U.S. FOOD & DRUG ADMINISTRATION

Memorandum

To: Administrative File to "Draft Guidance for Industry: The Declaration of Allulose and Calories from Allulose on Nutrition and Supplement Facts Labels"

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Date: February 28, 2019

Subject: Scientific Review of the Evidence on the Metabolism, Caloric Value, Glycemic Response, and Cariogenic Potential of Allulose

The purpose of this memorandum is to provide a summary of the scientific evidence related to the metabolism of allulose including the energy (caloric) value and glycemic response, as well as the cariogenic potential of allulose.[1] Our review is provided below.

Metabolism and Caloric Value

Two human studies (Atiee et al. 2015 and Iida et al. 2010) were identified that investigated the metabolism of allulose. Atiee et al. (2015) used a radioisotope label of allulose to determine the absorption, excretion, and fermentability of allulose. Iida et al. (2010) also measured the metabolism of allulose in four separate trials: The first trial evaluated the absorption of allulose in the small intestine, the second trial evaluated allulose excretion in urine, and the third (short-term) and fourth (long-term) trial evaluated the fermentability of allulose in the large intestine. Results on fermentability were used to estimate the caloric value of allulose. All studies are described below.

Atiee et al. (2015)

In an open-label study, eight U.S. healthy men received an oral solution (240 mL) containing a single dose of 15 g of unlabeled allulose and a minute tracer of ^{14}C-allulose (776 nCi, 99% purity, containing 1% of ^{14}C-fructose), after a light breakfast. Blood, urine and fecal samples were collected at several intervals up to 168 hours after dosing. Expired air was collected for 6 hours after dosing. The ^{14}C radiotracer in urine and feces samples was detected by liquid scintillation counter and accelerator mass spectrometer (AMS), while pooled 24-hour plasma and 48-hour fecal and urine samples were analyzed by high performance liquid chromatography followed by AMS analysis. The ^{14}C radiotracer peaked in plasma at 1 hour after administration of the dose and it was cleared from plasma within 24 hours. In the

[1] Allulose (D-psicose) is a monosaccharide C-3 epimer of D-fructose, naturally occurring in wheat plant and processed foods, e.g., molasses, maple syrup, and brown sugar (Oshima et al., 2006), or it can also be synthesized by enzymatic epimerization of D-fructose (He et al., 2016). Allulose is about 70% as sweet as sucrose (Binkley 1963) and has been generally recognized as safe (FDA GRAS notification No. 400, FDA GRAS notification No. 498, and FDA GRAS notification 693) to be used as a sugar substitute, or added to coffee mix, non-alcoholic beverages, medical foods, and in various low-calorie and sugar-free foods, (e.g., low-calorie pastries, cookies, cake, candies, frozen dairy desserts, yogurt, ready-to-eat cereals and chewing gum).

pooled 24-hour plasma sample, allulose accounted for 80.3% of the radioactivity recovered, while glucose and fructose accounted for more than 10% and the remaining were unknowns. Most of the ^{14}C radiotracer was eliminated in urine; 81-90% of the ^{14}C radiotracer was recovered in urine of seven subjects, while one subject had a recovery rate of 48%. Allulose was the predominant compound identified by the HPLC-AMS profiling in the 48-hour urine pooled sample. The ^{14}C radiotracer eliminated in feces ranged from 2 to 6%. Taken together, 70% 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, 12.5% were not identified (unknown), and the remaining 20.2% of radioactivity was lost during the process. Only 5 out of the 80 expired air samples (approximately 6%) had ^{14}C radiotracer above the validated lower limit of quantitation (LLOQ) of less than 50 dpm, meaning that the majority (94%) of the ^{14}C radiotracer in expired air samples were below the LLOQ. Therefore, expired air was not a major route of elimination for the ^{14}C radiotracer. No adverse events were reported in this study.

Iida et al. (2010)

Trial 1: Carbohydrate availability of allulose in the small intestine

In a crossover study, six Japanese men and women ingested 100-mL solution containing approximately 20 g (0.35 grams per kilogram (g/kg) body weight (BW)) of either allulose or starch hydrolysate dissolved in water, or control (water alone). Respiratory exchange was measured for 180 minutes during which urine was also collected and results were reported at 10-min intervals. The average respiratory quotient (RQ)[2] and the carbohydrate energy expenditure (CEE), calculated using the Weir method[3], were reported. There was no increase in the CEE and RQ after the allulose or water ingestion, while those values were significantly higher, starting at 40 minutes until 180 minutes, after ingestion of the starch hydrolysate used as a positive control, compared to allulose and water. The average RQ was 0.97 for starch hydrolysate and 0.89 for allulose and water. The CEE area under the curve (AUC)[4] was significantly higher after ingestion of the starch hydrolysate (2.33 ± 0.95 kilojoules per minute (KJ/minute/kg BW))[5] compared to that of allulose (0.02 ± 0.62 KJ/minute/kg BW).

Trials 2 and 3: Allulose excretion in urine (trial 2) and fermentability in the large intestine (trial 3)

In a crossover study, 14 Japanese men and women were randomly assigned to ingest three doses: 0.33 g/kg BW (~ 20 g), 0.17 g/kg BW (~10 g), and 0.08 g/kg (~ 5 g), of either allulose or fructooligossacharide (FOS). After a standardized dinner and an overnight fast, subjects receive the test doses. Breath was collected at baseline and in one-hour intervals for 10 hours, while urine was collected at baseline and in three intervals (0-12 hours, 12-24 hours, 24-48 hours) for 48 hours. Easily digestible meals (i.e., not forming hydrogen) containing 34 g of carbohydrate were provided at 4- and 8-hour post dose. The urine cumulative 48-hour excretion rate of allulose ranged from 66.2 ± 12.6 % (20 g) to 78.8 ± 11.7 % (5 g), with most of the dose being eliminated in the first 12 hours, ranging from 54.4 ± 10.5 % (20 g) to 62.7 ± 11.2 % (5 g). The breath hydrogen excretion was statistically significantly lower for allulose compared to FOS at several time points when doses of 10 and 20 g were administered ($P <$

[2] $RQ = (Vco_2/ Vo_2)$.
[3] Weir method: $CEE (KJ/minutes) = (71.80 \times Vco_2) – (50.8 \times Vo_2) – (44.4 \times UN)$, where Vco_2 = carbon dioxide production (L/minute), Vo_2 = oxygen consumption (L/minute), and UN = nitrogen excretion (g/minute).
[4] The area under the curve (AUC) refers to the incremental area under the curve (iAUC) and represents the overall carbohydrate availability over a period of time measured by the energy expenditure after carbohydrate ingestion.
[5] 1 kilocalorie (kcal) = 4.184 kilojoules (kJ) (NIST, 2006).

0.01), and at one time point when subjects received 5 g ($P < 0.05$). The breath hydrogen AUC was also statistically significantly lower for allulose compared to FOS at 20 g (56 ± 91 vs 507 ± 438 ppm/hour, respectively) and 10 g (27 ± 34 *versus* 290 ± 228 parts per million (ppm)/hour, respectively) ($P < 0.01$), but not at 5 g (25 ± 38 *versus* 148 ± 151 ppm/hour, respectively, $P > 0.05$). The authors used FOS as a positive control (energy value of 2 calories per gram for FOS (Oku and Nakamura, 2005)) to estimate the energy value for allulose. Based on this assumption, the energy value of allulose was estimated as 0.39, 0.20, and 0.21 kcal/g for 5, 10, and 20 g, respectively.

Trial 4: Allulose fermentability in the large intestine after an adaptation period

Eight subjects ingested 5 g of allulose three times a day at breakfast, lunch, and dinner for a total of 15 g per day of allulose for eight weeks. Experimental conditions were identical to those in trial 3, except that breath was collected at baseline, i.e., before the ingestion of allulose on the first day, and in one-hour intervals for 8 hours after ingestion of allulose on the first and last days of ingestion. Results of the breath hydrogen were corrected by the baseline values and no statistically significant differences were observed before and after the adaptation period.

Summary of the Evidence

Evidence from the two human studies (Atiee et al. 2015 and Iida et al. 2010) demonstrates that allulose is absorbed in the small intestine but most of the dose (80%) is excreted in urine within 48 hours, 70% being intact allulose (Atiee et al. 2015). The allulose that reaches the large intestine is poorly fermented, as demonstrated in the radiolabeled study using ^{14}C-allulose where 94% of breath samples contained less than the minimum detectable levels of carbon 14 (Atiee et al. 2015). Furthermore, Iida et al. (2010) demonstrated that there was no adaptation in the fermentability of allulose in the large intestine after consumption of 15 g per day of allulose for eight weeks. Based on Iida et al. (2010), the energy value generated from the fermentation of allulose in the large intestine as compared to that of FOS, a non-digestible carbohydrate, provided an estimated caloric value that is no more than 0.4 kcal/g.

Glycemic Response

Five human studies (Iida et al., 2008; Kendall et al., 2014; Wolever et al., 2014; Noronha et al., 2018; Braunstein et al., 2018) were identified that evaluated the impact of allulose on postprandial glycemic response.

Allulose added to Water

Kendall et al. (2014)

In a randomized, crossover study, 10 healthy Canadian men and women received, in two occasions, either 25 g of anhydrous glucose (control) or 25 g of allulose (test) dissolved in 250 mL of water after an overnight fast. Blood samples were collected by finger-prick at baseline (two fasting blood samples drawn 5 min apart) followed by 15, 30, 45, 60, 90, and 120 minutes after ingestion of either allulose or glucose. The blood glucose concentrations were significantly lower at 15, 30, 45, and 60 minutes, after ingestion of the allulose compared to that after ingestion of glucose. Compared to baseline, the blood glucose concentrations were not significantly different at 15, 30, and 45 minutes, and were significantly lower at 60, 90, and 120 minutes after ingestion of allulose. After ingestion of glucose, the blood glucose concentrations were significantly higher at 15, 30, 45 and 60 minutes, and significantly lower at 120 minutes than the blood glucose concentration at baseline. Overall, the blood glucose AUC was

statistically significantly lower after ingestion of allulose when compared to that after ingestion of glucose (5.2 ± 2.1 vs 149 ± 17 mmol × min/L, respectively, $P < 0.0001$).

Wolever et al. (2014)

In a randomized, double-blind, crossover study, 24 Canadian men and women (12 healthy subjects and 12 subjects with diabetes) consumed, in two occasions, either 25 g of glucose (control) or 25 g of allulose (test) dissolved in 240 mL of water after an overnight fast. Blood samples were collected by finger-prick at baseline (two fasting blood samples drawn 5 min apart) followed by 15, 30, 45, 60, 75[6], 90, and 120 minutes after ingestion of either allulose or glucose. Only water was allowed after one-hour post dose until the remaining of the study period. Blood samples were analyzed for glucose and insulin.

In healthy subjects, blood glucose concentrations were statistically significantly lower at 15, 30, 45 and 60 min. The peak blood glucose concentration was significantly lower, after allulose compared to that after glucose consumption (4.37 ± 0.11 vs 8.08 ± 0.32 mmol/L, respectively, $P < 0.001$). The blood glucose AUC after ingestion of allulose was statistically significantly lower than that after ingestion of glucose (2.2 ± 1.3 vs 150 ± 16 mmol x min/L, respectively, $P < 0.0001$). Insulin concentrations were statistically significantly lower at 15, 30, 45, 60, and 75 min, and the peak insulin concentration was significantly lower, after ingestion of allulose compared to that after ingestion of glucose (6.2 ± 0.6 vs 40.3 ± 8.7 µU/mL, respectively, $P = 0.0018$). The insulin AUC after ingestion of allulose was statistically significantly lower than that after ingestion of glucose (57 ± 14 vs 1361 ± 281 µU x min/mL, respectively, $P = 0.0007$).

In diabetic subjects, blood glucose concentrations were statistically significantly lower at all time-points. The peak blood glucose concentration was significantly lower, after ingestion of allulose compared to that after ingestion of glucose (7.39 ± 0.58 vs 13.0 ± 0.72 mmol/L, respectively, $P < 0.001$). The blood glucose AUC after ingestion of allulose was significantly lower than that after ingestion of glucose (3.5 ± 2.0 vs 404 ± 33 mmol x min/L, respectively, $P < 0.0001$). Insulin concentrations were statistically significantly lower at 30, 45, and 60 minutes, however, the peak insulin concentration was not significantly different after ingestion of allulose when compared to that after ingestion of glucose (13.1 ± 1.9 vs 27.3 ± 8.9 µU/mL, respectively). The insulin AUC after ingestion of allulose was statistically significantly lower than that after ingestion of glucose (98 ± 31 vs 1068 ± 359 µU x min/mL, respectively, $P = 0.023$).

Allulose added to Beverages

Iida et al. (2008)

In a randomized, single-blind, crossover study, 20 healthy Japanese men and women were randomly assigned to consume four beverages containing 75 g of maltodextrin with either 0 (control), 2.5, 5.0, or 7.5 g of allulose after an overnight fast. Blood was collected at baseline, and 30, 60, 90 and 120 minutes after subjects ingested the beverage. The blood glucose level was statistically significantly lower at 30 ($P < 0.001$), 60 ($P < 0.001$), and 90 ($P < 0.05$) minutes after subjects ingested a beverage containing 7.5 g of allulose, and at 60 minutes ($P < 0.05$) after ingesting a beverage containing 5 g of allulose, compared to the control beverage (0 g of allulose). The blood glucose AUCs were also statistically significantly lower when subjects ingested 5 g ($P = 0.017$) and 7.5 g ($P < 0.001$), but not 2.5 g ($P <$

[6] For subjects with diabetes no blood sample was collected at 75 minutes.

0.05), of allulose, compared to the control beverage. The insulin levels were statistically significantly lower at 60 minutes after subjects ingested 5 g ($P < 0.05$) and 7.5 g ($P < 0.001$), and at 90 minutes ($P < 0.05$) when subjects ingested 7.5 g of allulose. The insulin AUCs were also statistically significantly lower when subjects ingested 5 g and 7.5 g ($P < 0.05$), but not 2.5 g ($P > 0.05$), of allulose, compared to the control beverage.

Braunstein et al. (2018)

In a randomized, double-blind, crossover study, 25 healthy men and women were randomly assigned to receive six treatments consisting of allulose or fructose each at 0 g, 5 g, or 10 g added to a 75 g of glucose dissolved in 500 mL of water. Blood samples were collected by finger-prick at baseline (two fasting blood samples drawn 30 min apart) followed by 15, 30, 60, 90, and 120 minutes after ingestion of either allulose or fructose. No statistically significant effect of allulose was observed on peak blood glucose or insulin concentration at any dose for any time point ($P > 0.05$). Furthermore, there was no significant effect of allulose at any dose or when doses were pooled on AUC for plasma glucose ($P > 0.05$) and plasma insulin ($P > 0.05$).

Noronha et al. (2018)

In a randomized, double-blind, crossover study, 24 diabetic men and women were randomly assigned to receive six treatments consisting of allulose or fructose each at 0 g, 5 g, or 10 g added to a 75-g glucose solution dissolved in 500 mL of water. Blood samples were collected by finger-prick at baseline (two fasting blood samples drawn 30 min apart) followed by 15, 30, 60, 90, and 120 minutes after ingestion of either allulose or fructose. The plasma glucose AUC was statistically significantly reduced by 8% after ingestion of 10 g of allulose compared to when allulose was not added to the 75-g oral glucose tolerance test (717.4 38.3 vs 777.5 39.9 mmol x min/L, $P = 0.015$), while the plasma glucose AUC following the 5-g dose was of borderline significance ($P = 0.051$). A significant linear dose response on AUC was observed ($P = 0.016$). No statistically significant effect of allulose was observed at any dose for any time point on AUC for plasma insulin ($P > 0.05$).

Summary of the Evidence

When subjects ingested 25 g of allulose added to water the post-prandial blood glucose concentration was significantly lower than that after 25 g of glucose added to water, and it was not statistically significantly different from baseline (Kendall et al. 2014). Furthermore, the post-prandial blood glucose and plasma insulin AUC among healthy and diabetic subjects was significantly lower with ingestion of 25 g of allulose added to water compared to that after ingestion of 25 g glucose added to water ($P < 0.05$) (Wolever et al. 2014). Three studies investigated the impact of allulose on the glycemic response when allulose was added to a beverage, usually a solution containing 75 g of available carbohydrate. One study (Braunstein et al. 2018) showed no statistically significant difference in the post-prandial blood glucose and insulin AUC between beverages containing 5 g and 10 g of allulose and the control beverage. Another study (Iida et al. 2008) among healthy subjects showed a significant reduction in the post-prandial glucose and insulin AUC after ingestion of a beverage containing 5 g and 7.5 g of allulose compared to after ingestion of the control beverage ($P < 0.05$). Among diabetic subjects, 10 g of allulose added to a beverage showed an attenuation of post-prandial blood glucose AUC when compared to the control beverage (Wolever et al. 2018). Therefore, allulose has a negligible effect on the glycemic and insulinemic responses among healthy and diabetic subjects, and in some instances, allulose had an attenuation effect on blood glucose and insulin concentration when allulose was added to beverages.

Cariogenic potential

Two *in vivo* studies (Attin et al. 2016 and Hasturk et al. 2018) were identified that evaluated the cariogenic potential of allulose. One *in vitro* study (Iida et al. 2013) demonstrate the possible mechanism for the non-cariogenic effect of allulose.

Attin (2016)

The cariogenic potential of allulose was tested among three subjects who performed a total of four tests, representing dental plaques of 3, 4, 5, and 7 days old. Briefly, subjects rinsed with 15 mL of 10% D-allulose solution for 2 min, rested for 30 min, then rinsed with 15 mL of 10% sucrose solution for 2 min, resting for another 30 min. Subjects chewed paraffin prior to initiating the test and between rinses with the two solutions. A plaque-covered electrode was used to measure the pH of the dental plaque which was performed continuously, during and for 30 min after rinsing. The plaque pH among all subjects did not drop below pH 5.7 during or after 30-min rinsing with allulose regardless of the plaque age, while the plaque pH dropped during rinsing with the 10% sucrose solution (positive control) and it was below pH 5 after 30-min of rinsing.

Hasturk (2018)

In a randomized, double-blind, crossover study, seven subjects who had experienced a high number of caries in the past year (defined as DMFT[7] score > 5), were randomly assigned to a sequence of rinsing test solutions containing either 4.7% allulose (treatment), 4.7% sucrose (positive control) or water (negative control). The rinse sequences of the three arms (one treatment and two controls) varied among subjects and were separated by a 5-day washout period. On the test day, subjects rinsed with the respective solutions for 1 min and measurements of the dental plaque pH were conducted at the mesiobuccal[8] sites of 6 teeth at several intervals, 0 to 60 min after rising, by using a handheld touch electrode. The post-rinsing mean minimum pH was 6.43 ± 0.12 for allulose which was statistically significantly higher than 5.42 ± 0.11 for sucrose (positive control; $P < 0.0001$) and not statistically significantly different from water (negative control; $P = 0.824$).

Iida et al. (2013)

In an *in vitro* study, *Streptococcus mutans* strain MT 8148 (JCM5175) was inoculated in a phenol red broth with no addition of sugar (control) or supplemented with 1% of either D-allulose, L-allulose, glucose, fructose, sucrose, or xylitol. The culture medium pH and the bacterial growth (optical density (OD) at 660 nm) were measured after 48 hours of inoculation. The final medium pH was 6.1 for xylitol, 5.9 for control, 5.9 for D-allulose, 5.9 for L-allulose, 4.0 for fructose, 3.9 for glucose, and 3.9 for sucrose. The OD was 0.03 for xylitol, 0.03 for control, 0.02 for D-allulose, 0.02 for L-allulose, 0.20 for fructose, 0.26 for glucose, and 0.14 for sucrose. The culture medium containing D-allulose showed a higher pH and low bacterial growth when compared to medium containing either glucose, fructose, or sucrose. Furthermore, the culture medium pH and bacterial growth containing D-allulose was similar to that of the control medium, where no sugar had been added.

[7] DMFT, Decayed Missing Filled Teeth, (DMFT score = number of).
[8] Mesiobuccal is defined as related to the mesial (i.e., being or located in the middle or a median part) and buccal surfaces of a tooth. https://www.merriam-webster.com/medical/mesiobuccal (retrieved on September 6, 2018).

Summary of the Evidence

Two *in vivo* plaque pH studies demonstrated that exposure to allulose did not result in a decrease in the plaque pH levels below 5.7 either during consumption or up to 30 minutes after consumption. Supporting evidence from one *in vitro* study showed that, under the experimental conditions applied, D-allulose was not utilized by *S. mutans*, the main microorganism associated with dental plaque.

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