progressively impairs physical and mental activity. When the reaction rates various monosaccharides were examined in vitro with human hemoglobin, a model human protein, the rate of reactivity of D-tagatose was shown to be only one-half that of D-glucose (35). D-Tagatose experiments in which blood chemistry was monitored showed that normal, fasting rats administered D-tagatose had consistently lower plasma glucose and insulin concentrations after dosing than did control fasting rats administered glucose instead (36). These findings indicate a dual role for D-tagatose as a nutraceutical (or pharmaceutical) for reducing glycosylation.

TABLE 6
Occurrence of D-tagatose in edible formulations[1]

Foods and food products
Sterilized cow milk (2–3000 mg/kg)
Hot cocoa (≈1000 mg/kg)
Powdered cow milk (100–1000 mg/kg)
Parmesan, gjetost, feta, cheddar, and Roquefort cheeses (10–100 mg/kg)
Ultra-high-temperature milk (≈5 mg/kg)
Yogurt (broad variation depending on bacterial cultures used)
Dairy products prepared using *Lactobacillus gayonii* or *Lactobacillus pentosus* (both bacteria have L-arabinose isomerase, which converts D-galactose to D-tagatose)
Cephulac,[2] an orally ingested medication (120–244 g/d) for treatment of portalsystemic encephalopathy, contains ≈0.7% by wt D-tagatose; widely used in United States over past 11 y
Chronulac,[2] an orally ingested laxative (20–82 g/d), contains ≈0.7% by wt D-tagatose; widely used in United States over past 17 y
Generic products simulating Cephulac and Chronulac; in wide use
Other natural sources of D-tagatose
Common intracellular, metabolic intermediate in bacteria that utilize lactose (eg, various *lactobacilli* and dairy *streptococci*); occurs as D-tagatose-6-phosphate and D-tagatose-1,6-diphosphate
Sterculia setigera (tropical date tree) gum exudate component; ≤30% by wt of sugar fraction
Roccella hypomecha, *Roccella linearis*, and *Roccella fucoformis* (lichens); ≈3% by wt of carbohydrate fraction

[1] Information compiled by Biospherics Incorporated, Beltsville, MD.
[2] Marion Merrell Dow, Inc, Kansas City, MO.

SUMMARY AND STATUS OF D-TAGATOSE DEVELOPMENT

The attributes of D-tagatose may allow it to provide a full-bulk sweetener with zero available energy and sweetness and other important characteristics similar to those of sucrose. Thus, D-tagatose may play a significant dual role in helping people realize the health and longevity benefits of diet restriction, and also in deterring the specific aging effects of glycosylation.

An economic manufacturing process for D-tagatose has been developed starting with whey or lactose, both readily available materials, and using no chemicals other than lime (37, 38). Although D-tagatose occurs naturally, the amounts are too small for economic recovery. However, we are gathering information about the natural occurrence and human consumption of D-tagatose. These data are summarized in **Table 6**. So far, we have identified products that may be providing daily amounts of several tenths of a gram of D-tagatose to large populations for which no adverse effects have been reported. Additional analyses of foodstuffs are being conducted to look for consumptions that might be enough to qualify D-tagatose as a generally recognized as safe (GRAS) substance under FDA regulations.

We hope the use of D-tagatose as a limited substitute for sucrose will prove feasible. Chocolates and chewing gum, both of which were successfully formulated by substituting D-tagatose for the $> 50\%$ sucrose in each, are planned as initial products along with the use of D-tagatose as a tabletop sweetener for coffee and tea and in single-use packets for those

beverages. Early introduction is planned in countries where regulations permit. Baked goods would follow. Meanwhile, safety tests gathering additional data for the more stringent US FDA submittal for food additive approval, or for qualification as GRAS, continue.

REFERENCES

1. Masoro EJ. Aging and proliferative homeostasis: modulation by food restriction in rodents. Lab Anim Sci 1992;42:132–7.
2. Masoro EJ. Dietary restriction and aging. J Am Geriatr Soc 1993;41: 994–9.
3. Kubo C, Johnson BC, Day NK, Good RA. Effects of calorie restriction on immunologic functions and development of autoimmune disease in NZB mice. Proc Soc Exp Biol Med 1992;201:192–9.
4. Kari FW, Abdo KM. Sensitivity of the NTP bioassay for carcinogen hazard evaluation can be modulated by dietary restriction. In: Hart RW, Newmann DA, Robertson RT, eds. Dietary restriction: implications for the design and interpretation of toxicity and carcinogenicity studies. Washington, DC: ILSI Press, 1995:63–78.
5. Keenan K, Soper KA. The effects of ad libitum overfeeding and moderate dietary restriction on Sprague-Dawley (SD) rat survival, carcinogenesis and chronic disease, and the toxicological response to pharmaceuticals. In: Hart RW, Newmann DA, Robertson RT, eds. Dietary restriction: implications for the design and interpretation of toxicity and carcinogenicity studies. Washington, DC: ILSI Press, 1995:99–126.
6. Kritchevsky D. Fat, calories and cancer. In: Hart RW, Newmann DA, Robertson RT, eds. Dietary restriction: implications for the design and interpretation of toxicity and carcinogenicity studies. Washington, DC: ILSI Press, 1996:155–66.
7. Masoro EJ. Dietary restriction and aging. J Am Geriatr Soc 1993;41: 994–9.
8. Wolf NS, Pendergrass WR. In vivo cell replication rates and in vitro replication are modulated by calorie intake. In: Hart RW, Newmann DA, Robertson RT, eds. Dietary restriction: implications for the design and interpretation of toxicity and carcinogenicity studies. Washington, DC: ILSI Press, 1995:299–310.
9. Makinodan T, James SJ. Possible roles of apoptosis in immune enhancement and disease retardation with dietary restriction and/or very low doses of ionizing radiation. In: Hart RW, Newmann DA, Robertson RT, eds. Dietary restriction: implications for the design and interpretation of toxicity and carcinogenicity studies. Washington, DC: ILSI Press, 1995:311–26.
10. Murasko DM, Blank KJ. Effect of age and caloric restriction on endogenous and exogenous retrovirus expression. In: Hart RW, Newmann DA, Robertson RT, eds. Dietary restriction: implications for the design and interpretation of toxicity and carcinogenicity studies. Washington, DC: ILSI Press, 1995:327–40.
11. Pieri C. Food restriction slows down age-related changes in cell membrane parameters. In: Pierpaoli W, Fabris N, eds. Physiological senescence and its postponement. Ann N Y Acad Sci 1991;621:353–62.
12. Holehan A, Merry B. The experimental manipulation of aging by diet. Biol Rev 1986;61:329–68.
13. Beil L. Scientists seek to understand why underfed animals live significantly longer. Science News 1988;134:142–3.
14. Lane MA, Ingram OK, Cutler RG, Knapka JJ, Barnard DE, Roth GS. Dietary restriction in non-human primates: progress report on the NIA study. In: Fabris N, Harman D, Knook D, Steinhagen-Thiessen E, Zs-Nagy I, eds. Physiopathological processes of aging towards a multicausal interpretation. Ann N Y Acad Sci 1992;673:36–45.
15. Joint FAO/WHO Expert Committee on Food Additives. Evaluation of certain food additives and contaminants. Geneva: World Health Organization, 1992. (Report no. 683.)
16. Ellis FW, Krantz JD Jr. Sugar alcohols: XXII, metabolism and toxicity studies with mannitol and sorbitol in man and animals. J Biol Chem 1941;141:147–54.
17. Olinger P. Xylitol technical properties. Schaumburg, IL: American Xyrofin Inc, 1992.
18. Joint FAO/WHO Expert Committee on Food Additives. Evaluation of certain food additives and contaminants. Geneva: World Health Organization, 1986. (Report no. 733.)
19. Joint FAO/WHO Expert Committee on Food Additives. Evaluation of certain food additives and contaminants. Geneva: World Health Organization, 1983. (Report no. 696.)
20. Joint FAO/WHO Expert Committee on Food Additives. Evaluation of certain food additives and contaminants. Geneva: World Health
21. Joint FAO/WHO Expert Committee on Food Additives. Evaluation of certain food additives and contaminants. Geneva: World Health Organization, 1989. (Report no. 24.)
22. Joint FAO/WHO Expert Committee on Food Additives. Evaluation of certain food additives and contaminants. Geneva: World Health Organization, 1993. (Report no. 32.)
23. Hiele M, Ghoos Y, Rutgeerts P, Vantrappen G. Metabolism of erythritol in humans—comparison with glucose and lactitol. Br J Nutr 1993;69:169–76.
24. National Research Council. Recommended dietary allowances. 10th ed. Washington, DC: National Academy Press, 1989.
25. Levin G. L-Sugars: Lev-O-Cal. In: Nabors L, Gelardi R, eds. Alternative sweeteners. New York: Marcel Dekker, 1986:155–64.
26. Levin G, Zehner L. L-Sugars: Lev-O-Cal. In: Nabors L, Gelardi R, eds. Alternative sweeteners. 2nd ed. New York: Marcel Dekker, 1991:117–25.
27. Livesey G. The energy values of unavailable carbohydrates and diets: an inquiry and analysis. Am J Clin Nutr 1990;51:613–37.
28. Livesey G. Procedures for calculating the digestible and metabolizable energy values of food components making a small contribution to dietary intake. J Sci Food Agric 1989;48:475–81.
29. Levin G, inventor; Biospherics Inc, assingee. Sweetened edible formulations. US patent Re. 33,719. 1991.
30. Zehner L, inventor; Biospherics Inc, assignee. D-Tagatose as a low-calorie carbohydrate sweetener and bulking agent. US patent 4,786,722. 1988.
31. Senate Select Committee on Nutrition and Human Needs, US Senate. Dietary goals for the United States. 2nd ed. Washington, DC: US Government Printing Office, 1977.
32. Marigliano V, Bauco C, Campana F, et al. Normal values in extreme old age. In: Fabris N, Harman D, Knook D, Steinhagen-Thiessen E, Zs-Nagy I, eds. Physiopathological processes of aging towards a multicausal interpretation. Ann N Y Acad Sci 1992;673:23–8.
33. Heydari RH, Gutsmann A, You S, Takahashi R, Richardson A. Effect of caloric restriction on the genome function of cells: alterations in the transcriptional apparatus of cells. In: Hart RW, Newmann DA, Robertson RT, eds. Dietary restriction: implications for the design and interpretation of toxicity and carcinogenicity studies. Washington, DC: ILSI Press (in press).
34. Cerami A, Vlassara C, Brownlee M. Glucose and aging. Sci Am 1982;256:90.
35. Bunn H, Higgins P. Reactions of monosaccharides with proteins: possible evolutionary significance. Science 1981;213:222.
36. Zehner L, Levin G, Saunders J, Beadle J, inventors; Biospherics Inc, assignee. D-Tagatose as anti-hyperglycemic agent. US patent 5,356,879. 1994 October 18.
37. Beadle JR, Saunders JP, Wajda TJ, inventors; Biospherics Inc, assignee. Process for manufacturing D-tagatose. US patent 5,002,612. 1991 March 26.
38. Beadle JR, Saunders JP, Wajda TJ, inventors; Biospherics Inc, assignee. Process for manufacturing D-tagatose. US patent 5,078,796. 1992 January 7.