levels). Extrapolating this percentage to the entire U.S. population during that time, the prevalence of prediabetes can be estimated to be around 86 million American adults [2].

Two abnormalities that typically characterize the pathogenesis of type 2 diabetes are peripheral insulin resistance and progressive failure of pancreatic β-cell function leading to loss of insulin secretion. These complications eventually lead to chronic hyperglycemia and associated long-term disease complications [3]. Insulin resistance does not progress in parallel with loss of β-cell function, but occurs first. Most type 2 diabetes patients who are initially treated by diet management or oral blood glucose lowering agents eventually require insulin as loss of β-cell function progresses. None of the therapies currently available appear to be able to slow or stop the progression of β-cell loss once it begins. Therefore, there is clearly a need for therapies for the long-term treatment of type 2 diabetes that can prevent patients from reaching this current "point of no return" of loss of β-cell function.

Currently the approach to the treatment of type 2 diabetes is stepwise and systematic with early treatment consisting of having patients alter and manage their diet, adhere to a regular exercise program and, if overweight, control their weight. If the disease progresses and blood glucose remains uncontrolled, then pharmacologic therapy is initiated, usually with one or two oral anti-hyperglycemic drugs. Additional medications or insulin may be added if the disease continues to progress. Many, if not most, patients with type 2 diabetes will eventually require insulin as a primary therapy with or without adjuvant drug therapy in order to control their blood glucose [4].

Despite the introduction in recent years of new drugs for the treatment of diabetes, glucose control in many patients remains unsatisfactory. Metformin remains the first drug of choice for the treatment of type 2 diabetes. A recent review that included 140 controlled trials and 26 observational studies comparing diabetes medications, both as monotherapy and in two-drug combinations, concluded that the long-term benefits and harms of current drug treatments remain unclear [5]. The study concluded that there was not enough evidence to clearly support the use of one drug or drug combination over another for stemming the complications of diabetes, including macrovascular and microvascular complications, and for mortality. Metformin remains the initial drug of choice, however, because compared to the newer drugs it has the highest benefit-to-risk ratio for intermediate outcomes, such as moderate HbA1c reduction, less weight gain and less risk of hypoglycemia. Many of the other therapies have limiting side effects such as weight gain, hypoglycemia, and edema, and have restrictions for use [6].

This information illustrates the limitations of drug therapies currently available for the progression of diabetes. Further, even with aggressive intervention, it is estimated that 60% of diabetics do not achieve target blood sugar levels with their current treatment regimen [4]. Moreover, current drugs available for treatment of diabetes may result in unwanted weight gain (long and rapid acting Insulin, sulfonylureas, thiazolidinediones, repaglinide, nateglinide), hypoglycemia (insulin, sulfonylureas), gastrointestinal distress (metformin, α-glucosidase inhibitor, amylin mimetics, bile acid sequestrant, bromocriptine), or more serious adverse events such as pancreatitis (short and long-acting glucagon-like peptide-1 (GLP-1) agonists and dipeptidyl peptidase-4 (DDP-4) inhibitors) [7,8]. From the statistics

showing a high percentage of diabetics are not able to consistently control their blood sugar levels within recommended limits, it is evident that there is a medical need for a drug that can slow and/or halt the progression of diabetes. Preferably, such a drug should exhibit a unique mode of action to enable additive or synergistic use with current therapies; produce no weight gain, hypoglycemia, or other limiting or unmanageable side effects; preserve or enhance β-cell function; and reduce cardiovascular risk factors that potentially lead to morbidity and mortality.

D-tagatose is an isomer of fructose that is ~90% as sweet as sucrose. In 2001 D-tagatose was designated as a Generally Recognized as Safe (GRAS) product by the United States Food and Drug Administration, and subsequently has been used as a nutritive or low-calorie sweetener [9]. Currently, D-tagatose may be used as a sweetener in diet beverages, light ice creams or yogurts, and regular or dietetic hard candies [10]. Subsequently it was hypothesized from observations in food use, and then demonstrated experimentally that, when consumed, D-tagatose functions as a "sugar blocker" and inhibits lipid formation from carbohydrates without stimulation of pancreatic beta cells for insulin production or secretion [11]. Preliminary animal and pre-clinical studies of D-tagatose demonstrated its ability to lower blood glucose and lipoprotein levels. D-tagatose has been shown to reduce total cholesterol and VLDL and LDL-cholesterol when compared to sucrose [12], and increase HDL-cholesterol levels [13]. A number of clinical trials demonstrating the ability of D-tagatose to blunt postprandial rises in blood glucose and reduce HbA1c have been conducted in both healthy subjects and diabetic patients [13–18]. A phase 2 study designed to estimate the lowest dose of D-tagatose capable of lowering HbA1c found that the dose was 5 g TID [18]. Single-dose and repeated-dose studies in healthy and diabetic human subjects have shown that the predominant adverse effects associated with excessive consumption of D-tagatose are gastrointestinal disturbances attributed to osmotic effects from incomplete absorption [13–17]. Such effects are also commonly associated with excessive consumption of other poorly digestible carbohydrates including polyols. In short, D-tagatose provides glycemic and lipoprotein control through a mechanism of action unlike any agent that is currently available on the market in the United States.

Here we report the results of a phase 3 clinical trial with D-tagatose and demonstrate statistically significant reductions in hemoglobin A1c levels (HbA1c) in patients with mild type 2 diabetes. HbA1c levels are an indicator of glycolated hemoglobin in the blood and reflect a patient's glycemic control over a six to twelve week period [19]. If blood glucose levels have been elevated over recent weeks, there will likely be a concomitant rise in HbA1c levels.

The primary objective of this Phase 3 clinical trial was to evaluate the placebo-controlled effect of D-tagatose on glycemic control and safety in subjects with type 2 diabetes over the course of a 10-month treatment. The secondary objectives of this clinical trial were to evaluate the placebo-controlled effects of D-tagatose on fasting blood glucose, insulin, lipid profiles, and changes in BMI. D-tagatose effectively lowered HbA1c levels in type 2 diabetic patients compared to placebo; thus, results of this phase 3 clinical trial illustrate the potential for D-tagatose to fulfill a need in current diabetes treatment.

PATIENTS AND METHODS

Ethical Conduct of the Study

The protocol and protocol amendment(s) were reviewed and approved by an Institutional Review Board (IRB) before the study was initiated. This trial was conducted in accordance with regulations governing clinical trials including the US Code of Federal Regulations (CFR), Title 21, Part 50; regulations governing IRBs, Title 21, Part 56; and the Declaration of Helsinki concerning medical research in humans (Recommendations Guiding Physicians in Biomedical Research Involving Human Patients: adopted by the 18th World Medical Assembly (WMA), Helsinki, Finland, June 1964 and amended by the 29th WMA, Tokyo, Japan, October 1975, the 35th WMA, Venice, Italy, October 1983, the 41st WMA, Hong Kong, September 1989, the 48th WMA, Somerset West, Republic of South Africa, October 1996, and the 52nd WMA, Edinburgh, Scotland, October 2000). Additional governing regulations included US CFR Title 21, Part 54 and US CFR Title 21, Part 312. This study was also conducted according to International Conference on Harmonization (ICH) Good Clinical Practices (GCP).

Eligibility

Subjects were required to meet the following criteria for inclusion in this study: male or female between the ages of 18 and 75 diagnosed with type 2 diabetes (according to WHO criteria) who were being treated with diet and exercise alone, and not on any medication for diabetes; a HbA1c level at screening and baseline greater than 6.6% and less than 9.0%; a fasting glucose concentration less than 240 mg/dL (13.3 mmol/l); a BMI of less than or equal to 45 kg/m^2; and a stable weight (±10%) for 3 months prior to entry into the study. Exclusion criteria included: treatment with any sulfonylureas, or other antidiabetic medications (e.g., thiazolidinediones, metformin, acarbose, exenatide, or insulin) within the prior 3 months; chronic (lasting longer than 14 consecutive days) systemic glucocorticoid treatment within 4 weeks of the baseline visit; use of any weight loss drugs within the prior 3 months; proliferative retinopathy; known or suspected abuse of alcohol or narcotics; any experience with hypoglycemic unconsciousness; impaired hepatic, renal or cardiac function; uncontrolled hypertension; pregnancy, breastfeeding, or intention of becoming pregnant or judged to be using inadequate contraception; documented gastrointestinal disease, or taking of medications to alter gut motility or absorption; and treatment with any investigational drug within 30 days of the screening visit.

Three populations were evaluated:

- The Intent-to-Treat (ITT) population consisted of all randomized subjects who received at least one dose of their randomized treatment and had at least one post-treatment visit evaluating efficacy.
- The Per Protocol (PP) population consisted of all ITT subjects who had at least 80% compliance with medication for 75% of the dosing time points and had no major protocol violations. Subjects who had a screening and visit 2 HbA1c value ≤ 6.6% and ≥ 9.0% and who were put on other diabetes medications were excluded from PP population.

- The Safety population consisted of all randomized subjects who received at least one dose of their randomized treatment and had at least one post-treatment visit evaluating safety.

Treatment protocol

This was a Phase 3, multicenter, randomized, double-blind, placebo-controlled, parallel-group study to evaluate the efficacy, safety, and tolerability of D-tagatose.

Subjects were screened for eligibility using physical examination and clinical laboratory tests. The basic physical examination included physical measurements, general examination by observation (inspection), palpation, percussion, auscultation, blood pressure measure, and heart rate check. The clinical laboratory tests included: (1) hematology (hematocrit, hemoglobin, MCH, MCHC, MCV, total white blood cells, platelets, and differential); (2) clinical chemistry (sodium, chloride, potassium, carbon dioxide, BUN, uric acid, albumin, creatinine clearance, SGOT, SGPT, bilirubin (total and direct), phosphorus, calcium, alkaline phosphatase, total protein, and glucose (fasting); (3) HbA1c; (4) serum lipid profile including total cholesterol, HDL, LDL, and triglycerides; (5) urinalysis (appearance, volume, specific gravity, pH, glucose, protein, and microscopic evaluation of urinary sediment). Those determined to be provisionally eligible participated in an 8-week lead-in period prior to the start of the study during which diabetes education was provided and diet and exercise treatment stabilized.

Patients recorded their food intake and exercise in nutrition diaries. After the 8 week lead-in period, fasting (minimum of 8 hours) subjects returned to the study sites and underwent medical history review followed by baseline tests including ECG (or EKG), pregnancy test for females, and hematology, clinical chemistry, and urinalysis tests. The clinical laboratory tests were the same as those performed during the screening visit. Subjects continued on a weight-maintaining diet plus exercise under physician's recommendation. In addition, subjects received their randomized study treatment and detailed instructions about its use. A total of 494 subjects were randomized into the study. Randomization was stratified according to screening HbA1c values (<7.5% and ≥7.5%) to achieve a balanced distribution of subjects across two arms (treatment and placebo).

The treatment period consisted of 12 monthly visits, the first (Visit 2, designated month 0 in figures) of which was used to gather the baseline data for the efficacy and safety parameters and also included the first distribution of test and placebo treatments. Baseline was defined as the last available value before the first randomized treatment. Subsequent visits occurred monthly and were of two types: (1) Supply Visits and (2) Supply and Procedures Visits. HbA1c was monitored at baseline (Visit 2) and every 2 months thereafter as were the secondary end-point parameters (described below). Efficacy analyses were conducted on data from 2, 6, and 10 months.

There were two treatment groups in this trial: drug (D-tagatose), and placebo (Splenda). The dose of D-tagatose was 15 g dissolved in 125 to 250 mL of water TID; the dose of placebo was 1.5 g dissolved in 125 to 250 mL of water TID. The placebo amounts were chosen to match sweetness for blinding. The powder packets were the same size and bore the same

labeling with the exception of the designation "Substance A" or Substance B". The entire drug/placebo solution was consumed prior to each main meal (breakfast, lunch, dinner). If severe gastrointestinal (GI) effects were seen, the tagatose dosage was reduced to 10 g TID temporarily and further reduced to 5 g TID if severe GI side effects were not resolved within 24 hours. The dosage of D-tagatose would then be increased to 10 g TID and further increased to 15 g TID when patients had adapted to the treatment (*i.e.*, GI effects reduced to mild). The placebo dosage would be reduced in a similar fashion if severe GI effects were seen. That is, the dose could be reduced to 1.0 g TID temporarily and then further reduced to 0.5 g TID if severe GI side effects were not resolved within 24 hours. The dosage of placebo was then to be increased to 1.0 g TID and further increased to 1.5 g TID when patients had adapted to the treatment (i.e., GI effects reduced to mild).

Removal of Subjects from Therapy or Assessment

Premature end-point—A premature end-point of the efficacy analysis for the trial occurred when additional antidiabetic medications were prescribed to a patient at the sole discretion of the their primary care physician. However, patients were advised to continue in the trial if an antidiabetic medication was added after the start of the trial, even though the HbA1c data were not used in efficacy analyses at the time points subsequent to the initiation of the additional medication(s). The continuation of the trial was solely for the safety analysis of D-tagatose and patient's data were withdrawn from the efficacy analysis.

Treatment failures—Subjects were categorized as treatment failures if both of the following criteria were met:

1. HbA1c change of +1.0% from baseline at any clinic visit or an HbA1c ≥10% or required additional antidiabetic medication for glycemic control;
2. Received at least 80% of all study drug doses within the initial 3 month dosing period.

The need for additional antidiabetic medication was determined by an elevated fasting blood glucose value (>240 mg/dL) not secondary to a readily identified illness or pharmacological treatment. Investigators were to withdraw subjects from study treatment (and therefore the evaluable population for assessment of efficacy as measured by HbA1c) after additional antidiabetic medication had been prescribed. However, subjects were advised to continue the rest of the trial procedures for the assessment of safety parameters.

Criteria for Evaluation

Efficacy—The primary efficacy variable was the change from baseline in HbA1c level when measured at pre-specified time points. Efficacy analyses were conducted on data from 2, 6 and 10 months (visit 4, 8 and 12). Statistical analyses were conducted on the 2-month, 6-month and 10-month data separately. The primary efficacy endpoint was at 2 months in the Intent-To-Treat population. Secondary efficacy endpoints for change in HbA1c were at 6 and 10 months. Additional secondary efficacy end-points were measured at the same time points and included changes from baseline in (1) fasting blood glucose, (2) serum insulin

levels, (3) lipid profiles (LDL, triglycerides, total cholesterol, HDL), and (4) BMI, as well as the proportion of subjects achieving HbA1c targets of <7%.

Safety—The safety parameters assessed for each subject included adverse events, serious adverse events, vital signs, results of physical exams, 12-lead ECG, and clinical laboratory tests (hematology, chemistry, and urinalysis).

Statistical Methods—Three analysis populations were evaluated: (1) The Intent-to-Treat (ITT) population, (2) the Per Protocol (PP) population, and (3) the Safety population. The ITT population was the focus of the efficacy analyses for regulatory purposes, with the PP population being used in supportive analyses. For the ITT population, missing data (including missing values at intermediate visits) were to be imputed from scheduled visits using the last-observation-carried-forward (LOCF) method.

The Safety population was used for analyses of all safety parameters. Secondary efficacy end-points were evaluated using the closed test procedure (in order to preserve the type I error rate, α, of 0.05). Accordingly, the analysis of end-points was conducted in the following order: (1) body mass index, (2) triglycerides, (3) LDL, (4) total cholesterol, (5) fasting blood glucose, (6) proportion of subjects with HbA1c concentration of <7%, (7) insulin, and (8) HDL. Analysis of continuous efficacy variables used mixed-model repeated-measures analysis of covariance controlling for covariates and stratification factors identified as significant in preliminary explorations. Categorical efficacy variables were analyzed using general estimating equation models.

RESULTS

Study population

There were 494 subjects randomized, 185 subjects in the US and 309 subjects in India. Of these, 480 were treated, 248 with placebo and 232 with D-tagatose (Table 1). The mean age of subjects in the ITT population was 52 with an age range between 22 and 74. The ITT population was approximately evenly divided between males and females and the racial distribution was 72% Asian, 12% Caucasian, 11% Latino and 5% Black, with approximately equivalent distributions in the D-tagatose and placebo groups. At baseline, approximately 90% of subjects in both treatment groups were controlling their diet and exercising in order to control their diabetes.

Efficacy Results

Primary Endpoint Results: Decrease in Hemoglobin A1c Level—The primary efficacy variable was the change from baseline in HbA1c level when measured at pre-specified time points. An efficacy analysis was conducted on data from 2, 6 and 10 months (Visits 4, 8 and 12). The primary efficacy end-point, set at the 2-month time point in the ITT population, showed a statistically significant difference ($p = 0.0198$) between D-tagatose and the placebo (Figure 1A). In the ITT population, the active treatment group, i.e., subjects who received D-tagatose, showed greater and statistically significant reductions in HbA1c levels at all post-baseline visits when compared to the placebo group (Figure 1B). The same mixed

model analysis conducted with the PP population data showed similar results insofar as the population receiving D-tagatose always showed greater decreases in HbA1c compared to placebo (Figure 1C). The differences in the PP population did not achieve statistical significance until month 6, however, but then remained significant throughout the remainder of the study.

Analysis of subgroups—In light of the above results, subgroup analyses were conducted to examine whether starting HbA1c levels (< 7.5% or ≥ 7.5%) influenced the effectiveness of D-tagatose. The results of these analyses for the ITT population (LOCF) are shown in Figure 2A and 2B. A significant difference between D-tagatose- and placebo-treated subjects was seen earlier in the subgroup entering with a baseline HbA1c < 7.5%., although both subgroups achieved greater changes from baseline than those observed in the placebo groups.

In the PP population the D-tagatose treatment for both subgroups demonstrated greater reductions in HbA1c compared to placebo (with the exception at 6 months in the ≥ 7.5% subgroup) (Figure 2C and 2D). However, a statistically significant difference between the D-tagatose and placebo-treatment groups was only achieved in the <7.5% subgroup (Figure 2C). Statistical significance between D-tagatose and placebo groups was achieved after 6 months of treatment in subjects with a baseline HbA1c of <7.5% (Figure 2C, p-values of 0.0497, 0.029, and 0.0121 after 6, 8, and 10 months of treatment, respectively), whereas statistical significance between D-tagatose and placebo groups was not achieved in patients with a higher baseline HbA1c at any time point (Figure 2D).

Further subgroup analyses were conducted to compare the ability of D-tagatose to effectively decrease HbA1c in the United States (US) population of subjects versus subjects of Indian origin. Interestingly, the effect on lowering HbA1c was more pronounced in US compared to Indian subjects (Figure 3). A subgroup analysis on the ITT US population (Figure 3A) showed a greater and statistically significant LS mean reduction in HbA1c in the D-tagatose groups than in the placebo group at all post-baseline visits: month 2 (reduction of 0.23 vs. an increase of 0.07, $\Delta = 0.3$ and $p = 0.0273$), month 4 (reduction of 0.15 vs. an increase of 0.28, $\Delta = 0.4$ and $p = 0.0011$), month 6 (reduction of 0.17 vs. an increase of 0.17, $\Delta = 0.3$ and $p = <0.0001$), month 8 (reduction of 0.10 vs. an increase of 0.29, $\Delta = 0.4$ and $p = <0.0001$), and month 10 (reduction of 0.07 vs. an increase of 0.37, $\Delta = 0.4$ and $p = <0.0001$). Analysis of the LOCF ITT data showed statistically significant reductions in the India population only at month 10 ($\Delta = 0.2$, $p = 0.0187$) (Figure 3B). PP analyses for the US population produced similar results with statistically significant differences being noted at all post-baseline time points (Figure 3C). The effect of D-tagatose in reducing HbA1c was evident by 2 months of treatment in the US subjects. However, the effect of D-tagatose in reducing HbA1c in the Indian subjects was not significantly different than placebo at any time point in the PP population (Figure 3D).

Proportion of Subjects Achieving HbA1c < 7%—The data from the ITT population with LOCF indicated that the D-tagatose group had a greater proportion of subjects achieving an HbA1c level of less than 7% at all post baseline time points compared to placebo, with the results being statistically significant at post-baseline months 6, 8 and 10

(Table 2). Thus, the percent of responders in the D-tagatose group was higher than in the placebo group at 6 months (38.37% vs 22.4%), 8 months (40.7% vs 25.68%) and 10 months (43.02% vs 26.23%, respectively). Similar results were observed in the analyses using the PP population except that in the latter case statistical significance was observed at months 6 and 10 (Table 3).

Secondary Endpoint Results: Changes in BMI, fasting blood glucose, insulin, blood lipids—D-tagatose was also evaluated for its placebo-controlled treatment effects on BMI, fasting blood glucose, insulin, blood cholesterol, and triglyceride levels. There was no observed effect of D-tagatose treatment on changes in body weight or BMI (body mass index) compared to placebo in either the ITT or PP populations.

For the ITT population better reductions in fasting blood glucose levels from baseline were observed in the D-tagatose group compared to the placebo group at all post baseline time points (Figure 4A), with the differences in LS mean being statistically significant for post-baseline month 6 and beyond, i.e., month 6 (reduction of 2.0 vs. an increase of 4.1, $\Delta = 6.0$ and $p = 0.0440$), month 8 (reduction of 0.44 vs. an increase of 2.5, Δ 2.9 and $p = 0.0340$), and month 10 (reduction of 0.25 vs. an increase of 6.6, $\Delta = 6.9$ and $p = 0.0079$). Reductions were also seen in PP population analyses; however, none were statistically significant (Figure 4B). Regardless of the statistically significant difference in fasting blood glucose between placebo and D-tagatose groups, there was no detectable consistent change in serum insulin concentrations in this clinical trial (data not shown).

Particularly interesting findings of this phase 3 clinical trial include the effect of D-tagatose to significantly reduce total cholesterol and LDL-cholesterol compared to placebo. The D-tagatose group (ITT population) showed better reductions in total cholesterol from baseline compared to placebo at all post baseline time points (Figure 5A), with the differences becoming statistically significant after 4 months of treatment. This effect was maintained for the duration of the trial (differences in LS means being statistically significant starting from month 4 5.4 to 5.3, month 6 (reductions of 6.9 3.8 to 3.7 and $p = 0.0217$), month 8 (reductions of 8.1 vs. 5.3, $\Delta = 2.8$ and $p = 0.0157$), and month 10 1.6 to 1.5. In the PP population analyses, reductions were also seen; however, none were statistically significant (Figure 5B).

Better reductions from baseline in LDL levels were observed in the D-tagatose-treated group (ITT population) than the placebo at post baseline time points (Figure 5C), with the differences in LS mean being at month 6 a reduction of 4.1 to 3.8; at month 8 a reduction of 3.1 to 3.0.; and at month 10 a reduction of 7.1 vs. 4.2, $\Delta = 2.9$ and $p = 0.0057$. Smaller reductions were seen in the PP population analyses and none were statistically significant (Figure 5D).

The results of analyses of serum HDL concentrations in the ITT population showed greater decreases in the D-tagatose group than in the placebo group with the differences being statistically significant at all post-treatment time points in the ITT population and at the month 4 time point in the PP population (Figure 5E and 5F). However, the mean HDL values always remained above the target value of 40 mg/dL, so it is unclear whether the

reduction in HDL concentrations observed in the D-tagatose group has any clinical significance.

Triglyceride levels showed no benefit from D-tagatose treatment, *i.e.*, the placebo group showed greater reductions in triglyceride levels than the D-tagatose group with the differences becoming statistically significant from month 8 onward (i.e., at month 8 a reduction of 12.7 vs. an increase of 1.46, $\Delta = 14.2$ and $p = 0.0301$; and at month 10 a reduction of 10.8 vs. an increase of 11.2, $\Delta = 22$ and $p = 0.0048$ (Figure 6A)). No statistically significant differences were seen in the PP population analyses (Figure 6B). Additionally, subgroup analyses for subjects who had baseline triglyceride levels 1) less than 200 mg/dL, 2) between 200 and 500 mg/dL, and 3) above 500 mg/dL, revealed no statistically significant differences between the D-tagatose and placebo treatment groups (data not shown).

Safety Results—D-tagatose was reasonably well tolerated by the subjects in this trial with most of the adverse events (AEs) experienced being mild to moderate in intensity. Adverse events in the D-tagatose treatment group were similar to that of the placebo group, with most instances noted as GI disturbances. Importantly, there were no reported episodes of hypoglycemia or pancreatitis.

The incidence of SAEs in the D-tagatose group was about one-half that seen in the placebo group. The incidences of AEs by system organ class were generally similar between the placebo and D-tagatose groups, the one exception being AEs in the gastrointestinal disorders class. Most of the subjects in both treatments experienced GI symptoms; however, the incidence was higher in the D-tagatose group, and the GI AEs were more frequently severe in the D-tagatose group. There were no remarkable effects in other safety parameters with very few clinically significant changes in safety laboratory values, ECG parameters, vital signs or physical examinations.

DISCUSSION

This trial met its primary objective of demonstrating that D-tagatose was effective at reducing the HbA1c level when administered for two months at doses of 15 g TID. The secondary end-point of significant reductions in the the HbA1c level at six and ten months were also met. Mixed model analyses using the ITT population and LOCF imputation of missing values indicated significantly greater reductions in the mean HbA1c levels in the D-tagatose group than in the placebo group at all post-baseline visits. The same mixed model analysis conducted with the PP population data showed similar results insofar as the population receiving D-tagatose always showed greater decreases in HbA1c compared to placebo. Additionally, subgroup analyses were done for the primary end-point on different subgroups of subjects to see if the reduction in HbA1c is significantly different between the treatment groups in the different subgroups *i.e.*

1) subjects whose baseline HbA1c level was less than 7.5%, 2) subjects whose baseline HbA1c level was greater than or equal to 7.5%, 3) subjects who were randomized in India, and 4) subjects who were randomized in US. In these analyses the results were generally

agreement with the primary results although in the India subgroup the results were statistically significant at far fewer time points. Additionally it was observed that a greater proportion of subjects in the D-tagatose group had achieved HbA1c targets of <7%; this difference was also found to be statistically significant for the majority of the post-baseline time points. Regardless of the subgroup analyzed, the effect of D-tagatose to decrease HbA1c levels was robust and could be seen with differing analytical approaches. These results demonstrate the ability of D-tagatose to effectively aid in the control of blood sugar levels for type 2 diabetics and suggest D-tagatose as a potential drug therapy in this area.

Statistically significant differences were observed between the D-tagatose and placebo treatment groups for the secondary end-points LDL, total cholesterol, and fasting blood glucose when examining the ITT population. Reductions were also seen in PP population analyses however, none were statistically significant. Numerous studies have demonstrated that increased total cholesterol and particularly elevated LDL-cholesterol are associated with amplified risk for the development of cardiovascular disease. Thus, this finding is especially important for type 2 diabetic patients that may also battle dyslipidemia and/or the metabolic syndrome [20].

There were also two end-points, triglycerides and HDL levels, where the D-tagatose treatment produced greater and statistically significant increases in the case of triglycerides and decreases in the case of HDL levels compared to the placebo group in the ITT population. The PP population however, showed no significant difference in triglycerides or HDL between placebo and D-tagatose. Of note, a recent study by Donner et al. [13] demonstrated a striking and significant effect of D-tagatose to increase HDL from a baseline level of 30.5 ± 15.8 to 41.7 ± 12.1 mg/dL ($p<0.001$) after 10 months of treatment in 6 subjects who did not take lipid medications during the study. Beginning HDL levels in the current study were higher (mean placebo 45.8, D-tagatose 44.5 in ITT and mean placebo 45.5, D-tagatose 45.5 in PP) than in the Donner study. The differences in results between these studies could be from this initial HDL level discrepancy, and any elevations in HDL that D-tagatose is able to provide may only manifest if HDL levels are below recommended values. Consequently, further studies are needed to elucidate the direct effects of D-tagatose on HDL cholesterol levels. HDL-cholesterol levels are inversely related to risk of developing cardiovascular disease.

There was no observed effect of D-tagatose treatment on changes in body weight or BMI (body mass index) compared to placebo in either the ITT or PP populations. In contrast to these findings, previous studies in humans [13] and rodents [12] have demonstrated significant weight loss and a reduction in body weight gain with D-tagatose, respectively. The mean body weight of subjects (both D-tagatose and placebo groups) included in this phase 3 clinical trial at baseline was 73.8 kg, or 162 pounds; with an average BMI of 28.3 mg/m^2 (overweight category). However, in previous studies which have demonstrated an effect of D-tagatose to promote weight loss, the mean body weight of all subjects at baseline was much higher (mean body weight at baseline ~109 kg, or 240 pounds [13]. Thus, it is plausible that the subject population within this phase 3 clinical trial was not adequately overweight to demonstrate an effect of D-tagatose to promote weight loss.

D-tagatose appeared to be reasonably well tolerated by the subjects in this trial with most of the AEs experienced being mild to moderate in intensity. The incidence of SAEs in the D-tagatose group was about one-half that seen in the placebo group. The incidences of AEs by system organ class were generally similar between the placebo and D-tagatose group, the one exception being AEs in the gastrointestinal disorders class. Most of the subjects in both treatments experienced GI symptoms; however, the incidence was generally higher in the D-tagatose group, and the GI AEs were more frequently severe in the D-tagatose group. There were no remarkable effects in other safety parameters with very few clinically significant changes in safety laboratory values, ECG parameters, vital signs or physical examinations. Previous single-dose and repeated-dose studies in healthy and diabetic human subjects showed that the predominant adverse effects associated with consumption of high levels D-tagatose were gastrointestinal disturbances attributed to osmotic effects from incompletely absorbed tagatose [13–18, 21–23]. At single doses of up to 25 g D-tagatose per meal, flatulence was generally the only side effect, with nausea, borborygmi (i.e., rumbling or gurgling noises, colic, and laxation noted at higher doses). Such effects are also commonly associated with excessive consumption of other poorly digestible carbohydrates.

In summary, results of this phase 3 clinical trial demonstrate an effect of D-tagatose to significantly decrease HbA1c levels over time compared to placebo. The longer subjects remained on the treatment, the further HbA1c was reduced. D-tagatose treatment also reduced fasting blood glucose levels and total and LDL-cholesterol concentrations.

CONCLUSIONS

- D-tagatose was effective at lowering HbA1c levels when administered at a daily dose of 15 g in 125–250 mL of water three times per day just prior to meals.
- Unlike many other diabetes drugs, the longer a patient is on D-tagatose therapy in compliance with instructions, the better the efficacy.
- The effect on HbA1c levels was robust and could be demonstrated in both subgroups, various subpopulations, and with differing analytical approaches.
- D-tagatose appeared to be reasonably well tolerated with most of the AEs experienced in both treatment groups being mild or moderate in severity.

Bloomgarden et al.'s [24] analysis of 61 studies shows that the degree of decrease in HbA1c produced by drugs for treating type 2 diabetes is dependent on the baseline HbA1c; the higher the baseline the greater the decrease. When the baseline HbA1c was <8.0%, the reduction from active therapy is only 0.1–0.2% greater than in the control group. When the HbA1c was >8.0 to 11.8 the reduction in HbA1c was between −0.6 and −1.2%, and the greater the HbA1c, the greater the reduction overall.

In this study the mean HbA1c baselines were 7.6% ± 0.75 (± Stdev) and 7.4% ± 0.59 for placebo and D-tagatose groups respectively in the ITT population, and 7.7% ± 0.64 and 7.5% ± 0.53 for placebo and D-tagatose groups respectively in the PP population. Based on Bloomgarden's study (see Table 1 in Bloomgarden), other drugs show an average of only

half the efficacy of D-tagatose in similar patients. Overall results of this phase 3 clinical trial suggest a strong potential for D-tagatose as an adjunct in the management of type 2 diabetes.

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ABBREVIATIONS

AE	adverse event
BMI	body mass index
BUN	blood urea nitrogen
ECG (or EKG)	electrocardiogram
GI	gastrointestinal
GRAS	generally recognized as safe
HbA1c	glycosylated hemoglobin A1c
HDL	high-density lipoprotein
ITT	intent-to-treat
LDL	low density lipoprotein
LSMean	least squares mean
LOCF	Last Observation Carried Forward
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
PP	per protocol
SAE	serious adverse event
SEM	standard error of the mean
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamic pyruvic transaminase
TID	three times daily
VLDL	very low density lipoprotein.

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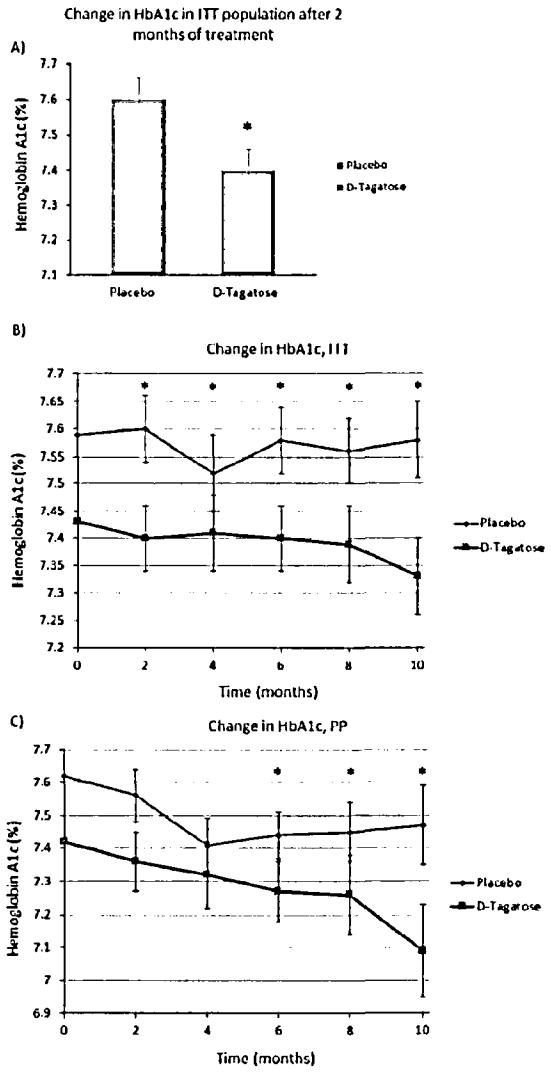

Figure 1.

(A) Change in HbA1c in ITT population after 2 months of treatment. The primary efficacy endpoint set at 2 months in the ITT population showed a significant difference between placebo and D-tagatose groups (p=0.0198) (placebo n=182, D-tagatose n=172). (B) Change in HbA1c in ITT population. Significant differences were seen at 2 (p=0.0198), 4 (p=0.0160), 6 (p=0.0015), 8 (p=0.0002), and 10 months (p=<0.0001). (C) Change in HbA1c in PP population. Significant differences were seen at 6 (p=0.0343), 8 (p=0.0148), and 10 months (p=0.0021). Zero time points plotted as means, remaining time points plotted as least squares means ±SEM.

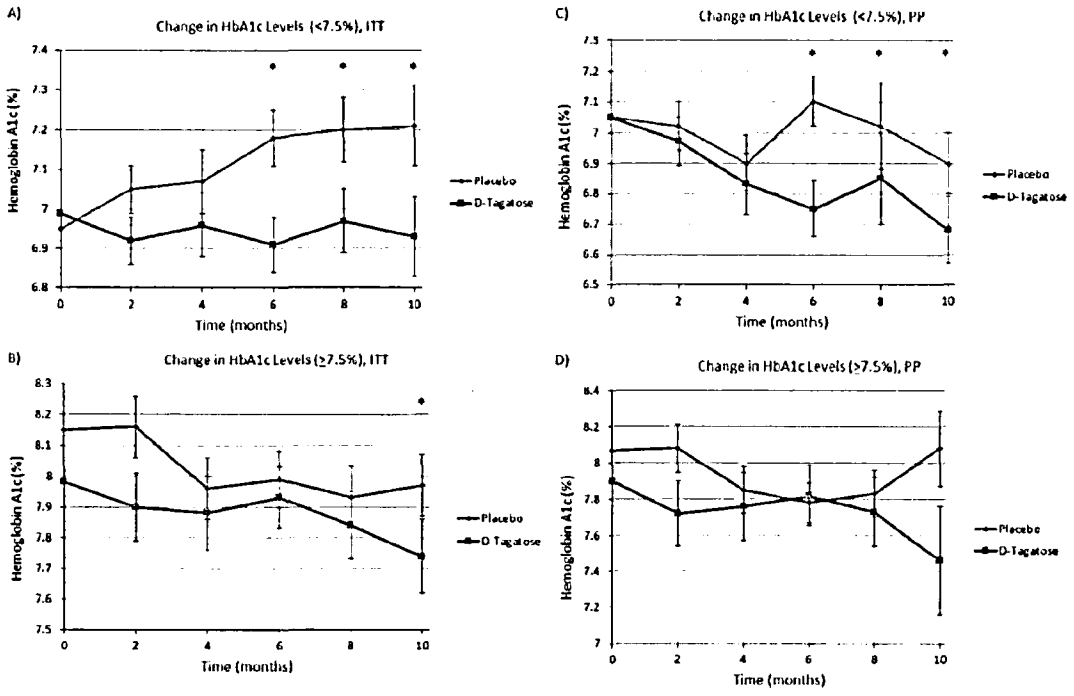

Figure 2.

(A) Results of analyses for the ITT subgroup with starting HbA1c baseline < 7.5%. Significant differences between D-tagatose and placebo were seen at 6 (p=0.0030), 8 (p=0.0004), and 10 months (p=<0.0001). (B) Change in HbA1c in ITT subgroup with baseline >7.5%. A significant difference between D-tagatose and placebo was seen at 10 months (p=0.0277). (C) Change in HbA1c in PP subgroup with baseline HbA1c <7.5%, (D) Change in HbA1c in PP subgroup with baseline >7.5%. Zero time points plotted as means, remaining time points plotted as least squares means ±SEM.

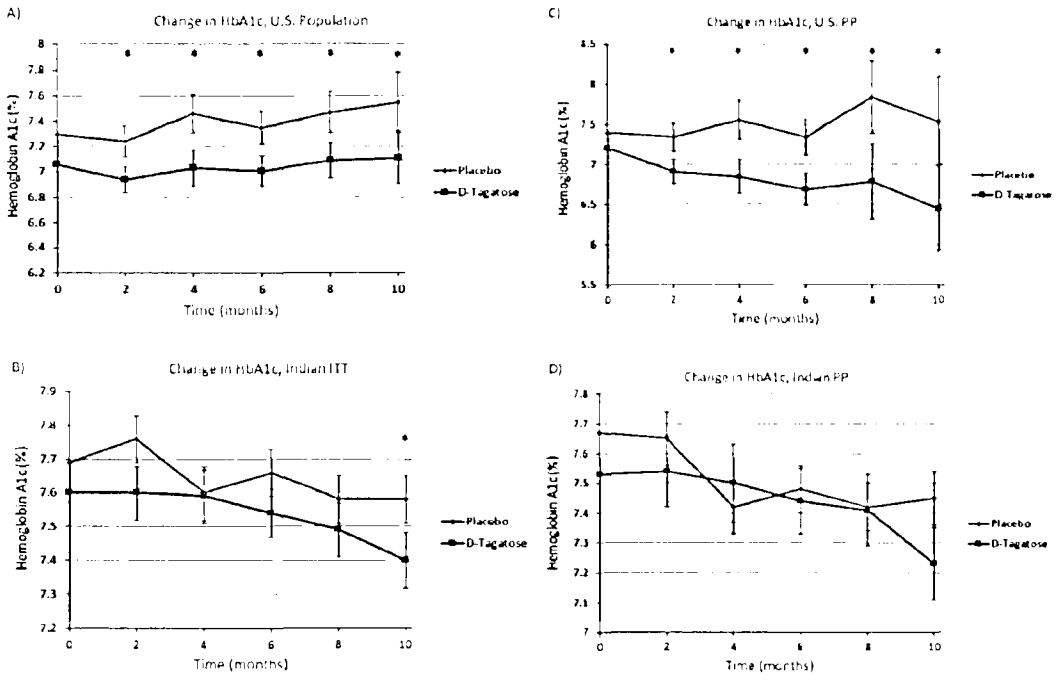

Figure 3.

(A) Change in HbA1c U.S. subjects, ITT population. (B) Change in HbA1c, Indian subjects, ITT population. (C) Change in HbA1c U.S. subjects, PP population. p-values for 2, 4, 6, 8, and 10 months of treatment are 0.0435, 0.0016, <0.0001, <0.0001, and <0.0001, respectively. (D) Change in HbA1c, Indian subjects, PP population. Zero time points plotted as means, remaining time points plotted as least squares means ±SEM.

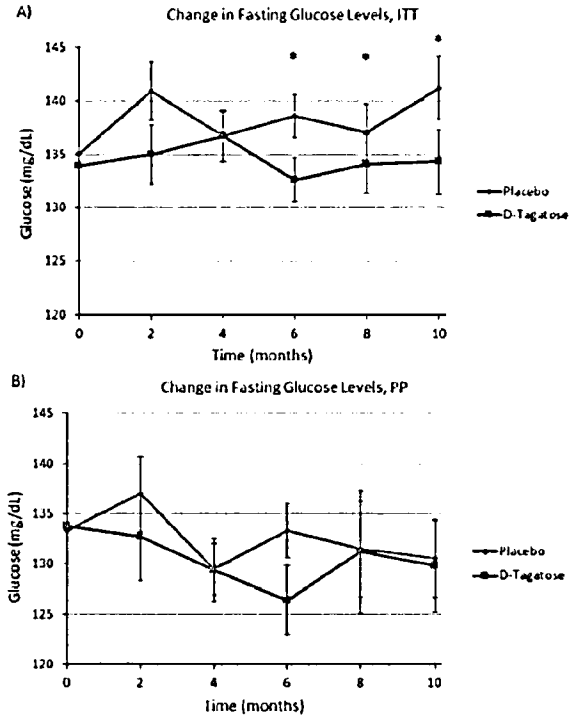

Figure 4.

(A) Change in fasting blood glucose, ITT population. D-tagatose significantly decreased fasting blood glucose compared to placebo after 6 months of treatment. (B) Change in fasting blood glucose levels, PP population. No significant difference between placebo and D-tagatose groups was observed at any time point. Zero time points plotted as means, remaining time points plotted as least squares means ±SEM.

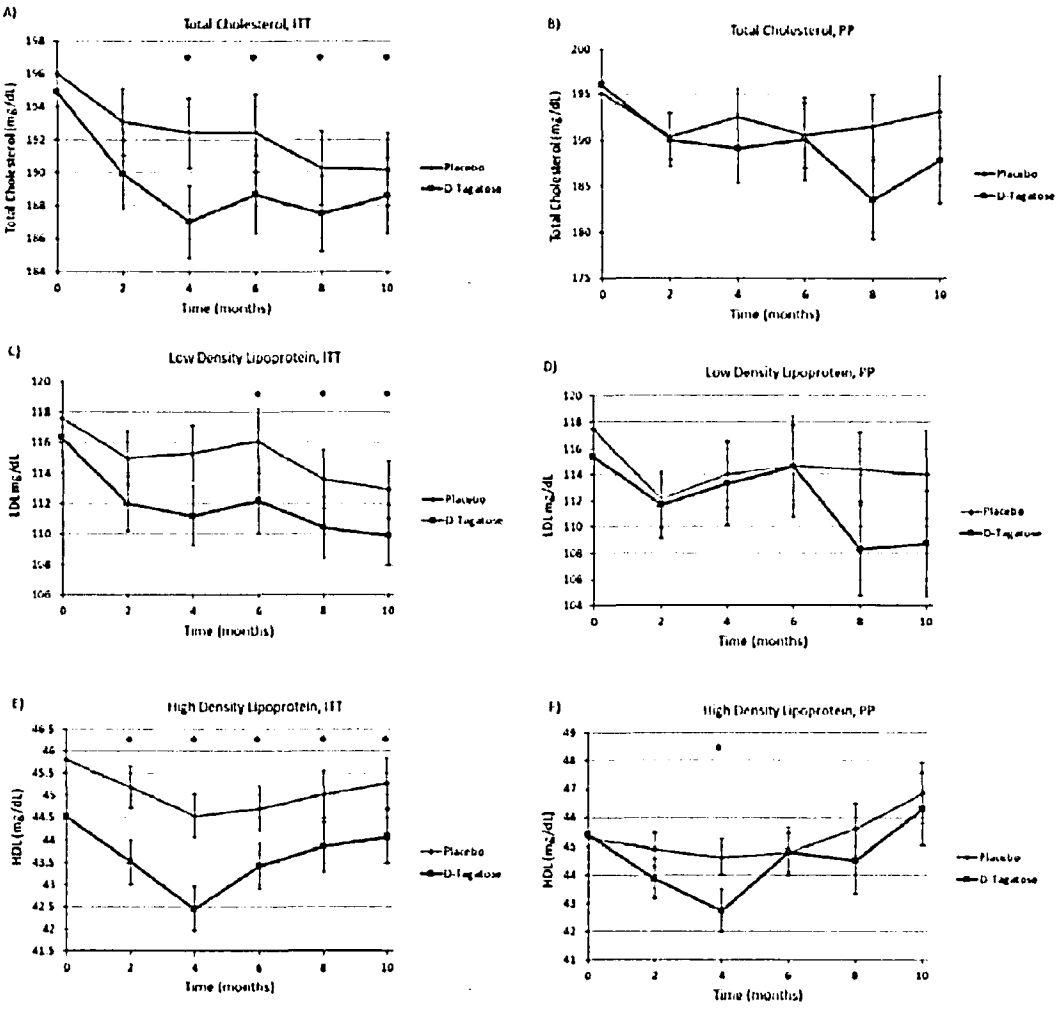

Figure 5.

(A) Total cholesterol, ITT population. (B) Total cholesterol, PP population. (C) LDL, ITT population. (D) LDL, PP population. (E) HDL, ITT population. Significant differences between D-tagatose and placebo were seen at 2 (p=0.0126), 4 (p=0.00040, 6 (p=0.0002), 8 (p=0.0003), and 10 months (p=0.0008). (F) HDL, PP population. The only significant difference was at 4 months (p=0.0397). Zero time points plotted as means, remaining time points plotted as least squares means ±SEM.

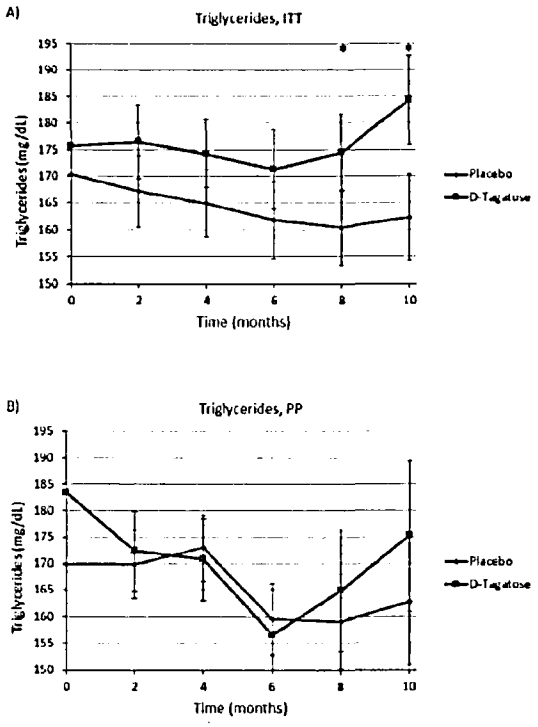

Figure 6.

Serum triglycerides. (A) ITT population. (B) PP population. Zero time points plotted as means, remaining time points plotted as least squares means ±SEM.

Table 1

Analysis Population.

Population	Placebo N =253 n (%)	D-tagatose N = 241 n (%)	Total N = 494 n (%)
ITT	184 (72.7%)	172 (71.4%)	356 (72.1%)
Per Protocol	119 (47.0%)	85 (35.3%)	204 (41.3%)
Safety	207 (81.8%)	185 (76.8%)	392 (79.4%)

Table 2

Proportion of Subjects Achieving HbA1c <7%, ITT Population.

Month	Statistic	Placebo	D-tagatose	Delta	P-values
2	Responder Non-responder	49 (26.92%) 133 (73.08%)	65(38.01%) 106 (61.99%)	11.09	0.0913
4	Responder Non-responder	53 (28.96%) 104(60.47%)	68 (39.53%) 84 (59.57%)	10.57	0.1580
6	Responder Non-responder	41 (22.40%) 106(61.63%)	66 (38.37%) 76 (60.32%)	15.97	0.0048
8	Responder Non-responder	47 (25.68%) 136 (74.32%)	70 (40.70%) 102 (59.30%)	15.01	0.0133
10	Responder Non-responder	48 (26.23%) 135 (73.77%)	74 (43.02%) 98 (56.98%)	16.79	0.0052

Table 3

Proportion of Subjects Achieving HbA1c <7%, PP Population.

Month	Statistic	Placebo	D-tagatose	Delta	*P*-values
2	Responder Non-responder	31(26.50%) 86(73.50%)	32(38.10%) 52(61.90%)	11.06	0.3787
4	Responder Non-responder	31(30.39%) 71(69.61%)	29(43.94%) 37(56.06%)	13.55	0.5260
6	Responder Non-responder	19(21.11%) 71(78.89%)	27(48.21%) 29(51.79%)	27.10	0.0073
8	Responder Non-responder	21(28.00%) 54(72.00%)	21(46.67%) 24(53.33%)	18.67	0.1052
10	Non-responder Responder	14(25.93%) 40(74.07%)	22(57.89%) 16(42.11%)	31.97	0.0044

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Contains Nonbinding Recommendations

The Declaration of Allulose and Calories from Allulose on Nutrition and Supplement Facts Labels: Guidance for Industry

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U.S. Department of Health and Human Services
Food and Drug Administration
Center for Food Safety and Applied Nutrition

October 2020

Contains Nonbinding Recommendations

Table of Contents

Contains Nonbinding Recommendations

The Declaration of Allulose and Calories from Allulose on Nutrition and Supplement Facts Labels: Guidance for Industry[1]

This guidance represents the current thinking of the Food and Drug Administration (FDA or we) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff responsible for this guidance as listed on the title page.

I. Introduction

This guidance provides our current view on the declaration of allulose on Nutrition and Supplement Facts labels, as well as on the caloric content of allulose. This guidance also advises manufacturers of our intent to exercise enforcement discretion for the exclusion of allulose from the amount of "Total Sugars" and "Added Sugars" declared on the label and the use of a general factor of 0.4 calories per gram (kcal/g)[2] for allulose when determining "Calories" on the Nutrition and Supplement Facts labels pending review of the issues in a rulemaking.

In arriving at our decision to consider the exercise of enforcement discretion, we considered data and information provided in citizen petitions and in comments to the draft guidance, and other information submitted to us. We received a citizen petition requesting the exemption of allulose from the declaration of "Total Carbohydrate," "Total Sugars," and "Added Sugars" on the Nutrition Facts label (Docket Number FDA-2015-P-1201) (Ref. 1). We also considered a citizen petition requesting the use of a general factor for caloric value of allulose of 0.4 calories per gram (kcal/g) (Docket Number FDA-2016-P-2030) (Ref. 3), and another citizen petition requesting the use of a general factor for the caloric value of allulose of 0.2 kcal/g (Docket

[1] This guidance has been prepared by the Office of Nutrition and Food Labeling in the Center for Food Safety and Applied Nutrition at the U.S. Food and Drug Administration.

[2] Calories per gram or kilocalories per gram (kcal/g) has been defined as the amount of heat energy needed to raise the temperature of a kilogram of water 1°C (determined at 14.5°C to 15.5°C) and is the unit that has been traditionally used for expressing the energy value of foods (Ref. 2).

Number FDA-2017-P-1463) (Ref. 4). We considered these citizen petitions (as well as comments submitted to the dockets for these citizen petitions) in conjunction with comments to a proposed rule titled "Food Labeling: Revision of the Nutrition and Supplement Facts Labels" (79 FR 11880, March 3, 2014) ("the proposed rule"). We also conducted an independent review of the scientific evidence related to the cariogenic potential, metabolism, and caloric value of, and glycemic response to, allulose.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe our current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in FDA guidances means that something is suggested or recommended, but not required.

II. Background

Allulose or D-psicose (D-ribo-2-hexulose) is a monosaccharide with a molecular formula of $C_6H_{12}O_6$, and is an epimer of D-fructose. D-psicose occurs naturally and is present in small amounts in wheat, fruits (e.g., raisins, dried figs) and in many other foods (e.g. molasses, maple syrup, and brown sugar). It can also be synthesized from fructose by enzymatic epimerization which converts fructose to D-psicose. FDA has not objected to three Generally Recognized as Safe (GRAS) notifications regarding the use of this substance as a sugar substitute in certain conventional foods and beverages (GRAS Notification Number (GRN) 400 (Ref. 5), GRN 498 (Ref. 6), and GRN 693 (Ref. 7)).

On April 10, 2015, we received a citizen petition from Tate & Lyle Ingredients America LLC (Tate & Lyle) (Docket Number FDA-2015-P-1201) (Ref. 1) requesting that we amend 21 CFR 101.9 to exempt allulose from being included as a carbohydrate, sugar,[3] or added sugar on the Nutrition Facts label for foods and beverages. The citizen petition provided data and other information suggesting that allulose is different from other sugars in that it is not metabolized by the human body, has negligible calories (0.2 kcal/g or less), and does not contribute to increases in blood glucose or insulin levels.[4]

The citizen petition was submitted after the comment period closed for the proposed rule. The proposed rule did not specifically address allulose; however, in a supplemental proposed rule (80 FR 44303, July 27, 2015), we proposed (among other things) to establish a Daily Reference Value (DRV) of 10 percent of total energy intake from added sugars and to require the declaration of the percent Daily Value (DV) for added sugars on the label.

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[3] The petition was submitted at a time when the Nutrition Facts label used the term "Sugars." On May 27, 2016, FDA issued a final rule to amend the Nutrition Facts and Supplement Facts label regulations. The final rule, among other things, replaced the term "Sugars" with "Total Sugars." See 81 FR 33742 and 21 CFR 101.9(c)(6)(ii) (requiring declaration of "Total Sugars" on the Nutrition Facts label). Therefore, in this guidance, we refer to "Total Sugars."

[4] The petition did not include data or other information on the association between consumption of allulose and dental caries.

While neither the proposed rule nor the supplemental proposed rule addressed the labeling of allulose, we received comments on allulose (81 FR 33742 at 33795-96). According to the comments, allulose is approximately 70 percent as sweet as sucrose and contributes less than 0.2 kcal/g to the diet. The comments stated that allulose is added to foods and beverages as a partial replacement for sugars or high-fructose corn syrup because of its low, near zero, calorie content and other organoleptic properties (e.g., mouthfeel, texture, etc.). One comment said we should not include allulose in the declaration for "Total Carbohydrate" and "Added Sugars" because of the properties mentioned above. In contrast, another comment said we should include allulose in the declaration of "Total Carbohydrate" for nutrition labeling purposes, but not in the declaration of "Total Sugars" or "Added Sugars" because allulose does not have the metabolic properties of fructose or other sugars and does not contribute calories or raise blood sugar levels like other sugars. The comments said that, upon ingestion, approximately 70 percent of allulose is absorbed in the small intestine, passes into the bloodstream and is then excreted intact in the urine, without significant metabolism; the other 30 percent, which is not absorbed in the small intestine, is transported to the large intestine where it is not fermented and is then excreted intact.

On May 27, 2016, we issued a final rule titled "Food Labeling: Revision of the Nutrition and Supplement Facts Labels" (81 FR 33742) ("final rule"). In the final rule, we stated that we needed additional time to fully consider the information provided in the citizen petition and the comments we received to the proposed rule (81 FR 33742 at 33796). Therefore, we did not reach a decision as to whether allulose should be excluded from the declaration of "Total Carbohydrate," "Total Sugars," and/or "Added Sugars." We stated that allulose, as a monosaccharide, must be included in the amount of the declaration of "Total Carbohydrate," "Total Sugars," and "Added Sugars" pending any future rulemaking that would otherwise consider excluding allulose from the declaration.

After we issued the final rule, we received two citizen petitions regarding allulose. One citizen petition, submitted by the Food Lawyers (Docket Number FDA-2016-P-2030) (Ref. 3), requested, among other things, that we amend 21 CFR 101.9(c)(1)(i) by adding the following:

> "(G) Using the following general factor for caloric value of Allulose (also known as D-Allulose, D-psicose): Allulose — 0.4 calories per gram."

The citizen petition included information to support the caloric value of 0.4 kcal/g for allulose.

Another citizen petition, submitted by Tate & Lyle (Docket Number FDA-2017-P-1463) (Ref. 4), requested, among other things, that we amend 21 CFR 101.9(c)(1)(i) to include the following in a new section (G):

> "(G) Using the following general factor for caloric value of Allulose (also known as, D-allulose, D-psicose): Allulose – 0.2 calories per gram."

The petition included information to support the caloric value of 0.2 kcal/g for allulose.

In a comment to the Tate & Lyle citizen petition, the Food Lawyers stated that they fully supported a caloric value for allulose of 0.2 kcal/g.

On August 13, 2018, Tate & Lyle submitted the results from a clinical trial that assessed the impact of allulose on dental plaque pH. On August 22, 2018, Matsutani Chemical Industry Co. LTD submitted the results of a clinical trial that assessed the impact of allulose consumption on dental plaque pH as well as a copy of U.S. Patent No. 8,496,915, including an *in vitro* study that examined the final medium pH and the growth of a bacterial strain that causes dental caries when cultured with allulose.[5] We considered these studies in our review of the scientific evidence related to the cariogenic potential of allulose (Ref. 8).

In the *Federal Register* of April 18, 2019 (84 FR 16272), we announced the availability of a draft guidance titled "The Declaration of Allulose and Calories from Allulose on Nutrition and Supplement Facts Labels: Guidance for Industry," in which we announced our tentative views on the declaration of allulose on Nutrition and Supplement Facts labels and caloric content of allulose. The draft guidance also advised manufacturers of our intent to exercise enforcement discretion for the exclusion of allulose from the amount of "Total Sugars" and "Added Sugars" declared on the label, and for use of a general factor of 0.4 kcal/g for allulose when determining "Calories" on the Nutrition and Supplement Facts labels pending review of the issues in a rulemaking. We have considered comments to the draft guidance, and we have made modifications, where appropriate, that are reflected in this final guidance. We also conducted an independent review of the scientific evidence on the cariogenic potential, metabolism, and caloric value of, and glycemic response to, allulose (Ref. 8).

III. Declaration of Allulose on the Nutrition and Supplement Facts Labels

1. FDA's Consideration of the Caloric Value of Allulose

We did not determine a specific caloric value for allulose in the final rule. Therefore, under 21 CFR 101.9(c)(1)(B), a caloric value of 4 kcal/g must currently be used for allulose because it is a carbohydrate. The citizen petitions from the Food Lawyers (Ref. 3) and from Tate & Lyle (Ref. 4) identified two human studies on the metabolism of allulose. Our search of the literature did not reveal any additional human studies that determined a caloric value of allulose. We provide a summary of the studies in our memorandum to the file (Ref. 8).

The two citizen petitions (Refs. 4 and 3) provided data supporting the use of 0.2 kcal/g for allulose and 0.4 kcal/g for allulose.[6] Based on our review of the evidence (Ref. 8), we conclude that the caloric contribution of allulose is very low (i.e., no more than 0.4 kcal/g) because the majority of allulose is excreted intact in the urine, and because allulose is poorly fermented in the gut. We have limited evidence from human studies, using different methodologies, upon which to determine the caloric value of allulose. Therefore, we intend to exercise enforcement discretion for the use of a caloric value of 0.4 kcal/g for allulose because, based on the range of data we have, such a caloric value would not underestimate the caloric contribution. We intend

[5] Both the Tate & Lyle and Matsutani Chemical Industry submissions were in support of the 2015 Tate & Lyle citizen petition. The submissions can be found in docket FDA-2015-P-1201.

[6] One petitioner subsequently supported a caloric value of 0.2 kcal/g rather than 0.4 kcal/g.

to exercise enforcement discretion with respect to the use of a caloric value of 0.4 kcal/g when calculating declarations on Nutrition and Supplement Facts labels pending rulemaking to consider amending 21 CFR 101.9(c)(1)(i) regarding the use of a general factor for the caloric value of allulose.

2. FDA's Consideration of Allulose as a Carbohydrate

Total Carbohydrate content is determined for the purposes of nutrition labeling by subtraction of the sum of the crude protein, total fat, moisture, and ash from the total weight of the food (21 CFR 101.9(c)(6)). The calculation method is described in A.L. Merrill and B.K. Watt, "Energy Value of Foods--Basis and Derivation" (Ref. 2). "Carbohydrate" as a class captures a variety of substances ranging from mono and disaccharides to numerous types of non-digestible carbohydrates, some of which are dietary fibers. As previously mentioned, allulose is a monosaccharide. It has the chemical composition of a carbohydrate and is captured under the method for determination of Total Carbohydrate in 21 CFR 101.9(c)(6).

The 2015 Tate & Lyle citizen petition (Ref. 1) suggested that allulose should not be included in the Total Carbohydrate definition because, in part, it does not raise blood glucose levels, and thus could be confusing for individuals who are interested in monitoring their blood glucose levels, such as diabetics. We have traditionally based our decision on labeling of carbohydrates on whether a substance is chemically a carbohydrate (79 FR 11880 at 11900). As we explained in the final rule, we had invited comment on and considered:

> whether carbohydrates should be classified and declared in nutrition labeling based on their chemical definition (which is the current method) or on their physiological effect (e.g., attenuation of blood sugar or laxation), and whether additional types of carbohydrates (e.g., starch) should be listed separately on the Nutrition Facts label. We explained that carbohydrates include starch, sugars, sugar alcohols, and dietary fibers and that different carbohydrates have different physiological effects [79 FR 11879 at 11901]. Within the different types of carbohydrate (i.e., starch, sugars, sugar alcohols, and dietary fibers), too, specific carbohydrates may have different physiological effects (e.g., different types of dietary fibers) making it difficult to apply a definition that is based on physiological effects across a category of carbohydrates. Furthermore, analytical methods for measuring different types of carbohydrates are based on chemical structure rather than physiological effect. Given the various components of total carbohydrate and different types of physiological effects of each, we decided not to change our provisions for the classification or declaration of carbohydrates specified in 21 CFR 101.9(c)(6).

81 FR 33742 at 33795.

In summary, we considered whether a physiological effect-based definition was appropriate for total carbohydrates and determined that it was not because of the wide variety of effects of different types of carbohydrates.

Furthermore, as discussed in the final rule, the information in the Nutrition Facts label is not targeted to individuals with acute or chronic disease (e.g., diabetes, chronic kidney disease, or cardiovascular disease). The nutrient declaration and percent Daily Values on the label are to help consumers make more informed choices to consume a healthy diet and not intended for the clinical management of an existing disease (81 FR 33742 at 33750). Inclusion of allulose in the declaration of "Total Carbohydrate" on Nutrition and Supplement Facts label is also consistent with how we have considered the declaration of other substances that are captured under the method that is currently used for the determination of "Total Carbohydrate" on the label, including those that provide few or no calories, such as sugar alcohols and dietary fibers.

Therefore, because allulose is a carbohydrate, it is captured under the calculation method for "Total Carbohydrate" described in 21 CFR 101.9(c)(6), like a number of other substances without significant caloric contribution. We have determined that our existing definition of carbohydrate is the most appropriate and allulose must be included in the amount of "Total Carbohydrate" declared on the label under the existing regulations.

3. FDA's Consideration of Allulose as a Sugar

Total Sugars are defined in 21 CFR 101.9(c)(6)(ii) as the sum of all free mono-and disaccharides (such as glucose, fructose, lactose, and sucrose). Allulose is a monosaccharide that is an epimer of D-fructose. In the final rule, we said that consumption of sugars continues to be associated with an increased risk of dental caries; thus, the "Total Sugars" declaration continues to be necessary to assist consumers in maintaining healthy dietary practices (81 FR 33742 at 33798).

The 2015 Tate & Lyle citizen petition (Ref. 1) suggested that allulose should not be included in the "Total Sugars" declaration because it is not metabolized like a sugar, does not raise blood glucose levels, and inclusion in the "Total Sugars" declaration would be confusing to consumers, particularly those who monitor their blood glucose levels. A summary of the evidence related to the cariogenic potential, metabolism, and caloric value of, and glycemic response to, allulose is provided in a memorandum to the file (Ref. 8).

We have traditionally determined what is captured under the "Total Sugars" declaration on the label by chemical structure. Due to advances in food technology, novel sugars are now available that are not metabolized and that do not contribute 4 kcal/g to the diet like other traditional sugars. Consequently, we need to consider how information about sugars like allulose should be captured on the label.

Our current thinking is that, consistent with the goal of section 403(q) of the Federal Food, Drug, and Cosmetic Act for the nutrient declarations to assist consumers in maintaining healthy dietary practices, we should consider not only the chemical structure of sugars, but also other evidence, including their association with dental caries and how they are metabolized in the body (e.g., caloric contribution and their effect on blood glucose and insulin levels), when determining whether a sugar should be included in the declaration of "Total Sugars" on the label.

Sugars are known to be associated with an increased risk of dental caries (21 CFR 101.80). Sugars that are metabolized by oral bacteria produce polymers that adhere to the tooth surface

(i.e., dental plaque) and generate acids resulting in a decrease in the pH (Ref. 9) of dental plaque. The low pH provides an environment that allows for decalcification of the teeth (e.g., solubilization of calcium from dental enamel), increasing the risk of dental caries (or tooth decay) (Ref. 10). The "Total Sugars" declaration provides consumers with information that they can use to evaluate the contribution of sugars in their diet to the risk of dental caries. We therefore believe that evidence related to the association between consumption of a sugar and dental caries is an important consideration when determining whether the amount of a particular sugar in a serving of a product should be excluded from the "Total Sugars" declaration on the label.

Mono and disaccharides typically provide 4 kcal/g (Ref. 2). If a consumer wishes to determine how many calories are contributed by sugars in their diet, they can multiply the grams of Total Sugars per serving by 4 kcal/g. Current dietary recommendations suggest that Americans should limit their consumption of calories from sugars, and particularly added sugars, and stay within calorie limits (Ref. 11). As a result, manufacturers are substituting sugars that provide much less than 4 kcal/g, such as allulose, for sugars that provide 4 kcal/g in an effort to reduce caloric content. Including sugars that contribute much less than 4 kcal/g to the diet in the "Total Sugars" declaration would not accurately reflect the caloric contribution to the diet of sugars like allulose that contain much less than 4 kcal/g. Therefore, we consider the caloric contribution of a sugar to be an important consideration when determining if the sugar should be excluded from the amount of the "Total Sugars" declaration.

During digestion, disaccharides are hydrolyzed into two monosaccharide units in the upper small intestine. Primarily, the body breaks sugars down into glucose, which is used as energy by cells in the body or stored as glycogen (Ref. 12). Consuming sugar increases circulating glucose in the blood stream. The presence of glucose in the blood triggers the release of the hormone insulin from the pancreas. Insulin stimulates the uptake of glucose by muscle and adipose tissue. Therefore, when traditional sugars like glucose, fructose, lactose, and sucrose are consumed, there is a rise in blood glucose and insulin levels (Ref. 12). The "Total Sugars" declaration provides consumers with information that they can use to determine whether a product contains sugars that are likely to cause an increase in circulating blood glucose and insulin levels. Some consumers expect that when they eat sugars, the result will be an increase in blood glucose and insulin levels. Therefore, we consider a sugar's effect on blood glucose and insulin levels to be important considerations when determining whether a sugar should be excluded from the "Total Sugars" declaration.

Allulose, like other non-cariogenic carbohydrate sweeteners listed in 21 CFR 101.80(c)(2)(ii), does not result in a decrease in dental plaque pH below 5.7, which is associated with decalcification of the dental enamel (Ref. 10). Therefore, given the low cariogenic potential of allulose, we conclude that allulose does not promote dental caries. Furthermore, based on our review of the evidence, we conclude that allulose, once ingested, is rapidly absorbed (within 1 hour) and cleared from plasma in 24 hours, and 70% of orally consumed allulose is eliminated

intact in urine and feces within 48 hours (Ref. 8).[7] Allulose produces only a negligible increase in glycemic and insulinemic responses and is not readily fermented in the large intestine, providing no more than 0.4 kcal/g (Ref. 8). Based on the totality of the available evidence from which scientific conclusions can be drawn, allulose does not promote dental caries and is virtually unmetabolized in the human body (Ref. 8).

As previously discussed, allulose does not result in a decrease in the dental plaque pH below 5.7, at which decalcification of dental enamel may begin, and thus, does not promote dental caries. It provides much less than 4 kcal/g. Additionally, the consumption of allulose produces only a negligible increase in glycemic and insulinemic responses. Therefore, we intend to exercise enforcement discretion with respect to the exclusion of allulose from the amount of "Total Sugars" declared on the label pending future rulemaking regarding amending the definition of "Total Sugars."

Finally, we note that allulose must be declared in the ingredient statement in accordance with 21 CFR 101.4 if it is present in a product so that consumers can determine when it is an ingredient in a food.

4. FDA's Consideration of Allulose as an Added Sugar

Added sugars are sugars that are either added during the processing of foods, or are packaged as such, and include sugars (free, mono- and disaccharides), sugars from syrups and honey, and sugars from concentrated fruit or vegetable juices that are in excess of what would be expected from the same volume of 100 percent fruit or vegetable juice of the same type.

As previously discussed, we did not decide whether allulose should be excluded from the amount of "Added Sugars" declared on the label in the final rule and stated that allulose must be included in the declaration of "Added Sugars" pending any future rulemaking regarding excluding allulose from the declaration.

A summary of the evidence related to the metabolism of allulose is provided in our memorandum to the file (Ref. 8). Based on our review of the scientific evidence, we conclude that allulose is virtually unmetabolized in the human body. Based on this, as well as the evidence regarding caloric value and dental caries, as previously discussed, we intend to exercise enforcement discretion if allulose is not included in the amount of "Total Sugars" declared on the label. The "Added Sugars" declaration is a subset of the "Total Sugars" declaration. Applying similar logic, through the exercise of our enforcement discretion, we consider that allulose should not be included in the "Added Sugars" declaration, including the %DV declaration. Furthermore, we note that, based on information about usage levels provided in GRAS notices (Refs. 5-7), we expect that the caloric contribution of allulose will be insignificant in most cases and will substantially reduce the amount of total calories and calories from added sugars in products where it replaces those added sugars.

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[7] In this mass-balance study, 70.4% of the ^{14}C radiotracer orally administered to seven subjects was eliminated as intact allulose in urine and feces, while 1.5 % was identified as glucose and fructose, 11.7% were not identified (unknown), and the remaining radioactivity was lost during the process (Ref. 8).

Therefore, we intend to exercise enforcement discretion with respect to the exclusion of allulose from the gram amount of and the %DV for "Added Sugars" declared on Nutrition and Supplement Facts labels, pending future rulemaking.

We again note that allulose must be declared in the ingredient statement in accordance with § 101.4 if it is present in a product so that consumers can determine when it is an ingredient in a food.

IV. Paperwork Reduction Act of 1995

This guidance contains no collection of information. Therefore, clearance by the Office of Management and Budget (OMB) under the Paperwork Reduction Act of 1995 (PRA) (44 U.S.C. 3501-3521) is not required.

However, this guidance refers to previously approved FDA collections of information. These collections of information are subject to review by OMB under the PRA. The collections of information in 21 CFR part 101 have been approved under OMB control number 0910-0381.

V. References

The following references marked with an asterisk (*) are on display in the Dockets Management Staff, Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. You may see them at that location between 9 a.m. and 4 p.m., Monday through Friday. They also are available electronically at https://www.regulations.gov. References without asterisks are not on public display at https://www.regulations.gov because they have copyright restriction. Some may be available at the website address, if listed. References without asterisks are available for viewing only at the Dockets Management Staff. FDA has verified the website addresses, as of the date this document publishes in the *Federal Register*, but websites are subject to change over time.

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Food labeling: health claims; D-tagatose and dental caries. Final rule

Food and Drug Administration, HHS

- PMID: 12848171

Abstract

The Food and Drug Administration (FDA) is adopting as a final rule, without change, the provisions of the interim final rule that amended the regulation authorizing a health claim on sugar alcohols and dental caries, i.e., tooth decay, to include the sugar D-tagatose as a substance eligible for the dental caries health claim. FDA is taking this action to complete the rulemaking initiated with the interim final rule.

nutrients

MDPI

Article

Alteration of Microbiome Profile by D-Allulose in Amelioration of High-Fat-Diet-Induced Obesity in Mice

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Abstract: Recently, there has been a global shift in diet towards an increased intake of energy-dense foods that are high in sugars. D-allulose has received attention as a sugar substitute and has been reported as one of the anti-obesity food components; however, its correlation with the intestinal microbial community is not yet completely understood. Thirty-six C57BL/6J mice were divided in to four dietary groups and fed a normal diet (ND), a high-fat diet (HFD, 20% fat, 1% cholesterol, w/w), and a HFD with 5% erythritol (ERY) and D-allulose (ALL) supplement for 16 weeks. A pair-feeding approach was used so that all groups receiving the high-fat diet would have the same calorie intake. As a result, body weight and body fat mass in the ALL group were significantly decreased toward the level of the normal group with a simultaneous decrease in plasma leptin and resistin. Fecal short-chain fatty acid (SCFA) production analysis revealed that ALL induced elevated total SCFA production compared to the other groups. Also, ALL supplement induced the change in the microbial community that could be responsible for improving the obesity based on 16S rRNA gene sequence analysis, and ALL significantly increased the energy expenditure in Day(6a.m to 6pm). Taken together, our findings suggest that 5% dietary ALL led to an improvement in HFD-induced obesity by altering the microbiome community.

Keywords: D-allulose; obesity; metagenomics; microbiome; sugar substitute

1. Introduction

Obesity is a serious global health issue that, in combination with other risk factors, is increasing the prevalence of metabolic diseases [1–5]. Besides fat, sugar is one of the dietary factors responsible for obesity in modern society [4,5]. Among the different types of sugar, high fructose intake is particularly problematic because, unlike glucose, fructose does not stimulate insulin secretion from pancreatic β-cells or circulating leptin levels and fails to stimulate satiety signaling [6–8]. Persistent dysregulation of food intake and energy homeostasis due to reduced insulin and leptin signals can accelerate energy intake, weight gain, and obesity [9].

Numerous studies targeting the prevention and treatment of obesity have incorporated prebiotics, probiotics, or synbiotics as co-adjuvants [10–14]. The basis for this approach is the experimental

evidence showing that modifying the gut microbiome in rodents ameliorates insulin sensitivity and decreases body weight and fat mass [12–14]. Furthermore, there is mounting evidence that the gut microbiota plays an essential role in energy harvesting and host metabolism, suggesting a link between the gut microbiota composition and metabolic diseases, such as obesity and type 2 diabetes [12,15,16]. The production of short-chain fatty acids (SCFAs) through non-digestible carbohydrates is one of the ways in which the microbiome regulates the energy expenditure (EE) and metabolism within the host [17,18]. These findings support the idea that prebiotics, probiotics, or synbiotics may ameliorate obesity through the modulation of the gut microbiome.

D-allulose, a C-3 epimer of D-fructose, is a sugar substitute, which has 70% of the sweetness of sucrose but almost zero calories and is rarely found in nature [19]. It is only present in small quantities in commercial mixtures of D-glucose and D-fructose obtained from the hydrolysis of sucrose or the isomerization of D-glucose. Several studies have provided preliminary evidence on the impact of D-allulose on lipid metabolism in animal and human models [20–22]. However, the mechanism underlying the microbial action of D-allulose is still not clear. In our past study with a diet-induced obesity (DIO) mouse model, D-allulose supplementation suppressed lipid absorption in the small intestine and increased the fecal lipid contents [22]. Thus, we assumed that D-allulose would improve DIO by altering the gut microbiome profile due to the gut bacteria and diet interactions, and then we performed an animal feeding study and evaluated both the biochemical composition of the microbiome by differentially abundant genera [23–25].

2. Methods

2.1. Animals and Diets

A total of 40 male C57BL/6J mice (4 weeks old) were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). The animals were maintained in a room with a controlled temperature (20–23 °C) and lighting (12/12 h light–dark cycle) and fed a pelletized commercial non-purified diet for 1 week after arrival. The mice were then randomly divided into four groups (n = 9) and fed the respective experimental diets for 16 weeks, as shown in Table 1: normal diet control (ND, American Institute of Nutrition AIN-76 semi-synthetic diet); high-fat diet control (HFD, 20% fat plus 1% cholesterol based on the AIN-76 diet); 5% erythritol (ERY, 5% erythritol substituted for sucrose in HFD, w/w), and 5% D-allulose (ALL, 5% D-allulose substituted for sucrose in HFD, w/w). D-allulose was purchased from Sigma–Aldrich (St. Louis, MO, USA). The HFD was formulated to provide 39.5% of the total energy from fat, by replacing carbohydrate energy with lard and corn oil, and had the same amounts of vitamins and minerals per kilojoule as the ND. The ALL group was fed the D-allulose diet. The HFD and ERY groups were fed iso-caloric diets based on the energy intake of the ALL group in a pair-fed manner. The mice had free access to distilled water during the experimental period. Food intake was recorded daily and body weight was monitored every 2 weeks. The SYRCLE's risk of bias was performed as described in the Supplementary Table S1. All animal procedures were approved by the Ethics Committee for Animal Studies at Kyungpook National University, Daegu, Republic of Korea (Approval No. KNU-2016-130).

Table 1. Composition of experimental diets (% of diet, w/w).

Ingredient (g)	ND	HFD	ERY	ALL
Casein	200.00	200.00	200.00	200.00
D,L-Methionine	3.00	3.00	3.00	3.00
Corn Starch	150.00	111.00	111.00	111.00
Sucrose	500.00	370.00	320.00	320.00
Cellulose Powder	50.00	50.00	50.00	50.00
Corn Oil	50.00	30.00	30.00	30.00
Lard	-	170.00	170.00	170.00
Mineral Mix (AIN-76) [1]	35.00	42.00	42.00	42.00
Vitamin Mix (AIN-76) [2]	10.00	12.00	12.00	12.00
Choline Bitartrate	2.00	2.00	2.00	2.00
Cholesterol	-	10.00	10.00	10.00
tert-Butylhydroquinone	0.01	0.04	0.04	0.04
D-Allulose				50.00
Erythritol			50.00	
Total (g)	1000.0	1000.0	1000.0	1000.0
Calorie (kcal/kg)	3902	4584	4384	4384
Calorie (kcal/g)	3.902	4.584	4.384	4.384

ND, normal diet (AIN-76); HFD, high-fat diet (AIN-76, 20% fat, 1% cholesterol); ERY (HFD + 5% erythritol); ALL, (HFD + 5% D-allulose). [1] Mineral mix (AIN-76) (g/kg): calcium phosphate, 500; sodium chloride, 74; potassium citrate, 2220; potassium sulfate, 52; magnesium oxide, 24; manganous carbonate, 3.5; ferric citrate, 6; zinc carbonate, 1.6; cupric carbonate, 0.3; potassium iodate, 0.01; sodium selenite, 0.01; chromium potassium sulfate, 0.55; sucrose 118.03. [2] Vitamin mix (AIN-76) (g/kg): thiamin HCL, 0.6; riboflavin, 0.6; pyridoxine HCL, 0.7; nicotinic acid, 0.003; D-calcium pantothenate, 0.0016; folate, 0.2; D-biotin, 0.02; cyanocobalamin (vitamin B12), 0.001; retinyl palmitate premix, 0.8; DL-α-tocopheryl acetate, premix, 20; cholecalciferol (vitamin D3), 0.0025; menaquinone (vitamin K), 0.05; antioxidant, 0.01; sucrose, finely powdered, 972.8.

2.2. *Plasma Lipid Profile Analysis*

The plasma-free fatty acid, apolipoprotein A-I (ApoA-1), and apolipoprotein B (ApoB) levels were measured using a Nittobo enzymatic kit (Nittobo Medical Co., Tokyo, Japan). The plasma HDL-cholesterol (HDL-C), triglyceride (TG), and total cholesterol (total-C) levels were determined using commercially available enzymatic kits (Asan, Seoul, South Korea).

2.3. *Plasma Adipokines Measurement*

Plasma leptin, resistin, and adiponectin were determined using a multiplex detection kit from Bio-Rad (Hercules, CA, USA). All of the samples were assayed in duplicate and analyzed using a Luminex®200 LabMAP™ system (Luminex, Austin, TX, USA). The data analyses were performed using the Bio-Plex Manager software version 4.1.1 (Bio-Rad).

2.4. *EE and Whole-Body Oxygen Consumption*

EE was measured using an indirect calorimeter (Oxylet; Panlab, Cornella, Spain). The mice were placed into individual metabolic chambers at 25 °C, with free access to food and water. O_2 and CO_2 analyzers were calibrated with highly purified gas standards. Whole-body oxygen consumption (Vo_2) and carbon dioxide production (Vco_2) were recorded at 3-min intervals using a computer-assisted data acquisition program (Chart 5.2; AD Instruments, Sydney, Australia) over a 24-h period. The data were averaged for each mouse. EE was calculated as follows: EE (kcal/day/ [kg of body weight × 0.75]) = $Vo_2 \times 1.44 \times [3.815 + (1.232 \times Vo_2/Vco_2)]$.

2.5. Histopathology Analysis

Liver and epididymal white adipose tissue (eWAT) were removed from mice and fixed in a buffer solution of 10% formalin. All fixed tissues were processed routinely for paraffin embedding. Sections of 4 mm in thickness were prepared and stained with hematoxylin–eosin and Masson's trichrome (MT). The stained areas were viewed under an optical microscope (Nikon, Tokyo, Japan) with 200× magnification.

2.6. SCFA Analysis

All SCFAs were extracted from 50 mg of mice fecal sample in 500 µL of extraction buffer (0.1 M oxalic acid, 40 mM NaN_3) with 0.3 g of zirconium beads. After bead-beating the sample for 3 min, the samples were incubated in a shaking incubator at room temperature for 1 h and centrifuged at 16,000× *g* at 25 °C for 5 min. according to Schwiertz et al. [25]. The supernatant of the centrifuged samples was collected and transferred to a transparent gas chromatography vial for analysis using a Shimadzu GC2010 (Agilent, Santa Clara, CA, USA) equipped with a flame ionization detector (FID) and HP INNOWax column (30 m × 32 mm). The operating conditions were as follows: column heated from 100 to 180 °C at the rate of 25 °C/min; splitter temperature 260 °C; FID temperature, 260 °C; pressure, 27.1 psi.

2.7. Microbiota Analysis

Fecal DNA was extracted using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany). Briefly, 50 mg of feces was mixed vigorously with zircon/silica beads (BioSpec Products, Oklahoma, USA) for 3 min, and the remaining protocol proceeded as described in the manufacturer's instructions. Microbial community profiling by 16S ribosomal RNA amplicon sequencing of 5 ng/µL of fecal DNA was performed using primers specifically targeting the 16S rRNA V3/V4 region. The PCR products were purified by PCR clean-up, according to the Illumina (San Diego, CA, USA) 16S Metagenomic Sequencing Library Preparation protocol. Dual indices were attached to the samples by using the Nextera XT Index Kit (FC-131-1002), and the indexed samples were sequenced on an Illumina MiSeq system. The raw data were visualized and analyzed for beta diversity, alpha diversity, and taxonomy using QIIME [26].

2.8. Statistical Analysis

All phenotype data are presented as the mean ± SE or SD. Statistical analysis was performed using SPSS software version 11.0 (SPSS, Inc., Chicago, IL, USA). The statistical differences between the ND and HFD results were determined by the Student's *t*-test. One-way ANOVA was performed to compare the HFD groups, and Turkey's multiple-range test was performed when significant differences were identified between the groups ($p < 0.05$).

3. Results

3.1. Anti-obesity Effects of D-Allulose Supplement in DIO Mice

At the end of the experimental period, HFD-fed mice were drastically increased in body weight relative to the ND group (Figure 1A). However, the ALL-fed animals had a lower body weight than the HFD and ERY groups due to the suppression of the total body weight gain (Figure 1A,B), which was similar among the animals fed HFD, ERY, and ND. Muscle weight increased after D-allulose supplementation for 16 weeks, whereas the spleen weight was comparable before and after the ALL diet (Figure 1C,D). In the comparison of fat mass, all types of adipocyte tissue in the HFD group weighed significantly more than in the ND group (Figure 1E) and, except for mesenteric fat, were dramatically lower in the ALL group than HFD group. These results are consistent with the epididymal morphology (Figure 1F).

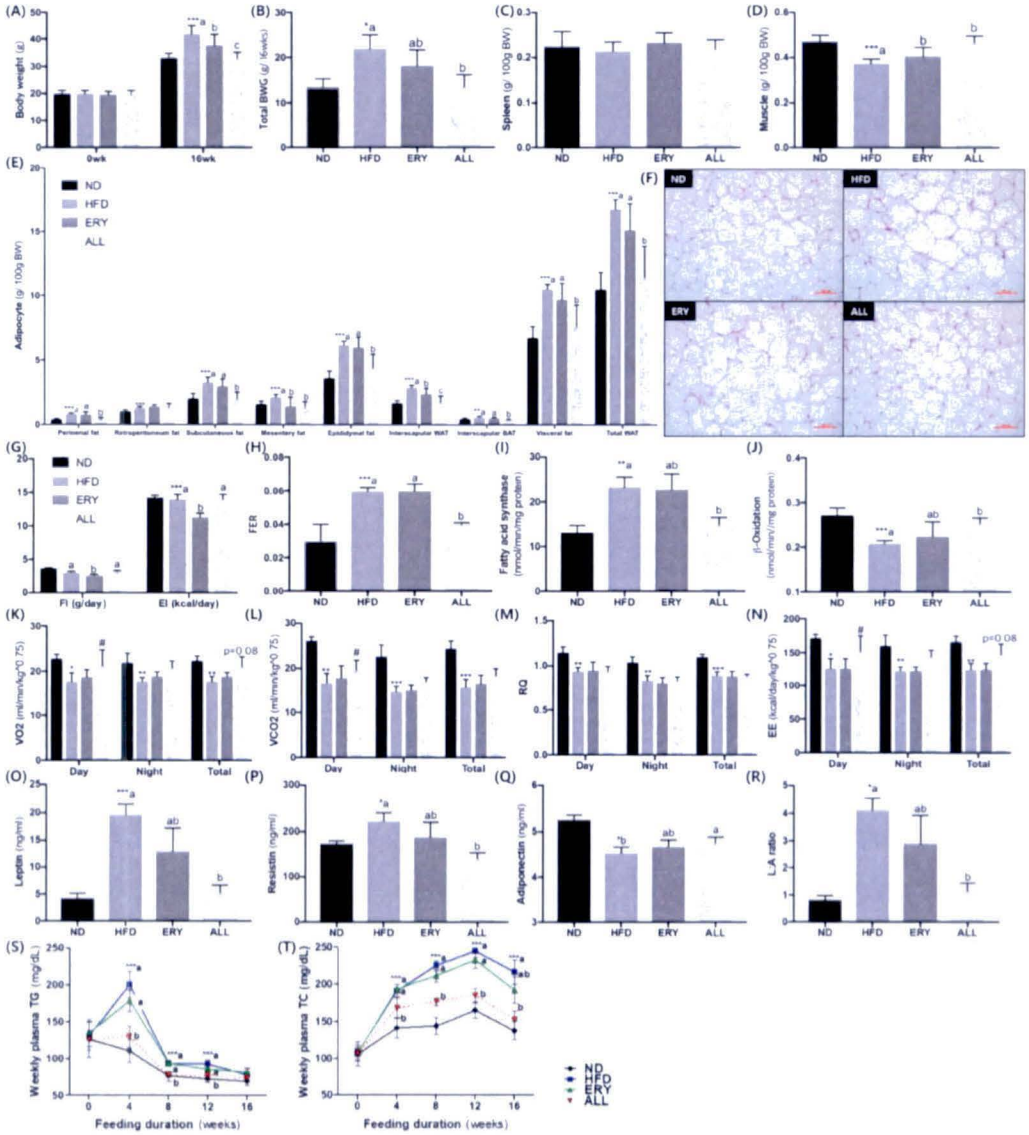

Figure 1. Effects of D-allulose supplementation for 16 weeks on; (**A**) Body weight; (**B**) Body weight gain; (**C**,**D**) Spleen weight and muscle weight; (**E**,**F**) Adipocyte weight and morphology; (**G**) Food intake and energy intake; (**H**) Food efficiency ratio; (**I**,**J**) Adipocyte enzyme activity-related lipid metabolism; (**K**–**N**) Metabolic rate, (**O**–**R**) adipokine levels and weekly plasma (**S**) TG and (**T**) TC. Data are mean ± SE; ND, normal diet (AIN-76); HFD, high-fat diet (AIN-76, 20% fat, 1% cholesterol); ALL, (HFD + 5% D-allulose). Mean values are significantly different for ND vs HFD, *** $p < 0.001$; Mean values are significantly different for HFD vs ALL, $^{\#\#}$ $p < 0.01$, $^{\#\#\#}$ $p < 0.001$. (B) BWG, body weight gain; (C) FI, food intake; EI, energy intake; (D) FER, food efficiency ratio = body weight gain/food intake; (E) WAT, white adipose tissue; BAT, brown adipose tissue; (F) hematoxylin and eosin (H&E)-stained transverse section of epididymal fat and liver; Representative photomicrographs of the liver are shown at ×200 magnification; (G) FI, food intake; EI, energy intake; (K) VO_2, oxygen consumption, (L), VCO_2, carbon dioxide production, (M) RQ, respiratory quotient, (N)EE, energy expenditure, (R) L:A ratio, Leptin:adiponectin ratio, (S) TG, triglyceride, (T) TC, total cholesterol.

The food intake and energy intake were significantly lower in the ERY group than the HFD and ALL groups, while the food efficiency ratio in the ALL group was significantly lower compared with the HFD and ERY groups (Figure 1G,H). Regarding the plasma lipid profiles among the groups of animals, the HFD group presented markedly elevated total-C, HDL-C, and non-HDL-C. In contrast, these three variables, as well as ApoA-1, were significantly decreased in the ALL group (Table 2). In eWAT, the activity of fatty acid synthase and β-oxidation activity was significantly decreased and increased, respectively, in the ALL group (Figure 1J). The metabolic rate measurements of VO_2, VCO_2, and EE per day were significantly increased in the ALL group relative to the HFD group (Figure 1K–N). While the adiponectin level was significantly higher in the ALL group, the leptin and resistin levels and the leptin–adiponectin ratio were significantly decreased compared with the HFD group.

We measured the concentration of TG and TC in collected plasma from the tail vein every 4 weeks during the experiment, and these results are shown in Figure 2S–T. From the fourth week to the 12th week of the D-allulose supplement, plasma TG concentration was significantly decreased in the ALL group compared to the other HFD groups. From the fourth week of the D-allulose supplement to the end of the experiment, plasma TC concentration was significantly decreased in ALL group compared to HFD group. The lipid profiles with plasma obtained after a 24-hour fast at sacrifice was showed in Table 2. Total-C, HDL-C, nonHDL-C and apo A-I levels were significantly decreased in ALL group compared to HFD group. Also, the FFA, TG and apo B levels and HTR showed a decreasing tendency in the ALL group.

Table 2. Effect of D-allulose supplementations for 16 weeks on plasma lipid profiles in C57BL/6J mice fed a high-fat diet.

	ND	HFD	ERY	ALL
FFA (mmol/L)	0.20 ± 0.00	0.20 ± 0.00	0.19 ± 0.00	0.16 ± 0.00
TG (mg/dL)	0.86 ± 0.31	0.86 ± 0.09	0.93 ± 0.31	0.81 ± 0.16
Total-C (mmol/L)	3.56 ± 0.32	5.17 ± 1.21 ^{***a}	4.48 ± 0.80 ^{ab}	3.94 ± 0.32 ^{b}
HDL-C (mmol/L)	0.95 ± 0.15	1.46 ± 0.34 ^{***a}	1.15 ± 0.21 ^{a}	0.88 ± 0.13 ^{b}
Non-HDL-C (mmol/L)	2.61 ± 0.38	3.72 ± 0.93 ^{***a}	3.33 ± 0.69 ^{a}	2.65 ± 0.39 ^{b}
ApoA-I (mg/dL)	31.52 ± 1.88	30.25 ± 0.90 ^{a}	26.97 ± 1.18 ^{ab}	25.53 ± 1.56 ^{b}
ApoB (mg/dL)	6.03 ± 2.81	8.04 ± 2.92	6.51 ± 3.05	5.87 ± 2.64
ApoA-I/ApoB	6.54 ± 3.59	4.41 ± 2.18	5.02 ± 2.52	6.09 ± 4.71
HTR ^{1}	25.75 ± 4.39	28.32 ± 3.58	25.92 ± 4.46	22.30 ± 2.48
AI ^{2}	2.84 ± 0.72	2.58 ± 0.45	2.96 ± 0.69	3.54 ± 0.52

Data are mean ± SD. Significant differences between HFD vs ND are indicated; *** $p < 0.001$; $^{a, b}$ Mean not sharing a common letter are significantly different among the groups at $p < 0.05$. ND, normal diet (AIN-76); HFD, high-fat diet (AIN-76, 20% fat, 1% cholesterol); ALL, (HFD + 5% D-allulose). FFA, free fatty acid; TG, triglyceride; C, cholesterol; ApoA-I, apolipoprotein A-I; ApoB, apolipoprotein B; non-HDL-C = (Total-C) − (HDL-C); HTR ^{1}, (HDL-C/Total-C) × 100; AI ^{2}, atherogenic index = [(Total-C) − (HDL-C)]/HDL-C.

3.2. Suppression of Fatty Liver by D-Allulose Supplement in DIO Mice

The increased liver weight and hepatic lipid levels, including TG, fatty acids, and cholesterol, caused by HFD feeding, were significantly suppressed by ALL supplementation (Figure 2A). Moreover, the enzyme activities related to lipid metabolism, such as fatty acid synthase, β-oxidation, cholesterol acyltransferase, and 3-hydroxy-3-methylglutaryl-CoA reductase, were significantly decreased by D-allulose supplementation (Figure 2E–H). Consistent with these results, the ALL treatment reduced the accumulation of lipid droplets in the hepatic tissue (Figure 2I). MT staining of liver tissue revealed fibrotic stained a blue color in the HFD and ERY group, whereas it was absent in the ND and the ALL group. In particular, the ALL group showed a similar appearance to that of the ND group.

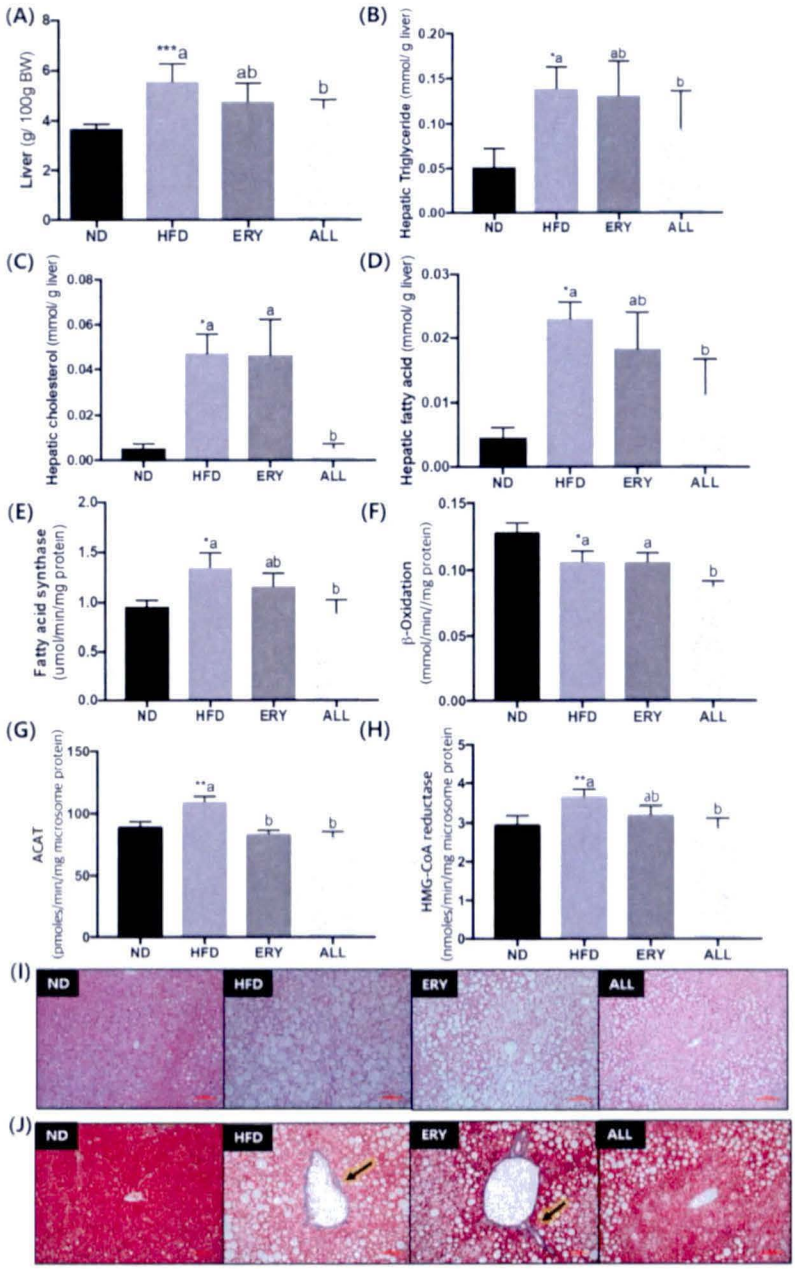

Figure 2. Effects of D-allulose supplementation for 16 weeks on; (**A**) Liver weight; (**B–D**) Hepatic lipid profiles; (**E**,**F**) Enzyme activities related to lipid metabolism; and (**I**) morphology. Data are mean ± SE; ND, normal diet (AIN-76); HFD, high-fat diet (AIN-76, 20% fat, 1% cholesterol); ALL, (HFD + 5% D-allulose). Mean values are significantly different for ND vs HFD, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; [a, b] Mean not sharing a common letter are significantly different among the groups at $p < 0.05$. (H) L: A ratio, leptin:adiponectin ratio; (I) Hematoxylin and eosin (H&E)-stained transverse section of epididymal fat and liver; Representative photomicrographs of the liver are shown at ×200 magnification; (J) Fibrillar collagens, primarily collagen I and III, are stained blue, as indicated by arrowheads; Representative photomicrographs of the liver are shown at ×200 magnification.

3.3. *Effects of D-Allulose on SCFA Production in DIO Mice*

The results of SCFA production are presented in Figure 3. Although the production of butyrate showed an increasing tendency in the ALL group, there was no significant difference in the SCFA production between the ALL and HFD groups.

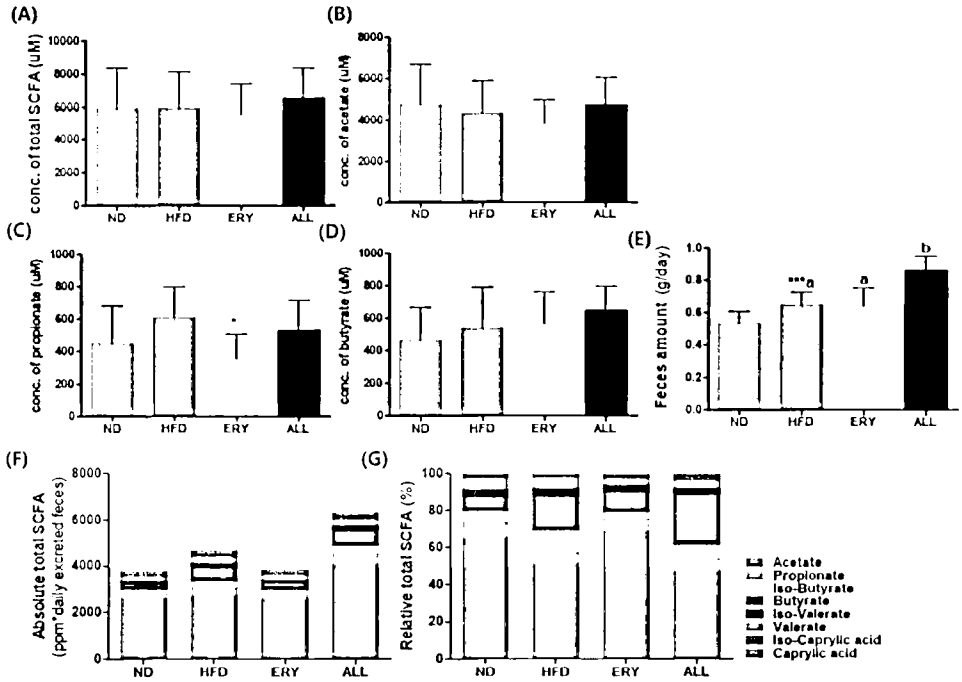

Figure 3. Effects of D-allulose on SCFA production. (**A**) Total SCFA production (**B**) Acetate; (**C**) Propionate; (**D**) Butyrate; (**E**) Daily fecal weight; (**F**) Absolute total SCFA adjusted with daily fecal weight (**G**) Relative total SCFA. Data are mean ± SE; ND, normal diet (AIN-76); HFD, high-fat diet (AIN-76, 20% fat, 1% cholesterol); ALL, (HFD + 5% D-allulose); SCFA, short-chain fatty acid.

3.4. *Effects of D-Allulose on Microbiome Modulation in DIO Mice*

The microbiome taxonomy result is shown in Figure 4A, B. At the genus level, there was a significant increase in *Lactobacillus*, *Coprococcus*, and *Coprobacillus*, in addition to a significant reduction in *Turicibacter*, *Clostridiaceae*, *Dorea*, and *Erysipelotrichaceae* in the ALL group compared with the HFD control (Figure 4A,B). Animals fed ALL and ND had a significantly higher alpha-diversity relative to HFD in both the Chao 1 (estimated OTU (operational taxonomic unit) richness and evenness) and observed OTU (diversity richness), as seen in Figure 4C,D. Both the ALL and the ND group had a significantly different beta-diversity from HFD, according to PC3 of the coordinate plot (Figure 4E). All of the significantly changed bacteria in the ALL group compared with the HFD group were checked for Pearson's correlation with the body weight difference in the mice. Body weight had a significantly positive correlation with the *Turicibacter* and *Erysipelotrichaceae* genera (Figure 5C,D) and a significantly negative correlation with *Lactobacillus* and *Coprococcus* (Figure 5B,E). Changes in *Dorea* and *Clostridiaceae* did not show any significant correlation with body weight (Figure 5A).

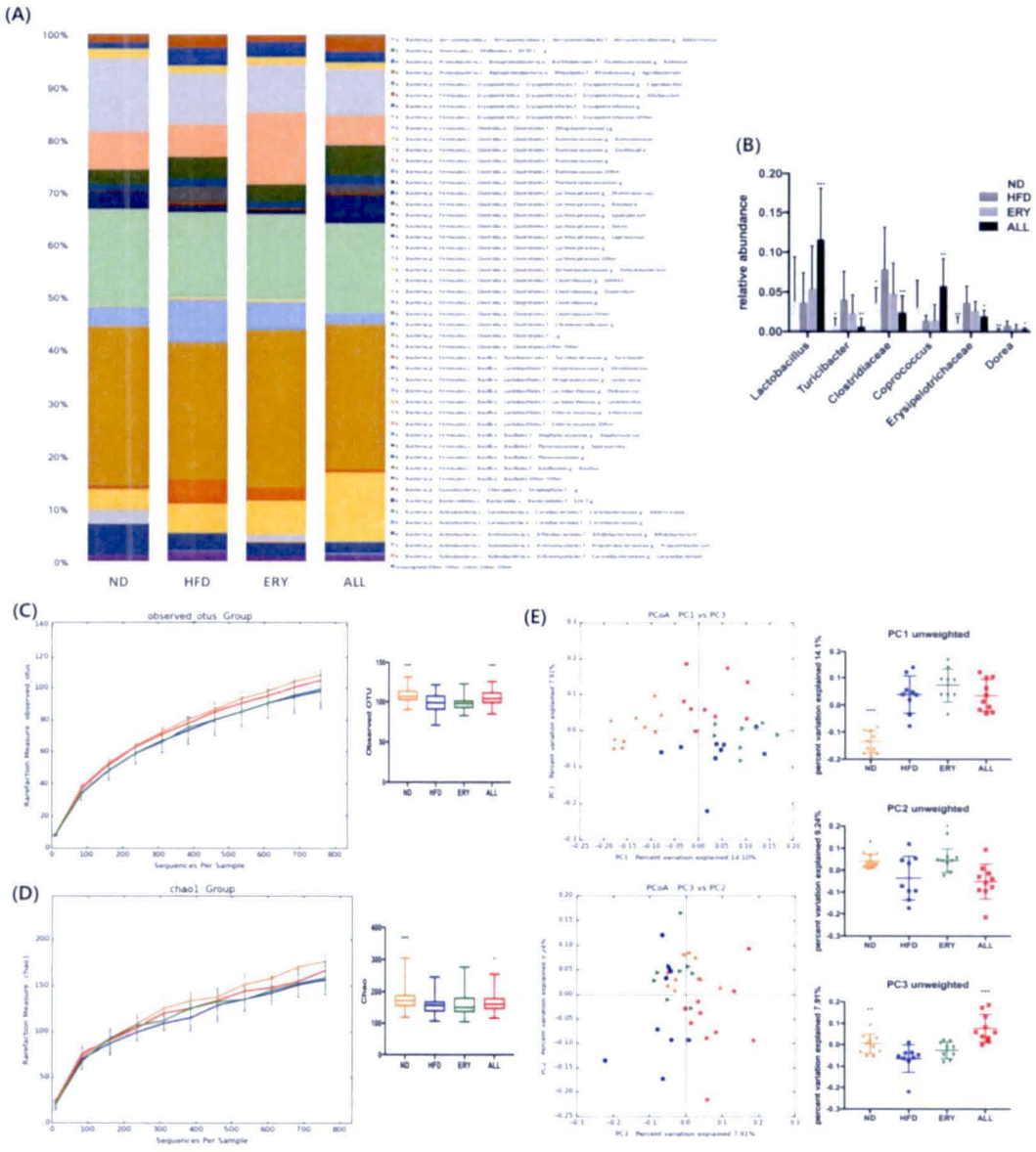

Figure 4. Effects of D-allulose on microbiota modulation. (**A**) Relative taxonomic abundance at the genus level. (**B**) Relative abundance of *Lactobacillus*, *Coprococcus*, *Coprobacillus*, *Turicibacter*, *Clostridiaceae*, *Dorea*, and *Erysipelotrichaceae*. (**C**) Observed operational taxonomic unit (OTU). (**D**) Chao 1. (**E**) Unweighted principal coordinates analysis and value of each principle coordinate dimension. Data are mean ± SE; ND, normal diet (AIN-76); HFD, high-fat diet (AIN-76, 20% fat, 1% cholesterol); ALL, (HFD + 5% D-allulose). Mean values are significantly different for HFD vs ALL, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

(A)

		g	Lactobacillus	Erysipelotrichaceae	Turicibacter	Coprococcus	Dorea	Clostridiaceae
g	Pearson Correlation	1	-.645**	.756**	.769**	-.607**	.350	.417
	Sig. (2-tailed)		.004	.001	.000	.010	.154	.085
	N	19	18	15	18	17	18	18

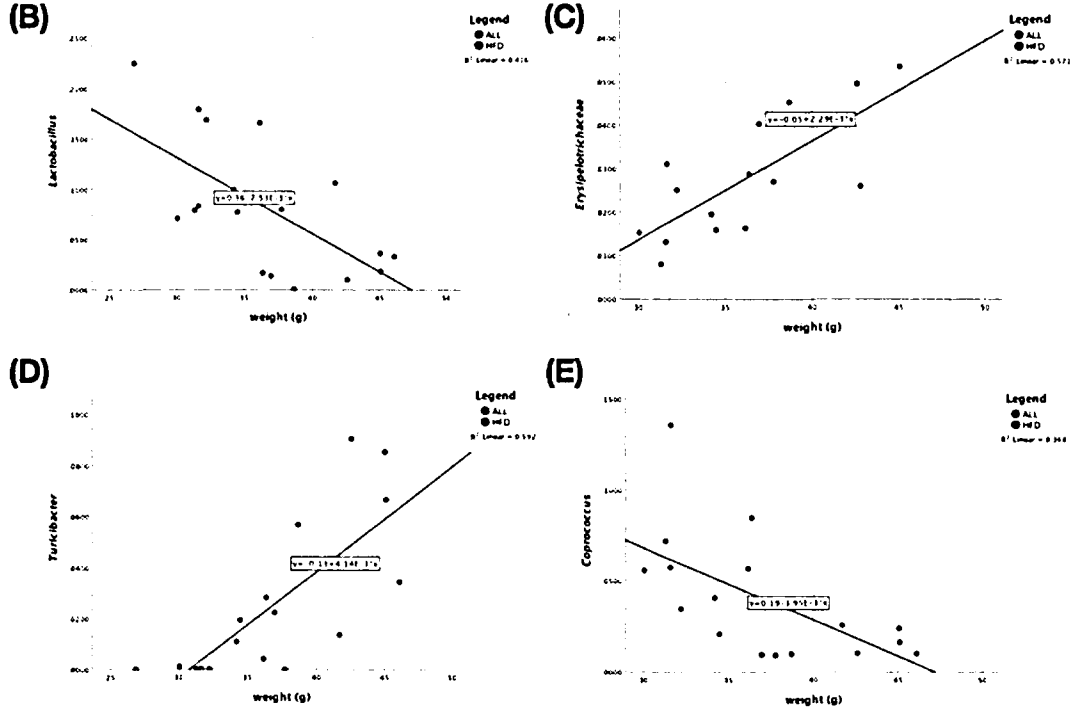

Figure 5. Correlation of microbiota with body weight. **(A)** Significance chart for Pearson's correlation between microbiota and body weight. Correlation between body weight and the relative abundance of **(B)** *Lactobacillus*; **(C)** *Erysipelotrichaceae*; **(D)** *Turicibacter*, and **(E)** *Coprococcus*. HFD, high-fat diet (AIN-76, 20% fat, 1% cholesterol); ALL, (HFD + 5% D-allulose). Significance calculated through bivariate Pearson's correlation analysis with body weight, * $p < 0.05$, ** $p < 0.01$.

4. Discussion

Previous studies have suggested that D-allulose can reduce body fat by regulating lipid metabolism [15,16]. In the same manner, the present study showed that D-allulose supplementation drastically decreased the body weight (Figure 1A,B) and body fat mass, without any change in food intake (Figure 1G,H)). D-allulose significantly increased FA oxidation and significantly decreased FA synthesis in eWAT (Figure 1J). To support these results, we measured the metabolic rate, which showed that VO_2 and EE were significantly increased in the D-allulose group (Figure 1K–N). Our results are in accordance with part of a past study, in which D-allulose significantly increased the β-oxidation activity and its related mRNA expression (CPT1α, CPT2) [22]. The acyl CoA, the metabolite of β-oxidation, can used as a fuel for energy expenditure [27]. Taken together, D-allulose may increase the energy expenditure via the regulation of the mRNA expression and enzyme activity of β-oxidation in eWAT.

SCFAs are produced through the complex interactions of diet and the gut microbiome [28]. The interaction of SCFAs can regulate the host energy homeostasis and be evaluated as novel therapeutic targets for DIO [29]. According to previous studies, SCFAs has diverse roles in DIO, such as enhancing FFA oxidation and promoting beige adipogenesis and mitochondrial biogenesis. In particular, SCFAs led to significant increases in the expressions of G-protein coupled receptor (GPR)43 and GPR41 in the adipose tissue, which may further result in body weight reduction by enhancing TG hydrolysis and FA oxidation. In our study, the SCFAs production per 50 mg of feces had an increasing tendency in the ALL group compared to the HFD groups. However, as the D-allulose supplement significantly increased the daily fecal weight, the total absolute amount of SCFA production, when adjusted with daily fecal weight, can be more than that of other groups. Thus, the increased SCFA production by D-allulose supplement may result in FA oxidation, which is in accordance with increased enzyme activity in FA oxidation in the ALL group.

An increased richness in the gut's microbial diversity has been negatively correlated with obesity and various disease states [30,31]. Furthermore, animal studies have indicated that treatment with probiotics or prebiotics can be a promising approach to alleviate these pathophysiological symptoms, by modulating the gut microbial ecology [28,29]. Firstly, we could observe the increase in the absolute total SCFA amount, a subset of key gut microbial metabolite, in the ALL group and the difference in relative total SCFAs among the groups in feces. Secondly, through the treatment of HFD feeding with D-allulose, there was an increase in both the alpha- and the beta-diversity in the gut microbiota when compared with the HFD control group (Figure 4E). Our study also found a significant increase in the relative abundance of *Lactobacillus*, which is known to improve gut barrier integrity, and *Coprococcus*, a known butyrate and propionate producer [32,33]. The abundance of both of these genera decreased with HFD-only consumption and increased in the D-allulose-consuming group compared to all the other groups (Figure 4A,B). Furthermore, some genera and families that were found to be at greater levels in obesity, such as *Dorea* and *Erysipelotrichaceae* [34,35], were significantly decreased in the D-allulose-fed animals relative to the HFD group. In addition, we observed a significant positive correlation between the abundance of *Erysipelotrichaceae* and body weight (Figure 5C).

Excessive cholesterol can build up in the arteries, which can lead to coronary heart disease and many other serious conditions such as stroke, insulin resistance and so on [1,36,37]. A high-cholesterol diet is a one of the predisposing factors for high cholesterol levels in blood [38]. The present study showed that HFD-fed mice increased the plasma and hepatic cholesterol and HMG-CoA reductase and ACAT activities compared to ND-fed mice;however, D-allulose supplement drastically decreased those values. Also, fecal cholesterol concentration was drastically increased by HFD with D-allulose. According to our previous study, D-allulose inhibits the dietary lipid absorption by the suppression of the CD36 expression, which is an important factor in the uptake of cholesterol and FA. In comparison of plasma TG levels with D-allulose, there was no significant difference in plasma collected after sacrifice; however, from the fourth week to the 12th week of the D-allulose supplement, the plasma TG concentration was significantly decreased in the ALL group compared to the other HFD groups. Although D-allulose could reduce the plasma TG levels, plasma TG concentration in HFD groups

at the endpoint of the experiment was decreased overall, which may be due to various factors. In order to elucidate the exact mechanism of its action, an additional study is required for the correlation among D-allulose, HFD and TG metabolism. Our findings suggest that D-allulose ameliorated the HFD induced dyslipidemia by altering the lipid metabolism and increasing the excretion of fecal lipids.

High-fructose-fed animal studies have suggested that an increase in hepatic insulin resistance and increased glucose tolerance lead to non-alcoholic fatty liver disease (NAFLD) and the loss of tight junction proteins [39–41]. The loss of integrity of the tight junctions allows the leakage of endotoxins, induction of hepatic inflammation, and is associated with decreased levels of *Lactobacillus* and *Bacteroides* in the gut microbiota [40–44]. Several studies have also proved that the administration of *Lactobacillus* species protected the host against the onset of fructose-induced NAFLD [42–44]. In our study, the decrease in hepatic steatosis coincides with the increasing *Lactobacillus* population in the D-allulose-fed mice (Figure 2J, Figure 4B). It may have contributed to the amelioration of fatty liver disease and weight loss, by strengthening the epithelial barrier, inhibiting endotoxin translocation, and alleviating overall systemic and hepatic inflammation. Accordingly, a future experiment will be performed to find out whether the increasing *Lactobacillus* population associated with D-allulose supplementation can improve the inflammation.

5. Conclusions

In the present study, D-allulose supplementation was markedly effective in protecting the host against HFD-induced obesity and hepatic steatosis. It is plausible that these pathologies are mediated by the alteration of the gut microbiome profile and enhanced energy expenditure. Our findings suggest that D-allulose can exert its biological effects through modulating the gut microbiome.

Supplementary Materials: The following are available online at http://www.mdpi.com/2072-6643/12/2/352/s1.

Author Contributions: Y.H. and H.P. performed the experiments, analyzed the data, and wrote/edited the manuscript. B.-R.C. performed the experiments. E.-Y.K. and Y.J. reviewed the manuscript. M.-S.C. supervised this work and had full access to all the data and therefore takes full responsibility for the integrity of the results and accuracy of the data analysis. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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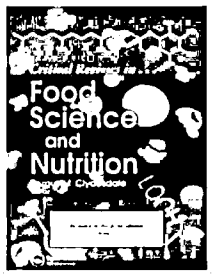

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The scientific basis for healthful carbohydrate profile

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ABSTRACT

Dietary guidelines indicate that complex carbohydrates should provide around half of the calories in a balanced diet, while sugars (i.e., simple carbohydrates) should be limited to no more than 5–10% of total energy intake. To achieve this public health goal a collective effort from different entities including governments, food & beverage industries and consumers is required. Some food companies have committed to continually reduce sugars in their products. Different solutions can be used to replace sugars in food products but it is important to ensure that these solutions are more healthful than the sugars they replace. The objectives of this paper are, (1) to identify carbohydrates and carbohydrates sources to promote and those to limit for dietary intake and food product development, based on current knowledge about the impact of carbohydrates on the development of dental caries, obesity and cardio-metabolic disorders (2) to evaluate the impact of food processing on the quality of carbohydrates and (3) to highlight the challenges of developing healthier products due to the limitations and gaps in food regulations, science & technology and consumer education.

KEYWORDS

Dental caries; Obesity; Type 2 diabetes; Cardiovascular diseases; Food formulation & processing

Introduction

Dietary carbohydrate should provide 45–65% of total energy intake (Institute of Medicine 2005; EFSA journal 2010). Accordingly, carbohydrates (see classification, Table 1) represent the main macronutrient in most people's diets. Among the different carbohydrates, sugars have been the subject of a lot of attention. In 2010, the European Food Safety Authority (EFSA) recognized the role of added sugars in the risk of developing dental caries, obesity and type 2 diabetes (T2D), but indicated that the available data were insufficient to set an upper limit of intake (Table 2). In 2015, the World Health Organization (WHO) set upper limits by recommending that the intake of free sugars be less than 10% of the total energy intake (strong recommendation) and a further reduction to less than 5% of total energy intake (conditional recommendation) throughout the lifespan for preventing both dental caries and obesity (Table 2). The US Dietary Guidelines Advisory Committee (DGAC) has also proposed a 10% upper limit for added sugars intake (Table 2). More recently, the Scientific Advisory Committee on Nutrition (SACN) has associated sugars and foods containing sugars with a greater risk of tooth decay and increase in body weight, resulting in a recommendation of free sugars intake of less than 5% of total energy (Table 2).

In order to achieve this public health target, consumers will need to make significant changes to their diets. At the same time, it results in the need to develop healthful technological solutions to reduce sugars in packaged foods. Different solutions can be applied to replace sugars to help consumers to follow dietary guidelines, but it is important to ensure that they are truly supportive of the expected health benefits, i.e, contribution to reduce the risk of dental caries, obesity, T2D and cardiovascular diseases (CVD). For that reason, it is pertinent to develop healthful carbohydrate blends, possibly by using better sources of carbohydrates and avoiding ultra-processing of manufactured products. However, in order to achieve this shift, some major limitations and scientific gaps need to be overcome.

Dietary carbohydrates, health & diseases

Recently, the current evidence of the role of dietary simple and complex carbohydrates in the development of four public health issues, i.e, dental caries, obesity, T2D and CVD have been extensively reviewed through systematic review or meta-analysis (SACN 2015). However, some of the evidence that describes the negative impact of excessive intake of sugars on health is based on association studies, not proving causality. It is therefore important to identify the potential mechanisms of action for the postulated health effects to reinforce futher support the plausibility of the observed associations and the various intrinsic properties of the carbohydrates linked to a specific health outcomes.

Dental caries

Dental caries occur due to tooth demineralization by organic acids produced by the combination of orally fermentable carbohydrates (e.g., glucose, sucrose, digestible oligosaccharides and starches) and dental plaque bacteria (Moynihan and Petersen 2004). Therefore, the main property linking a given sugar or complex carbohydrate to dental caries is their fermentability by specific bacteria in the oral cavity.

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Table 1. Dietary simple and complex carbohydrates classification (adapted from FAO/WHO 1997 and Cummings and Stephen 2007).

	Class	Sub-groups	Components
Simple	Sugars (DP 1–2)	-Monosaccharides	-Glucose, galactose, fructose, tagatose
		-Disaccharides	-Sucrose, lactose, maltose, isomaltulose
		-Sugars alcohols (polyols)[1]	-Sorbitol, mannitol, xylitol, erythritol. Maltitol, isomalt, lactitol
Complex	Oligosaccharides (DP 3–9)	-Malto-oligosaccharides	-Maltodextrins (hydrolysed starch)
		-Non-digestible oligosaccharides	-Raffinose, stachyose, fructo- and galacto-oligosaccharides, polydextrose, inulin
	Polysaccharides (DP >9)	-Starch	-Amylose, amylopectin, modified starches
		-Non-starch polysaccharides	-Cellulose, hemicellulose, pectins, hydrocolloids (gums, ß-glucan)

DP: degree of polymerization; [1]Regulatory-wise, polyols are not labeled as "sugars".

Sugars (mono- and disaccharides)

Both cohort studies and human trials have shown an association between the consumption of total sugars and foods or beverages containing sugars with a greater risk of dental caries (SACN 2015). Among the sugars, there is no clear evidence of differences in the cariogenicity between fructose, glucose and sucrose. However, lactose is fermented more slowly in the oral cavity than sucrose (Birkhed et al. 1993). In agreement, early studies of plaque pH in human subjects have shown that plaque bacteria produce less acid from lactose compared with other sugars (Jenkins and Ferguson 1966). Interestingly, rare sugars, such as tagatose (an isomer of fructose) and isomaltulose (a structural isomer of sucrose) are recognized to be non-cariogenic and have obtained an EFSA positive opinion and health claim approval by the US Food & Drug Administration (FDA) on the prevention of dental caries when replacing sucrose (EFSA 2011; FDA 2017). However, under food regulations, tagatose and isomaltulose are not exempted from the definition of sugars. This means that they are labeled as such and counted as free/added sugars when used in food manufacturing. Interestingly, sugar alcohols (e.g., xylitol and sorbitol), which have been also shown to be non-cariogenic (Hayes 2001) and are associated to positive dental health claims in Europe (EFSA 2011) and in the US (FDA 2017) are not declared as added sugars but under total carbohydrates.

Oligo- and polysaccharides

The cariogenenicity of digestible oligo- and polysaccharides has been less extensively studied. Nevertheless, there is some evidence that foods rich in maltodextrins (starch partially hydrolyzed) (Levine 1998; Al-Khatib et al. 2001) or rapidly digested starches (e.g., highly gelatinized) (Lingström et al. 2000) may also contribute to dental caries, since easily available to salivary enzymes, which results in maltose and to a lesser extent, glucose release. Therefore, depending on the residence time in the mouth and the nature and extent of chewing, some complex carbohydrates, such as maltodextrins, might have a non negligeable cariogenicity. To our knowledge, non-digestible oligo- (e.g., fructo- and galacto-oligosaccharides) and polysaccharides have not been reported to be cariogenic.

Key messages:

-Not all sugars are cariogenic (e.g., tagatose, isomaltulose)

-Some complex carbohydrates, but not fibers, might be cariogenic

Obesity

Obesity is an excess of body fat that occurs when energy intake from foods is greater than total energy expenditure during a

Table 2. Associations of free/added sugars intake as foods (F) and beverages (B) and recommendations of intake according to different health organizations/authorities.

	Sugars definition	Dental caries	Obesity	Type 2 diabetes	CVD	Recommendations (% of total energy)
EFSA (2010)	Added sugars: "Sucrose, fructose, glucose, starch hydrolysates (glucose syrup, high- fructose syrup) and other isolated sugar preparations used as such or added during food preparation"	F & B	B	B	NC	None
DGAC (2015)	Added sugars: « Mono- and disaccharides added during the processing of foods or packaged as such, and include syrups, naturally occurring sugars that are isolated from a whole food and concentrated and other caloric sweeteners »	F & B	F & B*	B	F & B*	< 10
WHO (2015)	Free sugars: "all monosaccharides and disaccharides added to foods and beverages by the manufacturer, cook, or consumer, plus sugars naturally present in honey, syrups, fruit juices and fruit juice concentrates"	F & B	F & B*	ND	ND	< 10 (strong); <5 (conditional)
SACN (2015)	As WHO	F & B	F & B*	B	NC	< 5

F:, Foods; B: Beverages; ND, not determined; NC, not conclusive; CVD: cardiovascular diseases; *more consistent in beverages than foods.

prolonged period of time. Therefore, the main property linking a given sugar or complex carbohydrate to obesity is their caloric content.

Sugars

A meta-analysis of randomized controlled trials indicates that reducing or increasing intake of free sugars promotes, respectively, loss or gain in body weight (Te Morenga et al. 2012). However, no evidence of difference in body weight change was found when the group interventions were isocaloric through isoenergetic exchanges of dietary sugars with other carbohydrates or other macronutrient sources. This suggests that calories, rather than the sugars *per se*, are the main driver of the body weight gain. Indeed, there is a growing consensus that high intake of free/added sugars, especially in sugars-sweetened beverages, increases overall energy intake. The mechanism behind this calorie overconsumption might be different between liquids and solid foods. Today, most of the evidence linking sugars and obesity have involved sugars-sweetened beverages rather than solid foods. The mechanism might be explained by the less satiating effect of liquid vs solid foods (DiMeglio and Mattes 2000; de Graaf 2011). Regarding solid foods, the potential mechanism is less clear. It has been proposed that foods rich in sugars are also often high in energy density (Te Morenga et al. 2014) and that such foods are less satiating than those that are less energy dense (Rolls 2009). One potential direct role of sugars might come from its sweetness, even though the role of sweet taste in energy intake and appetite regulation in humans is controversial (Yeomans 2012). This research field certainly deserves more attention, and will be key to drive nutritional recommendations and product (re) formulation strategies.

Sugar alcohols being less absorbed than sugars show a lower energy density, ranging from 0.2 to 3 kcal/g (Livesey 2003). Consequently, they can reduce the energy density of a food and potentially calorie intake, when used as sugar replacer.

Oligo- and polysaccharides

Regarding the risk of obesity, there is no evidence, when total energy is matched and in excess, that digestible oligo- and polysaccharides are less detrimental than sugars. Maltodextrins, which are commonly used in beverages (Hofman et al. 2016), have the same caloric value as glucose or sucrose but a lower sweetness (Table 3).

Dietary fibers provide few calories and almost no sweetness (Table 3). Consequently, they represent a good alternative for reducing the energy density of certain foods and the risk of overconsumption. Indeed, some randomized control trials have shown a positive association between dietary fiber intake, as foods or supplements, and weight loss. Several cohort studies have also shown an inverse association with weight gain (EFSA 2010). However, using a more systematic search strategy, the SACN (SACN 2015) has recently concluded that at present there is no consistent evidence of an association between dietary fiber intake and body weight change.

Key messages:

-High intake of free/added sugars is associated with higher risk of obesity, particularly when consumed under liquid form

-The potential underlying mechanisms seem to favor an indirect role of sugars and highlight the issue of caloric beverages and energy dense foods

-Some sugars (e.g., allulose and tagatose) have lower caloric value than 4 kcal/g

-The role of sweetness as a potential contributor of calorie overconsumption deserves further investigation

-Intake of dietary fibers might have some protective effects against weight gain

Type 2 diabetes

T2D is characterized by the body's inability to respond to insulin action and/or produce enough insulin, which causes elevated levels of glucose in the blood (hyperglycemia). Obesity is a major risk factor for the development of T2D and CVD (Pi-Sunyer 1991). The sequence of biological events that leads from obesity to T2D and CVD is known (Saltiel and Olefsky 1996). Briefly, excess adipose tissue, especially visceral fat, promotes insulin resistance, which may lead to impaired glucose tolerance characterized by an excessive glycemic response (GR) after carbohydrate ingestion. The transition from this prediabetic state to T2D can take many years and is often associated with a decline in the capacity of beta cells to secrete enough insulin (Fonseca 2009). The potential role of carbohydrates in the development of T2D is their ability to increase glycemic and inulinemic response after ingestion. The postprandial GR can be translated into a standardized index (Jenkins et al. 1981), which allows the ranking of different carbohydrates or carbohydrate-containing foods on a scale called the glycemic index (GI). The lower and higher the GI is, a lower and higher GR is expected, respectively. In addition, the glycemic load (GL), calculated as the product of GI and the quantity of available carbohydrates in a serving of the test food (divided by 100) estimates both the "quality" and the quantity and therefore represents a better proxy of GR than GI (Salmeron et al. 1997). Mirroring the GI concept, the insulin index (II) was developed to quantify the postprandial insulin response to different carbohydrates or foods (Holt et al. 1997). The II values for carbohydrate and food correlate with their GI (low GI → low II; high

Table 3. Caloric value and average relative sweetness of different carbohydrates (Shallenberger 1993; Livesey 2003; Lê et al. 2016).

	Sugars (mono and disaccharides)										Polysaccharides	
	Gluc	Fruc	Galac	Allu	Taga	Suc	Lac	Iso	S. Al	Oligosaccharides Maltodextrins	Digestible Starches	Non digestible Fibers**
Caloric value (kcal/g)	4	4	4	0.2	1.5–2.4*	4	4	4	0.2–3	4	4	≤ 2
Relative sweetness	0.6	1.2	0.5	0.7	0.9	1.0	0.3	0.5	0.4–1	0.1–0.2	<0.05	<0.05

Gluc: glucose; Fruc: fructose; Galac: Galactose; Allu: Allulose; Taga: tagatose; Suc: sucrose; Lac: Lactose; Iso: isomaltulose; S. Al: Sugars alcohols.
*labeled as 1.5 kcal/g in the US and 2.4 kcal/g in Europe,**include non digestible oligosaccharides and resistant starch.

GI → high II), with some exceptions for foods containing high levels of proteins, especially dairy proteins, where a low GI can be associated to a higher II than expected.

Sugars

The association between free/added sugars and T2D was not addressed by the WHO but it was addressed by the US dietary and the UK nutrition advisory committees (DGAC and SACN, respectively). These committees found moderate to strong evidence associating an increased risk of T2D with a higher consumption of free/added sugars in the form of sugars-sweetened beverages (SACN 2015; DGAC 2015). According to the DGAC, this relationship is not completely explained by change in body weight. One potential mechanism by which the excess of sugars consumption might promote the development of T2D, independently of overweight and obesity, is the capacity of certain sugars to trigger a high postprandial glycemic or insulinemic response. However, not all sugars will promote a high GR as indicated by their GI (Table 4); glucose and sucrose triggering the highest response, lactose, isomaltulose, fructose and galactose an intermediate one, while tagatose and allulose having almost no effect on blood glucose levels.

Sugar alcohols have lower GI and II than glucose or sucrose (Livesey 2003) and therefore represent a good alternative to limit the glycemic and insulinemic response of a food. In 2011, their use as sugar replacers received a positive opinion from EFSA for their property to decrease the glycemic response (EFSA 2011).

Oligo- and polysaccharides

Based on cohort studies, no association was found between total starch intake and T2D. But this analysis does not take into account the different types of starch; i.e., rapidly or slowly digested starches. Interestingly, when starchy foods are considered, some cohort studies have associated high consumption of cooked potatoes and rice (white but not brown) with higher incidence of T2D (Halton et al. 2006; Hu et al. 2012). As shown in Table 4, complex carbohydrates such as malto-oligosaccharides and some starches (i.e., those poor in amylose or highly processed) can have a higher GI than sugars (e.g., fructose and lactose). For example freshly boiled white rice and potato have GI values of 89 and 98%, respectively (Atkinson et al. 2008) with an II as elevated as 79 and 121 (Holt et al. 1997).

There is adequate evidence from cohort studies that higher consumption of dietary fibers is associated with reduced risk of T2D (SACN 2015). As non-digestible moieties, dietary fibers have a GI of 0, and thus their intake does not contribute to postprandial glycemia and insulinemia. In addition, some viscous fibers such as ß-glucan, pectin, guar gum or glucomannan, when incorporated into high glycemic foods, have the capacity to lower the glycemic and insulinemic responses (Jenkins et al. 1986).

High GI and GL foods

The plausibility of causality behind the association between some carbohydrates or carbohydrate-rich foods and T2D can be reinforced by the analysis of cohort and clinical studies examining the impact of the intake of low vs high GI foods.

According to the SACN report (2015), the analysis of prospective cohort studies shows that a diet higher in GI or GL is associated with a greater risk of T2D. Interestingly, sugars-sweetened beverages which are positively associated with T2D (DGAC 2015; SACN 2015) have a GI ranging from moderate to high (i.e, 40 to 78), the highest being sport drinks, and have a GL higher than 10.

However, randomized controlled trials have not provided consistent evidence of an effect of GI or GL on surrogate markers or risk factors of T2D such as fasting blood glucose and insulin (SACN 2015). The heterogeneity of the macronutrient composition of the tested low *vs* high GI/GL diets may explain this inconsistency. In addition, most studies have not measured the GR and when they have been measured they do not always achieve meaningful blood glucose differences (Blaak et al. 2012). Therefore, the use of more direct measures of glucose and insulin postprandial exposure than GI/GL and II, such as for instance continuous glucose monitoring or urine c-peptide, are needed to better define a healthful digestible/glycemic carbohydrate intake.

Key messages

-Greater intake of free/added sugars is associated with higher risk of T2D, particularly when consumed as sugars sweetened beverages.

-Not all sugars have the same GI and II. Some complex carbohydrates (e.g., maltodextrins, refined & highly processed starches) have higher GI and II than sugars (e.g., lactose, fructose, isomaltulose)

-Diet higher in GI or GL is associated with a greater risk of T2D but the evidence based on randomized control trials is not consistent

-Intake of dietary fibers is associated with reduced risk of T2D

-Some soluble viscous fibers lower the glycemic and insulinemic response of foods

Cardiovascular diseases

CVD are a group of disorders related to the heart and blood vessels. Myocardial infarction and cerebrovascular accident,

Table 4. Average GI of different carbohydrates. Low GI < 55; High GI > 70 (adapted from Atkinson et al. 2008; Livesey 2003; Lê et al. 2016).

	Sugars (mono and disaccharides)										Polysaccharides	
	Gluc	Fruc	Galac	Allu	Taga	Suc	Lac	Iso	S. Al	Oligosaccharides Maltodextrins	Digestible Starches*	Non digestible Fibers**
Glycemic Index (%)	100	23	25	~0	~0	65	46	32	6-50	105	40-105	~0

Gluc: glucose; Fruc: fructose; Galac: Galactose; Allu: Allulose; Taga: tagatose; Suc: sucrose; Lac: Lactose; Iso: isomaltulose; S. Al: Sugars alcohols.
*Quality and process dependent.; **include also non digestible oligosaccharides and resistant starch.

which are the acute and often fatal phases of the diseases, are due to the blockage by atherosclerotic lesions of the blood, from flowing to the heart or brain (WHO 2016). The combination of high levels of LDL cholesterol and triglycerides (hyperlipidemia) accelerates atherosclerosis increasing the risk of heart attack and stroke. Diabetes is also a prime risk factor for CVD since chronic high blood glucose levels promote macro and micro-vascular damage.

Other risk factors include tobacco use, excessive consumption of alcohol, unhealthy diet, obesity, hypertension, fatty liver and physical inactivity (World Heart Federation 2016). Therefore, as CVD have multifactorial causes, it is difficult to point out one specific property linking sugars/carbohydrates to their development. However, a simplified view may be that their main direct effects rely in their ability to modulate lipid homeostasis.

Sugars

According to the SACN report (2015), there is insufficient evidence from cohort or randomized controlled studies to conclude on the impact of high sugars intake and CVD risks. However, the limited evidence tends to favor a positive association. Indeed, a recent study of a prospective cohort in the US, showed a significant relationship between added sugars consumption and increased risk for CVD mortality (Yang et al. 2014). In addition, a meta-analysis of randomized controlled trials concluded that greater intake of sugars (especially sucrose or high-fructose corn syrup) raises blood pressure, cholesterol and triglycerides (independently of body weight) (Te Morenga et al. 2014). With respect to the DGAC, their report concludes on a moderate evidence that higher intake of added sugars, especially as beverages, is associated with CVD (DGAC 2015). They also found a consistent relationship between higher consumption of added sugars and increased blood pressure and triglycerides.

Among the different sugars, fructose, either alone or as a component of sucrose or high-fructose corn syrup, seems to be the most detrimental to metabolic and cardiovascular health. Indeed, when ingested in excessive amounts (>50 g/day) and compared to the same amount of glucose, fructose has been shown to increase blood postprandial triglycerides (Livesay and Taylor 2008) and LDL-cholesterol (Stanhope et al. 2011). It also promotes the accumulation of fat in the liver increasing the risk of developing hepatic insulin resistance as well as non-alcoholic liver steatosis (Faeh et al. 2005; Lê et al. 2009). A recent meta-analysis suggests that these various effects may only appear when fructose is consumed in excess of energy requirements, by providing 24% or more excess calories (Chiavaroli et al. 2015). It is known that high consumption of sugars-sweetened beverages is associated with excess energy intake, with some beverages having up to 30g of fructose per 500 ml (Ventura et al. 2011). Therefore, the consumption of two 33 cl bottles will exceed the detrimental threshold of 50g fructose/day while at the same time providing 480 kcal. Even though some fruits are as rich in fructose, it is unlikely that someone would consume more than 50 g of fructose from fruit alone. For example, it would require about 1.0 kg of apples to get 60 g of fructose and it would take about 30 min, to ingest this quantity of apples (Haber et al. 1977). In comparison, an intake of one liter of a sweetened beverage could lead to a consumption of 60 g fructose, while providing an additional 60 g of glucose.

Oligo- and polysaccharides

There is a lack of available evidence on the potential association of starch or starchy foods intake with CVD. On the other hand, observational studies have shown inverse associations between high intake of dietary fibers and cardiovascular diseases, coronary events and stroke. When the type or origin of the dietary fiber was analyzed, insoluble fiber, vegetable and cereal fibers were, or tended to be, protective against cardiovascular diseases and coronary events (SACN 2015).

Randomized controlled trials show that higher intake of the soluble fiber beta-glucans and one of their main natural source, oat bran, promotes a reduction of several CVD risk factors, including blood LDL cholesterol, triglycerides and blood pressure (SACN 2015).

High GI and GL foods

Prospective cohort studies indicate that there is a relationship between high GL, but not GI, diet and increased risk of CVD. Based on randomized controlled trials, a low *vs* high GL diet might promote a reduction of blood pressure and serum triglycerides (SACN 2015). Nevertheless, the evidence regarding GI/GL foods and CVD remains very limited.

Key messages

- Greater intake of free/added sugars might be associated with higher risk of CVD, probably through an increase in blood pressure and triglyceride levels

-Fructose seems to be the main sugar associated with the increased CVD risk

-An excessive daily fructose intake is not readily achievable by consuming whole fruits but can be easily reached with fructose/sucrose rich beverages.

-Elevated postprandial glycemic and/or insulinemic responses might contribute in increasing CVD risk, but more evidence is needed to confirm this hypothesis.

-Higher intake of different types of dietary fibers is inversely associated with cardio-vascular and coronary diseases.

-Intake of viscous soluble fibers, such as beta-glucans, decrease CVD risk factors

Conclusions

The current scientific evidence regarding the role of dietary carbohydrates on health and diseases tends to allow the distinction between two categories of carbohydrates:

(1) "The healthful", composed of no/low cariogenic and/or non/slowly-digestible carbohydrates, such as slowly digestible starch, dietary fibers, lactose, isomaltulose or tagatose.
(2) "The health sensitive", consisted of cariogenic, high GI or dyslipidemic carbohydrates, such as fructose, glucose, sucrose, maltodextrins and rapidly digested starch.

In order to improve the nutritional value of food products, formulation of carbohydrate blends specific and adapted to different food matrices need to be explored. The utilization of the healthful carbohydrates at the expense of the health sensitive

ones should be favored. The main challenge is to find, in different food matrices, the right ratio between these 2 categories of carbohydrates, taking into account the nutritional needs of the individual and product application (e.g., general population, sport or clinical nutrition), as well as the physiological impact and limitations in terms of safety/tolerability, production and organoleptic properties. Given that the average intake of dietary fiber in most Western countries is only half of the recommended levels, it is imperative that this challenge is addressed. Although recommendations differ, the recommended fiber intake for adults usually ranges from 25–38 g/d (EFSA 2010; IOM 2005; SACN 2015).

The healthful carbohydrates listed above are commercially available as ingredients that have been isolated from their natural sources. However, the components of whole foods that constitute healthful carbohydrates, are considered as such because they also provide phytochemicals, vitamins and minerals. The following section highlights the main types of healthful carbohydrates; those which should be consumed as part of a healthy diet and thus should be favored in the development of food products with a healthier carbohydrate profile. Furthermore, an important component of food product development that is often overlooked is food processing. As in the nutrition field, research in food science is evolving constantly and the impact of processing on the organoleptic properties of food has been extensively characterized. Here, we also highlight the importance of studying the impact of food processing on the nutritional properties of food products as well as discuss its potential on preserving and potentiating the nutritional value of healthful carbohydrate sources.

Types of healthful carbohydrates and their sources

Carbohydrates comprise a wide range of saccharides and current nutritional recommendations are based on their classical chemical classification; simple carbohydrates are constituted by mono- and disaccharides commonly referred to as sugars, and complex carbohydrates includes all the rest, oligo- and polysaccharides (Table 1). However, this chemical classification does not always translate equally when evaluating their nutritional quality. In fact, emerging research has shown that both healthful and "health sensitive" (when consumed in excess) carbohydrates can be found within the same group of chemical classification. For instance, the soluble fiber β-glucan, which is known for its health benefitting property, is categorized as a polysaccharide based on its chain length. In the same group, maltodextrins derived from starch can also be found if their dextrose equivalent (DE) value is low enough to be classified as polysaccharides. The following subsections describe the specific types of carbohydrates considered healthful based on the ways in which they are metabolized and not on their chemical classification.

Dietary fibers

EFSA (2010) defines dietary fibers as carbohydrates plus lignin, including all carbohydrate components occurring in foods that are non-digestible in the human small intestine and pass into the large intestine. In addition, the CODEX definition specifically refers to carbohydrate polymers of 10 or more monomeric units and states that dietary fiber is constituted by a) edible carbohydrate polymers naturally occurring in the food as consumed (e.g. whole grain), b) carbohydrate polymers obtained from food raw material by physical, enzymatic, or chemical means (e.g. fructooligosaccharide), and c) synthetic carbohydrate polymers (e.g. polydextrose). Furthermore, carbohydrates that fall into categories b and/or c, must show a proven physiological benefit to health as demonstrated by generally accepted scientific evidence to competent authorities before they can be labelled as dietary fibers. Therefore, dietary fiber includes a wide range of carbohydrates such as non-digestible oligosaccharides (NDOs), non-starch polysaccharides (NSPs, such as pectins, and ß-glucans), hydrocolloids such as mucilage, and resistant starches. To further illustrate the complexity of dietary fibers, in the case of resistant starches (RS) there are 4 types, namely, RS1, RS2, RS3 and RS4. RS1 refers to the portion of starch naturally found in raw/unprocessed seeds, legumes, or whole cereal grains that is resistant to digestion, and RS2, in grain mutants that have been bred to produce starches with a particular structural conformation that is resistant to digestive enzymes. RS3, on the other hand, is produced in cooked and cooled starchy foods where the starch has recrystallized or retrograded (i.e., stale bread and cold potato salad). Finally, the RS4 is constituted by chemically modified starches that resist digestion. Furthermore, non-starch polysaccharides in cell wall tissues are usually found intricately entangled with lignin, which is not a carbohydrate but a phenolic polymer, and is therefore considered a component of dietary fiber.

As can be deduced from the examples provided, dietary fibers, natural or synthetic, are predominantly of plant origin. Cereal grains, pseudocereals and pulses, which are important components of the diet, are good sources of dietary fiber (Table 5). The majority of the dietary fiber from these sources is insoluble because the dietary fiber is mainly concentrated in outer layers, bran and hulls, of the grain or seed. In addition,

Table 5. Total fiber and non starch polysaccharide (NSP) content (% dry weight) of different food sources of plant origin. (Adapted from Anderson and Bridges 1988; Knudsen 2014).

Food Source	Total Fiber	NSP*	Soluble NSP	Insoluble NSP
Cereals (raw)				
Corn	10.1	9.0	1.1	7.9
Wheat	13.1	11.3	2.4	8.9
Barley	21.8	18.6	4.8	13.8
Oats	29.8	23.2	3.1	20.1
Wheat bran	43.4	36.4	2.7	33.7
Vegetables (raw)				
Cabbage	23.2	22.4	8.7	13.7
Carrots	23.8	22.8	11.4	11.4
Lettuce	21.0	19.0	4.7	14.3
Spinach	28.8	24.7	6.6	18.1
Legumes (canned)				
Green beans	34.0	31.4	8.1	23.3
White beans	18.2	17.2	5.3	11.9
Green peas	21.3	20.4	3.0	17.4
Lentils (cooked)	15.7	12.6	1.7	10.9
Fruits (raw)				
Apple	12.7	10.5	4.5	6.0
Banana	7.3	4.1	2.1	2.0
Orange	11.5	11.1	6.7	4.4

*Non starch polysaccharides: total fiber minus resistant starch.

resistant starches (RS) are usually insoluble due to their structural conformation and large molecular weight. Different physiological effects have been attributed to insoluble (e.g. improve laxation) and soluble dietary fiber (e.g. lowering of blood cholesterol or glucose); however, this difference of solubility does not systematically predict physiological effects. Among the natural sources of fibers, cereal fibers have been more strongly associated with a reduction of T2D than fibers from fruits and vegetables (Lê et al. 2016). Interestingly, the interaction between dietary fiber and the gut microbiome is an emerging mechanism to explain the positive association between dietary fiber intake and cardiometabolic health. However, more research is needed to demonstrate causality and identify the most beneficial fiber and gut microbial composition.

Slowly digestible carbohydrates

Starch is a major source of energy in the human diet and its specific macro- and fine-structural features, such as crystallinity or amylose:amylopectin ratio, largely determine its susceptibility to digestion in the small intestine (Behall et al. 1988; Zhang and Hamaker 2009). Starches that have higher proportions of amylose have slower digestion rates and/or are more resistant to digestion (Jane et al. 1999; Chung et al. 2011). In order to differentiate the digestive properties of starches in foods, a classification into rapidly digested starch (RDS), slowly digested starch (SDS) and resistant starch (RS), the latter being classified as a dietary fiber, was proposed by Englyst et al. (1996). Most raw cereal, pulse and tuber starches contain considerable amounts of both SDS and RS. Table 6 shows the content of these three types of starch in different food sources.

The nutritional relevance of SDS is based on its slow rate of digestion, which in turn, elicits a lower, plateaued GR (Ells et al. 2005). When applied to food manufacturing, this results in products with lower GI values (Goñi and Valentín-Gamazo 2003). The quantification of RDS, SDS, and RS is the result of the Englyst *in vitro* digestibility analysis that is based on simulating the action of digestive enzymes on starch or starchy food samples analyzed 'as eaten'. Thus, it is important to note that the levels of RDS, SDS and RS may vary for each food source depending on the specifics of the cooking method (temperature, pressure, moisture content, pH, etc.) used (Bravo et al. 1998; Mishra et al. 2008). Furthermore, subsequent storage conditions of cooked starchy foods have a direct impact on the amount of SDS that the food contains. For instance, Monro et al. (2009) have shown that cooling freshly cooked potatoes result in significantly greater amounts of SDS in particular genotypes.

Table 6. Rapidly digested starch (RDS) slowly digested starch (SDS), resistant starch (RS) and non starch polysaccharides (NSP) content of starchy foods (g/100 g as eaten) (from Englyst et al. 1996). Values might differ between cultivars.

Food Source	RDS	SDS	RS	NSP
Cereals				
Pearled barley	8.0	7.0	2.1	4.8
Buckweat	11.8	8.5	1.8	0.8
Sweetcorn	15.4	1.4	0.3	3.3
Freshly-boiled white rice	17.4	5.6	0	0.2
Brown rice	14.6	9.2	0	0.8
Legumes and tubers				
Butter beans	9.4	0.8	1.2	5.9
Haricot beans	4.1	5.8	8.3	6.6
Kidney beans	4.7	9.8	2.5	6.3
Red lentils	7.3	6.1	2.4	1.6
Potato	15.2	0.7	0.1	1.4

Other than slowly digestible starches, sugars with slowly digestible properties also exist. Isomaltulose is a slowly-digestible disaccharide (Holub et al. 2010) composed of glucose and fructose linked by an ($\alpha 1 \rightarrow 6$)-glycosidic linkage that naturally occurs in honey and sugar cane extract in very small quantities (Low and Sporns 1988). It is available as a commercial ingredient produced by the enzymatic isomerization of sucrose (Mu et al. 2014). It has about 50% the sweetness of sucrose and its expected physiological response is similar to that of SDS but their techno-functional properties differ greatly. Indeed, studies of its digestibility *in vitro* have shown that its rate of hydrolysis is significantly slower than that of sucrose and maltose (Tsuji et al. 1986). In agreement, clinical studies have shown that the postprandial glycemic and insulinemic responses to isomaltulose rise at a slower rate, and maximum concentrations reached are lower than sucrose (Maresch et al. 2017).

Lactose is another disaccharide that has a low GI (Table 3), which is attributed, in part, to a slow rate of hydrolysis of its components, glucose and galactose and to the low GI of galactose itself (Gray and Santiago 1966; Rerat et al. 1984). Milk is the highest food source of lactose (about 5 to 7% in bovine and human milk, respectively) and also provides proteins, vitamins and minerals, especially calcium.

Low calorie sugars

Low calorie sugars represent interesting candidates for sucrose replacement. There exists more than 30 mono- and disaccharides, among which 24 have a caloric content lower than 4 kcal/g (Vafeiadi et al. 2015). The method to establish available energy for such sugars yield different results from those obtained by the traditional bomb calorimeter. Indeed, the principle of the latter rests on the energy released by complete combustion of the molecules. In this case, all carbohydrates have an energy content similar to that of glucose, i.e. around 4 kcal/g (Livesey 1990). In contrast, available energy relies on the amount of energy that is available for cell metabolism. In vivo, this can be measured by a combination of tracers and indirect calorimetry methods. Only few studies have investigated the effect of low caloric sugars in humans, and most studies refer to tolerance/digestive comfort. To our knowledge, only arabinose, xylose, tagatose and allulose have been studied in humans for metabolic outcomes. Today, tagatose and allulose are the two low caloric sugars with the highest potential for sugar replacement, as the technical developments to incorporate them into food products are the most advanced and both have been granted a GRAS status by the US FDA.

Both tagatose and allulose are naturally found in some foodstuffs (Oshima et al. 2006; Levin 2002), such as dairy products and cereals, albeit in very small quantities. Ingredient suppliers are currently developing biotransformation processes for their industrial production. Tagatose is manufactured from galactose through enzymatic and chemical processes (Oh 2007), while allulose is produced at an industrial scale by the enzymatic

isomerization of fructose (Takeshita et al. 2000). These sugars have similar physicochemical properties as sucrose, glucose or fructose, therefore they can be easily used to replace sugars. Their main difference lie in their absorption and metabolism.

Tagatose has an available energy content between 07–1.5 kcal/g (Levin et al. 1995). Its low caloric content is mostly due to its incomplete intestinal absorption. Rats studies have shown that only 15–20% of ingested tagatose was absorbed (Levin 2002), which at doses above 30 g can yield intestinal discomfort in humans. For metabolic health, it has been shown that moderate doses of tagatose (5–10 g) decreased glucose-induced postprandial glucose and insulinemic reponses Kwak et al. 2013). The mechanism involved is not clearly understood. In addition to the GRAS status by the US, tagatose has an EFSA-approved health claim for tooth demineralization and lower glucose response (EFSA 2011).

Allulose has an available energy content of 0.2 kcal/g. In contrast to tagatose, after oral administration, ≈70% of allulose is absorbed and excreted via urine. This suggests that the absorbed allulose is not or minimally metabolized. The proportion of allulose that is unabsorbed may pass through the colon and be fermented by gut bacteria (Hossain et al. 2015). Oral administration of allulose decreases maltodextrin-induced glucose excursion, both in healthy subjects and in subjects with impaired glucose tolerance (Hayashi et al. 2010). The postulated mechanism may involve inhibition of a-glucosidases (Hayashi et al. 2010). Despite these promising effects, some early toxicity studies in rats showed increased liver and kidney weight, which warrants caution and further investigations to determine chronic effects in humans (Matsuo et al. 2003; 2012)

In summary, a healthful carbohydrate profile is constituted by dietary fibers, slowly digestible carbohydrates and, when a certain level of sweetness is required for palatability, low amounts of sugars, preferably those of low-caloric value. Opting for the consumption of cereal grains, pulses, fruits and vegetables in their whole form will significantly contribute to the intake of healthful carbohydrates. These sources should contribute to a healthy diet and should be preferred for the development of food products with "blends of healthful carbohydrates" as previously described. However, the recalcitrant nature of insoluble dietary fibers present in whole foods and the susceptibility of raw starch crystallinity to cooking pose considerable technological challenges.

Processing healthful carbohydrates: Current challenges and opportunities

Historically, humans have employed a variety of cooking techniques in order to improve the organoleptic and nutritional quality of food (e.g., increased food digestibility) (Carmody et al. 2011). Although industrial food processing generally tends to be negatively perceived by the general population (Cardello 2003), it is often key for preservation by achieving reductions of anti-nutritional factors (Hotz and Gibson 2007), toxins, or pathogenic microorganisms (Beuchat 2002). The knowledge in the field of food processing is vast and each type of macronutrient imparts specific changes to foods during processing. Here, we focus on examples of food processing techniques that are relevant for sources of healthful carbohydrates.

As in traditional cooking settings where for instance fruits are peeled or grains are dehulled, sources of healthful complex carbohydrates (e.g., whole grain) are often refined to circumvent the technological challenges, poor organoleptic quality and consumer acceptance of the final products (Poutanen et al. 2014). Nonetheless, healthful carbohydrates can be incorporated into food products with minimal impact on the organoleptic properties of food products and potentially improved nutritional value with the aid of processing. Carefully selected or designed food processing techniques can effect changes on the physicochemical characteristics of healthful carbohydrates, which may allow for their incorporation into food at nutritionally relevant quantities (Wen et al. 2017; Agama-Acevedo et al. 2016). However, when sources of healthful carbohydrates are processed as part of a recipe, the impact on the other ingredients must be considered as well. It is well known that processes of high heat, moisture and shear conditions convert raw starch into a rapidly digestible nutrient that triggers an elevated glycemic and insulinemic responses, when consumed (van Amelsvoort and Weststrate 1989; Wang and Copeland 2013). Therefore, the specific changes to physicochemical properties of nutrients in foods should be carefully considered when investigating their physiological impact. The processing technologies that are widely used for sources of healthful carbohydrates include mechanical fractionation or milling, thermal treatment or cooking, extrusion and enzymatic hydrolysis.

Mechanical fractionation or milling

Dehulling has been traditionally used in households to remove the tough outer layers of cereal grains in order to improve their palatability and for the removal of anti-nutritional factors. Mechanical fraction or milling of cereal grains is the process by which flours are produced at a larger scale. It may be considered the industrial equivalent of dehulling because the production of refined or "white" flours consists of removing the bran and germ to isolate the starchy endosperm of the grain. The removal of such grain components produces flours that are more stable upon storage, have improved processability, and result in food products of preferred sensory quality (i.e., white bread versus whole grain bread). However, refined flours have a total dietary fiber content that is about four times less than that of whole grain flours (Slavin et al. 2000) and, thus, refined cereal flours lack other nutritionally relevant compounds that are found in the bran and germ components (Hemery et al. 2007). Due to the impact that bran and germ components have on stability and sensory quality of whole grain food products (i. e., rancidity, rough textures and darker colors), substantial research efforts have focused on understanding how to process whole grains to enhance their consumption (Slavin and Lloyd 2012). Currently, the recombination of individual whole grain constituents obtained via traditional milling is a widely used and accepted practice for the preparation of whole grain flours and products (van der Kamp et al. 2014; Ross et al. 2017). Similarly, fruits and vegetables are largely consumed without their peels, which are usually inedible, and this practice results in a 50% decrease of their dietary fiber content. Herein lies the

main difference in consuming fruit purees, juices, and/or concentrates instead of whole fruit. However, the nutritional value contained in the peels can be salvaged via various processing technologies. In fact, the extraction of dietary fibers such as pectins, which have been shown to have beneficial health effects, is aided by the mechanical fractionation of discarded peels from citric fruits and apples. Powders manufactured from fruit peels can also be used as ingredients for fiber fortification in a variety of food products (Figuerola et al. 2005; Ajila et al. 2008).

Thermal treatment

Thermal treatment or cooking is the main form of food processing carried out in households and is equally prevalent in the food industry. Cooking by heat application to raw foodstuffs generates the desired flavors and textures as well as ensure that they are safe for consumption. Heat treatment of native starch (as found in raw tubers, cereals and pulses) in the presence of water, such as boiling potatoes or baking bread, results in the loss of the starch granular structure and its subsequent gelatinization. Gelatinized starch is considerably more susceptible to digestive enzymes (Holm et al. 1988; Hernot et al. 2008). Factors such as concentration, fine structure, presence of dietary fibers and the food matrix in which it is embedded have an influence on the extent of starch gelatinization (Lovegrove et al. 2015). Conversely, thermal treatment can also be used to reduce the susceptibility of starch to digestion. Annealing of starch is a type of heat treatment with specific temperature and moisture conditions that alter the crystallinity of starch granules and stabilize their native structure (Tester and Debon 2000). Additionally, when thermal treatment is followed by cooling cycles, the gelatinized starch undergoes a second structural transformation, referred to as retrogradation, that renders the starch more resistant to digestion (Ottenhof and Farhat 2004). For example, the starch in boiled potatoes undergoes retrogradation when the potatoes are stored at refrigeration temperatures overnight (Nayak et al. 2014). Thus, the processing of starchy-foods should target a balance between preserving or effecting slow-digestion profiles as well as preferred organoleptic properties.

Besides starch, thermal treatment can also impact non-starch polysaccharides. The preparation of oat porridge, for instance, solubilizes beta-glucans contained in the oat flakes. For this reason, it is important to realize that viscous and soluble dietary fibers such as beta-glucans or pectins, which bear approved health claims related to cholesterol and blood glucose, are sensitive to heat and shear. Indeed, when pectin and beta-glucans are heated or exposed to shear they tend to depolymerize and such changes can impact the health benefits they confer (Regand et al. 2009; Gunness and Gidley 2010). Non-starch polysaccharides in their insoluble form are also changed by thermal treatment. Cereal brans, which are predominantly insoluble and used for animal feed, undergo a certain level of solubilization when subjected to high heat and pressure conditions (Rose and Inglett 2010). Through targeted processing, cereal brans can be a source of soluble fibers or prebiotic oligosaccharides.

Finally, thermal treatment is applied extensively for drying purposes. Although this tends to be the final step in a processing line, its effects on carbohydrate physicochemical structures should not be overlooked. Depending on the conditions, drying has been shown to change the molecular and digestibility properties of flours in products such as pasta (Stuknyte et al. 2014). Different types of drying processes can also impact the functionality of carbohydrates by changing their hydration capacities and texture properties (Mandge et al. 2014).

Baking

Baking is another type of thermal treatment that is extensively used in the processing of many cereal-based products such as breads and biscuits. However, the effects of baking on the nutritional value of baked goods is largely dependent on the recipe (i. e. water, proteins and fiber content) (Garsetti et al. 2005). The "gentle" form of heat application that occurs during baking does not always effect a dramatic change to the native starch and, if designed purposefully, can actually result in products composed of cereal starches that have a reduced rate or degree of digestibility (Lehmann and Robin 2007). In addition, resistant starch can be generated during the baking and subsequent cooling processes (Sanchez-Pardo et al. 2007; Hallstrom et al. 2011).

Extrusion

Food extrusion is a process where a combination of raw materials is forced through a screw under different conditions of heat, moisture, pressure and mechanical shear (Alam et al. 2016). The impact of extrusion on carbohydrates depends on the specific conditions and the physicochemical features of the ingredients used. The combination of heat, pressure and shear during extrusion can result in the complete or partial gelatinization of starch, or on the generation of resistant starches when other processing aids such as organic acids are used (Hasjim and Jane 2009). Similar to the effects of thermal treatment, non-starch polysaccharides also undergo depolymerization during extrusion (Hernot et al. 2008; Yan et al. 2015). Specific modulation of extrusion parameters in combination with tailored formulations can improve the organoleptic properties of starch and dietary fiber blends (Redgwell et al. 2011). For example, it was reported that the expansion properties of extruded wheat flour was impacted by the addition of wheat bran due to an increase in melt viscosity (Robin et al. 2011). Altan et al. (2009) have also shown that the incorporation of fruit pomace in extruded blends of cereal flours resulted in lower digestibility of starch. Thus, the optimization of extrusion parameters and product recipes is of interest for the generation of carbohydrate blends with acceptable organoleptic properties while maintaining or improving the nutritional value of the carbohydrates.

Enzymatic treatment

Common examples of the application of enzymatic treatment in food processing is the baking of yeast-leavened and sourdough breads (Linko et al. 1997) and the production of alcoholic beverages (Bamforth 2009). The food industry applies

enzymatic treatment in a variety of ways. For instance, one of the ways to produce common food ingredients such as maltodextrins and glucose syrups is via enzymatic hydrolysis of starch (Guzman-Maldonado and Paredes-Lopez 1995). Another example is the hydrolysis of complex carbohydrates for ease of processing because large polymers generate highly viscous pastes that are difficult to pump. As previously discussed, changes to the physicochemical properties of native starches have a direct impact on their digestibility, and hydrolysis of the high molecular weight, starch structure into low molecular weight polymers significantly increases the rate at which it is digested. On the other hand, emerging research has shown the potential of using enzymatic treatment to reduce the digestibility of processed starches (Wu et al. 2015). The generation of highly-branched starch hydrolyzates have been shown to have a comparably slower digesting property both *in vitro* and *in vivo* (Lee et al. 2013), however, further research on how these enzymatically branched starches behave in food products is required.

As previously mentioned, isomaltulose, a slowly digestible disaccharide is produced by the enzymatic isomerization of sucrose. Sucrose can also be enzymatically converted into fructooligosaccharide (FOS), a non-digestible oligosaccharide, by means of fructosyl transferases (Singh et al. 2010). These particular enzymatic reactions convert a rapidly-digested disaccharide into a slowly-digested disaccharide or an indigestible oligosaccharide that does not elicit a high GR. Such enzymatic transformations occur in nature for different purposes and their large-scale application in the food industry can be an opportunity for the development of healthful carbohydrate blends.

In the case of dietary fibers, enzymatic treatments are predominantly used to solubilize fiber components from biomass or cell wall material in order to potentiate the value of plant material that is traditionally discarded. Isolated fiber ingredients have lower impact on the organoleptic properties of food products they are used in. FOS can also be produced from the enzymatic hydrolysis of inulin, which is a fructan polymer of longer chain length. Thus, enzymatic treatment of carbohydrates, in some cases, can be beneficial by changing structural features that result in carbohydrates of low GR or that allow for the enrichment of fiber in food products. A balance between ease of processing and nutritional/physiological values should be always considered.

Conclusions

The impact of food processing on the physicochemical properties of carbohydrates is very complex and, contrary to popular belief, it can contribute significantly to the consumption of healthful carbohydrates by providing means to create them during the manufacture of the product. More specifically, the selection of carbohydrates sources can be tailored to the type of process and vice versa. The optimal combinations can only be possible if the changes that healthful carbohydrates undergo during processing are properly assessed. Such changes should be analyzed in terms of nutritional value but also organoleptic properties because a carbohydrate blend with good nutritional quality can only be healthful if consumed. Therefore, to have a better understanding of how to design or choose the best carbohydrate sources and processing parameters to create healthful carbohydrate blends, the physiological responses elicited should be evaluated in parallel to the impact of such factors on the organoleptic properties of food product, while preserving or increasing nutritional quality as much as possible.

Proposals for future developments

Current knowledge, and evidence linking carbohydrate quality to health, provides scientific support for a number of future developments, including avenues for future research as well as projects linked to product development.

1. This paper provides the rationale for not simply classifying carbohydrates based only on their chemical basis. Carbohydrates should also be classified according to their physiological impact since not all sugars are detrimental to health and not all complex carbohydrates are neutral. Indeed, from a health perspective current dietary recommendations for sugars should be based on their functional properties and physiological effects and not simply on their chemical classifications. For these reasons, well-characterized sugars with both no/low cariogenicity, low GI and lipogenic capacity (i.e., lactose, isomaltulose, tagatose and allulose) might be considered differently regarding the "free/added sugars" labeling as done for sugar alcohols. By contrast, dietary recommendations to prevent excess intake could be extended to oligo- and polysaccharides with potential cariogenic properties and/or promoting a high glycemic and insulin response.
2. Further work on the role of sweet taste perception and exposure on eating behaviour and energy intake could help to identify the drivers of over-consumption of sweet foods, especially sweet energy dense foods.
3. Further research is needed to demonstrate the underlying mechanisms linking postprandial glycemia to cardiometabolic disorders and to identify very early predictive risk biomarkers.
4. This review has also highlighted the need for relevant reformulation of manufactured foods. This includes the three following activities:
   a. Firstly, studying and developing healthful carbohydrate blends that will contribute to the effort of improving consumers' diets.
   b. Secondly, characterizing the physico-chemical properties of alternative carbohydrate sources (e. g., cereal brans, pulses and/or legumes) or their isolated fractions (e. g., soluble fibers and resistant starches) in order to develop optimized blends that have improved ratios between glycemic and non-glycemic carbohydrates. This would also need to take account of nutritional needs, tolerance as well as technological and taste limitations.
   c. Thirdly, studying the impact that structural features (chain length, type and frequency of branches) of glycemic carbohydrates have on their rate of digestibility.
5. The impact of product formulations and industrial processing technologies on energy intake and postprandial

glycemic/ insulinemic and lipid responses is not fully understood, and so further research would be useful.

6. Finally, this review supports the case for developing evidence-based and consumer-friendly communication to guide consumers towards healthier manufactured food and beverage products.

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D-Tagatose Is a Bulk Sweetener with Zero Energy Determined in Rats[1,2]

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ABSTRACT The ketohexose D-tagatose is readily oxidized but contributes poorly to lipid deposition. We therefore examined whether this sugar contributes to energy requirements by determining its net metabolizable energy value in rats. All substrate-induced energy losses from D-tagatose, with sucrose as reference standard, were determined as a single value accounting for the sum of the energy losses to feces, urine, gaseous hydrogen and methane and substrate-induced thermogenesis. A randomized parallel design involving two treatment periods (adaptation to D-tagatose and subsequent energy balance) and two control groups (to control for treatment effects in each period) was used. Rats consumed 1.8 g test carbohydrate daily as a supplement to a basal diet for a 40- or 41-d balance period after prior adaptation for 21 d. Growth, protein and lipid deposition were unaffected by supplementary gross energy intake from D-tagatose compared with an unsupplemented control, but sucrose significantly ($P < 0.05$) increased all three. Based on the changes induced in protein and fat gain during the balance period it was calculated that D-tagatose contributed −3 ± 14% of its heat of combustion to net metabolizable energy, and therefore this ketohexose effectively has a zero energy value. D-Tagatose would potentially be helpful in body weight control, especially in diabetic subjects because of its antidiabetogenic effects. J. Nutr. 126: 1601–1609, 1996.

INDEXING KEY WORDS:

- *D-tagatose* • *net energy value* • *rats*
- *body composition*
- *supplement-induced energy loss*

A method of body weight control that has gained wide acceptance is the reduction of the energy content of foodstuffs. Reduced fat or fat-free products are now commonplace in grocery stores in both the U.S. and Europe. Efforts have also been made to find carbohydrates of reduced energy content. Because at least half of food energy comes from carbohydrates, significant reduction in food energy can potentially be acheived by the introduction of carbohydrates of low energy value. In recent publications (Life Sciences Research Office 1994, Livesey, 1992) the energy content of commonly used dietary fibers and sugar alcohols was reported to vary between 0 and 15 kJ/g. The combined intakes of these carbohydrates is, however, limited by their tolerance in the gastrointestinal tract.

During the last 7 years a ketohexose, D-tagatose, has been examined as a possible bulk sweetener. Nutritional and physiological properties of this sugar that render it potentially useful for such a purpose have been reported (Levin et al. 1995). D-Tagatose has a physical appearance and sweetness essentially identical to sucrose and can be found in trace quantities in some milk products (Levin et al. 1995). The manufacturer of D-tagatose (Biospherics, Beltsville, MD) state that the compound has no toxic, carcinogenic or teratogenic effects in tests conducted to date under conditions specified by the U.S. Food and Drug Administration. In addition, D-tagatose has a lower glycosylating power than glucose, lowers the glycosylating power of glucose by promoting lower postprandial glucose concentrations (Bunn and Higgins 1994, Syrovy 1994) and has been patented as an antidiabetic agent (Zehner et al. 1994 and 1995).

Although D-tagatose was shown to be readily oxidized to CO_2 in vivo, unpublished research showed that it did not support fat deposition and appeared to deliver less usable energy than sucrose (Levin et al. 1995, Livesey and Brown 1995). Evidence of thermogenic ef-

[1] Supported in part by Biospherics, Beltsville, MD.

[2] The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked as "advertisement" in accordance with 18USC section 1734 solely to indicate this fact.

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fects of any carbohydrate other than possible dietary-induced thermogenesis is scant; we were intrigued to discover whether D-tagatose contributes to energy requirements and whether it had an energy value less than might be assumed for a carbohydrate that is completely metabolized. We present evidence that in rats the energy value of D-tagatose is effectively zero.

MATERIALS AND METHODS

General considerations. The net metabolizable energy value of D-tagatose was determined in rats on the basis of its influence on body composition, essentially as described by Brown and Livesey (1994) and Livesey and Brown (1995). The same equations were used to calculate energy values, but modifications to the experimental design were made in an attempt to keep precision and accuracy while fully adapting rats to the test carbohydrate before a balance period. The design is therefore described in detail.

Materials. D-Tagatose was from Biospherics, Beltsville, MD, and sucrose was Silver Spoon (beet) originating from British Sugar, Peterborough, UK.

Animals. The routine animal care and experimental procedures used in this study were in accordance with the statutory regulations and ethical guidelines of the United Kingdom Home Office. One hundred fifty specific pathogen-free male Wistar rats weighing 80–100 g (A. Tuck and Son, Battlebridge, Essex, UK) were housed individually in polypropylene cages with wire mesh floors and roofs (Type RB3, North Kent Plastics, Dentford, UK). Room temperature was maintained at 21 ± 1°C, lighting was from 0600 to 1800 h and drinking water was freely available. Rats were fed a basal diet (Table 1) for 5 d to secure their rehydration before starting the study.

Experimental design. A randomized parallel design was used in which rats were allocated using random numbers to one of five groups of 30 (Fig. 1). Each group was used for the energy value determination of a single test carbohydrate. Results from two groups are reported here and are for sucrose, a known reference standard, and D-tagatose. The remaining three groups were used to determine energy values of three other carbohydrates not included in this report because they are proprietary data. Nevertheless, the statistical analysis included data from all these rats to improve the reliability of variance estimates.

In each dietary group all 30 rats consumed the test or reference carbohydrate for 21 d (Fig. 1), which we have called the adaptation period, although rats need not adapt to sucrose. Afterward three subgroups of 10 rats per group were selected at random; one for immediate body composition analysis, one from which the test (or reference) carbohydrate was removed from the diets for 40 or 41 d and one that continued to receive the test (or reference) carbohydrate for 40 or 41 d.

TABLE 1

Composition of the basal diet either consumed alone, as control or with sucrose and D-tagatose as dietary treatments in rats

Component	Amount
	g
Maize starch[1]	310
Sucrose	310
Casein[2]	200
L-Methionine	2
Maize oil	80
Cellulose	40
Mineral mix[3]	40
Vitamin mix[4]	20
Total weight	1002

[1] Contained ~10% moisture and was Snowflake from Corn Products, Manchester, UK.

[2] Edible, mesh 30, from G. Fiske, Richmond Surrey, UK.

[3] Produced in mg per kg basal diet: $CaHPO_4$, 13; $CaCO_3$, 8.3; KCl, 7.04; Na_2HPO_4, 7.4; $MnSO_4 \cdot H_2O$; $ZnCO_3$, 0.1; $FeSO_4 \cdot 7H_2O$, 0.144; $CuSO_4$, 0.023; KIO_3, 0.001.

[4] Produced in mg per kg basal diet: nicotinic acid, 60; cyanocobalamin in mannitol, 50; calcium D-pantothenate, 40; thiamine hydrochloride, 10; riboflavin, 10; α-pteroyl-monoglutamic acid, 10; pyridoxine, 10; biotin, 1; menadione, 2; Rivomix E-50 (containing 7.5 mg RRR-tocopherol acetate, Roche, Welling Garden City, Herts, UK), 150; Rivomix A-500 (containing 3.75 mg retinol, Roche), 25; Rivomix D_3-500 (containing 0.19 mg cholecalciferol, Roche), 15; choline bitartrate, 1800; maize starch carrier, 17.8 g.

Diets and prescribed intakes. The feeding protocol is shown in Table 2. During both the adaptation and balance periods rats were given individually weighed portions of food daily that contained a fixed amount of the basal diet (15.2 g/d, Table 1) with or without carbohydrate supplement. The basal diet was based on AIN-76 modified to include the starch (Wise 1982). Adaptation to the test carbohydrate was stepwise to avoid possible diarrhea and reached 1.8 g per rat daily. During adaptation sucrose was fed at half the rate of other carbohydrates in anticipation of lower energy values. Food was provided daily, and food spillage was carefully measured each day; when it occurred, an equal amount of fresh diet was added the following day.

Carcass preparation and analyses. Rats were killed by cervical dislocation after deep surgical anesthesia with intraperitoneal sodium pentobarbital (120 g/L; 1 mL/kg body weight). After removal of digesta the analysis of carcasses for fat and lean dry matter was as described before (Brown and Livesey 1994, Livesey and Brown 1995). Protein was the difference in lean dry matter and ash content determined gravimetrically after charring 1-g samples and heating to 480°C for 72 h in a muffle furnace. The heat of combustion of carbohydrates and diets was determined by adiabatic bomb calorimetry (Gallenkamp, Loughborough, UK) by using

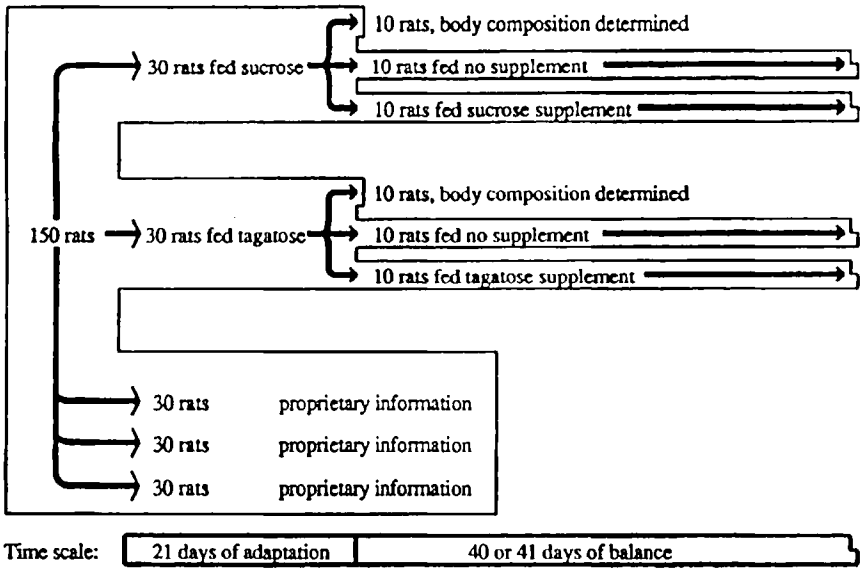

FIGURE 1 Experimental protocol used for the determination of net metabolizable energy values in the rat. Rats were divided into groups of 30, each with subgroups of 10. In each group all 30 rats were adapted to the test or reference carbohydrate for 21 d before a balance period of 40 or 41 d. Body compositions were determined at the start and end of the balance period, in which time one subgroup of rats received basal diet alone and another received the same amount of basal diet plus the test or reference material.

0.5- to 1-g dry samples and benzoic acid thermochemical standard (Brown 1993).

Model for calculating net metabolizable energy. The net metabolizable energy value of a supplement (NEV_s)[4] was the energy available to the rats from the supplement after accounting for all supplement-induced energy losses (SIEL). The SIEL included losses to feces, urine, combustible gases (H_2 and CH_4) and supplement-induced energy expenditure. The NEV_s was calculated as described below in four steps (Equations 1–4) as suggested (Livesey 1993) and applied previously (Brown and Livesey 1994, Livesey 1990, Livesey and Brown 1995). The calculations are briefly described below beginning with the calculation of the sum of the metabolizable energy for maintenance (ME_m, kJ) and the supplement-induced energy loss (SIEL; kJ), for each diet in the balance period according to Equation 1:

$$ME_m + SIEL = IE - IE_b \cdot (1 - Q_b) - 1.36 \cdot EF - 2.25 \cdot EP \quad (1)$$

The SIEL was zero when there was no supplement in the diet, IE was the total intake of gross energy, IE_b was the intake of gross energy from the basal portion of the diet alone, Q_b was the metabolizability of the basal portion of the diet and was 0.9, the coefficients 1.36 and 2.25 were the energy costs (kJ/kJ) of fat and protein deposition from the semipurified diet (Pullar and Webster 1977) and EF and EP were the energies (kJ) depos-

[4] Abbreviations used: AP, average protein content of rats; DEIT, dietary energy-induced thermogenesis; EF, energy accretion as fat; EP, energy accretion as protein; IE, intake of energy; I_s, intake of supplement; ME_m, metabolizable energy for maintenance; NEV_s, net metabolizable energy value; Q_b, metabolizability of a basal diet; RSD, residual standard deviation; SIEL, supplement-induced energy loss.

TABLE 2

The rates of consumption of basal diet, sucrose and D-tagatose supplements by rats during both adaptation and balance periods[1]

	Consumption rate	
	Sucrose	D-Tagatose
	g/(rat·d)	
Basal diet		
All rats consumed 15.2 g/(rat·d) of basal diet every day of the adaption and balance periods		
Days 1–7 of adaptation	0.45	0.9
Days 8–21 of adaptation	0.9	1.8
Days 1–40 or 41 of balance		
Supplemented subgroup	1.8	1.8
Unsupplemented subgroup	0	0

[1] Supplements were mixed with the basal diet. Degrees of freedom were zero.

ited in fat and protein, respectively, during the balance period. For these calculations 39 kJ/g fat deposited and 23.6 kJ/g protein deposited were used to convert weights to energies stored in fat and protein, respectively. Body fat and protein were determined directly at the end of the balance period, but at the start they were estimated from linear regression relationships between fat and live weight and between protein and live weight, relationships that were derived for each group by using carcass compositions in the corresponding subgroup killed at the end of the adaptation period.

Second, the difference in ME_m + SIEL between rats on the test (t) and basal (b) diet, when SIEL was zero, gave the supplement-induced energy loss (SIEL). The SIEL was obtained with greater precision by normalizing to the average mass of (metabolically active) lean tissue, i.e., average protein content of the rats over the treatment period (AP; g):

$$SIEL/AP_t = [(ME_m + SIEL)/AP]_t - [ME_m/AP]_b \quad (2)$$

Third, the value of SIEL per unit mass of the supplement was obtained by dividing by the weight of the supplement eaten during the treatment period (I_s, g), where I_s was also normalized to AP. Because metabolizable energy intake and the lean mass of the rats are determinants of ME_m/AP and $(ME_m + SIEL)/AP$ in Equation 1 (Brown and Livesey 1994), these outcomes were adjusted toward equal values of AP for each animal (see below) before calculation of $SIEL/AP_t$:

$$SIEL/I_s = (SIEL/AP_t)/(I_S/AP_t) \quad (3)$$

Finally, the difference between the heat of combustion of a supplement (ΔH_c; kJ/g) and the supplement-induced energy loss ($SIEL/I_s$; kJ/g) gave the net metabolizable energy value of the supplement (NEV_s; kJ/g):

$$NEV_s = \Delta H_c - SIEL/I_s \quad (4)$$

Adjustment of NEV_s for the effect of the supplementary energy on ME_m. Provision of the extra energy from the supplement may increase ME_m because of possible dietary energy-induced thermogenesis (DEIT). Adjustment for such an increase in ME_m was derived, assuming the true value of SIEL for sucrose was zero and the experimental value obtained was actually due to DEIT. Thus adjustments to $(ME_m + SIEL)/AP_t$ for the test carbohydrate were made possible from Equation 5 in which DEIT is equal to the experimentally determined value of $SIEL_{sucrose}$:

$$[(ME_m + SIEL)/(AP_t]_{adj} = [(ME_m + SIEL)/AP_t] - I_s \cdot NEV_s \cdot (DEIT_{sucrose}/ME_{sucrose})/AP_{sucrose} \quad (5)$$

NEV_s was calculated again with iterations until a constant value of NEV_s was reached.

Statistics. The statistical methods used (linear regression, ANOVA, ANCOVA, *F*-test, least significant difference test) were as described in Mead and Curnow (1983) and conducted using the Minitab statistical package (Ryan, Joiner and Ryan 1985). Results were expressed as means and residual standard deviations (RSD) or means and SEM. When *F*-tests were significant, differences between subgroup means (contrasts) were tested using the least significant difference test (LSD, $P < 0.05$; Mead and Curnow 1983).

Live weight gains were adjusted for initial live weights by covariance analysis (ANCOVA). The ME_m/AP (Equation 2) was expected to vary with lean mass or protein content of the rats (AP). Such associations were approximately equal for each diet, and so minor adjustments toward means at equal AP were made by covariance analysis (ANCOVA). Similarly, half of the rats in each subgroup had a balance period of 40 d and half of 41 d. Mean values were obtained after adjustment to 40.5 d by use of blocking factors (Mead and Curnow 1983).

Missing and outlying data. One rat in the D-tagatose group failed to thrive and was removed from the study. Inspection of studentized residuals (Mead and Curnow 1983) indicated possible outlying values (> 2.5 RSD) that were omitted from the computation of energy values without further analysis of discordancy.

RESULTS

Live weights. Rats grew satisfactorily throughout the adaptation and balance periods, and there was no evidence of diarrhea. Randomization of the rats before adaptation resulted in similar subgroup mean body weights, within 86 to 88 g (**Table 3**). Likewise, there was no significant difference in the live weights at the end of the adaptation period. However, during the balance period the sucrose supplement caused extra weight gain compared with rats fed the basal diet alone (15 g, $P < 0.05$; Table 3), but the D-tagatose supplement did not (−3 g, $P > 0.5$; Table 3).

Energy balance. Intakes of gross energy from the basal portion of the diet during the balance period were similar in all subgroups, at 11.2 MJ per rat-balance period (IE_b; **Table 4**). Likewise, the intake of gross energy from the sucrose and D-tagatose supplements was similar in both test groups, at 1.2 MJ per rat-balance period (IE_S; Table 4).

Although the sucrose supplement significantly elevated energy gain both as protein (by 72 kJ EP per rat-balance period, $P < 0.05$, Table 4,) and as fat (251 kJ EF per rat-balance period, $P < 0.05$, Table 4), the D-tagatose did neither. Likewise, at the end of the adaptation period rats consuming D-tagatose had significantly less body fat than those fed sucrose (23.7 vs. 28.1 ±2 SEM g fat per rat, $P < 0.01$).

Estimates of energy expenditure on maintenance (ME_m) plus substrate-induced energy losses (SIEL) were

TABLE 3

Mean live weights and live weight gains of rats consuming the basal diet alone or basal diet supplemented with either sucrose or D-tagatose during the period of adaptation and balance

	n[1]	Live body weights			Weight gains during balance[4]
		Start[1]	Postadaptation[2]	Postbalance[3]	
		g			g/40·5 d
Sucrose					
Control	10	86	207	—	—
Basal	10	86	203	305[a]	102[a]
+Sucrose	10	88	204	320[b]	117[b]
Tagatose					
Control	10	86	208	—	—
Basal	10	87	209	305[a]	96[a]
+Tagatose	9	86	207	309[a]	99[a]
RSD[1]		7	7	11	9
df[1]		145	144	89	89

[1] The degrees of freedom exceed the sum of n shown because additional information was gained from those study groups not reported to improve the reliability of the variance estimates (see experimental design, Fig. 1). One missing datum explains $n = 9$ for the tagatose + tagatose subgroup.

[2] Live weights before feeding.

[3] Live weights 2–3 h after feeding less weight of digesta.

[4] Adjusted for covariance with live weight at the start of the experiment. The coefficient of covariance was −0.98 ± 0.08 g/g starting live weight.

[a,b] Values with different superscripts in the same column differ significantly (least significant difference test, $P < 0.05$).

Abbreviations: RSD, residual standard deviation; df, degrees of freedom.

elevated by both sucrose and D-tagatose but significantly only for D-tagatose ($P < 0.05$). This was observed whether these estimates were adjusted or not for differences in both the duration of the balance period and the average lean mass of the rats during the balance period (Table 4). The adjustments had only small effects on the mean values but increased the power of the study by lowering the residual variance. After both adjustments had been made, the increase in ME_m + SIEL due to the D-tagatose supplement (by 24 kJ/g AP

TABLE 4

Components of energy balance in rats consuming basal diet either alone or with supplements of sucrose and D-tagatose

Treatment			Intake of gross energy				Calculated metabolisable energy for maintenance (ME_m) plus substrate-induced energy losses (SIEL)			
Adaptation period	Balance period	$(n$[1]$)$	Basal diet IE_b	Supplement IE_s	Fat gain EF	Lean gain EP	ME_m + SIEL (unadjusted)[2]	ME_m + SIEL (adjusted)[2]	ME_m + SIEL / AL (unadjusted)[3]	ME_m + SIEL / AL (adjusted)[3]
			kJ/40.5 d						kJ/g	
Sucrose	0	(10)[1]	11,202	0	48[a]	589[a]	8668[b]	8668[b]	175[b]	175[b]
Sucrose	Sucrose	(9)	11,187	1204	299[b]	661[b]	9379[c]	9394[c]	183[c]	183[c]
Tagatose	0	(9)	11,218	0	321[b]	560[a]	8402[a]	8388[a]	163[a]	163[a]
Tagatose	Tagatose	(7)	11,222	1146	387[b]	544[a]	9492[c]	9473[c]	187[c]	187[c]
RSD			—	—	201	71	241	200	8	4
df			0	0	77	77	77	76	76	75

[1] The degrees of freedom exceed the sum of n shown because additional information was gained from those study groups not reported to improve the reliability of the variance estimates (see experimental design, Fig. 1). Values of $n = 10$ minus missing datum (see Materials and Methods) and minus outlying values (Fig. 2).

[2] Adjustments to 40.5 d of balance by using blocking factors.

[3] Adjustments by covariance to similar values of AP. The coefficient of covariance was −3.5 ± 0.3 kJ/g.

[a–c] Values with different superscripts in the same column are significantly different using the least significant difference test ($P < 0.05$).

Abbreviations: IE, intake of gross energy with subscripts b (basal diet) and t (test supplement); EF, energy gain as fat; EP, energy gain as protein; ME_m, metabolizable energy for maintenance; SIEL, substrate-induced energy loss; AP, average protein mass of animals in the balance period; RSD, residual standard deviation; df, degrees of freedom.

TABLE 5

Calculation of the first estimates of substrate-induced energy loss (SIEL) and net metabolizable energy value (NEV_s) for sucrose and D-tagatose in the rat

Terms[1]:	ME_m + SIEL	ME_m	SIEL			I_s	SIEL		NEV_s	
	AP_t	AP_b	AP	AP_t	I_s	AP	I_s	ΔH_c	1st estimate	10th iteration[2]
Relationships:	A	B	C = A − B	D	E	F = E/D	G = C/F	H	I = H − G	See Table 6
	kJ/g			*g*		*g/g*	*kJ/g*			
Sucrose	183	175	8.0	50.9	73.7	1.45	5.5	16.3	10.8	16.3
Tagatose	187	163	23.5	49.7	74.2	1.49	15.8	15.4	−0.4	−0.5
SD[3]	4	4	5.6	2.7	—	0.04	3.8	—	3.8	5.7

[1] Terms used in Equations 1–6.

[2] For the iteration procedure see Table 6 and Equation 5.

[3] Degrees of freedom attached to values of SD are >70. They exceed the sum of rats used in the reported groups because additional information was gained from those study groups not reported, to improve the reliability of the variance estimates (see experimental design, Fig. 1).

Abbreviations: ME_m, metabolizable energy for maintenance; SIEL, substrate-induced energy loss; AP, average protein content of rats during the balance period; I_s, intake of supplement; ΔH_c, heat of combustion of the supplement; NEV_s, net metabolizable energy value of the supplement.

in each rat balance period, $P < 0.05$, Table 4) was greater than that due to the sucrose supplement (8 kJ/g AP for each rat-balance period, Table 4). The incremental values (24 vs. 8 kJ/gAP in each rat-balance period) were significantly different ($P < 0.05$).

Adaptation and energy balance. Among those rats fed the unsupplemented diets during the balance period, the low body fat at the end of adaptation period noted above (23.7 vs. 28.1 ± 2 SEM g fat per rat, $P < 0.01$) was associated with a higher fat gain during the subsequent balance period (EF, 321 vs. 48 g per rat-balance period; Table 4, $P < 0.05$) and a lower value for metabolizable energy for maintenence (ME_m + SIEL, where SIEL is zero, 8.4 vs. 8.7 MJ /rat-balance period, Table 4). This implied that energy status at the start of the balance period was a determinant of ME_m.

TABLE 6

The net energy value (NEV_s) of D-tagatose and sucrose in the rat and their heats of combustion[1]

Iteration (Equation 5)	NEV_s of D-tagatose	NEV_s of sucrose
	kJ/g	
1st	−0.4	10.8
2nd	−0.5	14.4
3rd	−0.5	15.7
4th	−0.5	16.1
5th	−0.5	16.2
6th	−0.5	16.3
10th	−0.5	16.3
SEM	2.2	1.8
n, basal[2]	9	10
n, test[2]	7	9

[1] Heats of combustion were 15.7 and 16.3 kJ/g for D-tagatose and sucrose.

[2] Values of $n = 10$ minus missing datum (see Materials and Methods) and minus outlying values (Fig. 2).

The net energy values of the supplements. The calculation of net energy values is shown in **Tables 5** and **6**. The value of $NEV_{tagatose}$ was slightly negative (−0.4 kJ/g), but with an SEM of 2.2 it was not significantly different from zero. The calculated values of $NEV_{sucrose}$ and $NEV_{tagatose}$ during iteration (Equation 5, Table 6) show the value for sucrose to change as expected from the first to sixth iteration (from 10.8 to 16.3 kJ/g). However, the $NEV_{tagatose}$ changed relatively little (from −0.4 to −0.5 kJ/g).

Outlying values. In deriving energy values for D-tagatose, data from certain rats were discarded because they were regarded as unreliable statistical outliers (> 2.5 RSD). Both outlying and inlying values of the ME_m + SIEL (per g AL) are shown in Figure 2. In the box-dot plot (Fig. 2) the boxes show ± 2 RSD based on all rats in the study while the dots show individual data for each rat in the sucrose and D-tagatose subgroups. The dots distribute along the boxes, indicating similar variances between reported data (dots) and the study as a whole (boxes). The dot-box variance ratio or *F*-ratio was 1.18, which with $n_1 = 35$ (dots) and $N_2 = 100$ (box) was not significantly different (*F*-distribution tables). The outliers occurred at both the upper and the lower end of the range for D-tagatose in the balance period, thus their omission had minimal impact on the derived energy value. Conserving all observations gave a net metabolizable energy value for D-tagatose of −2.2 kJ/g after iteration. Eliminating only the single most outlying value, which had an RSD > 6, resulted in a more negative energy value for D-tagatose.

With inclusion or exclusion of the outlying values, the data give evidence that the energy value of D-tagatose in the growing rat was effectively zero.

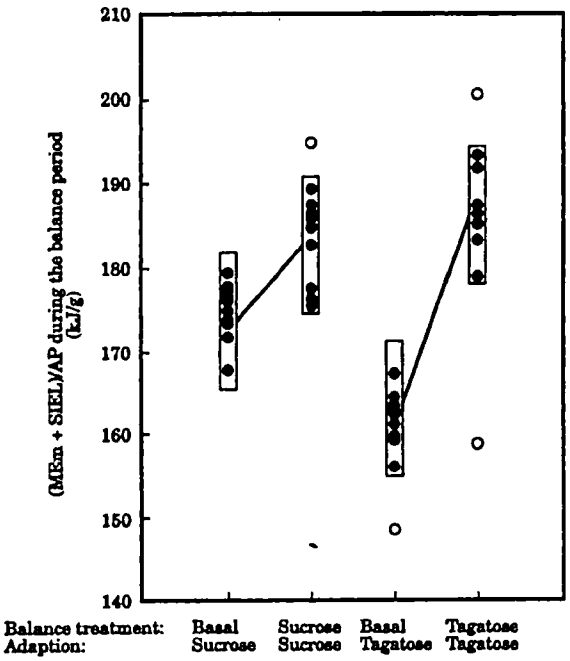

FIGURE 2 Distribution of the calculated metabolizable energy for maintenence (ME_m) plus substrate-induced energy loss (SIEL) when expressed per g average protein (AP) content of rats over the balance period. Data are adjusted to a balance period of 40.5 d and for variation in AP. Boxes show ± 2 residual standard deviations (derived from all rats in the study), dots show data for individual rats (data reported in the study) and lines join mean values for the basal and test subgroups. Data omitted from the calculation of energy values are shown outside the boxes, with open symbols. Treatment and corresponding basal mean values are significantly different (LSD test, $P < 0.05$).

DISCUSSION

We previously reported a method for the determination of energy values of carbohydrates based on their influence on fat and protein deposition in rats (Brown and Livesey 1994, Livesey 1990 and 1993, Livesey and Brown 1995). The validity of the method was shown with both starch (Brown and Livesey 1993) and sucrose (Livesey and Brown 1995) as fully available energy yielding reference materials and with cellulose (Brown and Livesey 1994) and L-glucose (Livesey and Brown 1995) as zero energy reference materials. Likewise, with this approach it was shown that both L-fructose and L-gulose supply energy to metabolism in amounts expected from fecal and urinary excretions (Livesey and Brown 1995). By contrast, the viscous polysaccharide guar gum supplies energy via fermentation (Davies, Brown and Livesey 1991) but equally increased energy expenditure, resulting in a net metabolizable energy value that is close to zero. Contributing to the increased energy expenditure on consumption of guar gum is an increase in the size of digestive organs (Brown and Livesey 1994).

Studies of the thermogenic effects of carbohydrates caused by other than dietary energy induction are scant, and we were intrigued by observations of decreased fat deposition in rats fed D-tagatose while experiments with ^{14}C-labeled D-tagatose indicated the carbohydrate is fully or nearly fully oxidized in the rat (Levin et al. 1995). This implied either an increased energy expenditure or incomplete coupling between D-tagatose oxidation and ATP generation. We questioned, therefore, whether D-tagatose contributed any useful energy to the rat even though it is used completely in oxidative metabolism. We find that D-tagatose, like the guar gum mentioned earlier, appears not to supply significant amounts of useful energy because the derived net metabolizable energy value (−0.5 kJ/g) was not significantly different from zero (± SEM 2.2 kJ/g). The energy yield from D-tagatose corresponds to −3 ± 14% of its heat of combustion, and this ketohexose, therefore, did not contribute to the rats' energy requirements.

Although the energy evaluation approach used in this paper has proved valid, there have been difficulties with rats when previously attempting to adapt them to the test carbohydrate before the balance period. The influence of adaptation on the result has previously been minimized by the choice of relatively long balance periods. An attempt to introduce a period of adaptation to a test carbohydrate before the balance (Livesey and Brown 1995) did not work because rats gained too variable amounts of body fat before the balance period began, and it was difficult to accurately predict body compositions after the adaptation period. Those rats had free access to food during the adaptation period. Minimizing food intake during the adaptation period in this study appears to have limited fat deposition in the adaptation period and allow an adequate estimate of body composition from live weight at the start of the balance period. The limited intake of energy in the adaptation period may also have had an additional advantage, a lowering of the subsequent expenditure on maintenance. Thus the lower supply of energy from D-tagatose compared with sucrose in the period of adaptation was associated with a lower subsequent value of ME_m in the balance period (Table 4).

The lower fat accretion in the adaptation period when feeding D-tagatose in place of sucrose and the subsequent higher fat deposition and lower metabolizable energy for maintenance (Table 4) in control rats during the balance is consistent with D-tagatose supplying substantially less energy than sucrose. That a decreased fat deposition in one period due to lower energy intake can be accompanied by an adaptation that favors a higher fat deposition in a subsequent period is not new (Dulloo and Girardier 1990) and implies the preservation of body composition through alteration in energy expenditure. However, the present observations give evidence that decreased energy intake due to replacing sucrose by twice the weight of D-taga-

tose during the adaptation is sufficient to elicit such adaptive changes.

Several factors that affect the derived energy value are controlled in these studies. One is an increased thermogenesis due to increasing dietary energy intake from supplementary carbohydrate, an increase that appears more extensive at ambient temperatures below the thermal neutral 28°C in the rat (Brown and Livesey 1994, Brown, Livesey and Dauncey 1991). A distinction had to be made between such dietary energy-induced thermogenesis and supplement-induced energy loss, which includes increases of energy expenditure through inefficient oxidation of the supplement (e.g., through inefficient capture of ATP) or hypertrophy of metabolically active tissues and so forth. In previous work the influence of increased energy intake on energy expenditure has been accounted for in different ways, as a covariance between metabolizable energy for maintenance and metabolizable energy intake (Livesey and Brown 1995) and as blocking factors grouping rats together with equalized intakes (Brown and Livesey 1994). Neither of these approaches was possible in the current design; instead, an iterative approach was used in which it was assumed that sucrose was fully available to metabolism, and the first derived value of the supplement-induced energy loss for sucrose indicated the magnitude of the supplementary energy-induced thermogenesis. In the present instance any error introduced by this procedure in the derivation of the energy value of D-tagatose must be negligible; this is because from the beginning of the iteration this carbohydrate showed no useful energy was yielded to cause supplementary-energy induced thermogenesis.

The observations with guar gum (Brown and Livesey 1994) and with D-tagatose (Table 5) show that some relatively rare carbohydrates can have thermogenic effects. In drawing this conclusion for D-tagatose, we safely assume negligible energy losses from D-tagatose to feces and urine (0 to 15%; Levin et al. 1995). However, such thermic effects cannot be expected of all rare carbohydrates because L-gulose and L-fructose supply metabolizable energy and have no thermogenic influences other than supplementary energy-induced thermogenesis (Livesey and Brown 1995). Isomalt, a disaccharide alcohol, does not have thermic effects that can be allocated to SIEL because the present approach to energy evaluation yields the expected net metabolizable energy value of 8 kJ/g (Livesey 1992).

Finally, the addition of dietary fiber components to the diet is limited to 3–7% of the diet (Livesey 1990). The use of D-tagatose as a bulk sweetener within human tolerance levels could likely replace another 5–10% of the diet. A daily intake of 75 g of D-tagatose is readily tolerated by humans when equally distributed in three meals (Zehner, L., personal communication) Also, substances such as glucose and especially fructose that promote lipogenesis (Szepesi and Michaelis 1986) and have high glycosylation indices (McPhearson, Shilton and Walton 1988) could be replaced with D-tagatose with lower fat accumulation (Levin et al. 1995), lower glycosylation index (Bunn and Higgins 1994, Syrovy 1994) and strong antidiabetic effects in rats (Szepesi 1996). These observations, together with the zero energy value found in the present study, make D-tagatose a carbohydrate with physiological properties potentially valuable for the control of both body weight and symptoms of the metabolic syndrome as seen in diabetes.

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ORIGINAL ARTICLE

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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.

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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 (www.

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 pathophysiologic 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 ($HbA_{1c} < 7.3\%$), ($7.3\% \leq HbA_{1c} = 8.4\%$), ($8.5\% \leq HbA_{1c} = 9.2\%$), ($9.3\% \leq HbA_{1c} = 10.2\%$) 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 H_2 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 ^{14}C-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 CO_2 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 unadaped 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	(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
Jørgensen and Lærke, 1998*	6 pigs with an average weight of 34 ± 4 kg. Two pigs per diet	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	(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
Jensen and Laue, 1998*	Five castrated male pigs fitted with permanent cannulas in the portal vein, a mesenteric vein and a mesenteric artery	Pigs were fed a diet containing 20% sucrose (control) and were switched to a second diet containing 20% 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
Normén *et al.* [52]	Six people (2♂, 4♀) with well-functioning ileostomies with <10 cm of terminal ileum removed	A controlled diet was served during two 2-day periods. In one of the periods, 15 g tagatose was added to the diet daily	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]

(Continued)

Table 1. *(Continued)*

Study	Subjects	Administration	Results
Buemann *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
Buemann *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	Urinary excretion of tagatose was 1.45% of ingested

*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 administrated 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 hyperglycaemia 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
Donner *et al.* [2]; Donner *et al.* [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 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)
Donner *et al.* [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
Donner *et al.* [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
Buemann *et al.* [18]	8 normal persons (8♂)	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 1st 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 *et al.* [60]	12 normal persons (12♂)	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.
Sugirs 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 *et al.* [4]	8 NIDDM patients (4♂, 4♀): 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 4th 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 *et al.* [19]	12 persons (10♂, 2♀) 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; OAAs, 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 ± 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 ± 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/m^2 (range = 17.4–26.9 kg/m^2) 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 administrated 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 administrated with the first bite of each meal.

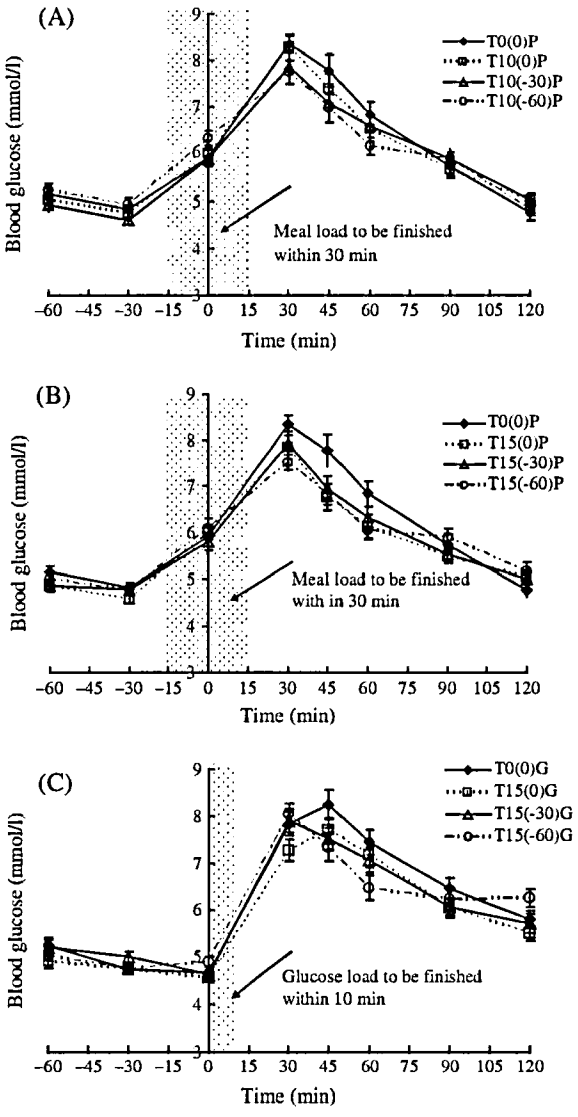

Fig. 1 Effect of time-of-administration of tagatose on the efficacy of blunting the rise of plasma glucose after 90 g meal (instant mashed potato containing 75 g available carbohydrate) load (P) or 75 g glucose load (G). 0, 10 or 15 g tagatose (T) dissolved in 60 ml water were administered at 60, 30 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 ± 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 OAAs, tagatose is GRAS for human oral ingestion. The main side effects of currently available orally administrated 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 intestine. 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.

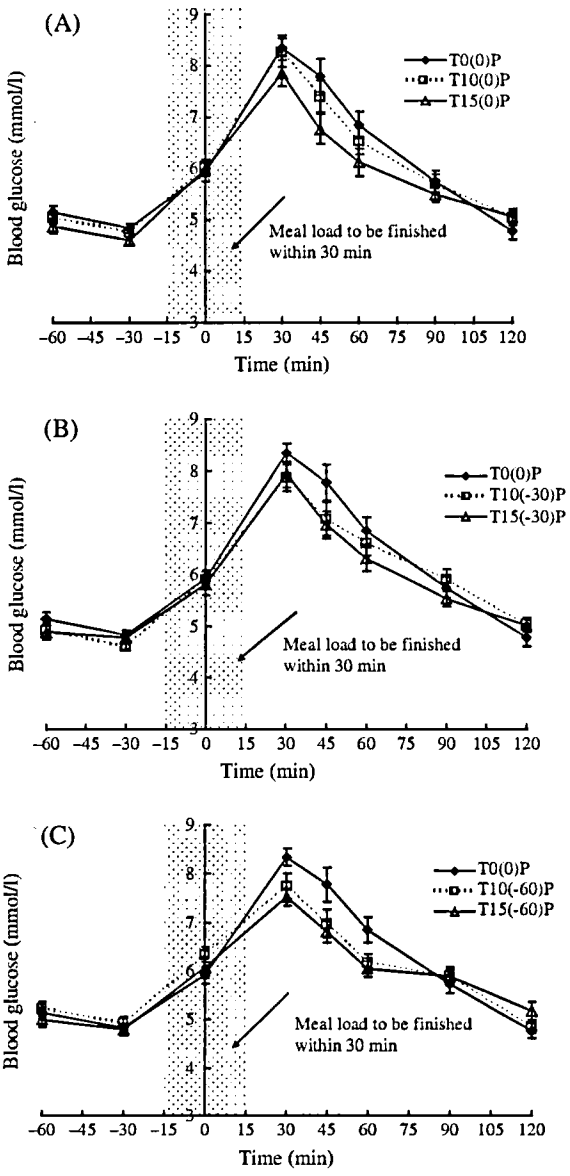

Fig. 2 Dose of tagatose on the efficacy of blunting the rise of plasma glucose after 90 g meal (instant mashed potato containing 75 g available carbohydrate) load (P). 0, 10 or 15 g tagatose (T) dissolved in 60 ml water were administered at 60, 30 or 0 min (immediately) before the planned meal load (0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

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 thiazolinediones. 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 OAAs), 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 contract 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 antihyperglycaemic 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).

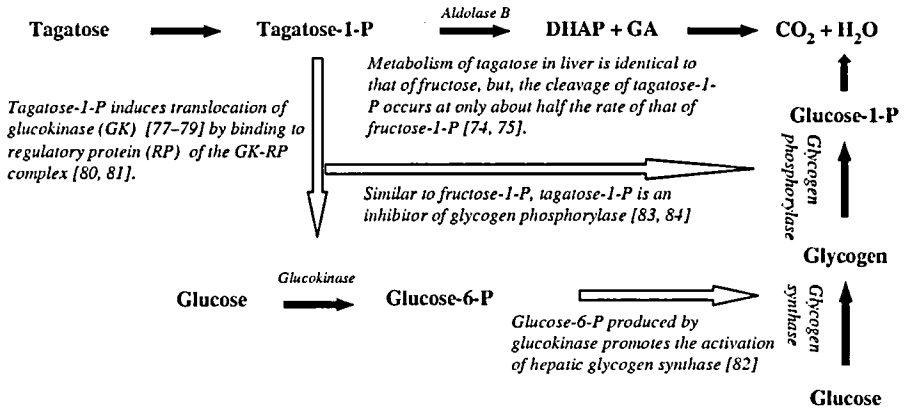

Fig. 3 Plausible mechanism of actions for tagatose (Part One).

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 μmol/kg/min, which raised the portal blood fructose concentration from <6 (basal) to 113, 209 and 426 μmol/l, respectively, increased net hepatic glucose uptake from 15 to 41, 54 and 69 μmol/kg/min, respectively, during a hyperglycaemic, hyperinsulinaemic clamp in 42-h-fasted dogs [85]. An inclusion of low amounts of fructose (2.22 μmol/kg/min) with an intra-duodenal glucose load (44.4 μmol/kg/min) significantly increased net hepatic glucose uptake (28 vs. 17 μmol/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 predominant 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

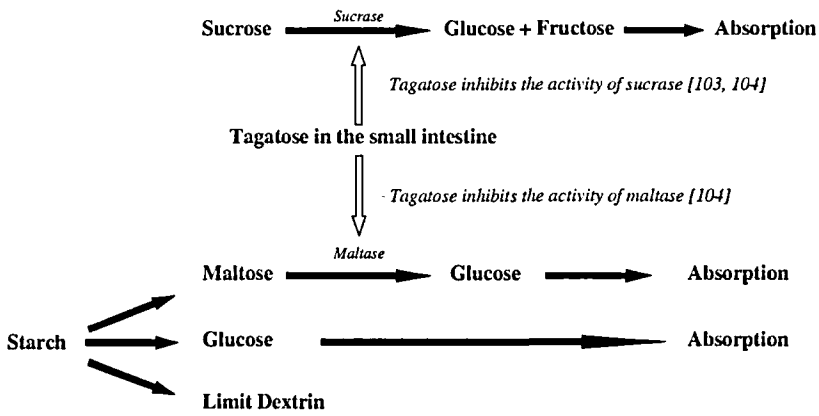

Fig. 4 Plausible mechanism of actions for tagatose (Part Two).

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 distil 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 ± 1.6% compared with a baseline of 8.5 ± 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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Review

Low Glycemic Index Prototype Isomaltulose—Update of Clinical Trials

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Abstract: Low glycemic index diets are supposed to achieve a more beneficial effect on blood glucose control in people with diabetes mellitus and may also provide metabolic benefits for the general population. A prototype of a low-glycemic index carbohydrate is the natural occurring disaccharide isomaltulose that can be commercially produced from sucrose (beet sugar) to industrial scale. It is currently used in various food and drink applications as well as special and clinical nutrition feeds and formula diet as a food ingredient and alternative sugar. Here we provide an overview on clinical trials with isomaltulose including an analysis of its effects on glycemia and fat oxidation as compared to high glycemic index sugars and carbohydrates. In addition, we discuss recent reports on beneficial effects in weight-loss maintenance and pregnancy.

Keywords: glycemic index; isomaltulose; glucose metabolism; diabetes mellitus; weight-loss maintenance; clinical trials; fertility and pregnancy outcome; sweetened beverages

1. Carbohydrates with High and Low Glycemic Index on Postprandial Glucose Homeostasis

Plasma glucose levels change according to food supply but are maintained within a narrow range at 5 mmol/L. Glucose homeostasis is the process of maintenance of plasma glucose at a constant concentration (normoglycemia). The rate of glucose entering the circulation and the rate of glucose leaving are tightly regulated. Postprandial glucose excursions are determined by coordinated processes.

After absorption of ingested carbohydrates from the small intestine, glucose is transported to the liver via the portal vein. Simultaneously, the liver converts from production to uptake and storage of glucose [1]. About one-third of the consumed glucose is absorbed by splanchnic tissues while the larger quantity enters the systemic circulation. Systemic glucose load is determined by the rates of intestinal transfer, splanchnic glucose sequestration, and endogenous glucose production [2]. Glucose appears in the circulation already at 15 min after consumption, peaks at about 30 min and decreases gradually thereafter. Synchronous with rising blood glucose levels endogenous glucose production is suppressed [3].

Glycemic index (GI) and glycemic load (GL) are measures to quantitate the level of rising blood glucose by foods [4]. GI is calculated from the incremental area under the postprandial plasma glucose curve of a test food and compared to that following consumption of an equal carbohydrate amount (typically 50 g) from glucose or white bread expressed as percentage of the standard. GL is calculated by multiplying GI with the amount of available carbohydrates in a given food portion or serving size.