

Tagatose, a new antidiabetic and obesity control drug

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A potentially important new drug for treating type 2 diabetes, tagatose, is now in phase 3 clinical trial. The history, development, additional health benefits, mechanisms of action and the potential for the drug are presented in context with a review of the rapidly growing epidemic of type 2 diabetes and treatments for it. An epimer of fructose, the natural hexose tagatose was originally developed by Spherix Incorporated (formerly Biospherics Inc.) as a low-calorie sugar substitute. Only 20% of orally ingested tagatose is fully metabolized, principally in the liver, following a metabolic pathway identical to that of fructose. Following a decade of studies, tagatose became generally recognized as safe for use in foods and beverages under US FDA regulation. The simple sugar is commercially produced by isomerization of galactose, which is prepared from lactose. Early human studies suggested tagatose as a potential antidiabetic drug through its beneficial effects on postprandial hyperglycaemia and hyperinsulinaemia. A subsequent 14-month trial confirmed its potential for treating type 2 diabetes, and tagatose showed promise for inducing weight loss and raising high-density lipoprotein cholesterol, both important to the control of diabetes and constituting benefits independent of the disease. Furthermore, tagatose was shown to be an antioxidant and a prebiotic, both properties cited in the maintenance and promotion of health. No current therapies for type 2 diabetes provide these multiple health benefits. The predominant side effects of tagatose are gastrointestinal disturbances associated with excessive consumption, generally accommodated within 1- to 2-week period. The health and use potentials for tagatose (branded Naturlose® for this use) are given with respect to current type 2 diabetes drugs and markets. Under an FDA-affirmed protocol, Spherix is currently conducting a phase 3 trial to evaluate a placebo-subtracted treatment effect based on a decrease in HbA_{1c} levels. Side effects, contraindications and possibly beneficial new findings will be carefully monitored. It is hoped that early results of the trial may become available by mid-2008. If a subsequent NDA is successful, tagatose may fill a major health need.

Keywords: antidiabetic drugs, diabetes, HbA_{1c}, obesity

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Introduction

Initially researched and developed as a full-bulk, low-calorie sweetener, the rare, but naturally occurring hexose, tagatose, was found to have an antidiabetic property in an early animal feeding study [1]. This finding was followed up with human studies that confirmed its promise as a potential treatment for type 2 diabetes and,

also, obesity [2–4]. An economic method for manufacturing tagatose from lactose was developed [5,6]. Tagatose as a possible treatment for type 2 diabetes and other health problems has begun to receive attention as demonstrated in a recent paper [7]. The present paper brings current status of tagatose, branded 'Naturlose®', for medical and health applications. The pharmacokinetics and a plausible mechanism of action

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against type 2 diabetes are presented, along with a comparison to other drugs treating type 2 diabetes.

Background

Tagatose, or more precisely, D-tagatose is an epimer of D-fructose. It is a highly soluble white crystal or powder, and may be produced with a physical bulk similar to ordinary table sugar, and is 90% as sweet. Tagatose occurs naturally in small amounts in dairy products [8,9]. FDA-approved as having only 1.5 kcal/g compared with table sugar's 4 kcal/g (1 kcal = 4.187 kJ) [10], tagatose was originally developed as a sugar substitute for calorie and weight control. In the US, based on over 10 years of animal, human and other relevant use and safety data, tagatose qualified as generally recognized as safe (GRAS) for use in foods under the FDA-regulated program [11], and has since developed a long history of use in food and beverage products with no reported incident of toxic events. An estimated intake of 6.6 g/person/day at the mean, and 14.9 g/person/day at the 90th percentile is currently permitted by the FDA. On 23 July 2003, the Korean Food & Drug Administration authorized the use of tagatose in foods [12]. In 18 February 2004, Food Standards Australia New Zealand issued a favourable final assessment report permitting the use of tagatose as a novel food ingredient [13]. The joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated tagatose at its 55th, 57th, 61st and 63rd meetings. At the 63rd meeting in June 2004, JECFA stated that there is no need to limit the allowed daily intake (ADI) of tagatose. JECFA has, therefore, established an ADI of 'not specified,' the safest category in which JECFA can place a food ingredient [14]. On 14 December 2005, tagatose was formally approved as a 'novel food ingredient' in the European Union (EU) without any restriction on usages [15].

The pathogenesis of type 2 diabetes is generally characterized by two principal abnormalities: peripheral insulin resistance, which alone rarely results in clinical diabetes, and progressive failure of pancreatic β -cell function that leads to inadequate insulin secretion [16]. The insufficient production of insulin and/or insulin resistance causes hyperglycaemia that is the principal cause of diabetes complications or sequelae including retinopathy, neuropathy, nephropathy and arteriosclerosis. Although the cause of diabetes remains elusive, both genetics and environmental factors, such as excessive caloric intake leading to obesity and lack of exercise, promote the disease.

Because we observed tagatose to produce exceptionally low glycaemic and insulin responses, only 3% of that ascribed to glucose [17], we early proposed it as an ideal

sugar substitute in foods for those with diabetes. The development of tagatose as an antidiabetic drug began with the discovery that animals on a tagatose diet showed alleviation of diabetic symptoms including polydipsia in SHR/N-cp rats [1]. Short-term clinical trials run at the University of Maryland School of Medicine, jointly funded by Spherix Incorporated (formerly Biospherics Inc.) and the Maryland Industrial Partnerships Program, showed that the pre-administration of tagatose blunts the rise in blood glucose and insulin otherwise observed after glucose or sucrose loading in both healthy and diabetic subjects [2,3]. The inhibition of postprandial glucose increase by tagatose was seen even when tagatose was administered 4 h and 15 min before lunch in healthy subjects [18]. This blunting effect was also seen in subjects with mild fasting hyperglycaemia (110–140 mg/dl), when tagatose was administered with glucose [19]. Further studies showed that the daily intake of tagatose by type 2 diabetic patients results in a decline in glycosylated haemoglobin (GlyHb) in both short-term and long-term trials [2,4].

These several studies along with the safety data of tagatose were submitted by Spherix Inc. to the US FDA with a request for the activation of a phase 3 clinical trial. The FDA accepted the studies as having satisfied the requirements for phases 1 and 2, and authorized Spherix to undertake a phase 3 clinical trial, which is currently underway through Anaclim LLC contracted by Spherix in the USA and Australia.

Prevalence and Trends of Type 2 Diabetes and Obesity

According to the American Diabetes Association (www.diabetes.org), nearly 9 out of 10 people with newly diagnosed type 2 diabetes are overweight. In 2005, among the total US adult population surveyed, 60.5% were overweight, 23.9% were obese and 3.0% were extremely obese [20]. Thus, the causal connection between obesity and type 2 diabetes has been nicknamed 'Diabesity' [21]. Currently, there are 20.8 million children and adults in the US, or 7% of the population, who have diabetes, and an additional 54 million are at risk. The World Health Organization (www.who.int) estimated that at least 171 million people worldwide have diabetes. This figure is likely to double by 2030 to reach 366 million. Type 2 diabetes comprises 90% of the people with diabetes around the world. Diabetes is associated with an increased risk for a number of serious, debilitating complications, sometimes life-threatening and resulting in death. In the US, the total annual cost of diabetes in 2002 was estimated to be \$132 billion ([110 | Diabetes, Obesity and Metabolism, 10, 2008, 109–134](http://www.</p></div><div data-bbox=)

diabetes.org). The global diabetes drug market, including both oral antidiabetic agents (OAAs) and insulin products, was valued at \$15 billion in 2005. Oral antidiabetics were the leading category of drugs, constituting \$8.19 billion, and showed a growth rate of 6.3% from the total global sales in 2004 [22].

The current approach to the treatment of type 2 diabetes is generally stepwise and systematic. Early treatment may consist of diet management, exercise and weight control. As glucose control deteriorates, pharmacological therapy is initiated with one or two OAAs. Various classes of OAAs are now available that target different pathophysiological factors contributing to diabetes, including defects in muscle, liver, adipose tissue and pancreas. α -Glucosidase inhibitors delay intestinal carbohydrate absorption, biguanides increase central and peripheral insulin sensitivity and decrease hepatic glucose production, insulin secretagogues or sulphonylureas increase pancreatic insulin secretion, insulin sensitizers or thiazolidinediones target adipocyte (to inhibit free fatty acid release) and muscle insulin resistance, and intestinal lipase inhibitors (orlistat) inhibit fat absorption and promote weight loss in obese patients. Recently, sitagliptin phosphate (Januvia™) was approved by the FDA and is the first of a new class of diabetes medications called dipeptidyl peptidase-4 inhibitors, and the sixth class of oral medications now available to treat diabetes [23].

β -Cell failure is progressive and relentless despite therapy with insulin, sulphonylurea or metformin agents [24,25]. No currently available therapy has been shown to slow the decline in β -cell function in established type 2 diabetic patients. Fortunately, insulin resistance does not appear to progress in parallel with β -cell failure [26]. Thus, many, if not most, individuals with type 2 diabetes ultimately require insulin as primary therapy with OAA adjunctive therapy often necessary to achieve targeted glycaemic goals [27]. However, glucose control in type 2 diabetics remains unsatisfactory because average GlyHb A_{1c} (HbA_{1c}) values well above 8% (4.5 to 6.5% being the normal range) are reported in many epidemiologic studies [28]. An analysis of The National Health and Nutrition Examination Survey data show that the percentage of US patients with controlled diabetes (HbA_{1c} < 7%) did not improve from 1988–1994 (44.3%) to 1999–2000 (37%, $p = 0.11$) [29]. Poor diabetes control persisted during this interval despite compelling new evidence that long-term glycaemic control reduces microvascular and macrovascular complications [30–32]. HbA_{1c} is a function of both fasting and postprandial hyperglycaemia. After examining diurnal glycaemic patterns in type 2 diabetic patients, stratified into quintiles by the quality of their glycaemic control (HbA_{1c}

< 7.3%), (7.3% ≤ HbA_{1c} = 8.4%), (8.5% ≤ HbA_{1c} = 9.2%), (9.3% ≤ HbA_{1c} = 10.2%) and (HbA_{1c} > 10.2%), Monnier *et al.* [33] found that the relative contribution of postprandial glucose to the total diurnal hyperglycaemia decreased progressively from the lowest quintile (69.7% in the patients with HbA_{1c} < 7.3%) to the highest quintile of HbA_{1c} (30.5% in patients with HbA_{1c} > 10.2%). However, the relative contribution of fasting glucose to the total diurnal hyperglycaemia increased gradually with increasing levels of HbA_{1c} (30.3% in the lowest quintile vs. 69.5% in the highest of HbA_{1c}). In addition to improving glucose control, therapy targeting postprandial hyperglycaemia has also been shown to reduce the progression of atherosclerosis and cardiovascular events [34]. A meta-analysis of seven randomized, double-blind, placebo-controlled long-term (with a minimum treatment duration of 52 weeks) studies in type 2 diabetic patients revealed a 35% reduction in the development of any cardiovascular events, and a 64% reduction in relative risk of myocardial infarction for patients on acarbose treatment compared with those on placebo [35]. To date, acarbose (Precose®) and miglitol (Glyset®), both belonging to a class of drugs called α -glucosidase inhibitors, remain the two US-approved agents specifically designed to reduce postprandial hyperglycaemia. Another such agent, voglibose (Basen®), is not approved for use in the US. Other once promising agents, discussed in the Multiple Benefits section, have been withdrawn or have been required to carry 'black box' warning labels. In the face of the growing worldwide epidemic of type 2 diabetes, it is imperative that new agents be developed to achieve postprandial glycaemic control. Tagatose may fill that need and offer significant additional benefits.

Tagatose regulates blood glucose through inhibiting the postprandial glucose rise. Because its postprandial glucose effect is independent of insulin secretion, tagatose should also be useful in treating type 1 diabetes by lowering insulin requirements. Tests remain to be made. In addition to the use of tagatose as a monotherapy for patients who are under diet control, or for patients with pre-diabetes, initial studies [2,4] also showed tagatose to be a potential adjunct therapy to other oral drugs, like sulphonylureas and metformin. However, one study found that the acute effect of tagatose as an adjunct therapy to insulin or sulphonylurea in attenuating postprandial glucose and insulin rise is insignificant [36].

Compared with many therapies that have side effects, such as weight gain, hypoglycaemia and oedema, and therefore have restrictions imposed on use [37], tagatose is safe to humans for oral ingestion. Although tagatose is not expected to arrest or reverse declining β -cell

function, it produces no weight gain or hypoglycaemia. It helps in weight control and may reduce cardiovascular risks associated with type 2 diabetes by increasing high-density lipoprotein cholesterol (HDL) levels [4]. In addition, it is prebiotic [38,39], which is beneficial to human health. Tagatose can be taken without the worry of serious side effects. The only questionable drawback to tagatose, compared with other OAAs, is its relatively large dose. Tagatose might be administered in doses up to as much as 15 g tid (although lesser doses are being investigated), much larger than a pill of regular OAA. However, this issue can be resolved by putting the tagatose dose on cereal, in juice, other foods, mints or candy bars. The sweet sucrose-like taste of tagatose will enhance the flavour.

Manufacture of Tagatose

Tagatose is an epimer of fructose (inverted at C-4) widely used in foods and beverages as high-fructose corn syrup (HFCS), or crystalline fructose. Both fructose and tagatose are ketoses that can be produced from their corresponding hexoses, glucose and galactose, by isomerization, either chemically or biologically. Current commercial production of fructose adopts immobilized glucose isomerase (more precisely, xylose isomerase) catalyzing the reversible isomerization of glucose to fructose. Similarly, studies revealed the potential of using L-arabinose isomerase to catalyze the conversion of galactose to tagatose [40–43]. However, before the commercial process for the production of tagatose using L-arabinose isomerase become economically feasible, there are many technical issues to be resolved, such as enzyme yield, activity, immobilization and shelf life. Thus, the current production process of tagatose is based on the chemical isomerization of galactose developed by Spherix Incorporated [5,6]. Galactose is isomerized to tagatose under alkaline conditions using a hydroxide, preferably calcium hydroxide, as a complexing agent. Calcium hydroxide shifts the isomerization equilibrium between galactose and tagatose in the direction of tagatose because it forms an insoluble complex with tagatose at elevated pH. Treatment of the suspension with acid, preferably carbon dioxide, liberates tagatose by neutralizing the mixture and precipitating calcium as calcium carbonate. The tagatose is further purified, crystallized from water and dried. The raw material, galactose, is prepared by the hydrolysis of lactose using immobilized lactase as a biocatalyst, yielding galactose and also glucose as an economic by-product. Lactose is prepared from whey, a by-product of the cheese manufacturing industry.

Pharmacokinetics of Tagatose

Feeding studies in pigs and rats showed that 25% of the amount of tagatose ingested is absorbed into the bloodstream passively [12,13,44–47]. In humans, the absorption of tagatose cannot be measured directly. Indirect evidence for the incomplete absorption is provided by gastrointestinal side effects [48–50], and by an increased H₂ expiration after the ingestion of tagatose [51]. In an ileostomy study, a median of 19% ingested tagatose (15 g) was recovered from the 24-h ileal effluent, suggesting an 81% intestinal absorption [52]. However, similar high absorption rates were suggested early also for sorbitol, maltitol and isomalt from studies in ileostomates, although these polyols are poorly digested and absorbed [53]. Several factors may explain the excessive absorption rates in ileostomic patients, such as fermentation of the test compounds in the small intestine, incomplete analytical recovery of the test compounds from the ileal effluent and altered permeability of the intestinal mucosa.

Approximately 20% of the systemically absorbed tagatose (or 5% of that ingested) is excreted in urine based on results from several studies. These include a rat metabolic study with ¹⁴C-tagatose that showed excretion of 4.4% of the ingested tagatose [46], and two metabolic studies in pigs that showed excretion values from 4.7 to 5.3% of the ingested tagatose [12,13,45]. Although, human studies have shown substantial inter-individual variation in urinary excretion (0.7 to 5.3% ingested) [18,51], the maximum percentage of urinary excretion of tagatose is consistent with results seen in the animal studies. Of the 75% of ingested tagatose that is not absorbed into the bloodstream, almost all is fermented by intestinal microorganisms yielding short-chain fatty acids (SCFAs). This is supported by the finding that no tagatose was found in the faeces of pigs [12,13,44,45,47], and only a small amount (1.8% of ingested tagatose) was recovered from the faeces of tagatose-adapted rats [46]. The above studies and results are summarized in table 1. It should be noted that tagatose's 1.5 kcal/g (1 kcal = 4.187 kJ) caloric value is calculated upon the assumption of 100% absorption and energy utilization of SCFAs produced by the fermentation of tagatose in the large intestines. This is likely an overestimate, and actual caloric value may be less than 1.5 kcal/g.

Systemically absorbed tagatose, 20% of that ingested, is metabolized to CO₂ in a manner similar to that of fructose. The liver appears to be the primary site of tissue uptake, with little tagatose reaching the systemic

Table 1 Evidence of intestinal absorption, urinary and faecal excretion upon ingestion of tagatose

Study	Subjects	Administration	Results
Saunders et al. [46]	17 conventional rats (CV) and 2 germ-free rats (GF) with (adapted) or without (unadapted) prior exposure to tagatose	A single oral dose of ¹⁴ C-tagatose to 3 CV and 2 GF unadapted rats and to 4 CV rats adapted to tagatose at 100 g/kg of diet for 28 days. A single intravenous dose of ¹⁴ C-tagatose to 2 unadapted CV rats. A single oral dose of ¹⁴ C-tagatose to 8 unadapted CV rats sacrificed at various time intervals to obtain blood and cecum contents	(1) Absorption of tagatose in small intestine was about 20.3%; (2) Urine and faecal excretion of tagatose was 4.4% and 1.8%, respectively, of that ingested in rats adapted to tagatose; (3) Microbial adaptation to tagatose in the gut increased fermentation of tagatose evidenced by the 25.7% of faecal excretion of tagatose in unadapted rats compared with 1.8% of that in adapted rats; 4) Absorbed tagatose was quickly metabolized to CO ₂
Lærke and Jensen [47]; Johansen and Jensen [44]	16 pigs (62–75 days old castrated pigs)	8 pigs were fed a diet containing 15% sucrose (control diet) and the other 8 pigs were fed a diet containing 5% sucrose and 10% tagatose. Each diet was given over 18 days Diet 1 contained 20% sucrose, Diet 2 contained 10% sucrose and 10% tagatose and Diet 3 contained 20% tagatose. Two pigs were fed one of the diets for 2 weeks and then switched to another diet Pigs were fed a diet containing 20% sucrose (control) and were switched to a second diet containing 20% tagatose A controlled diet was served during two 2-day periods. In one of the periods, 15 g tagatose was added to the diet daily	(1) Absorption of tagatose in small intestine was 25.8%; (2) Tagatose was not seen in faeces suggesting complete fermentation of tagatose in the large intestine (1) Approximately 5% of ingested tagatose were excreted in urine; (2) Only negligible amount of tagatose were found in faeces of pigs fed 20% tagatose and none was found in pigs fed 10% tagatose (1) 26.3 to 27.6% of ingested tagatose was absorbed; (2) urine excretion of tagatose was 4.7 to 5.3% of that ingested The apparent absorption of 15 g tagatose/day was 81%. Similar high-absorption rates were suggested early also for sorbitol, maltitol and isomalt from studies in ileostomates, although these polyols are poorly digested and absorbed in normal subjects [53]
Jørgensen and Lærke, 1998*	6 pigs with an average weight of 34 ± 4 kg. Two pigs per diet		
Jensen and Laue, 1998*	Five castrated male pigs fitted with permanent cannulas in the portal vein, a mesenteric vein and a mesenteric artery		
Normén et al. [52]	Six people (2♂, 4♀) with well-functioning ileostomies with <10 cm of terminal ileum removed		

(Continued)

Table 1. (Continued)

Study	Subjects	Administration	Results
Buermann et al. [51]	8 healthy people (3♂, 5♀)	Eight people in a respiration chamber consumed a piece of cake containing 30 g tagatose or 30 g sucrose per day. 24-h energy expenditure was measured	(1) Urinary excretion of tagatose was in the range of 0.7 to 5.3% of ingested; (2) A significant increase of about 35% in H ₂ production with tagatose compared with sucrose indicated that a substantial part of the consumed tagatose escapes absorption and is fermented in the large intestine Urinary excretion of tagatose was 1.45% of ingested
Buermann et al. [18]	8 healthy people (8♂)	A single oral dose of 30 g tagatose or 30 g fructose dissolved in 400 ml water or plain water	

*Jørgensen H, Lærke HN. The influence of D-tagatose on digestibility and energy metabolism in pigs; and Jensen BB, Laue A. D-tagatose, absorption of tagatose and fermentation products of tagatose from the gastrointestinal tract of pigs. These are two unpublished reports by Danish Institute of Agricultural Sciences in 1998, which were submitted to and reviewed by the US FDA [45]. EU Novel Foods Regulation [12] and Food Standards Australia New Zealand [13].

circulation. When 30 g tagatose were administered to eight healthy subjects, serum concentrations peaked after 50 min, with a range of 0.05 to 0.28 mmol/l. No tagatose could be detected in the serum of any subject after 420 min [18]. In a glucose tolerance test, plasma tagatose levels were measured in four normal subjects who took 75 g of tagatose 30 min before another dose of 75 g glucose (both doses given orally). Tagatose reached peak values at 90 min, with mean levels of 3.6 mg/dl or 0.2 mmol/l and did not exceed 5 mg/dl in any subject [3].

Toxicity Studies

The toxicity of tagatose was examined in standard *in vitro* and *in vivo* toxicity tests. Tests for bacterial gene mutation, chromosomal aberration, micronucleus formation and TK-locus mutation gave uniformly negative results, demonstrating that tagatose is not genotoxic [54].

A 90-day sub-chronic toxicity study was conducted on male and female (20/sex/group) of Crl:CDBR rats at dietary doses of 0, 5, 10, 15 and 20% tagatose in Purina Certified Lab Chow [55]. There were no treatment-related effects at 5% tagatose in the diet. At higher doses, treatment-related effects included transient soft stools in male and female animals from the 15 and 20% dose groups during the first few days of the study, which were attributed to the incomplete absorption of tagatose but are not considered toxic effects. Body weights were about 12% below controls in the 20% dose group at the end of the study. Serum cholesterol was increased in the 15 and 20% dose groups. Liver enzyme levels (ALT, AST, GGT and ALP) were not increased in response to the tagatose treatments. No toxicological relevance was attributed to a slight, yet statistically significant, reduction of haemoglobin and hematocrit in males and females of the 15 and 20% dose groups (A separate *in vitro* study demonstrated that tagatose, unlike L-sorbose, does not cause haemolysis of dog erythrocytes, but rather has a stabilizing effect on these cells [56]). Statistically significant, dose-related reversible liver enlargement was noted in male and female animals in the 10, 15 and 20% dose groups compared with the dietary control. No gross pathological findings correlated with these increased liver weights. Minimal hepatocellular hypertrophy was observed in male and female animals in the 15 and 20% dose groups. An independent review of the liver slides concluded that histomorphologic changes associated with tagatose were restricted to hepatocyte hypertrophy and hepatocyte glycogen accumulation. The reversible liver enlargement, characterized

by increased glycogen deposition, was not considered as a toxic effect [57]. It was concluded that increased liver weights and minimal hypertrophy were the result of adaptation to the high dietary levels (greater than 5% in the diet) of tagatose, and that, at 5% in the diet, tagatose produced no treatment-related increase in liver weight.

The biochemical and morphological characteristics of tagatose-induced liver enlargement were examined in a 4-week study with male 10 to 11-week-old Crl:CDBR rats [58]. Groups of 20 rats received a diet containing 0, 5, 10 and 20% tagatose. The animals were killed in the non-fasted condition. Liver weights were significantly increased in linear relation to the tagatose intake. Except for an increased glycogen accumulation, no ultrastructural changes were seen on electron microscopic examination of livers of the control and treatment groups. Acyl-CoA oxidase and CYP4A1 activity were increased but the magnitude of this increase was small and not accompanied by electron microscopic evidence of peroxisome proliferation. The Ki-67 index (DNA synthesis) did not differ between the groups, but a dose-related decrease of the number of nuclei per unit area signified some hepatocellular hypertrophy or swelling, probably caused by the increased glycogen deposition. It was concluded that the liver enlargement seen in response to the consumption of tagatose is a physiological response to the treatment-induced increased glycogen deposition. No hepatocellular growth was seen at the 5% dietary level of tagatose (corresponding to an intake of 2.6–2.8 g/kg bw), suggesting that the increase of liver glycogen at this dose remained within normal limits.

Embryotoxicity and teratogenicity studies were conducted in Crl : CDBR rats with administration of tagatose by gavage at levels of 4, 12 and 20 g/kg bw/day from day 6–15 of gestation [59]. There were no signs of maternal toxicity, embryotoxicity or teratogenicity. Reproductive performance was not affected by the treatment. Relative liver weights were increased in the mid and high dose groups, which was not considered toxicologically significant because of the lack of any corresponding histopathology. No morphological changes were seen on microscopic examination of the livers.

Early Indications of Efficacy

In a 6-month study [1], nine obese diabetic rats (SHR/N-cp) were fed a diet consisting of 24% fructose, 10% glucose, 10% starch, 16% fat, 10% lactalbumin, 10% casein, 5.9% cellulose, 3.1% AIN salt mix and 1.0% vitamins. To this diet, 10% fructose or 10% tagatose

was added. The tagatose diet resulted in normal water intake, while rats given the fructose diet had marked polydipsia during the first 3 months. In a second part of this study, groups of five lean and five obese diabetic rats were fed the diet with fructose for 2 weeks, the diet with tagatose for 2 weeks and then the sequence was repeated. Tagatose reduced food efficiency (weight gain per food intake) in the lean rats but increased that in the obese, diabetic rats, which also had reduced polydipsia and urinary glucose. However, during the second rotation, food efficiency was reduced even in the diabetic obese rats, and urinary glucose was normal. No pathological changes were observed in any groups. The obese diabetic rats given tagatose had an increased calcium concentration in the kidney, probably, reflecting improved calcium retention resulting from the absence of polydipsia.

Human studies showed that oral administration with tagatose alone causes low glycaemic and insulin responses in both normal subjects and patients with type 2 diabetes. Studies performed at the University of Maryland showed that oral intake of 75 g of tagatose produced no increase in blood glucose or insulin in eight healthy subjects or in eight people suffering from type 2 diabetes [2,3]. The above findings were confirmed in a study with eight healthy volunteers at the Research Department of Human Nutrition, Copenhagen, where oral intake of 30 g of tagatose did not lead to changes in blood glucose or insulin [18]. A study with 12 healthy subjects conducted at Sydney University showed that tagatose, when a single dose of 50 g was taken orally, caused an exceptional low glycaemic and insulin responses of 3% compared with that of glucose [17]. A similar study conducted in Japan with 12 subjects with mild hyperglycaemia (i.e. fasting glucose of 110–140 mg/dl) showed that the oral administration of 7.5 g tagatose led to no increase in blood glucose [19].

Several studies suggested that tagatose helps in postprandial glycaemic control in type 2 diabetic patients, and shifts or controls blood sugar, GlyHb and body weight to healthier levels. In eight healthy subjects and in eight type 2 diabetic patients, tagatose (75 g) ingested 30 min prior to an oral load of glucose (75 g) blunted the rise in blood glucose and insulin otherwise observed after glucose loading [2,3]. The study in five type 2 diabetic patients demonstrated that the increase of blood glucose after the ingestion of sucrose (75 g) was also reduced by tagatose (75 g), which was ingested 30 min prior to the sucrose [3]. Moreover, an oral glucose tolerance test (OGTT) in 10 type 2 diabetic patients with an oral administration of 10, 15, 20 or 30 g of tagatose 30 min preceding 75 g doses of glucose showed that the

glucose-attenuating effects of tagatose were dose-related. The lowering effect on blood glucose was significant at even the lowest dose of 10 g of tagatose [3]. Unaccountably, pre-treatment with 15 g tagatose was less effective than 10 g tagatose in attenuating postprandial glycaemia [3]. Among those 10 patients, 8 of them were under diet control and the remaining two patients were under treatment with sulphonylurea. These patients took their usual morning dose of this medication 1 h before the OGTT. The inversion in dose response of the 10 g tagatose dose over that of 15 g might have been produced by OAA on interfering with tagatose's effect on the rise of postprandial glucose. A study in eight healthy volunteers showed that the post-lunch increases in plasma glucose and insulin were attenuated by the ingestion of 30 g tagatose, even when tagatose was administered 4 h and 15 min before lunch [18].

The above studies demonstrated that the pre-administration of tagatose blunts/attenuates the rise in blood glucose otherwise observed after carbohydrate loading (glucose, sucrose or a controlled lunch). A study in 12 subjects with mildly elevated fasting glucose levels (110–140 mg/dl) demonstrated that the administration of as little as 7.5 g of tagatose blunted hyperglycaemia following glucose ingestion, when the tagatose was administered with 75 g glucose [19]. Another study in 12 healthy subjects showed that postprandial increase in insulin was attenuated by 15 g tagatose when tagatose was taken with a breakfast containing 99 g of starch [60]. However, 1 h after the intake of the breakfast with tagatose or sucrose, the serum glucose was slightly elevated above baseline, but there was no difference between the two treatments at that time, which suggests that blood glucose had peaked before the first postprandial sample was collected. Therefore the data neither supported nor refuted the blood glucose blunting effect of tagatose seen in other studies.

Glucose and meal tolerance tests showed that both the pre-administration of tagatose and the administration of tagatose with glucose blunt glucose and insulin rises in normal subjects, in subjects with mildly elevated fasting glucose (110–140 mg/dl), and in type 2 diabetic patients. This blunting effect was seen in type 2 diabetic patients who were either under diet control or took an OAA 1 h before the start of the OGTT [2,3]. However, when insulin or a sulphonylurea were taken with glucose in the OGTT, the blunting effect on postprandial hyperglycaemia after the ingestion of 7.5 g tagatose and a carbohydrate source was small and not statistically significant in patients treated with a sulphonylurea. The blunting effect of tagatose on postprandial hyper-

glycaemia was not seen in patients treated with insulin [36]. The lack of effect seen in this study may be ascribed either to the smaller amount of tagatose ingested or to the tight glycaemic control already provided by the antidiabetic agents used.

The long-term glycaemic control by tagatose leads to a decline in GlyHb. Initial results from the study at the University of Maryland showed that the daily intake of tagatose of 75 g (25 g tid) for 8 weeks by four people suffering from type 2 diabetes produced a 0.7% decline in GlyHb [2], a change similar to that seen with acarbose. Among those patients, three were on sulphonylureas, suggesting tagatose as a potential adjunct therapy. In a subsequent long-term pilot study [4], 8 of 12 enrolled type 2 diabetic subjects completed the full 14-month trial (2-month run-in period followed by a 12-month tagatose therapy). Among these eight patients, three patients were under diet control and five patients were under stable doses of OAAs. Subjects in this study received 45 g of tagatose daily (15 g tid) for 12 months. The study subjects were in a state of deteriorating glycaemic control when they began tagatose therapy, which was demonstrated by a 1.1% increase in GlyHb during the 2-month run-in period. However, by the fourth month, their GlyHb had stabilized or the increase of GlyHb had been overcome by the tagatose therapy which, thereafter, produced an overall decrease of 1% GlyHb for the entire 12-month tagatose therapy. If the fourth month is considered as the starting point, the decrease of GlyHb would be 2.2% in 8 months. Unfortunately, this study was not placebo controlled, as it will be in the current phase 3 trial.

All the above human studies are summarized in table 2. These study results suggest a larger, placebo-controlled study is needed to confirm whether and to what degree, tagatose improves glycaemic control in patients with type 2 diabetes. Spherix hopes to develop tagatose as a monotherapy for glycaemic control, although tagatose is also a potential adjunct therapy as supported by initial results [2,4].

Dose and Time-of-Administration Study

A small dose and time-of-administration study was conducted to verify the dosing plan to be used in the phase 3 trial. It was a randomized, open-label, 3×3 factorial design for an instant mashed potato load, and a 2×3 factorial design for a glucose load in which each subject participated in all 11 separate 2-h meal or glucose tolerance tests. This study was conducted at Info Kinetics Sdn Bhd, Gleneagles Clinical Research Center in Penang, Malaysia. The study protocol was approved by the Joint Penang Independent Ethics Committee.

Table 2 Early indications of tagatose as an antidiabetic drug

Studies	Subjects	Methods	Results
Domner <i>et al.</i> [2]; Domner <i>et al.</i> [3]	8 normal persons (4♂, 4♀); 8 NIDDM persons (4♂, 4♀), among them, 4 were under diet control and 4 were on SU	3-h plasma glucose and insulin measured after the oral administration of 75 g glucose or 75 g tagatose 30 min preceding 75 g glucose. SU given 1 h before each study for patients on SU	(1) Oral loading with tagatose itself led to no changes in blood glucose or insulin in normal or NIDDM subjects. (2) Pre-treatment with tagatose attenuated the rise in blood glucose and insulin from baselines following oral glucose in both normal ($p = 0.25$ for glucose AUC and $p = 0.07$ for insulin AUC) and NIDDM subjects ($p = 0.002$ for glucose AUC and $p = 0.66$ for insulin AUC)
Domner <i>et al.</i> [2]	7 normal persons; 8 NIDDM persons, among them 5 were under diet control and 3 were on SU	Metabolic parameters during 8 weeks of daily tagatose (25 g tid with each meal) in both normal ($n = 3$) and NIDDM ($n = 4$) subjects, or daily sucrose (25 g tid with each meal) in 4 normal subjects, or no supplement in 4 NIDDM subjects. SU given 1 h before each study for patients on SU	(1) Normal subjects receiving either 75 g tagatose or sucrose daily for 8 weeks showed no significant changes in either FBG, insulin, GlyHb, BP, weight, lipids, or LFTs. (2) Compared with NIDDM subjects receiving no supplement who had GlyHb unchanged, all 4 NIDDM subjects on daily tagatose had a decrease in GlyHb at 4 weeks (9.4% vs. 10.1%); Two of three NIDDM subjects who completed the 8 weeks of tagatose had a continued fall in GlyHb, and one NIDDM subject had a 2.4% increase in GlyHb during the last 4 weeks associated with a five pound weight gain and excessive caloric intake by subjects breaking normal diet regimen
Domner <i>et al.</i> [3]	5 NIDDM patients	3-h plasma glucose and insulin measured after the oral administration of 75 g sucrose or 75 g tagatose 30 min preceding 75 g sucrose	Pre-treatment with tagatose did not lead to a statistically different glucose AUC or insulin AUC in NIDDM patients receiving oral sucrose. However, it attenuated the rise in blood glucose from baseline at 30 min (30 vs. 72 mg/dl, $p < 0.01$) and at 60 min (57 vs. 108 mg/dl, $p < 0.02$)
	10 NIDDM patients (6♂, 4♀), among them, 8 were under diet control and 2 were on SU	3-h plasma glucose measured after the oral administration of 0, 10, 15, 20 or 30 g tagatose 30 min preceding 75 g glucose. SU given 1 h before each study for patients on SU	Pre-treatment with tagatose attenuated the rise in blood glucose from baseline by significantly reducing glucose AUC in a dose-dependent manner ($p < 0.05$ for 10 g tagatose, $p < 0.001$ for 20 g tagatose and $p = 0.0001$ for 30 g tagatose vs. 0 g tagatose). Pre-treatment with 15 g tagatose was less effective than 10 g tagatose in AUC reduction

(Continued)

Table 2. (Continued)

Studies	Subjects	Methods	Results
Buermann <i>et al.</i> [18]	8 normal persons (8:3)	7-h metabolic response to 30 g tagatose or 30 g fructose in water, or plain water as a control. A 4.0-MJ lunch was consumed between 255 and 275 min after starting the 7-h trial	(1) Tagatose administration led to no increase in plasma glucose and insulin (pre-lunch period). (2) Increases in plasma glucose and insulin (pre-lunch) after fructose ingestion were not significant. (3) The post-lunch increase of plasma glucose and insulin (the 1 st sample at 45 min after the start of the meal, i.e. 300 min after the administration of tagatose) was attenuated by tagatose. The glucose and insulin levels (at 95 min after the lunch) remained somewhat lower with the pre-ingestion of tagatose. (4) Fructose blunted the postprandial glucose and insulin levels, but was much less effective than tagatose.
Boesch <i>et al.</i> [60]	12 normal persons (12:3)	7-h plasma glucose and insulin measured before and after the intake of a standard breakfast containing 99 g starch and 15 g tagatose or sucrose	(1) The serum insulin levels for a 3-h period after the tagatose breakfast were significantly lower than those after the sucrose breakfast. (2) One hour after the intake of the breakfast with tagatose or sucrose, the serum glucose was slightly elevated above baseline, but there was no difference between the two treatments at that time, which suggests that blood glucose had peaked before the first postprandial sample was collected.
Sugrfs 2004 [17]	12 normal persons	2-h plasma glucose and insulin measured after the oral administration of 50 g glucose or 50 g tagatose	Tagatose produced very low glycaemic and insulin responses of 3% compared with glucose.
Donner <i>et al.</i> [4]	8 NIDDM patients (4:3, 4:3); 3 were diet control; other 5 on OAA(s)	Metabolic parameters measured during 12 months of daily tagatose, 15 g tid with each meal (dissolved in liquids, used in baking or added to prepared foods)	Compared with mean baseline measurements, (1) Patients had a progressive weight loss after the 4 th month, which became significant at month 12 (103.3 vs. 108.4 kg patients, $p = 0.001$); (2) Mean GlyHb fell, becoming significant after 12 months (9.6 vs. 10.6%, $p = 0.08$); (3) HDL levels progressively rose from a baseline level of 30 to 41.7 mg/dl at month 12 in the 6 subjects who had no lipid-modifying medications added during the study ($p = 0.0001$).
Madenokoji <i>et al.</i> [19]	12 persons (10:3, 2:3) with mild hyperglycaemia (fasting glucose of 110–140 mg/dl).	3-h plasma glucose and insulin after the oral administration of 75 g glucose, 7.5 g tagatose or a mixture of 7.5 g tagatose and 75 g glucose.	(1) Oral loading with 7.5 g tagatose alone led to no changes in glucose or insulin. (2) Tagatose blunted the average rise in blood glucose from baseline by significantly reducing glucose AUC by 18.6% ($p < 0.006$). (3) An increased, but not statistically significant, insulin response to a combination of tagatose and glucose was seen over glucose ingested alone.

(Continued)

Table 2. (Continued)

Studies	Subjects	Methods	Results
Adamsen et al. [36]	23 NIDDM patients: 11 on SU (9♂, 2♀); other 12 on insulin (8♂, 4♀)	4-h plasma glucose and insulin after the intake of a regular medication (SU or insulin) and 75 g glucose together with a test substance (7.5 g tagatose, 5 g ingestible dextrin, or Palsweet as a control) at $t = 0$ h followed by a repeated intake of test substance at $t = 3$ h.	(1) Tagatose, at a dose of 7.5 g, reduced, but insignificantly, the rise of glucose in patients who received 75 g glucose with SU. (2) There was no statistically significant difference in glucose AUC or insulin AUC between treatments for either SU patients or insulin patients. (3) Tagatose, at a dose of 7.5 g, together with a regular medication, did not increase the risk of hypoglycaemia (blood glucose of 54 mg/dl or lower) in either SU patients or insulin patients.

AUC, incremental area under the curve; BP, blood pressure; FBG, fasting blood glucose; GlyHb, glycosylated haemoglobin; HDL, high-density lipoprotein cholesterol; LFT, liver function test; NIDDM, Non-insulin-dependent diabetes mellitus or type 2 diabetes; OAA, oral antidiabetic agents; Palsweet, a combination of erythritol, aspartame and acesulfame K by Ajinomoto, Japan; SU, sulphonylurea.

The 11 treatment codes are: T0(0)P and T0(0)G represent treatments in which subjects ingested 0 g tagatose 0 min prior to 90 g instant mashed potato load (containing 75 g available carbohydrate) or 75 g glucose load respectively. T10(0)P, T10(-30)P and T10(-60)P represent treatments in which subjects ingested 10 g tagatose 0, 30 or 60 min prior to 90 g instant mashed potato load respectively. T15(0)P, T15(-30)P and T15(-60)P represent treatments in which subjects ingested 15 g tagatose 0, 30 or 60 min prior to 90 g instant mashed potato load respectively. T15(0)G, T15(-30)G and T15(-60)G represent treatments in which subjects ingested 15 g tagatose 0, 30 or 60 min prior to 75 g glucose load respectively.

A total of 33 non-diabetic, healthy subjects (18 males and 15 females) aged 18–44 years (mean \pm s.e.m.; 24 ± 0.8) who had satisfied the screening evaluation were enrolled into the study and were randomly assigned to 1 of the 11 treatment sequences. One female and two males withdrew from the study for personal reasons. The remaining 30 subjects (16 males and 14 females) completed the clinical portion of the study. One subject's data were regarded as an outlier and, therefore, was not included in the analysis. The average body weight and average body mass index (mean \pm s.e.m.) of the remaining 29 subjects were 59.3 ± 1.9 kg (range = 42.8–82.8 kg) and 21.5 ± 0.48 kg/m² (range = 17.4–26.9 kg/m²) respectively.

Glucose and meal (mashed potato) tolerance tests were spaced 2 days apart for each subject. After an overnight fast of 10 h, subjects consumed 75 g glucose (dissolved in 200 ml water) or 90 g instant mashed potatoes (Hungry Jack instant mashed potatoes prepared in 550 ml of boiling water) containing 75 g available carbohydrate. The effects of the timing of tagatose ingestion were evaluated by having the subjects consume 10 or 15 g of tagatose dissolved in 60 ml of water at 60, 30 or 0 min (immediately) before ingesting the meal (instant mashed potato meal) or glucose load. After consuming the study medication, an additional 20 ml of drinking water were used to rinse the cup and the subject drank that water too. Subjects received 60 ml of plain water at each time point when tagatose was not consumed, that is, control meal or glucose with no supplemental tagatose. Finally, all subjects were allowed to consume an additional 240 ml of water to facilitate their consumption of the meal or glucose load. They consumed the glucose dose within 10 min. Because subjects could not consume instant mashed potatoes within 10 min, subjects started the potato meal 15 min prior to its reference (glucose) challenge, and finished instant mashed potato within 30 min. This was the most practicable approach to making the tests comparable that could be reasonably achieved.

To ensure that subjects have similar glycogen stores on the study days, they were instructed to consume a high carbohydrate diet (150 g carbohydrate/day) for 3 days before the first meal or glucose tolerance test, and to avoid exercise 24 h before the study. Adequate carbohydrate intake was verified for each subject by 3-day diet record. Subsequently, all subjects maintained the high carbohydrate diet throughout the study. In the evening before each meal or glucose tolerance test, all subjects consumed a low-residue dinner provided to them. After the low-residue evening meal, they were instructed to fast overnight, during which only the consumption of water was allowed.

Finger-prick capillary blood glucose samples were obtained at -60, -30 and 0 min before the meal or glucose load, as well as at 30, 45, 60, 90 and 120 min after the start of the meal or glucose load, that is, a total of eight finger-pricks per study day. Blood glucose measurements were performed using a portable glucometer (Advantage III Meter, Roche Diagnostics). Glucose measurement was always given precedence, that is, the finger-prick was always performed prior to tagatose dosing or meal loading.

The time-of-administration effect of tagatose on blunting the plasma glucose levels after a meal (instant mashed potato) or glucose load is presented in figure 1. The treatment effects were compared based on incremental area under the curve (AUC). The baseline glucose chosen to calculate the incremental AUC was that at -30 min for the meal load while it was that at 0 min for the glucose load, since the actual meal load started 15 min before the planned time at 0 min. Thus, for the treatments at times 0 min, tagatose was actually administered 15 min after the start of the meal load, while it was given immediately before the glucose load.

A single comparison model was used to determine whether the time-of-administration has an effect on treatment efficacy. The highest mean incremental AUCs were for T0(0)P (figure 1A,B) and T0(0)G (figure 1C), representing meal and glucose controls respectively. Treatment with tagatose, whether it was at a dose of 10 g or 15 g, whether it was taken with or before the meal or glucose load, blunted the rise of plasma glucose, although the reductions in incremental AUC, when compared with corresponding controls, were not statistically significant. Furthermore, at either 10 or 15 g tagatose for the meal load or 15 g tagatose for the glucose load, there were no statistical significances among different time-of-administrations, in terms of the reductions in incremental AUC compared with the corresponding controls. Thus, considering the difficulty of pre-administration of tagatose in clinical practice, tagatose will be administered with the first bite of each meal.

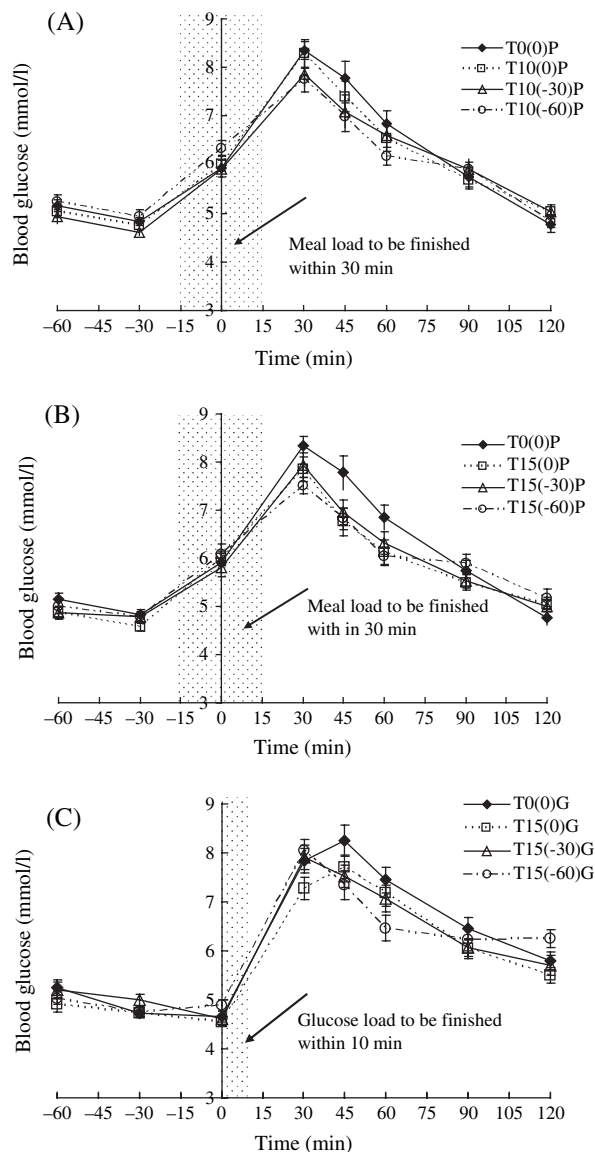


Fig. 1 Effect of time-of-administration of tagatose on the efficacy of blunting the rise of plasma glucose after 90 g meal (instant mashed potato containing 75 g available carbohydrate) load (P) or 75 g glucose load (G). 0, 10 or 15 g tagatose (T) dissolved in 60 ml water were administered at 60, 30 or 0 min (immediately) before the planned meal or glucose load (0 min). Actual meal load started 15 min before the planned time (see text for explanation). Plasma glucose (mean \pm s.e.m.) data represent average glucose levels of 29 subjects.

The dose effect of tagatose on plasma glucose after a meal (instant mashed potato) or glucose load is presented in figure 2. Again, a single comparison model was used to see whether 15 g of tagatose are more effective on glycaemic control than are 10 g of tagatose. The

comparisons were performed separately for each time-of-administration. Administration of 15 g of tagatose led to a larger reduction in incremental AUC compared with the corresponding control than did administration of 10 g tagatose, although the difference was not statistically significant. It is expected that a larger dose of tagatose would be more effective in postprandial glucose control than a lower dose. Thus, this finding suggests that the result from a previous study showing that a dose of 15 g of tagatose was less effective than 10 g tagatose in postprandial glycaemic control occurred by chance [3]. Considering the fact that 15 g of tagatose is the practical maximum dosage because of mild gastrointestinal side effects [2–4,48–51,61,62], we are giving the patients with type 2 diabetes 15 g tagatose tid with the first bite of each meal. This regimen is included in the phase 3 clinical trial.

Multiple Benefits and Safety of Tagatose Compared with Current Antidiabetic Drugs

Unlike currently available OAs, tagatose is GRAS for human oral ingestion. The main side effects of currently available orally administered antidiabetic agents are listed in table 3, where they are compared with those of tagatose. On 14 August 2007, the FDA required updated labels with a 'boxed' warning-FDA's strongest, on the risk of heart failure be added to the labels on the entire thiazolidinedione class of antidiabetic drugs [63]. This class includes Avandia® (rosiglitazone, GlaxoSmithKline), Actos® (pioglitazone, Takeda), Avandaryl™ (rosiglitazone and glimepiride, GlaxoSmithKline), Avandamet® (rosiglitazone and metformin, GlaxoSmithKline) and Duetact™ (pioglitazone and glimepiride, Takeda Pharmaceutical). Troglitazone (Rezulin®), also a member of the class of the thiazolidinediones, was withdrawn from the US market on 21 March 2000 [64,65]. Troglitazone (Romolin®) was withdrawn 3 years earlier in the UK after reports of severe adverse hepatic events [65]. The GRAS status of tagatose and its wide use without any adverse reports augers well for the safety of tagatose as a drug, a very important and promising advantage in entering a phase 3 clinical trial.

Also, hypoglycaemia is not expected even should an overdose of tagatose be taken. In addition, tagatose induces weight loss at medically acceptable rates, rendering the drug effective against obesity, a major and growing health problem that is epidemic among diabetic patients. Tagatose is also a potential antioxidant, helps raise HDL that is known as the 'Good' cholesterol because a high level of it seems to protect against heart attack, and promotes beneficial microorganisms in the large intes-

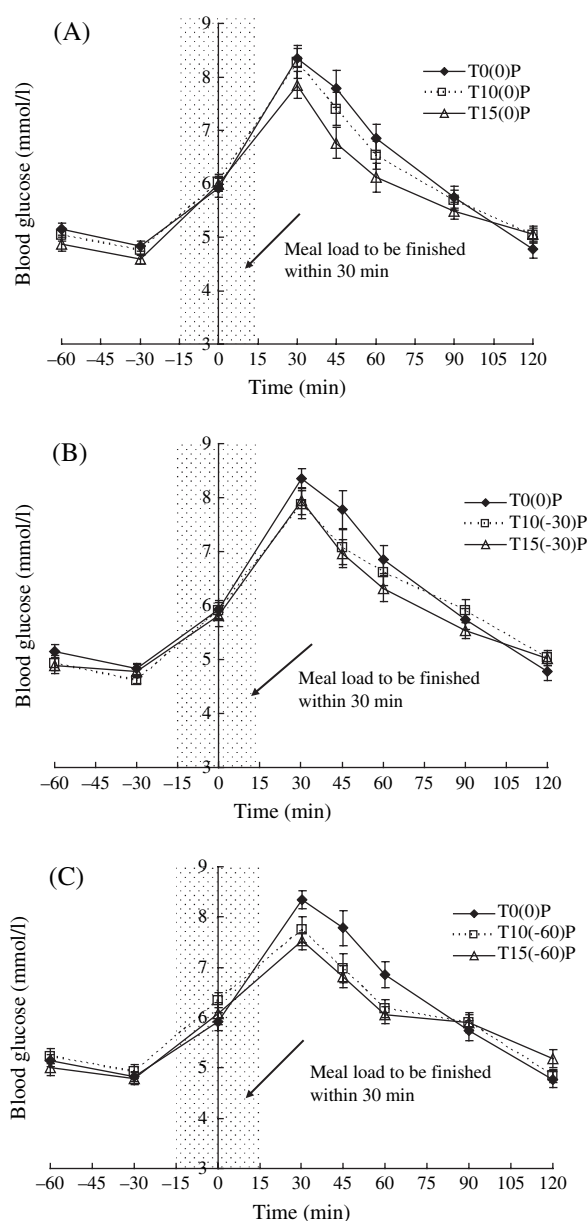


Fig. 2 Dose of tagatose on the efficacy of blunting the rise of plasma glucose after 90 g meal (instant mashed potato containing 75 g available carbohydrate) load (P). 0, 10 or 15 g tagatose (T) dissolved in 60 ml water were administered at 60, 30 or 0 min (immediately) before the planned meal load (0 min). Actual meal load started 15 min before the planned time (see text for explanation). Plasma glucose (mean \pm s.e.m.) data represent average glucose levels of 29 subjects.

tine. The sweet taste of tagatose, identical to that of table sugar, and its oral rather than IV route of administration, make tagatose as a potentially unique antidiabetic drug.

Table 3 Main side effects of tagatose and currently available orally administrated antidiabetic agents

Drug class	Principal side effects reported	GRAS?
α -Glucosidase inhibitors (acarbose, miglitol and voglibose)	Gastrointestinal	No
Biguanide (metformin)	Gastrointestinal, lactic acidosis (rare)	No
Insulin secretagogue. Sulphonylureas (gliclazide, glimepiride and glyburide)	Hypoglycaemia, weight gain	No
Insulin secretagogue. Non-sulphonylureas (repaglinide and nateglinide)	Hypoglycaemia, weight gain	No
Insulin sensitizers or thiazolidinedione (rosiglitazone and pioglitazone)*	Weight gain, oedema, anaemia, pulmonary oedema, congestive heart failure	No
Intestinal lipase inhibitor (orlistat)	Gastrointestinal, reduced absorption of fat-soluble vitamins	No
Dipeptidyl peptidase-4 inhibitor (sitagliptin)	Upper respiratory infection, stuffy or runny nose and sore throat, headache	No
Tagatose	Gastrointestinal (until adapted)	Yes

*This class of antidiabetic drugs was currently required to carry 'boxed' warning on the risk of heart failure by the FDA [63].

Tagatose and Obesity

Obesity has reached epidemic proportions globally. This is important because of the associated co-morbidities, which include cardiovascular disease, type 2 diabetes and osteoarthritis. In the US, nearly 9 out of 10 people with newly diagnosed type 2 diabetes are overweight. For many people with diabetes, weight loss is the key to getting control of blood sugar, and may eliminate the need for medication. However, weight gain is the main side effect of insulin secretagogues like sulphonylureas, which is likely related to increases of insulin [16]. Weight gain is also one of the major side effects of thiazolidinediones. However, metformin, also an insulin sensitizer, leads to weight loss [16]. Tagatose does not increase insulin levels for glucose control. Instead, it blunts increase in postprandial insulin (table 2). In a 14-month study of eight diabetic patients using tagatose as a monotherapy (three of the patients under diet control) and as an adjunct therapy (for the other five patients under stable doses of OAs), patients had a progressive average weight loss after the fourth month that became significant at month 12 (103.3 vs. 108.4 kg at beginning; $p = 0.001$) [4]. In a separate double-blind study of 19 normal weight men, tagatose was shown to reduce food

intake [61], apparently one of the causes of weight loss observed.

Tagatose Increases HDL

Dyslipidaemia is one of the factors associated with diabetes [16]. It is also a major contributor to the increased coronary heart disease (CHD) risk in patients with type 2 diabetes and is characterized by elevated levels of triglycerides and low-density lipoprotein (LDL) cholesterol and low levels of HDL cholesterol. Medications to lower elevated LDL cholesterol levels have demonstrated significant reductions in cardiovascular events in patients with diabetes and CHD [66]. There is similar convincing evidence in relation to the importance of HDL cholesterol. HDL is the scavenger lipoprotein. Its function is to transport excess cholesterol back to the liver for further metabolism. Results of some clinical trials have demonstrated that treating patients who have low HDL with therapies that raise HDL can reduce major coronary events, leading to the development of novel therapeutics to raise HDL levels [67]. In a 14-month study on eight type 2 diabetic patients taking tagatose 15 g tid, HDL levels progressively rose from a mean baseline level of 30 to 41.7 mg/dl ($p = 0.0001$) at month 12 in the 6 subjects who did not have lipid-modifying medications added during the study [4]. Reduction in body weight might partially contribute to the improvement in HDL. No significant changes were observed in total cholesterol, LDL or triglycerides during the study period.

Tagatose is Prebiotic

Advances in biosciences support the hypothesis that diet modulates various body functions, and may maintain well-being and reduce the risk of some diseases. Such discoveries have led to the concept of 'functional foods' or 'nutraceuticals', which include probiotics and prebiotics. A probiotic is defined classically as a viable microbial dietary supplement that beneficially affects the host through its effects in the intestinal tract. A prebiotic is defined as 'a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon that can improve host health'. Modification by prebiotics of the composition of the colonic microflora leads to the predominance of a few of the potentially health-promoting bacteria, especially, but not exclusively, lactobacilli and bifidobacteria. Although work with prebiotics is still evolving, studies with inulin-type fructans have generated sufficient data suggesting

potential benefits of prebiotics in constipation relief, suppression of diarrhoea and reduction of the risks of osteoporosis, atherosclerotic cardiovascular disease associated with dyslipidaemia and insulin resistance, obesity and possibly type 2 diabetes [68]. Tagatose shows promise in this area.

Only 25% of the ingested tagatose is absorbed into the bloodstream through passive absorption. The remaining 75% is fully fermented in the large intestine yielding SCFAs. In animal studies [47,69], tagatose altered the composition and population of colonic microflora, as evidenced by changes in the proportion of SCFAs produced. *In vitro* fermentation of 10 g/l tagatose for 4 h with colonic samples from pigs adapted to tagatose for 17 days showed 46 mol% of butyrate in SCFAs. This is in sharp contrast to the normal 17 mol% of butyrate resulting from corresponding tagatose fermentation with colonic samples from pigs fed a sucrose control diet [69]. A human, double-blind, crossover study with 30 volunteers supported this effect. There was a higher *in vitro* production of butyrate in a 4-h fermentation of tagatose (10 g/l) with fresh faecal samples from volunteers after 2-week consumption of 7.5 g (33 vs. 17 mol% butyrate in SCFAs) and 12.5 g (28 vs. 17 mol% butyrate in SCFAs) tagatose at breakfast, compared with butyrate production by those undergoing a 2-week consumption of 15.1 g sucrose [39]. Another *in vitro* fermentation of 1% tagatose was conducted with human faeces obtained from 16 volunteers before (unadapted) and after the intake of 10 g tid of tagatose for 14 days (adapted) [38]. The rate of *in vitro* fermentation, in terms of SCFA production, was four times higher with faecal samples of adapted volunteers than that of unadapted volunteers, and similarly, the mol% of butyrate in SCFAs was higher (35 vs. 25 mol%) in the 4-h incubation of tagatose with faecal samples of adapted volunteers than that of unadapted volunteers. After 48 h the unadapted faecal incubation also showed increased mol% of butyrate to 38 mol%. Butyrate has many claimed beneficial effects including defence against colon cancer [38]. The stimulation of butyrate production by tagatose may be important in its own right.

In a human trial, tagatose ingestion of 10 g tid was also characterized by changes in microbial population species and densities. Pathogenic bacteria, such as coliform bacteria, were reduced in numbers, and beneficial bacteria, such as lactobacilli and lactic acid bacteria, were increased [38]. In another study, a number of 174 normal or pathogenic human enteric bacteria and dairy lactic acid bacteria, including potential probiotic bacteria, were screened for ability to ferment tagatose [70]. Only a few of the normal occurring enteric human bacteria

were able to ferment tagatose, but tagatose fermentation was common among lactic acid bacteria. In summary, the study indicated that the daily consumption of 7.5 g or more tagatose may lead to increased production of butyrate at the expense of acetate, to selectively stimulating the growth of lactobacilli and lactic acid bacteria and to reducing the numbers of coliform bacteria, without serious gastrointestinal complaints.

In addition, tagatose has been indicated to be a potential treatment for anaemia and haemophilia, for medical problems related to infertility [9], and appears to have antioxidant and cytoprotective properties [71,72]. There are no current therapies that provide such multiple health benefits along with the treatment of type 2 diabetes and the control of obesity.

Mechanism of Actions

While the mechanisms by which tagatose exerts its anti-hyperglycaemic effects are not entirely clear, based on the results of a number of studies with fructose and tagatose, the following plausible mechanisms of tagatose for glycaemic control were developed.

Absorbed tagatose is metabolized mainly in the liver, following a metabolic pathway identical to that of fructose. Tagatose is phosphorylated to tagatose-1-P by fructokinase, with a K_m 1.8 times higher than for fructose and a V_{max} virtually identical to that of fructose [73]. The higher K_m for phosphorylation of tagatose is probably of no relevance to the intracellular formation of fructose-1-P and tagatose-1-P *in vivo*, because the K_m for cellular uptake of fructose (and probably also of tagatose) is substantially higher. Uptake limits the rate of phosphorylation at physiological concentrations of fructose and tagatose less than 1 mmol/l [12]. Tagatose-1-P is split by aldolase to yield glyceraldehyde (GA) and dihydroxyacetone phosphate (DHAP). Although aldolase acts on both fructose-1-P and tagatose-1-P, the cleavage of tagatose-1-P occurs at only about half the rate of that of fructose-1-P [74,75]. The resulting transient accumulation of tagatose-1-P was observed directly by ^{31}P magnetic resonance spectroscopy of the human liver following ingestion of 30 g tagatose [76]. Like fructose-1-P, the increase of tagatose-1-P concentration stimulates glucokinase activity [77–81], leading to an increased phosphorylation of glucose to glucose-6-P, which further activates glycogen synthase [82]. A literature review also suggests that tagatose-1-P, similar to fructose-1-P, inhibits glycogen phosphorylase [83,84]. The net effects of the regulation of these enzymes are to increase glycogen synthesis, and to decrease glycogen utilization (figure 3).

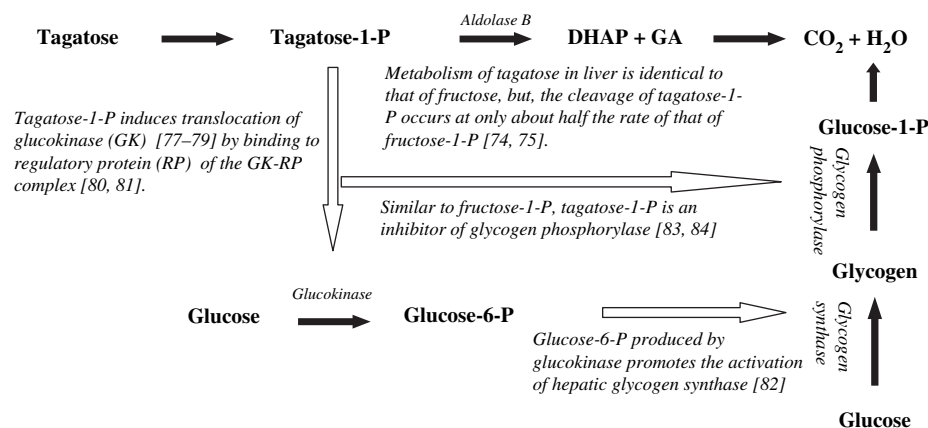


Fig. 3 Plausible mechanism of actions for tagatose (Part One).

The proposed mechanism suggests that fructose exerts an anti-hyperglycaemic effect similar to that of tagatose (table 2). Intraportal infusion of a small amount of fructose at 1.7, 3.3 or 6.7 $\mu\text{mol/kg/min}$, which raised the portal blood fructose concentration from <6 (basal) to 113, 209 and 426 $\mu\text{mol/l}$, respectively, increased net hepatic glucose uptake from 15 to 41, 54 and 69 $\mu\text{mol/kg/min}$, respectively, during a hyperglycaemic, hyperinsulinaemic clamp in 42-h-fasted dogs [85]. An inclusion of low amounts of fructose (2.22 $\mu\text{mol/kg/min}$) with an intra-duodenal glucose load (44.4 $\mu\text{mol/kg/min}$) significantly increased net hepatic glucose uptake (28 vs. 17 $\mu\text{mol/kg/min}$) and net glycogen deposition (3.68 vs. 2.44 mmol glucose equivalent/kg/bw) in conscious dogs, compared with that in the absence of fructose [86]. In a 2-h OGTT on healthy human subjects, the administration of 7.5 g fructose with 75 g glucose reduced plasma glucose response, that is, the positive incremental AUC, by 19%, without significantly enhancing the insulin or triglyceride response [87]. In a 3-h OGTT on patients with type 2 diabetes, the administration of 7.5 g fructose with 75 g glucose reduced plasma glucose response and insulin response (AUCs) by 14 and 21% respectively [88]. In contrast to the above studies demonstrating that the immediate administration of a small amount of fructose lowers the glycaemic response to glucose solution, a study of healthy adults found that fructose (7.5 g) must be consumed before the ingestion of a starchy food (containing 50 g available carbohydrate) in order to reduce postprandial glycaemia [89]. Compared with the control, the positive incremental AUC was reduced 25 and 27% when fructose was fed either 60 or 30 min before the meal respectively [89]. A slight improvement in glycaemic control, using both fasting serum glucose and GlyHb as clinical markers,

was seen in several small trials, suggesting patients with type 2 diabetes may benefit from the daily supplementation of fructose [90–92].

The above studies may suggest that small quantities of fructose appear to have a ‘catalytic’ effect to improve postprandial glycaemic control for patients with type 2 diabetes as a consequence of increased hepatic glucose uptake and storage as glycogen. Thus, nutritionists often recommend that people with diabetes use fructose. However, at least in the short-term, fructose at high doses is associated with increased lipid synthesis, dyslipidaemia, deposition of lipid in the liver and skeletal muscle, insulin resistance, obesity and diabetic complications [93–98]. Dietary fructose was found to be associated with increased fasting and postprandial plasma triacylglycerol concentrations in men, but not among women [99]. In a diet high in saturated fatty acids and cholesterol, fructose increases the levels of risk factors associated with heart disease, especially in hyperinsulinaemic men [100]. Also, fructose has potentially harmful effects on other aspects of metabolism, in particular, being a potent reducing sugar that promotes the formation of toxic advanced glycation end products. These have been cited to play a role in the aging process; in the pathogenesis of the vascular, renal and ocular complications of diabetes and in the development of atherosclerosis [101]. Because of fructose’s adverse effect on plasma lipids, in 2002, American Diabetes Association recommended avoiding fructose other than what occurs naturally in fruits [102].

Glycaemic regulation by increasing hepatic glycogen synthesis and decreasing glycogen utilization (figure 3) is accompanied by liver enlargement. A reversible liver enlargement, without the increase of liver enzymes, was seen in Sprague–Dawley rats fed tagatose at dietary levels

of 10 to 20% [55,58]. Different causes and consequences of asymptomatic liver enlargement in rats were reviewed, and the excessive increase of hepatic glycogen storage was concluded to be the reason [12,57]. Fructose ingestion produces similar effects to those of tagatose on liver glycogen and liver size, but at about four times higher doses to obtain the same response [57]. Based on the above information, tagatose is more effective than fructose in glycaemic control by regulating glycogen synthesis and utilization, which is accompanied with a temporary and reversible liver enlargement. This conclusion is supported by the evidence that the tagatose diet, but not the fructose diet, showed the alleviation of diabetic symptoms including polydipsia in SHR/N-cp rats [1]. It is also supported by the discovery that post-lunch increases in plasma glucose, and insulin were attenuated much more effectively by the ingestion of 30 g tagatose than by 30 g fructose [18]. Based on the graphs presented [18], it is estimated that the post-lunch increases in plasma glucose and insulin 45 min after the start of the meal (i.e. 300 min after the administration of tagatose) were attenuated by tagatose by 54 and 46% respectively. Similarly, the post-lunch increases in plasma glucose and insulin were attenuated by approximately 20 and 12%, respectively, by fructose. Only 20% of ingested tagatose is fully metabolized, principally in liver. Tagatose and its metabolic intermediates in the liver are more effective than fructose and its metabolic intermediates in regulating glycogen synthesis and utilization.

In addition to the effect on glycogen regulation, tagatose inhibits sucrase, leading to the suppression of sucrose digestion in the small intestine [103,104]. This unexpected potential benefit was confirmed by the finding that the small intestine's digestibility of sucrose (measured by the disappearance of sucrose in the small intestine) in pigs fed a low fibre diet containing 5% sucrose and 10% tagatose was 8% lower (90 vs. 98%) than that in the pigs fed a low fibre diet containing 15% sucrose [47]. An *in vitro* study found that tagatose also inhibits the activity of maltase derived from rabbit small intestine mucous membrane [104], and thereby delays the digestion of starch (figure 4).

Thus, perhaps together with a still unknown mechanism, tagatose depresses the rise in blood sugar by increasing glycogen synthesis, decreasing glycogen utilization, and, possibly, also by reducing the digestion of sucrose and other carbohydrates in the small intestine.

Side Effects of Tagatose

Single-dose and repeated-dose studies of tagatose in healthy and diabetic human subjects showed that the pre-

dominant side effects associated with excessive consumption are gastrointestinal disturbances attributed to osmotic effects from incompletely absorbed tagatose, one of the common effects of other diabetes drugs (table 3). In a single-dose tolerance test on 33 normal subjects, 29 g of tagatose were added as a sweetener to a continental breakfast with 29 g of sucrose as a control treatment. Although, on the first day, 'rumbling in the stomach', 'distension', 'nausea', 'rumbling in the gut', 'flatulence' and 'diarrhoea' scored significantly higher in the tagatose group, the sugar otherwise was well-tolerated. By the second day, no significant differences in symptom scores between tagatose and sucrose were found after the test load was taken [49]. Another tolerance study on 73 normal male subjects found that, after the consumption of 29–30 g of tagatose, nausea and diarrhoea were reported with an incidence of 15.1 and 31.5% respectively. Increased flatulence after tagatose ingestion was frequently reported during a 15-day period with a daily intake of 30 g of tagatose in a single dose. This indicates that single doses in excess of 30 g of tagatose should not be recommended for ordinary use [50]. However, in a study on tagatose metabolism [51] and a study on the effect of tagatose on food intake [61], volunteers took 30 g of tagatose in foods on the basis that most humans can tolerate 30 g of tagatose in a single dose without unacceptable gastrointestinal symptoms. The ingestion of 25 g of tagatose tid for a period of 8 weeks in both diabetics and normal subjects resulted in varying amounts of flatulence in seven of the eight subjects, with some degree of diarrhoea in six of the subjects [2,62]. The above gastrointestinal side effects are also commonly associated with excessive consumption of other poorly digestible carbohydrates including polyols, and with excessive consumption of sucrose. In a study with 50 normal subjects, consumption of 20 g of tagatose was not associated with a significant increase in the frequency of passing faeces or in the number of subjects passing watery faeces. However, 20 g of lactitol consumption was associated with an increase in either of these occurrences. Consumption of chocolate containing tagatose and lactitol resulted in significant increases in colic, flatulence, borborygmi and bloating compared with consumption of the sucrose-containing chocolate, but the majority of symptoms were described as only 'slightly more than usual', suggesting that a 20 g dose of tagatose is tolerated well in comparison to lactitol [48]. In an OGTT on patients with type 2 diabetes, the administration of a single dose of 75 g of tagatose led to diarrhoea, nausea and/or flatulence in 100% of the subjects. When tagatose was administered at lower doses, ranging from 10 to 30 g, only 3 of 10 patients with diabetes had

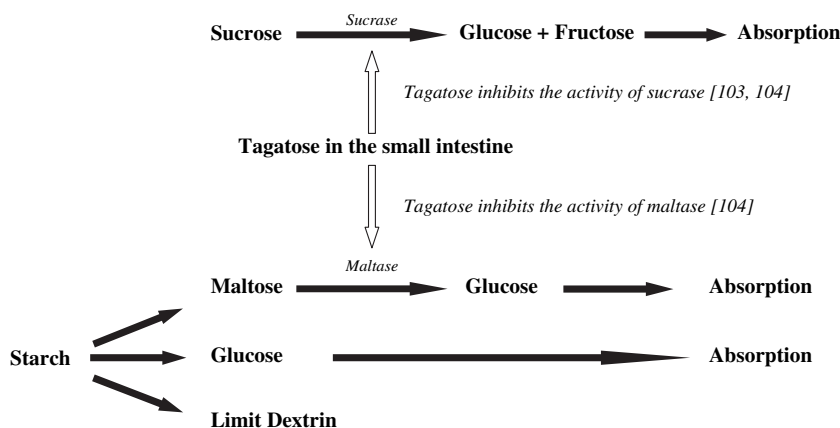


Fig. 4 Plausible mechanism of actions for tagatose (Part Two).

gastrointestinal symptoms, and these were much milder than those evoked by 75 g of tagatose [3]. In the 1-year trial on diabetic patients with tagatose, of the eight subjects who completed the 14-month study with 15 g tid, seven experienced gastrointestinal side effects which tended to be mild and limited to the first 2 weeks of therapy [4].

Because tagatose is currently manufactured by the isomerization of galactose, and galactose is derived from lactose, the potential allergenicity of tagatose bears consideration. Lactose, a disaccharide in the whey fraction of milk, is known to contain residual milk proteins, including several of the major allergens in cows' milk, principally β -lactoglobulin and α -lactalbumin. Although lactose often contains residual milk allergens, tagatose is much less likely to contain any milk allergens, because several steps in the tagatose production process denature or remove residual proteins in the lactose starting material. Therefore, tagatose should be safe for milk-allergic individuals [105]. Tagatose has, since being established as GRAS in 2001 [11], developed a history of uses in foods with no reported incident of allergic or any other toxic event.

Liver enlargement and elevated uric acid concentration had been two concerns during the safety evaluation of tagatose [14]. Standard toxicity tests with diets containing 10 to 20% tagatose showed reversible liver enlargement in Sprague–Dawley rats without increasing of liver enzymes [55,58]. The observed liver enlargement in tagatose-fed rats was found to have no relevance to the assessment of human safety of tagatose [57]. A clinical trial was conducted to study the potential effects of tagatose on the volume of the human liver and postprandial liver glycogen concentration [60]. Twelve healthy male subjects were studied in a double-blind

crossover study with the ingestion of tagatose (15 g tid) and placebo (sucrose, 15 g tid) for a period of 28 days each. Liver volume and glycogen concentration were determined by magnetic resonance imaging and spectroscopy. The ingestion of a standard breakfast providing 99 g of starch and 15 g of tagatose or sucrose had no effect on liver volume or liver glycogen measured 5 h after intake. This suggests that any liver glycogen build-up early after breakfast had been mobilized completely by the time the postprandial measurement was made. Examinations before and after the 28-day treatments revealed no effects of tagatose on liver volume or on glycogen concentration compared with sucrose. The treatment with tagatose was not associated with clinically relevant changes of the examined clinico-chemical and haematological parameters, including liver enzymes and uric acid [60]. However, steady increases in liver volumes, independent of the tagatose or placebo intake, were observed over the 28-day study, which is not fully understood. Seasonal effects (November to February) or the more regular food intake under supervision may play a role [60]. Continuing intake of tagatose beyond 28 days was predicted as unlikely to lead to any additional increase of liver volume [58].

The presence of chronically elevated plasma uric acid levels (i.e. hyperuricaemia) is one known risk factor for the development of gout, which is a group of disorders of purine metabolism. The ingestion of single high bolus doses of tagatose is associated with a mild, transient increase of plasma uric acid concentration in both healthy subjects and patients with type 2 diabetes [18,62]. However, repeated daily doses of 15 g of tagatose tid ingested with the main meals for a period of 28 days produced no effect on fasting plasma uric acid levels in 12 healthy volunteers [60]. Another clinical trial in both

normal subjects and type 2 diabetic patients with the ingestion of tagatose at 25 g tid for 8 weeks did not show an increase in fasting plasma uric acid [62]. A pilot study in eight patients with type 2 diabetes confirmed the non-effect of tagatose on fasting plasma uric acid at a dosage of 15 g tid taken with meals for a period of 1 year [4].

Hypoglycaemia is one of the major side effects of insulin and insulin secretagogues when they are overdosed. A recent study on 23 Japanese diabetic patients demonstrated that the oral ingestion of 7.5 g tagatose with regular antidiabetic medications (sulphonylurea or insulin) did not introduce hypoglycaemia [36]. In a separate 14-month study completed by eight diabetic patients, four were treated with a sulphonylurea and one with a combination of metformin and troglitazone. None of those five patients experienced hypoglycaemia during the 12-month period when tagatose, at 15 g tid, was used as an adjunct therapy [4].

Because tagatose is a simple hexose sugar, no adverse interactions with other drugs are likely.

Phase 3 Trial of Tagatose

Spherix Incorporated is currently conducting a phase 3 clinical trial of tagatose in subjects with type 2 diabetes. It is a 1-year, multi-centre, placebo-controlled, double-blinded, randomized, parallel clinical study to evaluate the effect of tagatose on glycaemic control and safety of tagatose in subjects with type 2 diabetes who take no medicines for the condition, but who attempt control of the diabetes through diet and exercise. Dose of up to 15 g tid are being administered. The add-on effect of tagatose as an adjunct therapy will be evaluated later. A placebo-subtracted treatment effect of tagatose will be evaluated based on a decrease in HbA_{1c} levels.

The Placebo Used in the Trial

Because tagatose tastes sweet, the placebo for the phase 3 clinical trial was developed using an alternative sweetener. Alternative sweeteners fall into two categories: non-nutritive and nutritive. Non-nutritive sweeteners, also called high-intensity or artificial sweeteners, do not contribute calories and, because of their intense sweetness, are generally used at very low levels. They include saccharin, aspartame, acesulfame K, sucralose and many others. Nutritive sweeteners are reduced in calorie compared with sucrose. They include tagatose and various polyols, such as sorbitol, mannitol, maltitol, isomalt, lactitol and erythritol. In addition to sweetness on taste, in the formulation of the test drug, tagatose and its placebo,

several other factors were considered. These included caloric value, glycaemic index, gastrointestinal effects, appearance and bulk, in order to meet the double-blind requirement and to eliminate any test drug to placebo bias.

A lower ratio of energy intake to expenditure promotes weight loss and the improvement of metabolic syndrome. Therefore, it would be expected that foods and drinks containing alternative sweeteners would produce these desirable effects. Weight losses of 5 to 10% have been shown to have a significant impact on several aspects of the metabolic syndrome, including diabetes and well-recognized risk factors for cardiovascular disease [106]. Among 114 patients with type 2 diabetes, those who lost 5% or more of their baseline weight showed statistically significant decreases in serum HbA_{1c} levels [107]. In a study on patients with stage 1 hypertension, weight losses of 5% or more produced reductions in diastolic pressure that were equivalent to those produced by a single dose of antihypertensive medication [108]. It has been shown that weight losses of 5 to 10% improve lipid profile by the reduction in total cholesterol, the increase in HDL cholesterol and the decrease in LDL and very low-density lipoprotein (VLDL) cholesterol [109,110]. Because tagatose imparts few calories, it seemed possible that its promotion of weight loss, HbA_{1c} reduction and HDL increase seen in the 14-month pilot study [4] might have resulted solely from the partial substitution of tagatose for sugar and the associated caloric reduction. Unfortunately, this study was not placebo-controlled. However, in another study, tagatose was shown to reduce food intake [61]. To address this question in the phase 3 clinical trial, the placebo was formulated to provide less caloric intake than that of the tagatose dose. In addition, weight changes are being recorded in a nutrition diary maintained for each subject. Thus, the phase 3 clinical trial should determine the true effects of tagatose on weight loss and on metabolic syndrome.

The clinical significance of glycaemic index remains the subject of intense debate. However, it is clear that not only the amount of carbohydrate but also its rate of absorption after a meal have significant effects on postprandial hormonal and metabolic responses. The consumption of foods that elicit low-glycaemic responses may help to reduce risk factors associated with obesity, type 2 diabetes and cardiovascular diseases. A meta-analysis reviewed 14 randomized controlled studies, involving a total of 356 subjects and 12 days to 12 months duration. It found that low-glycaemic-index diets reduced HbA_{1c} by 0.43% compared with high-glycaemic-index diets [111]. A review of 16 trials between 1981

and 2003 found that low-glycaemic-index diets significantly reduced fructosamine by 0.1 mmole/l, HbA_{1c} by 0.27%, total cholesterol by 0.33 mmol/l, and tended to reduce LDL cholesterol in people with type 2 diabetes by 0.15 mmol/l compared with high-glycaemic-index diets. No changes were seen in HDL cholesterol and triacylglycerol [112]. Another meta-analysis of 15 randomized controlled trials found, compared with high-glycaemic-index diets, no effect of low-glycaemic-index diets on CHD incidents, morbidity or mortality. It found only limited or weak evidence of a relationship between low-glycaemic-index diets and low total cholesterol. A small reduction in HbA_{1c} was noticed after 12 weeks on low-glycaemic-index diets, but not after 4–5 weeks. No impact of low-glycaemic-index diets on LDL, HDL, triglycerides, fasting glucose or fasting insulin was found [113].

Polyols have low to very low glycaemic indices [114]. In addition, seven studies between 1977 and 1987 on sorbitol and lactitol found interactions between these two polyols and sugar, and between these two polyols and foods, which reactions yielded glycaemic indices of foods containing them lower than predicted based only on glycaemic indices of the meal components [114]. Incomplete hydrolysis alone cannot explain this finding. Possibly, these two alcohols slow stomach emptying, or hasten the glucose to a distal site where absorption is less rapid, or, perhaps, they significantly dilute luminal glucose concentration through their osmotic effect [114]. The author concluded that polyols and other food glycaemic index values could be used to estimate the glycaemic index of food mixtures containing polyols without underestimation [114]. However, until now, none of these polyols was evaluated for its potential as an anti-hyperglycaemic agent, with the exception of erythritol. In a small trial involved 11 patients with type 2 diabetes, 20 g of erythritol per day were administered orally for 14 days. Fasting blood glucose, reported for nine subjects, decreased from its baseline of 181 ± 60 mg/dl to 165 ± 57 mg/dl after the trial, but was not statistically significant. The HbA_{1c} levels, reported for all 11 participants, were the same as those before treatment for four patients, decreased in six, and increased in one after erythritol treatment. Large decreases of HbA_{1c} in two subjects averaged $7.5 \pm 1.6\%$ compared with a baseline of $8.5 \pm 1.5\%$ [115]. Although tagatose and polyols have similar appearances, have similar gastrointestinal effects, both provide bulk and both have low glycaemic indices, polyols were not considered for the formulation of the placebo for the phase 3 clinical trial of tagatose because of the potential effects of polyols on postprandial glucose.

Although a bulking agent, such as glucose or maltodextrin, high in glycaemic index, is often mixed with artificial sweeteners in commercial products, artificial sweeteners, themselves, have a zero glycaemic index. In the US, five artificial sweeteners have been approved for use: saccharin, aspartame, acesulfame K, neotame and sucralose. One study [116] found that the consumption of a 5% w/v saccharin solution as drinking fluid deferred the development of hyperglycaemia and reduced hyperinsulinaemia and excess weight gain in young obese-hyperglycaemic (*ob/ob*) mice. A lower concentration of 1% w/v was ineffective. However, the daily amount of saccharin consumed as a 5% w/v solution in this study was 500–1000 times greater than that likely to be consumed by a diabetic patient [116]. Acesulfame K was found to stimulate insulin secretion in studies of rats [117] and rat islets [118]. Further study using rat islets found that artificial sweeteners with a bitter taste, such as sodium saccharin, sodium cyclamate, stevioside and acesulfame K, augmented insulin release from islets incubated in the presence of glucose. In contrast, aspartame, which has no bitter taste, failed to affect insulin secretion [119]. However, the above evidence is not sufficient to suggest chronic consumption of artificial sweeteners for regulating blood glucose. Acceptable daily intake levels of artificial sweeteners, including saccharin [120], aspartame [120–122], acesulfame K [123], neotame [124] and sucralose [125,126], have no significant effect on glycaemic control or on blood lipids in normal subjects or diabetics. Small studies suggested beneficial effects of stevioside on glucose metabolism [127,128]. However, this artificial sweetener is approved by the FDA only as a dietary supplement in the US.

Based on the above analysis, sucralose was selected as the sweetener in the placebo for the phase 3 clinical trial of tagatose. Because sucralose is about 600 times sweeter than tagatose, water is used as the bulking agent to formulate the test drugs in order to meet the double-blind requirement. The doses of tagatose and sucralose are dissolved in water.

Parameters to Be Monitored

The change in HbA_{1c} levels is the primary index to be monitored in the trial. Acute studies (table 2) indicate that tagatose is a potential antidiabetic drug by controlling postprandial hyperglycaemia in individuals with type 2 diabetes or pre-diabetes, and this will be the secondary index that will be monitored on a sub-group of patients. The weight loss associated with the use of tagatose is another important index. The weight loss is

considered to be a result of reduced food intake [61], and/or malabsorption of other nutrients [47,103,104]. Weight loss is not associated with a thermogenic effect of tagatose [51], which was previously proposed to explain the lack of net energy of tagatose when added to the basal diet seen in growth studies in rats [129]. Full assessment of the weight loss effect of tagatose in the 14-month pilot study was limited by the lack of a control group [4]. Patients might have lost weight merely because of participation in the trial. For example, in a meta-analysis of the effectiveness of orlistat, an intestinal lipase inhibitor that also promotes weight loss in obese patients, 35 and 16% of placebo-treated patients lost approximately 5 and 10% weight loss, respectively, in 1-year of treatment [130]. Weight changes will be recorded in a nutrition diary being maintained in the phase 3 clinical trial. The true effect of tagatose on weight loss will thus be determined.

Evidence that tagatose raised HDL was obtained from the study lacking a control group [4]. The mechanism which tagatose raises HDL is not clear. As mentioned previously, tagatose and fructose appear to have a similar effect in improving postprandial glycaemia. However, at high doses, the chronic use of fructose is associated with increased lipid synthesis, dyslipidaemia and insulin resistance [93–98], and benefits in glycaemic control were seen only in the short-term and at 'catalytic' (e.g. 7.5 g) doses [87–92]. Fructose is readily absorbed from the diet and rapidly metabolized, principally in the liver, bypassing the controlling phosphofructokinase step in glycolysis. This, in turn, causes activation of pyruvate dehydrogenase, and subsequent modifications favouring esterification of fatty acids, again leading to increased VLDL secretion [98]. Increases in VLDL secretion can then lead to chain reactions in other lipoproteins and lipids such as LDL. For thousands of years humans consumed fructose of 16–20 g per day, largely from fresh fruits. However, westernization of diets has resulted in significant increases in added fructose, leading to typical daily consumptions of 85–100 g of fructose per day [98]. Considering the fact that only 20% of ingested tagatose is fully metabolized in the liver, the proposed dose of 45 g per day (15 g tid) of tagatose is equivalent to only 9 g of ingested fructose. Thus, the ingestion of tagatose is not expected to cause dyslipidaemia as does fructose at high doses, even though tagatose is metabolized following a metabolic pathway identical to that of fructose. However, the change of lipid profiles is also being recorded during this 1-year phase 3 placebo-controlled clinical trial to evaluate the effect of tagatose on dyslipidaemia. For the same reason, it is not expected that the chronic intake of tagatose will cause insulin resistance. The potential effect

is also being evaluated by examining the effect of tagatose on postprandial hyperinsulinemia together with measuring the effect of tagatose on postprandial hyperglycaemia on a chronic basis. Although changes in blood pressure were not seen in either the short-term or long-term pilot study [2,4], blood pressure is also being monitored during this phase 3 clinical trial. Other safety-related parameters being monitored include plasma uric acid, liver enzyme activities and gastrointestinal side effects.

Conclusion

A candidate drug to treat type 2 diabetes, tagatose has entered a phase 3 clinical trial. In phases 1 and 2, tagatose showed strong evidence for the control of HbA_{1c}, postprandial hyperglycaemia, and hyperinsulinemia, while also inducing weight loss at a medically desirable rate. Further demonstrated benefits include increased HDL levels, enhanced butyrate production (reported to combat colon cancer), antioxidant and prebiotic properties. Tagatose is not expected to produce toxic side effects, avoiding the major pitfall in drug testing, which currently afflicts several major diabetes drugs in use or proposed. Tagatose has been declared GRAS under FDA food ingredient rules, and has been widely consumed in food products as a sweetener for many years with no toxic events being reported. Consumers and test subjects ingesting levels at or above those being tested for drug use have reported only gastrointestinal difficulties. These have generally been accommodated within 2 weeks of use. A natural, but rare, sugar, tagatose is economically synthesized by chemical and enzymatic treatment of lactose derived from deproteinated whey, a waste product of dairy operations or directly from galactose obtained from lactose.

The phase 3 clinical trial for chronic use is large-scale, placebo-controlled, double-blinded and randomized. Pertinent side effects, including those associated with chronic use of fructose or HFCS will be monitored.

The rising worldwide epidemic of type 2 diabetes has created an increasing need for safer and better prevention and treatment, of this debilitating and deadly disease. Should its phase 3 clinical trial, possibly yielding results in 2008 and a subsequent FDA NDA be successful, the unique benefits of tagatose might help fill this need and address its large market.

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