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Mulberry leaf extract reduces the glycemic indexes of four common dietary carbohydrates

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Abstract

Background: 1-Deoxynojirimycin (DNJ), a component of mulberry leaf extract (MLE), reduces postprandial hyperglycemia by inhibiting intestinal a-glycosidase. The aim of this exploratory study was to investigate the effects of MLE on the glycemic indexes (GI) of common dietary carbohydrates.

Methods: This single-center, randomized, open-label, 7-cycle self-controlled crossover study enrolled 15 healthy volunteers at the National Drug Clinical Trial Institution, Second Affiliated Hospital of Tianjin University of Traditional Chinese Medicine (June 2014 to December 2014). The participants were randomized to receive glucose (3 occasions), glucose+MLE, sucrose+MLE, maltose+MLE, and maltodextrin+MLE orally during 7 visits (every 3 days). Blood glucose level was tested at 15 minutes before and at 15, 30, 45, 60, 90, and 120 minutes after carbohydrate intake. The GI of each carbohydrate relative to glucose (GI = 100) was calculated using the incremental area under the curve method. Safety was assessed at each visit.

Results: All participants completed the protocol. After carbohydrate ingestion, blood glucose level peaked at 30 minutes (glucose, glucose+MLE, sucrose+MLE, and maltose+MLE) or 45 minutes (maltodextrin+MLE) before returning to preprandial levels at 120 minutes. At 30 minutes, the change in blood glucose level was lower for sucrose+MLE, maltose+MLE, and maltodextrin+MLE than for glucose or glucose+MLE ($P < .05$). GI was lowest for sucrose+MLE (43.22 ± 17.47) and maltose+MLE (49.23 ± 22.39), intermediate for maltodextrin+MLE (75.90 ± 26.01), and higher for glucose+MLE (91.88 ± 27.24). MLE reduced the GIs for maltose, sucrose, maltodextrin, and glucose by 53.11%, 33.51%, 31.00%, and 8.12%, respectively. MLE was well tolerated.

Conclusions: Coconsumption of MLE with sucrose, maltose, or maltodextrin can reduce the GI values of these carbohydrates.

Trial registration: Chinese Clinical Trial Registry Platform, no. ChiCTR-IPR-15006484. Registered on May 28, 2015.

Abbreviations: DNJ = deoxynojirimycin, GI = glycemic indexes, MLE = Mulberry leaf extract.

Keywords: glucose, glycemic index, maltose, Mulberry, plant leaf, sucrose

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Trial status: This clinical trial has been completed.

This clinical study was carried out in accordance with the principles of the Declaration of Helsinki and gained approval from the Ethics Review Committee of the Second Affiliated Hospital of Tianjin University of Traditional Chinese Medicine (no. 2014-040-01). All participants provided informed written consent before screening. Participation in the study was completely voluntary, and the participants were able to withdraw from the study at any time. The decision to participate or withdraw did not affect a participant's existing treatment or service received. All personal information collected was kept strictly confidential and used for research purposes only. Research team members were responsible for safe keeping of the personal data. Only the principal investigator and designated research staff had access to the interim results and the final trial dataset.

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1. Introduction

Diabetes mellitus has become a common disease in many countries due to changes in lifestyle and dietary habits.[1] In China, nearly half of all newly diagnosed patients with diabetes show only elevations in postprandial blood sugar.[2] A survey of global diet structures conducted by the United Nations Food and Agriculture Organization in 2013 reported that the proportion of carbohydrate in the Chinese diet was as high as 60% to 70%, whereas the carbohydrate intake in the American diet was 20% to 30%.[3] A study in China found that the diet of patients with type-2 diabetes was based mainly on carbohydrates.[4] A comparison of blood glucose levels between Chinese and western populations established that the consumption of glucose or 5 different types of rice resulted in higher postprandial blood sugar levels in Chinese people than in Europeans.[5]

The glycemic index (GI) is a number associated with the carbohydrates in a particular type of food that indicates the effect of these carbohydrates on a person's blood glucose level 2 hours after consumption of the food. A GI of 100 represents the standard value, which is that for an equivalent amount of pure glucose; high-GI, moderate-GI, and low-GI foods are considered to have GI values > 70, 55 to 70, and < 55, respectively. Low-GI foods produce smaller fluctuations in blood glucose and insulin levels than high-GI foods. Current guidelines for the management of diabetes recommend that glycemic control be improved through the consumption of a diet with a lower GI.[6]

Dietary GI values have been proposed as a potentially useful approach to informing food choices and benefitting health outcomes.[7] Six prospective cohort studies have identified high GI a positive predictor of type-2 diabetes, with the risk ratios ranging from 1.21 to 1.59.[8–13] Furthermore, in a 2008 meta-analysis of 7 prospective cohort studies, the highest quartile of GI was found to have a significant positive association with increased risk of developing type-2 diabetes (summary risk ratio: 1.40; 95% confidence interval [95% CI]: 1.23–1.59).[14] Findings from clinical trials have shown that a low-GI diet can alleviate the reduction in insulin sensitivity in those with impaired glucose tolerance[15] or type-2 diabetes.[16]

The leaves of the mulberry plant (*Morus alba* L) are regarded as both food and a traditional Chinese medicine in China. Its use was first recorded in Divine Husbandman's Classic of Materia Medica (Shennong Bencao Jing; 200–220 CE), and the mulberry leaf has been utilized in the treatment of fever, dizziness, headache, cough, and many other diseases since ancient times. The famous pharmacologist Li Shizhen recorded in his Compendium of Materia Medica (Bencao Gangmu: 1590) that mulberry leaf can be used to treat wasting and thirsting (xiao ke) syndrome, which is now recognized as typical of diabetes.

There is increasing evidence that mulberry leaf contains active constituents that may be of benefit at reducing blood glucose levels. Pharmacologic experiments have shown that mulberry leaf contains alkaloids, flavonoids, polysaccharides, amino acids, simple phenylpropanoids, phenols, and other hypoglycemic components.[17] Furthermore, Kimura et al[18] showed that food-grade mulberry powder suppressed the elevation of postprandial blood glucose in humans. Some alkaloids in mulberry leaf are effective inhibitors of digestive enzymes in mammals.[19] These alkaloids, and especially 1-deoxynojirimycin (DNJ),[20] can reduce the activity of α-glucosidase by competitively inhibiting the binding of natural substrates to the active site of the enzyme.[21] In addition, DNJ has been reported to inhibit intestinal glucose absorption and accelerate hepatic glucose metabolism by directly regulating the expressions of several proteins involved in glucose transport, glycolysis, and gluconeogenesis.[22] Recently, several animal and human studies have reported that mulberry or sericulture products containing DNJ suppressed postprandial increases in blood glucose.[18,23]

We hypothesized that oral consumption of mulberry leaf extract (MLE) together with common dietary carbohydrates would reduce postprandial glucose levels in healthy people. Therefore, the aim of the present exploratory study was to measure postprandial glucose levels in healthy individuals given different combinations of MLE, glucose, sucrose, maltose, and maltodextrin to determine whether the addition of MLE to the diet would reduce the GI of these four carbohydrates.

2. Methods

2.1. Study participants

This single-center, randomized, open-label, 7-cycle self-controlled crossover study enrolled 15 healthy volunteers at the National Drug Clinical Trial Institution, Second Affiliated Hospital of Tianjin University of Traditional Chinese Medicine, between June 2014 and December 2014. The enrolled participants were mainly students at the Tianjin University of Traditional Chinese Medicine. The inclusion criteria were: healthy male or female volunteer; age, 18 to 40 years; body mass index, 18 to 25 kg/m^2; no previous diagnosis of diabetes or prediabetes; fasting blood glucose, 3.9 to 5.5 mmol/L (70–100 mg/dL); not taking any medication for diabetes or insulin resistance; and willing to provide informed written consent for participation in the study. The exclusion criteria were: abnormal results for liver function tests (alanine transaminase and aspartate transaminase), renal function tests (blood urea nitrogen and creatinine), routine blood tests, urinalysis, fecal occult blood test, or electrocardiography; pregnant or breastfeeding women; recent participation in a clinical trial (within the last 3 months); history of malignancy or current malignancy; history of drug dependence, substance abuse, or alcoholism; history of drug or food allergy or allergy to any components of MLE; known history of primary disease of a major organ, such as the heart, liver or kidneys, or digestive, metabolic, neurologic, or psychiatric disorders; had taken drugs known to cause organ damage within the previous 3 months; and had taken any medications within the previous 2 weeks.

The participants received financial compensation after completion of all 7 study cycles; if a participant did not complete all 7 cycles, the financial compensation was reduced accordingly. This study was conducted in accordance with the principles of the Declaration of Helsinki. The informed consent form, study protocol, and sponsor qualifications were approved by the Ethics Committee of the Second Affiliated Hospital of Tianjin University of Traditional Chinese Medicine (no. 2014-040-01). All participants provided informed consent by signing a written consent form before screening. The study is registered in the Chinese Clinical Trial Registry (identifier: ChiCTR-IPR-15006484).

2.2. Determination of sample size

According to the provision of ISO 26642,[24] data from a minimum of 10 healthy volunteers were required. Assuming a dropout rate of 20%, a total of 15 volunteers needed to be enrolled to ensure full datasets from at least 12 participants. Data

Table 1

Experimental schedule for each study group.

Group	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
A	Glucose	Glucose	Glucose	Glucose+ MLE	Maltose+ MLE	Sucrose + MLE	Maltodextrin + MLE
B	Maltodextrin + MLE	Glucose	Glucose	Glucose	Glucose + MLE	Maltose + MLE	Sucrose + MLE
C	Sucrose + MLE	Maltodextrin + MLE	Glucose	Glucose	Glucose	Glucose+ MLE	Maltose+ MLE
D	Maltose+ MLE	Sucrose+ MLE	Maltodextrin+ MLE	Glucose	Glucose	Glucose	Glucose+ MLE
E	Glucose+ MLE	Maltose+ MLE	Sucrose+ MLE	Maltodextrin+ MLE	Glucose	Glucose	Glucose
F	Glucose	Glucose+ MLE	Maltose+ MLE	Sucrose+ MLE	Maltodextrin+ MLE	Glucose	Glucose
G	Glucose	Glucose	Glucose+ MLE	Maltose+ MLE	Sucrose+ MLE	Maltodextrin+ MLE	Glucose

MLE = Mulberry leaf extract.

from the per-protocol population set, defined as all participants who completed the study without any major protocol violations, were used for the analysis.

2.3. Study design

SAS 9.4 statistical software (SAS Institute, Cary, NC) and a random block design were adopted to randomly assign the 15 enrolled participants into 7 groups (A–G; see Table 1), so that 3 participants were allocated to group A and 2 participants were allocated to each of groups B to G. All participants were scheduled to receive 7 interventions in 7 test cycles (3 cycles of glucose alone; MLE plus glucose; MLE plus sucrose; MLE plus maltose; and MLE plus maltodextrin), the order of which depended on the allocated group (Table 1). Neither the participants nor the investigators were blinded to the agents being administered in each test cycle.

2.4. Study products

MLE was extracted from the leaves of *Morus alba* collected in Anhui province, China. The plant underwent species identification at the herbarium of the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, where a voucher specimen of *Morus alba* was kept. The samples were tested at Peking Union Medical College. MLE was prepared in the form of a powder by Botanic Century (Beijing) Co Ltd (Beijing, China) and Anhui Shangshan Biotechnology Co, Ltd (Chuzhou, China). The dried mulberry leaves were extracted by water, and the extraction solution was then ultrafiltered and spray-dried to obtain a powder. An Alliance HPLC system (Waters, Milford, MA) was used to verify that the DNJ content of the powdered extract was 1% by weight.

Glucose was used as reference food,[24] while glucose, maltose, sucrose, and maltodextrin combined with MLE were used as test foods. Sucrose (purity, 99.6%) and maltose (purity, 99.5%) were purchased from Shanghai Ruiyiyuan Food Co, Ltd (Shanghai, China). Anhydrous glucose (purity, 99.8%) and maltodextrin (purity, 99.0%) (manufactured in accordance with GB/T20884-2007 standards) were purchased from Xiwang Phamaceuticals Co, Ltd (Binzhou, China). For administration to the study participants, 50 g of carbohydrate powder with or without 750 mg of MLE (equivalent to 7.5 mg of DNJ) was dissolved in 150 mL of drinking water. Each 1 g of pure glucose, sucrose, maltose, or maltodextrin provides 4 kcal (16.75 kJ) of energy.

2.5. Operating procedures at each visit

The participants were asked to avoid drinking alcohol or partaking in intense physical activity on the day before each visit. Each participant came to the test center before 22:00 the night before the test and stayed overnight at the test center. The participant was fasted for food overnight for at least 10 hours (drinking of water was permitted) until the start of the test at 8:00 AM the next morning. At 8:00, the participant was asked to orally consume the test meal (carbohydrate with or without MLE dissolved in 150 mL warm water) within a 5 minutes period. The participant was then fasted for food and water for 2 hours after consumption of the meal. The admission, food intake, and water intake of the participants were all under the supervision of the investigators. The vital signs (body temperature, respiration, pulse, and blood pressure) of the participants were monitored during each visit, and any adverse reactions were noted.

Blood glucose concentration was measured by the dry glucose oxidase method at 15 minutes before and at 15, 30, 45, 60, 90, and 120 minutes after the test meal was administered. The participant's finger was sterilized with alcohol, the skin was pierced with a disposable blood-taking device, and 300 μL of capillary blood was collected. The blood sample was dropped onto test paper, and the glucose concentration was measured with a blood glucose meter (OneTouch Ultra, Johnson & Johnson, New Brunswick, NJ).

Each participant completed 7 test cycles, at separate visits, during the study period (Table 1). The washout period between cycles was chosen to be 5 to 7 half-lives of MLE to ensure that any influence from drug administered during the previous cycle had been eliminated. After oral administration, the plasma concentration of DNJ reaches a peak at 30 minutes, and complete elimination occurs after 4 to 8 hours.[25,26] On this basis, the visits were scheduled to occur every 3 days (Fig. 1) to allow for an adequate washout period.

2.6. Calculation of GI

The GI for each carbohydrate was calculated from the incremental area under the curve (IAUC) values using a method adapted from that described by Brouns et al[27] in accordance with ISO 26642:2010 standards.[24] The GI was calculated as: (IAUC of the test food/mean IAUC of glucose) × 100.[28,29] The GI was expressed as the mean value of 10 or more subjects ± the standard error of the mean, as recommended by ISO 26642:2010.[24]

2.7. Primary and secondary endpoints

The primary endpoints were the IAUC values under the blood glucose-time curve within the first 120 minutes after intake of the test food (glucose + MLE, sucrose + MLE, maltose + MLE or maltodextrin + MLE), in comparison with that of the reference food (glucose alone), and the GI of each test food in comparison

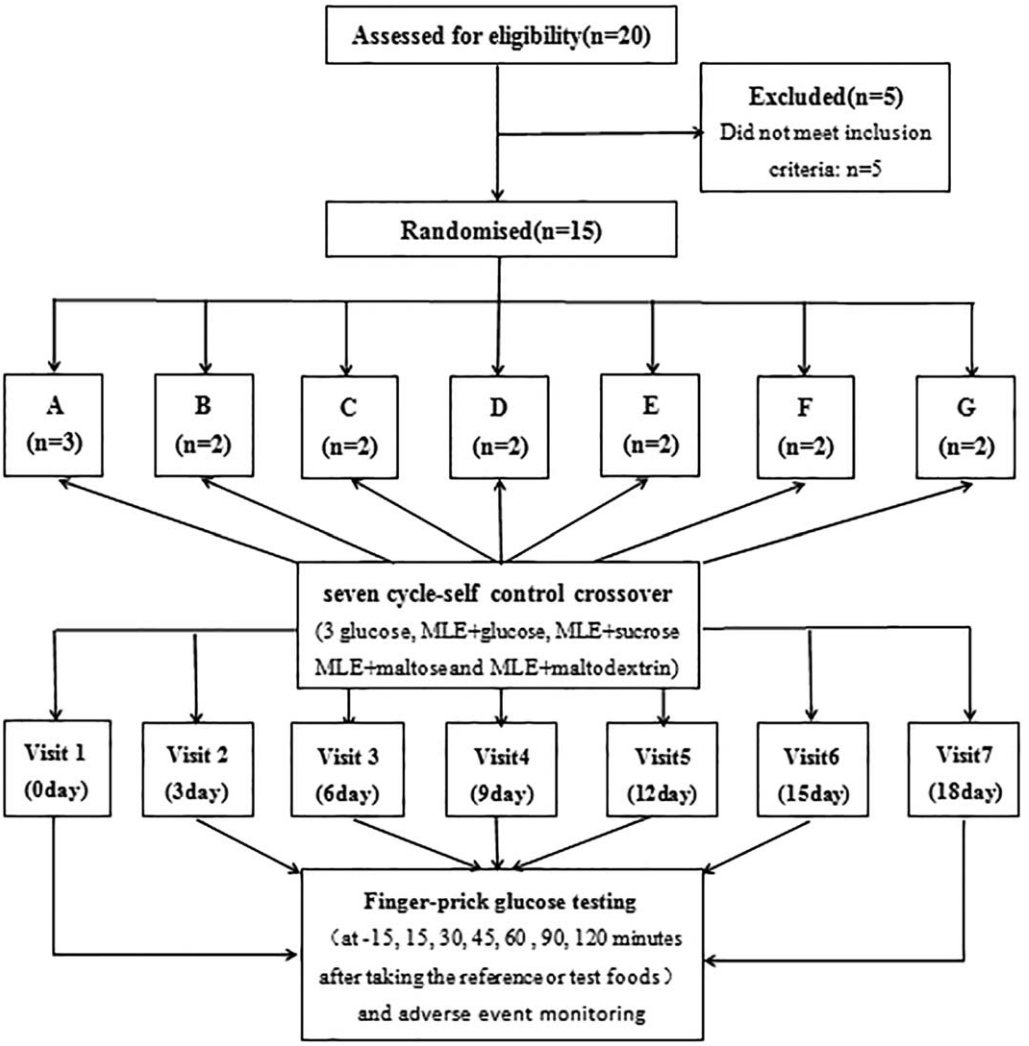

Figure 1. Patient disposition.

with that of the published GI of each carbohydrate in the absence of MLE. The secondary endpoints were the absolute values for the blood glucose levels at different time points and the changes in blood glucose concentration (relative to the preprandial level) at different time points.

2.8. Safety monitoring

Routine blood tests (including red blood cell count, hemoglobin content, packed cell volume, white blood cell count, and platelet count), urinalysis (including white blood cells, red blood cells, and protein), stool analysis, liver function tests (alanine transaminase and aspartate transaminase), kidney function tests (blood urea nitrogen and creatinine), and electrocardiography were performed at screening (before inclusion in the study) and at the last visit. Any abnormalities or changes between baseline and visit 7 were noted. In addition, any adverse events or reactions occurring during any of the visits were recorded.

2.9. Statistical analysis

All statistical analyses were performed using SAS version 9.4. Normality tests indicated that all data were normally distributed. The data are presented as the mean ± standard deviation, 95% CI, median and range, as appropriate. Differences between several groups were analyzed by one-way analysis of variance with the Bonferroni (homogeneity of variance) or Tambane's T2 (heterogeneity of variance) multiple range tests. The independent samples *t* test was used to compare the means between 2 groups. A value of $P < .05$ was considered statistically significant.

3. Results

3.1. Baseline characteristics of the study participants

Among 20 healthy volunteers screened for inclusion, 5 were excluded; therefore, 15 participants (9 males and 6 females) were enrolled in this study. All 15 participants completed the 7 visits and associated protocols (i.e., a dropout of 0). The baseline demographic and clinical characteristics of these 15 participants are listed in Table 2.

3.2. Blood glucose levels after intake of the reference meal (glucose only)

For each participant, glucose alone was used as a reference food on 3 separate occasions, allowing a mean value (n = 3) to be

Table 2

Baseline demographic and clinical characteristics of the study participants.

Characteristic	N	Mean ± SD	95% CI	Range	Median
Age, y	15	23.80 ± 1.47	22.98–24.62	22.00–26.00	23.00
Height, cm	15	167.86 ± 5.58	164.77–170.95	157.10–176.50	168.40
Weight, kg	15	62.35 ± 5.63	59.24–65.47	54.90–72.70	62.40
BMI, kg/m^2	15	22.05 ± 1.56	21.19–22.92	19.30–24.80	22.10
Temperature, °C	15	36.47 ± 0.33	36.28–36.65	35.80–36.90	36.50
Respiratory rate, breaths/min	15	17.93 ± 0.70	17.54–18.32	17.00–19.00	18.00
Systolic blood pressure, mm Hg	15	115.33 ± 7.67	111.09–119.58	100.00–130.00	115.00
Diastolic blood pressure, mm Hg	15	79.67 ± 4.42	77.22–82.11	70.00–85.00	80.00
Heart rate, beats/min	15	82.87 ± 7.34	78.80–86.93	64.00–96.00	84.00

95% CI = 95% confidence interval, BMI = body mass index, SD = standard deviation.

calculated for each participant at each postprandial time point (15, 30, 45, 60, 90, and 120 minutes). The changes in blood glucose relative to the preprandial levels (i.e., 15 minutes before oral intake of the glucose meal) at the various postprandial time points are presented in Table S1, http://links.lww.com/MD/C421 and Fig. 2. There were no significant differences between the 3 test cycles in the change in glucose level at any postprandial time point, indicating good repeatability of the results for each individual participant.

3.3. Comparisons of postprandial blood glucose levels between the test meals and reference meal

As recommended in the FAO Food and Nutrition Paper No. 66 (1998) and ISO 26642 (2010),[24,28] the reference test (glucose) was repeated 3 times to obtain a representative mean response from the 15 subjects. Table S2, http://links.lww.com/MD/C421 and Fig. 3 compare the mean blood glucose levels at the various time points between the 4 test foods (glucose + MLE, sucrose + MLE, maltose + MLE, and maltodextrin + MLE) and the reference food (glucose). After ingestion of the test or reference food, the blood glucose level increased progressively to reach a peak at 30 minutes (glucose, glucose + MLE, sucrose + MLE, and maltose + MLE) or 45 minutes (maltodextrin + MLE) before gradually falling back to preprandial levels at 120 minutes. The postprandial glucose level at 30 minutes was significantly lower for sucrose + MLE and maltose + MLE than for glucose, glucose + MLE or maltodextrin + MLE ($P < .01$).

The changes in blood glucose level relative to the preprandial level are presented in Table 3. Overall, the largest changes in postprandial blood glucose concentration occurred for glucose and glucose + MLE, while the smallest changes were seen for sucrose + MLE and maltose + MLE. At 30 minutes, the change in blood glucose level was significantly lower for sucrose + MLE ($P < .01$), maltose + MLE ($P < .01$), and maltodextrin + MLE ($P < .05$) than for glucose or glucose + MLE (see Table 3).

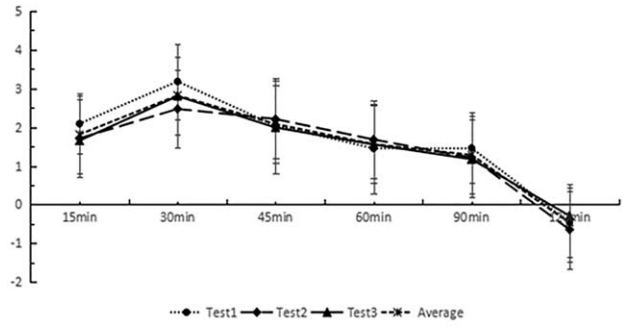

Figure 2. Change in blood glucose level (mmol/L) at each postprandial time point relative to the preprandial level. Data are presented as the mean ± standard deviation (n = 15 for each of Test 1, Test 2, and Test 3).

3.4. IAUC values after intake of the reference food (glucose only) and test foods

The average IAUC value was used as a reference standard for calculation of the GIs of the tested carbohydrates.[28,29] All 15 subjects received the reference food (glucose) on three separate occasions; the calculated IAUC values are shown in Table S3, http://links.lww.com/MD/C421. The calculated average IAUC value for the reference food was 181.28, the standard deviation was 10.73, and the coefficient of variation (standard deviation/mean) was 5.9%. The IAUC values for the test foods (Table S3, http://links.lww.com/MD/C421) were all numerically lower than that of the reference food. The IAUC value was numerically lowest for sucrose + MLE (43.22 ± 31.66) and maltose + MLE (89.25 ± 40.59), somewhat higher for maltodextrin + MLE (129.08 ± 26.01), and higher still for glucose + MLE (175.86 ± 59.71).

3.5. GI values for the test foods

The mean IAUC value of the reference food (glucose) was used as a standard for calculating the GI of the various test foods.[24,28] The GI values for the test foods are shown in Table S4, http://links.lww.com/MD/C421. The GI value was numerically lowest for sucrose + MLE (43.22 ± 17.47) and maltose + MLE (49.23 ± 22.39), somewhat higher for maltodextrin + MLE (75.90 ± 26.01), and higher still for glucose + MLE (91.88 ± 27.24). Compared with previously reported GI values for the various carbohydrates in the absence of MLE (Table S5, http://links.lww.com/MD/C421), the addition of MLE reduced the GIs for maltose, sucrose, maltodextrin, and glucose by 53.11%, 33.51%, 31.00%, and 8.12%, respectively.

3.6. Safety assessment

Adverse events, vital signs, biochemistry, hematology, and the electrocardiogram were monitored. There were no clinically significant differences in the vital signs or results of the biochemistry and hematology investigations between the start and end of the study. Similarly, there was little or no change in the results of routine analyses of urine and stool before and after the trial. Some subjects exhibited minor changes in the

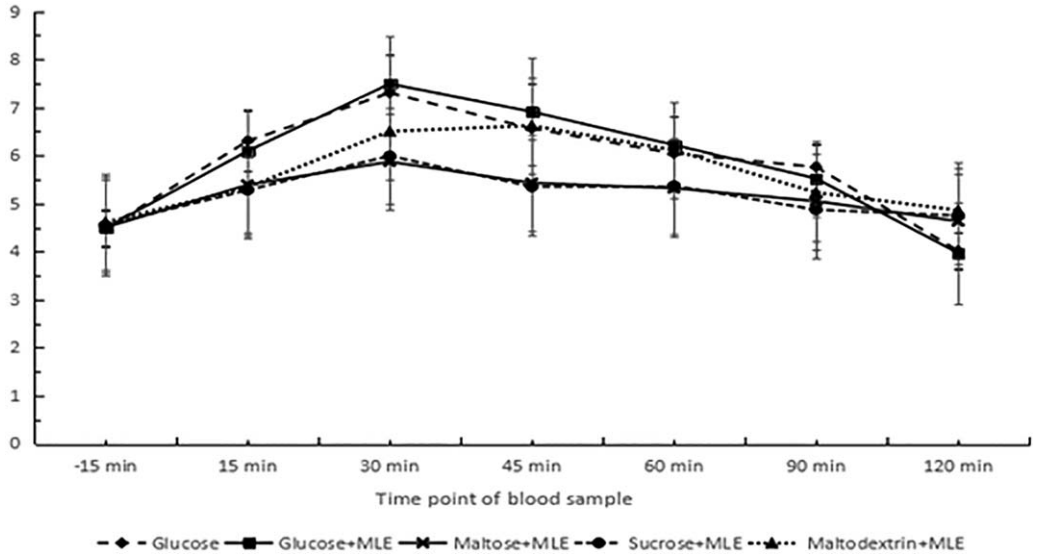

Figure 3. Blood glucose levels (mmol/L) at each time point for the various test foods and reference food.

electrocardiogram, such as sinus irregularity, PR interval prolongation, right axis deviation, and decreased heart rate; these changes were judged to be physiologic rather than pathologic. Throughout the trial period, all 15 participants reported no adverse events, and the reference and test foods were well tolerated.

4. Discussion

A notable finding of the present study was that the change in blood glucose level after oral consumption of carbohydrate was lower for sucrose + MLE, maltose + MLE and maltodextrin + MLE than for glucose + MLE or glucose alone. Furthermore, the GI value was lowest for sucrose + MLE (43.22 ± 17.47) and maltose + MLE (49.23 ± 22.39), intermediate for maltodextrin + MLE (75.90 ± 26.01), and highest for glucose + MLE (91.88 ± 27.24). Importantly, compared with published values, MLE reduced the GIs for maltose, sucrose, maltodextrin, and glucose by 53.11%, 33.51%, 31.00%, and 8.12%, respectively. In addition, MLE was well tolerated, with no adverse events. Our results indicate that coconsumption of MLE with sucrose, maltose, or maltodextrin can reduce the GI values of these carbohydrates. Since a low-GI diet is widely recognized as playing a role in the prevention and treatment of diabetes mellitus,[6–16] our findings indicate that MLE could potentially be used to help reduce postprandial blood glucose levels, which may be of particular benefit in patients with diabetes mellitus. However, it should be noted that this study was exploratory in nature and that the results are tentative. Therefore, further studies with larger sample sizes are needed to confirm and extend our observations.

Carbohydrates in food are absorbed into the bloodstream in the form of monosaccharides. Glycosidic linkage conformations are the main factors that determine the shapes of disaccharide, oligosaccharide, and polysaccharide molecules. Glucosidase enzymes, which are found in almost all organisms, hydrolyze the glycosidic bonds of sugar-containing compounds to form monosaccharides, oligosaccharides, or glycoconjugates. Glucosidase enzymes in the small intestine digest complex carbohydrates into monosaccharide constituents such as glucose and fructose, which can then be absorbed. Substances that inhibit the catabolic function of glucosidase can slow the breakdown of carbohydrates in the small intestine, thereby delaying the rate of glucose absorption and reducing the GI of the ingested food.

DNJ is an alkaloid azosugar[19] that acts as a competitive inhibitor of α-glucosidase in the brush border of the small

Table 3

Change in postprandial blood glucose levels relative to the preprandial level for the various test foods and reference food.

		Change in postprandial blood glucose versus 15 min before glucose intake (mmol/L and %)											
Food group	**n**	**15 min**		**30 min**		**45 min**		**60 min**		**90 min**		**120 min**	
Glucose	45	1.81 ± 0.77	40.4%	2.82 ± 0.98	62.9%	2.08 ± 1.23	46.4%	1.56 ± 1.16	34.8%	1.28 ± 0.91	28.6%	−0.47 ± 0.91	−10.5%
Glucose + MLE	15	1.60 ± 0.89	35.6%	2.99 ± 0.94	66.6%	2.42 ± 1.03	53.9%	1.73 ± 0.91	38.5%	1.03 ± 0.78	22.9%	−0.53 ± 1.14	−11.8%
Maltose + MLE	15	0.89 ± 0.38[†]	19.8%	1.37 ± 0.80[†,§]	30.4%	0.93 ± 0.89[†,§]	20.7%	0.82 ± 0.72	18.2%	0.55 ± 0.52[†]	12.2%	0.13 ± 0.48[*]	2.9%
Sucrose + MLE	15	0.73 ± 0.57[†,‡]	16.0%	1.44 ± 0.69[†,§]	31.6%	0.81 ± 0.51[†,§]	17.8%	0.82 ± 0.64[*,‡]	18.0%	0.33 ± 0.32[†,‡]	7.3%	0.20 ± 0.50[†]	4.4%
Maltodextrin + MLE	15	0.73 ± 0.62[†,‡]	15.8%	1.89 ± 0.90[*,‡]	41.0%	2.01 ± 0.78	43.6%	1.50 ± 0.91	32.5%	0.63 ± 0.66[*]	13.7%	0.25 ± 0.76	5.4%
P		<.001		<.001		<.001		.012		<.001		.003	

Data presented as mean ± standard deviation. Time points are relative to oral intake of test food.

[*] $P < .05$.

[†] $P < .01$ vs glucose.

[‡] $P < .05$.

[§] $P < .01$ vs glucose + MLE (analysis of variance with Bonferroni [homogeneity of variance] or Tambane's T2 [heterogeneity of variance] tests).

intestine. Natural DNJ was first isolated from mulberry trees by Yoshikaki et al[30] in 1976. DNJ is mainly distributed in the leaves, roots, and branches of mulberry trees; the leaves are currently the main source of natural DNJ due to its abundance there. DNJ can competitively inhibit the binding of carbohydrate substrate to the α-glucosidase enzyme, with a similar affinity to acarbose,[31] slowing the breakdown and absorption of ingested carbohydrates in the small intestine and thereby suppressing postprandial increases in blood sugar and insulin secretion. Importantly, prolonged oral administration of DNJ does not cause hypoglycemia.[18] In rats, DNJ is absorbed into the plasma in the intact form after oral intake, reaching a maximum concentration at 30 minutes; the half-life is around 30 to 60 minutes, with complete disappearance after 4 hours.[25,26] However, the majority of the DNJ administered to rats is excreted in the feces, with a smaller amount absorbed and excreted in the urine.[25,26]

In this study, comparison of the average blood glucose levels at various time points after oral ingestion of the reference or test foods revealed that MLE had only a small effect on the absorption of glucose from the gastrointestinal tract, as expected since glucose is directly absorbed without the requirement of α-glucosidase. By contrast, DNJ successfully inhibited the absorption of maltose, sucrose, and maltodextrin, suppressing the postprandial elevation of blood glucose. The inhibitory effect of MLE on the postprandial elevation of blood sugar after sucrose, maltose, or maltodextrin ingestion occurred mainly during the 15 to 60 minutes period after the meal. Furthermore, compared with known values for each carbohydrate alone, MLE reduced the GIs for maltose, sucrose, and maltodextrin by around 53%, 34%, and 31%, respectively. These data show that DNJ was able to retard the absorption of all 3 of these carbohydrates.

The findings of this study are in good agreement with previous reports that DNJ or MLE can inhibit α-glucosidase and impede the absorption of carbohydrates. In animal experiments, MLE has been found to inhibit postprandial hyperglycemia in rats,[23] exert hypoglycemic effects in mice,[32] and improve fasting blood glucose levels, glucose tolerance and insulin sensitivity in a rat model of diabetes mellitus.[33] In humans, MLE enriched with DNJ was observed to suppress the increase in postprandial blood glucose[18] and improve postprandial glycemic control in individuals with impaired glucose metabolism.[34] Interestingly, MLE but not DNJ was also found to inhibit glucose absorption in rats, particularly when the MLE was given 30 minutes before glucose loading rather than simultaneously.[35] This implies that constituents in MLE other than DNJ can inhibit glucose absorption from the gastrointestinal tract, and this may underlie the small effect of MLE on the GI of glucose observed in our study.

Maltose, produced by the action of amylase on starch, is a disaccharide composed of 2 glucose molecules linked by an α-1,4-glycosidic bond. Maltase, an α-glucosidase on the mucous membrane of the small intestine, hydrolizes maltose to 2 molecules of α-D-glucose by splitting the α-1,4-glycosidic bond.[36] Sucrose is also a disaccharide, composed of glucose and fructose linked by an α-1,2-glycosidic bond. Sucrose can be hydrolyzed to 1 molecule of α-D-glucose and 1 molecule of β-D-fructose by either of 2 enzymes, α-glucosidase or β-fructofuranosidase.[37] It was previously shown that DNJ could prevent the hydrolysis and absorption of sucrose through inhibition of α-glucosidase activity but not β-fructofuranosidase activity.[19] This might explain why the percentage reduction in GI was lower for sucrose (~34%) than for maltose (~53%). Maltodextrin, also known as water-soluble dextrin, is a polysaccharide made from various kinds of starch. It consists mainly of dextrin with a degree of polymerization above 10 and oligosaccharides with a degree of polymerization below 10; the molar mass is between that of starch and starch sugar.[38] The structure of maltodextrin is mainly that of a long chain formed by the linking of α-1,4-glycosidic bonds, with α-1,6-glycosidic bonds at branch points. Maltodextrin is mainly broken down into maltose and α-amylodextrin by α-amylase or β-amylase on the mucous membrane of the small intestine. The maltose is subsequently broken down by α-glucosidase. The α-amylodextrin, an intermediate between starch and glucose, is broken down mainly through the hydrolysis of α-1,6-glycosidic bonds[39] by α-dextrinase; ultimately, glucose is generated and absorbed. This multienzyme, multistep process for maltodextrin may explain why the peak postprandial glucose level was achieved more slowly than that for maltose or sucrose (45 minutes rather than 30 minutes) and why the effect of DNJ on the GI of maltodextrin was not as great as that on the GI of maltose.

Acarbose is an inhibitor of the α-glucosidase enzyme that has been reported to lower the GI values of sucrose, maltose, starch, and bread.[40–43] Although acarbose is recommended in some countries as a treatment for type-2 diabetes mellitus, its use is limited by side effects such as diarrhea and flatulence.[44] In our study, MLE was well tolerated, with no cases of diarrhea after its administration; thus, MLE may have advantages over acarbose as a potential therapy for diabetes mellitus.

This study has some limitations. First, this exploratory study was conducted at a single-center and the sample size was small; thus, the generalizability of the findings is unknown, and the results should be interpreted with a degree of caution. Second, measurements of the GIs of maltose, sucrose, and maltodextrin in the absence of MLE were not made, so direct comparisons of these GI values with those in the presence of MLE could not be made. Third, only 4 carbohydrates were assessed, so the effects of MLE on the absorption of other dietary carbohydrates, such as starch, were not determined. Fourth, it was not established whether MLE affected the GI values of common dietary foods. Fifth, the effects of MLE on insulin secretion and sensitivity were not explored. Sixth, the potential benefits of oral administration of MLE in patients with impaired glucose tolerance or diabetes mellitus were not examined.

In conclusion, the findings of the present study raise the possibility that supplementation of the diet with MLE or DNJ could potentially help to suppress postprandial blood glucose levels, which may be of benefit to patients with impaired glucose tolerance or diabetes mellitus. However, it should be noted that our study was preliminary in nature and that the results are tentative. The present study focused on structurally simple carbohydrates, so future studies are needed to explore whether MLE would reduce the postprandial glucose changes, GI values, and postprandial insulin secretion for complex carbohydrates and different foods. Furthermore, it will be important to extend the research to include patients with prediabetes or type-2 diabetes mellitus and determine whether MLE might have utility in the prevention and/or management of these conditions.

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