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The effect of Mankai plant consumption on postprandial glycaemic response among patients with type 2 diabetes: A randomized crossover trial

Gal Tsaban MD[1,2,3] | Genya Aharon-Hananel MD[4] | Shiran Shalem[4] | Hila Zelicha PhD[1] | Anat Yaskolka Meir PhD[1,5] | Dafna Pachter MSc[1] | Dana Tamar Goldberg MSc[1] | Omer Kamer MPH[1] | Liav Alufer MPH[1] | Meir J. Stampfer Dr.PH[6,7] | Dong D. Wang MD[7] | Lu Qi MD[6] | Matthias Blüher MD[8] | Michael Stumvoll MD[9] | Frank B. Hu PhD[6,7] | Iris Shai PhD[1,6,9] | Amir Tirosh MD[4]

[1]The Health and Nutrition Innovative International Research Centre, School of Public Health, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beersheva, Israel

[2]Department of Cardiology, Soroka University Medical Centre, Beersheva, Israel

[3]Department of Cardiovascular Medicine, Mayo Clinic, Rochester, Minnesota, USA

[4]Division of Endocrinology, Diabetes and Metabolism, Sheba Medical Centre, Ramat Gan, Israel

[5]Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA

[6]Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA

[7]Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA

[8]Medical Department III–Endocrinology, Nephrology, Rheumatology and Helmholtz Institute for Metabolic, Obesity and Vascular Research (HI-MAG) of the Helmholtz Centre Munich at the University of Leipzig and University Hospital Leipzig, Leipzig, Germany

[9]Faculty of Medicine, University of Leipzig, Leipzig, Germany

Correspondence
Gal Tsaban and Iris Shai, The Health and Nutrition Innovative International Research Centre, School of Public Health, Faculty of Health Sciences, Ben-Gurion University of the Negev, PO Box 653, Beer-Sheva 84105, Israel.
Email: tsaban.gal@mayo.edu and irish@bgu.ac.il

Amir Tirosh, Division of Endocrinology, Diabetes, and Metabolism, Sheba Medical Centre, Tel HaShomer, Ramat Gan, Israel.
Email: amir.tirosh@sheba.health.gov.il

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Abstract

Aim: To explore the effect of Mankai, a cultivated aquatic duckweed green plant, on postprandial glucose (PG) excursions in type 2 diabetes (T2D).

Methods: In a 4-week, randomized crossover-controlled trial, we enrolled 45 adults with T2D (HbA1c range: 6.5%-8.5%) from two sites in Israel. Participants were randomized to drink Mankai (200 mL of raw-fresh-aquatic plant + 100 mL of water, 40 kcal, ~10 g of dry matter equivalent) or water (300 mL) following dinner, for 2 weeks each, with a 4-day washout interval, without dietary, physical activity or pharmacotherapy alterations. We used continuous glucose monitoring (CGM) devices.

Results: Forty patients (adherence rate = 88.5%; 743 person-intervention-days, 68.9% men, age = 64 years, HbA1c = 6.8%) completed the study with a consistent

Gal Tsaban and Genya Aharon-Hananel have made equal contributions.

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diet and complete CGM reads. Only two-thirds of the individuals responded beneficially to Mankai. Overall, Mankai significantly lowered the PG peak by 19.3% (Δpeak = 24.3 ± 16.8 vs. 30.1 ± 18.5 mg/dL; $P < .001$) and delayed the time-to-peak by 20.0% (112.5 [interquartile range: 75-135] vs. 90 [60-105] min; $P < .001$) compared with water. The PG incline and decline slopes were shallower following postdinner Mankai (incline slope: 16.8 vs. water: 29.9 mg/[dL h]; $P < .001$; decline slope: −6.1 vs. water: −7.9 mg/[dL h]; $P < .01$). Mean postprandial net incremental area-under-the-glucose-curve was lowered by 20.1% with Mankai compared with water ($P = .03$). Results were consistent across several sensitivity and subgroup analyses, including across antidiabetic pharmacotherapy treatment groups. Within 2 weeks, the triglycerides/high-density lipoprotein cholesterol ratio in the Mankai group (−0.5 ± 1.3) decreased versus water (+0.3 ± 1.5, $P = .05$).

Conclusions: Mankai consumption may mitigate the PG response in people with T2D with an ∼20% improvement in glycaemic values. These findings provide case-study evidence for plant-based treatments in T2D to complement a healthy lifestyle and pharmacotherapy.

KEYWORDS

continuous postprandial glucose monitoring, glycaemic control, Mankai, randomized crossover trial, type 2 diabetes

1 | INTRODUCTION

Type 2 diabetes (T2D) is increasing globally and is strongly associated with microvascular and macrovascular morbidities and reduced life expectancy.[1] Glycaemic control, which refers to maintaining and sustaining normal plasma glucose levels, is associated with a lower risk of developing T2D-associated complications and death.[2] In particular, postprandial plasma glucose levels highly reflect glycaemic control and are closely associated with the long-term risk of T2D complications, primarily atherosclerotic cardiovascular risk and death.[3–6]

Nutrition plays a crucial role in the risk of developing T2D and in achieving glycaemic control among people with T2D.[7] Increased consumption of specific plant-derived micronutrients, such as dietary polyphenols and dietary fibres, is associated with improved glycaemic control, increased sensitivity to insulin and reduced cardiovascular risk.[8–11] Hence, the Academy of Nutrition and Dietetics and the American Diabetes Association recommend a balanced plant-rich diet to prevent obesity, cardiovascular disease and T2D and to improve glycaemic control among patients with T2D.[12,13] However, despite the great need for evidence-based nutritional recommendations, there is a paucity of data supporting specific food sources to benefit glycaemic control among people with T2D.

Mankai, a distinct *Wolffia globosa* strain cultivated under highly controlled supervised conditions, is characterized by high protein (more than 40% of the dry matter) and micronutrient content. Extensive research in recent years showed that Mankai is rich in bioavailable protein, iron and vitamin B12 and contains 200 different polyphenols, potentially affecting the metabolomic–gut–clinical axis.[14–17] In a previous study among healthy individuals, Mankai was shown to improve glucose control, partially mediated by elevation in fasting-state ghrelin levels that correlated with visceral adiposity regression and recovery of insulin sensitivity.[18] The metabolism of Mankai's polyphenol content by the gut microbiome and its potential metabolically beneficial effects were also shown independently in an ex vivo artificial gut trial.[17] Importantly, both in human and rodent-based models, Mankai consumption improved the microbiome composition and microbiome signature function, mainly by altering bacterial glucose metabolism.[19,20] The green-MED diet, distinct by the addition of Mankai and green tea, led to lowering of intrahepatic and visceral fat by one-half, a more significant reduction than a traditional Mediterranean diet.[21,22] Recently, we reported results from a 14-day crossover trial among 20 non-diabetic individuals using a continuous glucose monitoring (CGM) device, a highly reliable tool that is used clinically to reflect glycaemia[23]; that evening a Mankai shake improved the postprandial and overnight glucose excursion response compared with a yogurt shake.[24] In the current study, we sought to determine whether Mankai supplementation affects the postprandial glucose response among patients with T2D.

2 | METHODS

For this randomized crossover-controlled trial, we enrolled 45 participants with T2D who were recruited from two medical facilities: the Division of Endocrinology, Diabetes, and Metabolism at Sheba

Medical Centre, Ramat Gan, Israel, and the Nuclear Research Centre clinic in Dimona, Israel. These two sites allowed us to recruit representative patients across a broad spectrum of individuals with T2D. The trial was registered in clinicaltrials.gov (identifier number NCT04945109). The trial was performed in parallel in the two centres during November and December 2021. The inclusion criteria consisted of age 30 years or older with a formal diagnosis of T2D (at least two measures of fasting plasma glucose [FPG] ≥ 126 mg/dL or HbA1c ≥ 6.5%) and HbA1c levels lower than 8.5%. The exclusion criteria consisted of HbA1c lower than 6.5% or higher than 8.5%, treatment with coumadin (warfarin), advanced renal failure, pregnancy or lactation, presence of active cancer or chemotherapy treatment in the last 3 years, or participation in another trial. The sample size used to determine recruitment requirements for this study are provided in Supplement 1. The study was granted ethical approval and was supervised by the Soroka Medical Centre and Sheba Medical Centre institutional review boards.

2.1 | Interventions

Following enrolment, participants were randomly allocated to start in one of two intervention groups: (A) a daily Mankai drink (200 mL of fresh harvest raw Mankai aquatic plant +100 mL of water, equivalent to ~10 g of dry matter) after dinner; or (B) 300 mL of bottled water daily after dinner. Randomization was performed by GT, GAH, SS and HZ using a simple randomization method with sequentially numbered ballots. After 2 weeks of intervention, participants underwent a 4-day washout period, following which they switched to the other intervention group for an additional 2 weeks. We chose to test the intervention after dinner for three main reasons: (i) regular breakfast consumption is not common in Israel, where the study was conducted; (ii) many patients take their medications in the morning, which could introduce food–drug interactions affecting postprandial glycaemia; and (iii) most of the study population in the sites where the study was conducted perform physical activity in the morning, which could impact observed glucose excursions. Therefore, scheduling the intervention in the evening allowed minimal interruption with the participants' regular workout routines. All participants were provided with dietary and lifestyle recommendations according to the American Diabetes Association guidelines and were instructed to adhere to a constant diet throughout the trial. During the study, the participants were also instructed to maintain identical lifestyles (physical activity, smoking, medications, sleeping hours and eating hours). No diet was prescribed, nor was there any caloric restriction. Also, no physical activity prescriptions were provided; however, participants were encouraged to continue their same activity level, but not in the 4 hours before or after dinner (i.e. intervention). Mankai was provided free of charge, and a reusable 300-mL water bottle was also provided free of charge during the water intervention. Participants were requested to adhere to their current diabetes medical therapy without changing medication prescriptions, administration time or dosages. Participants were requested to record the meal composition and portion sizes for each meal during all days of intervention.

2.2 | Mankai drink

During the Mankai intervention phase, participants were provided with frozen bottles of 300 mL of flavoured fresh, raw, Mankai drinks each week during the Mankai intervention. The bottles were provided in a frozen form every week, two times for each participant. Participants were guided to store the bottles at 0°. The Mankai drink contained 200 mL of freshly harvested Mankai, with 100 mL of water flavoured with erythritol to produce three tastes: apple, passion fruit or coconut. The 300-mL Mankai boost drink, equivalent to ~10 g of dry matter, included 40 kcal, 0.8 g of carbohydrates, 4.1 g of protein and 0.8 g of fats. Each Mankai boost drink also contained 0.2 μg of vitamin B12, 113.9 μg of folic acid and 76.3 mg of polyphenols. The dietary fact sheet of the Mankai drink is provided in Supplement 2, and a summary of the nutrichemical and known health-related evidence on Mankai is provided in Supplement 3.

2.3 | Strategies to maintain and monitor adherence

Participants received daily text messages as a reminder to maintain study protocol instructions. We also telephoned participants to monitor and encourage adherence and enable them to log in to record their activity, medication use, appetite and symptoms through a dedicated web-based system.

2.4 | Measurements

2.4.1 | Continuous glucose monitor

A flash glucose monitoring device (Freestyle Libre 2.0; Abbott Diabetes Care, Witney, Oxon, UK; https://www.freestylelibre.com.au) was used.[25] The sensors are factory-calibrated and do not require further calibration. Each CGM sensor is valid for 2 weeks. The data are transferred to a reader when brought into proximity of the sensor. The reader then displays the current glucose level, a glucose trend arrow, and glucose readings over the previous 8 hours. Glucose data were automatically captured each minute and stored. All study participants were installed with CGM sensors immediately after the initial randomization (day 1) and after 18 days from randomization (after the 4 days of washout). The analyses did not include the first 24 hours after implementing the sensor. Analyses were performed on data generated from Saturday to Thursday, specifically excluding Fridays, to mitigate potential biases related to changes in dietary habits and lifestyle behaviours among the participants on that particular day. The sensors were installed on Thursdays, so the maximum number of observation days for each sensor was 12 per intervention. Additionally, days when

participants recorded a lack of adherence to their usual diet and lifestyle were excluded from the analyses.

2.4.2 | Clinical variables

We monitored basic clinical variables only within the first 2 weeks. To prevent potential bias related to a halo effect of the Mankai intervention, which our previous report indicated could have long-lasting effects on the gut microbiome composition and function, we decided not to collect anthropometric and laboratory data after the intervention crossover. Body weight was measured without shoes to the nearest 0.1 kg. Waist circumference was measured halfway between the last rib and the iliac crest to the nearest millimetre by standard procedures using a 150-cm anthropometric measuring tape. Blood pressure was measured using an automated system (Datascop acutor 4), following 5 minutes of rest. Clinical staff members were blinded to treatment assignment. Blood samples were drawn at 08:00 AM, after a 12-hour overnight fast at baseline, and after 2 weeks of the first parallel intervention phase. Glucose, HbA1c, plasma lipids and liver transaminases analyses were performed on the fresh samples in Soroka and Sheba University Medical Centres.

2.4.3 | Dietary and lifestyle logs

Participants recorded a detailed lifestyle log that the investigators provided. The logs included dietary consumption (including time, food description and description of portion size), daily activity, satiety scale (0-10) and symptoms during the study. The purpose of the dietary logs was to ensure that participants maintained a consistent diet throughout the study. To enhance adherence to self-reporting, participants were asked to describe the type of food and portion size they consumed instead of providing a detailed composition of their dietary intake. All dietary and lifestyle logs were scanned and categorized by the study staff's clinical dietitians, who were blinded to the intervention allocation and outcome measurements. Days considered unrepresentative of the participants' regular dietary or lifestyle behaviours, or when participants reported skipping or forgetting to consume the intervention (either water or Mankai), were coded and excluded from extraction and analysis. This approach was taken to minimize bias and skewness in the results resulting from deviations from regular lifestyle or diet rather than the intervention itself.

2.5 | Statistical analysis

Data are presented as means and standard deviations or medians and interquartile ranges (IQRs) for continuous variables. Categorical data are presented as numbers and percentages. The primary aim of the analysis was to compare the 5-hour postprandial glucose excursions across study interventions. Data were statistically analysed in a blinded manner as intervention allocations were revealed only after analyses were finalized. We conducted intention-to-treat analyses, including all the randomized participants who provided data for at least one observation day for both interventions. Patients' CGM values were based on repeated glucose measurements on a fixed 15-minute gap. Repeated measurement variables are reported as weighted means accounting for the number of observation days per intervention and participant.

Glycaemic response to interventions was assessed in each individual through five variables: (i) mean absolute change in glucose levels from baseline for each observation (i.e. every 15 minutes) during the 5-hour postprandial period; (ii) mean peak glucose; (iii) time to glucose peak; (iv) glucose excursion upslope and downslope; and (v) net postprandial glucose area under the curve (net-AUC). The mean absolute change in glucose levels from baseline was assessed using generalized estimating equations (GEE) for panel data analysis, also known as cross-sectional time-series analysis, accounting for the repeated glucose measurements per participant over time. A beneficial response at the individual level was defined by the mean postprandial glucose levels being lower by at least 5 mg/dL at four or more consecutive time points throughout the five postprandial hours. We assessed the within-person changes from baseline in each group using Wilcoxon pairwise tests. We compared peak-from-baseline glucose values and time-to-glucose-peak across interventions using GEE models with unstructured matrix correlation accounting for within-subject (observation day) and between-subject (participants) factors where the baseline was defined as the value indicating the start of the reported dinner time. Glucose upslopes and downslopes were compared across interventions using continuous non-linear regression curve-fit models with hinge function[26] where the upslope was extrapolated from the baseline to the peak mean glucose value, and the downslope from the peak mean glucose value back to the baseline. Mean 5-hour postprandial glucose excursion curves for each intervention arm were used to calculate the mean net-AUC'? Note that '(net-AUC). Specific sensitivity and subgroup analyses were conducted based on intervention allocation sequence, antidiabetic pharmacotherapy exposure and by excluding patients treated with insulin. According to the nature of the dependent variable, laboratory, anthropometric (weight and waist circumference) and metabolic laboratory (glucose, HbA1c, plasma lipids and transaminases) data were compared for the first 2-week parallel intervention period using Mann–Whitney or Chi-square tests. We focused on metabolic syndrome variables and liver transaminases (both as markers for safety and as surrogates of hepatic metabolism). Daily and postdinner satiety rankings (on a scale of 0-10) were compared using ordinal logistic GEE models accounting for differences between and within participants' observations over time. We also accounted for possible interactions between the intervention, study site and intervention sequence. For all analyses, a two-sided *P* value of less than .05 was considered statistically significant, and 95% confidence intervals (CIs) were calculated and reported, where applicable. Statistical analyses were performed using SPSS version 28.0.1.0 (IBM, Armonk, NY) and GraphPad Prism 9.5.1 (GraphPad Software Inc., San Diego, CA).

3 | RESULTS

3.1 | Baseline characteristics

The study included 45 participants from two sites (Sheba Medical Centre [$n = 23$] and the Nuclear Research Centre [$n = 22$]) who met the inclusion criteria. Baseline characteristics of the study population across the first intervention allocation are provided in Table 1. Overall, 24 participants were randomized to start the water intervention, and 21 were randomized to start with the Mankai intervention. Participants' median age was 65 years, and 31.1% were females. The median HbA1c was 6.8%, and the median FPG was 122.5 mg/dL. All patients had normal renal function with a median creatinine of 0.9. Metformin was the most used pharmacotherapy (51%), followed by glucagon-like peptide-1 analogues and sodium-glucose co-transporter-2 inhibitors (22% each). None of the participants were treated with sulphonylurea; four (9%) patients were on basal insulin therapy.

3.2 | Adherence to study protocol and intervention

The flowchart of the study is provided in Figure 1. Forty patients (89%) completed the study and were included in the final analysis. Two patients withdrew from the study because of a lack of adherence to a stable lifestyle and daily intervention (one during the first 2 weeks under the Mankai intervention and one during the last 2 weeks with the water intervention). Three participants were not included in the primary analysis because of repeated CGM sensor faults (two during the first intervention phase with water intervention and one during the second with water intervention). After considering compliance and adherence to keep a stable dietary regimen during the intervention, each of the 40 participants included in the final analysis provided a median of 10 (IQR: 7-12) water observation days and a median of 10 (IQR: 7-12) Mankai observation days, adding up to a total of 743 person-intervention-days.

3.3 | Postprandial glucose response to intervention

By individual response, only two-thirds of the individuals beneficially responded to Mankai, while others showed neutral responses. There were no differences in baseline characteristics between responders and non-responders ($P > .1$ for all comparisons, data not shown). Five-hour postprandial glucose excursions among all the study participants during the study are presented across interventions in Figure 2A. Overall, the Mankai intervention was associated with lower glucose levels starting from 30 until 135 minutes postprandially ($P < .05$ for all time points, GEE models accounting for repeated measurements). An

TABLE 1 Baseline characteristics of the T2D study population.

Variable	Water first ($n = 24$)	Mankai first ($n = 21$)	Total ($n = 45$)	*P* value
Age, y	65.0 (55.0, 68.0)	62.0 (52.0, 68.0)	64.0 (54.0, 68.0)	.64
Female sex	7 (29.2%)	7 (33.3%)	14 (31.1%)	
BMI (kg/m^2)	30.0 (26.6, 32.6)	30.3 (26.5, 33.4)	30.3 (26.5, 32.8)	.34
Waist circumference (cm)	109.0 (98.0, 112.0)	103.0 (96.0, 115.5)	107.0 (98.0, 112.0)	.42
HbA1c (%)	6.6 (6.2, 7.9)	6.9 (6.3, 7.8)	6.8 (6.2, 7.8)	.93
FPG (mg/dL)	122.0 (109.0, 133.0)	124.0 (110.0, 136.0)	122.5 (109.5, 134.5)	.98
Creatinine (mg/dL)	0.9 (0.7, 1.0)	0.9 (0.7, 0.9)	0.9 (0.7, 1.0)	.73
HDL-c (mg/dL)	47 (39, 55)	45 (40, 54)	47 (39, 55)	.87
TG (mg/dL)	128 (93, 168)	165 (121, 199)	143 (104, 191)	.14
AST (U/L)	24 (21, 28)	27.5 (23, 32)	25 (23, 30)	.06
ALT (U/L)	23 (21, 33)	28 (24, 38)	27 (21, 35)	.08
Antihyperglycaemic medications				
Metformin	12 (50.0%)	11 (52.4%)	23 (51.1%)	.87
GLP1a	7 (29.2%)	3 (14.3%)	10 (22.2%)	.23
SGLT2i	6 (25.0%)	4 (19.0%)	10 (22.2%)	.63
DPP4i	2 (8.3%)	2 (9.5%)	4 (8.9%)	1
TZD	1 (4.2%)	0 (0%)	1 (2.2%)	.34
SU	0 (0%)	0 (0%)	0 (0%)	NA
Insulin	3 (12.5%)	1 (4.8%)	4 (8.9%)	.36

Abbreviations: ALT, alanine transglutaminase; AST, aspartate transglutaminase; BMI, body mass index; DPP4i, dipeptidyl peptidase-4 inhibitors; FPG, fasting plasma glucose; GLP1a, glucagon-like peptide-1 agonists; HbA1c, glycated hemoglobin A1C; HDL-c, high density lipoprotein cholesterol; HDLc, high-density lipoprotein cholesterol; SGLT2i, sodium-glucose co-transporter-2 inhibitors; SU, sulphonylurea; T2D, type 2 diabetes; TG, triglycerides; TZD, thiazolidinediones.

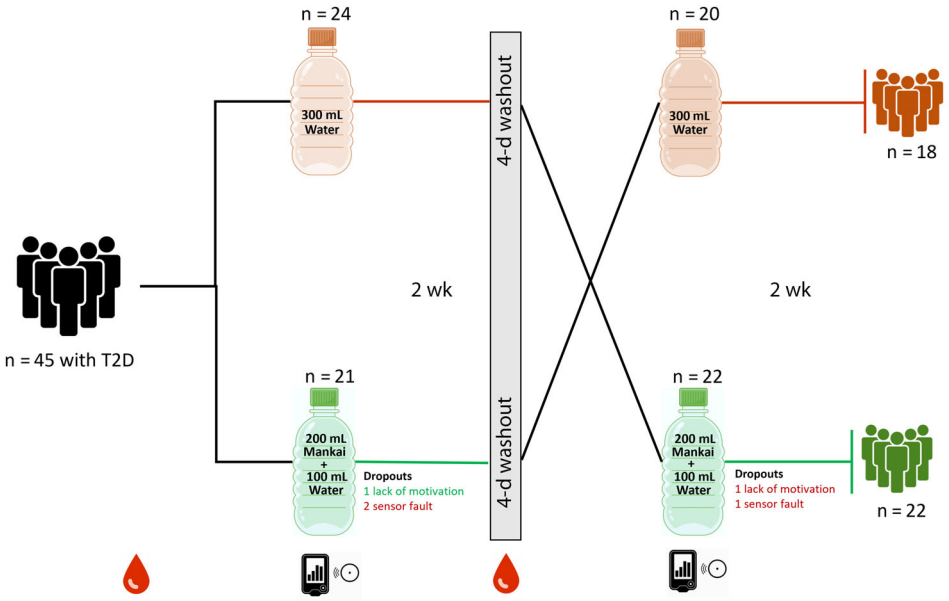

FIGURE 1 Study design and flowchart. T2D, type 2 diabetes.

estimation plot comparing the Mankai and water interventions across the observed postprandial time points is provided in Supplement 4. The Mankai intervention was associated with a longer time-to-glucose-peak compared with the water intervention (112.5 [IQR: 75, 135] vs. 90 [IQR: 60, 105] minutes, respectively; $P < .001$). Following the postprandial period and return to baseline glucose, the overnight glucose levels gradually lowered to 10 mg/dL below predinner levels across intervention groups and remained stable until awakening (Supplement 5).

Non-parametric derived hinge function graphical plots are provided in Supplement 6. The glucose excursion increase rate (upslope) was slower under the Mankai intervention compared with the water intervention (16.8 [95% CI: 14.5, 19.4] vs. 29.9 [95% CI: 25.6, 37.0] mg/dL/h, respectively; $P < .001$) and the glucose excursion elimination rate (downslope) during the Mankai intervention was also slower than that with the water intervention (−6.1 [95% CI: −6.5, −5.7] vs. −7.9 [95% CI: −8.2, −7.5] mg/dL/h, respectively; $P < .001$). Overall, the up-to-midnight netAUC of glucose after the Mankai intervention was 20.1% lower than the net-AUC of glucose with water intervention (50.9 [95% CI: 42.2, 59.5] vs. 63.7 [95% CI: 56.1, 71.3] mg/[dL min], respectively; $P = .03$).

A pooled within-participant comparison of postprandial peak delta glucose levels is presented in Figure 2B. Despite the additional calories from Mankai, the mean peak-from-baseline glucose levels were 19.3% lower with Mankai (+24.3 ± 16.8 mg/dL) than with water (+30.1 ± 18.5 mg/dL; $P < .001$).

3.4 | Sensitivity and subgroup analyses

A sensitivity analysis among patients not under a basal insulin regimen ($n = 38$) showed results that were consistent with those of the entire cohort, with significant improvements in all postprandial glycaemic parameters (Supplement 7). Glucose excursions across intervention groups based on the first intervention allocation (Mankai or water first) are presented in Supplement 8. There was no significant interaction effect at any tested time point between the intervention order (Mankai or water first) or the study site and the intervention itself (P for interaction > .1 for all time points).

Another sensitivity analysis among patients treated with antidiabetic drugs ($n = 22$) and those not treated ($n = 18$) showed that, despite different postprandial glucose metabolism patterns (Supplement 9), the effect of Mankai on postprandial glycaemic parameters was similar across these groups, as well as across age and sex criteria (P for interaction > 0.1 for all time points). Despite varying intergroup patterns, the magnitude of the observed effect was generally similar.

3.5 | Metabolic laboratory changes and medication usage

Changes in key metabolic markers during the first 2 weeks of intervention are illustrated in Figure 3. During the first part of the intervention, the only significant difference across groups was a reduction in the triglycerides (TG)/high-density lipoprotein cholesterol (HDL-c) ratio in the Mankai group (−0.5 ± 1.3) versus water (+0.3 ± 1.5, $P = .05$). Weight reduction was observed in both groups (Mankai first: −0.5 ± 1.0 kg; water first: −0.9 ± 1.1 kg; $P < .05$ for within-group changes), but there were no significant differences between the groups ($P = .2$). FPG did not significantly change in both interventions. Participants in both groups showed a trend towards a decrease in mean arterial pressure, which was significant within the Mankai intervention arm only ($P < .05$). Also, both interventions were associated with similar reductions in alanine transaminase, but only participants under the Mankai intervention showed parallel reductions in aspartate transaminase ($P < .05$).

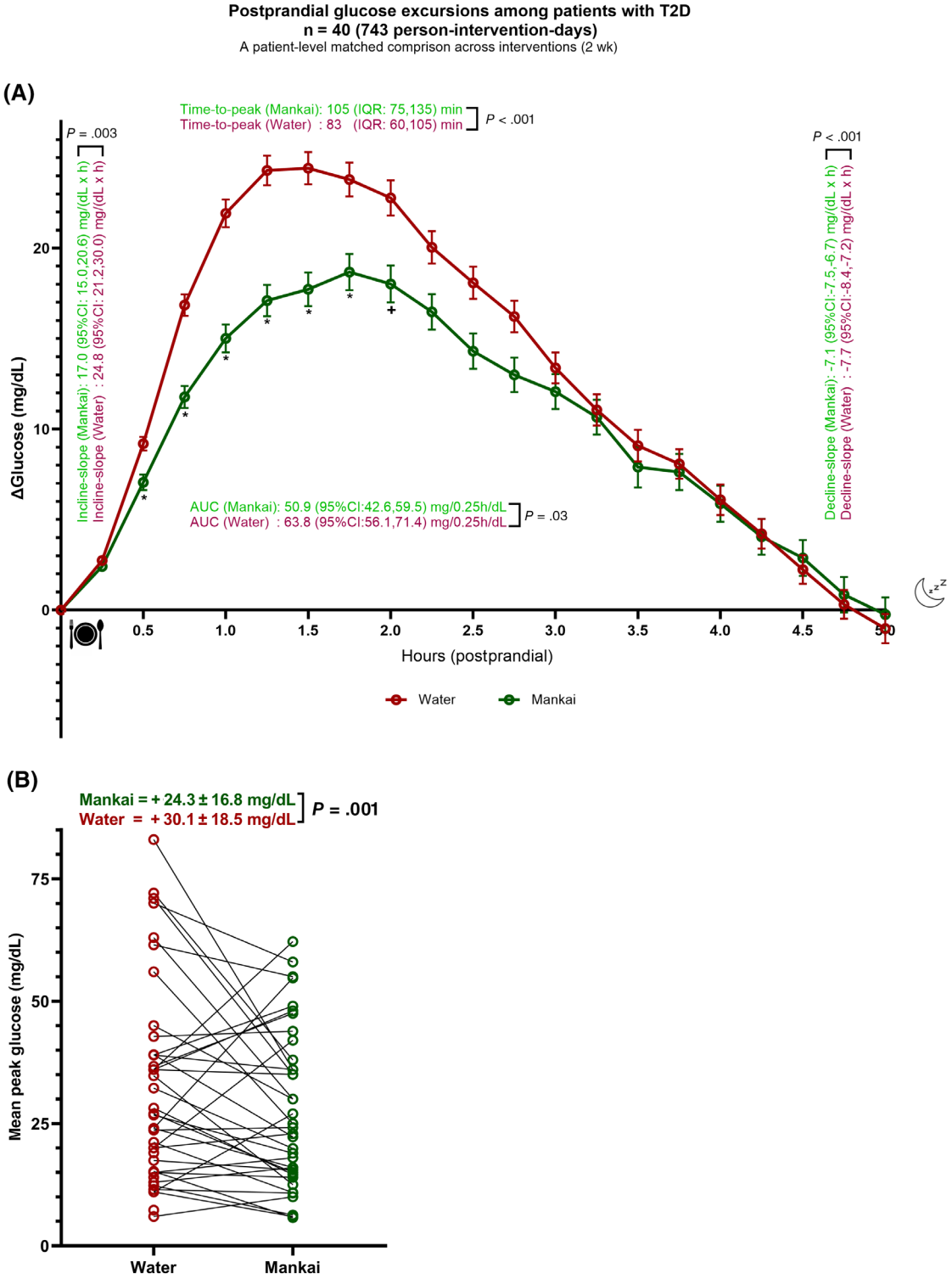

FIGURE 2 A, Postprandial glucose excursions with and without postdinner Mankai boost among patients with T2D. Postprandial glucose excursions among patients with T2D ($n = 40$; 743 person-intervention-days). A patient-level matched comparison across interventions (2 weeks each). B, Personal peak glucose excursions. T2D, type 2 diabetes.

During the study, participants maintained their non-insulin antihyperglycaemic therapy. Among the four patients under insulin therapy, two were not included in the primary CGM analysis because of a failure to comply with CGM; however, these participants did adhere to the nutritional intervention. Of these two participants, both started with the water intervention; one reported no changes in basal insulin requirements during the entire study term, while the other reported stable basal insulin requirements during the water intervention

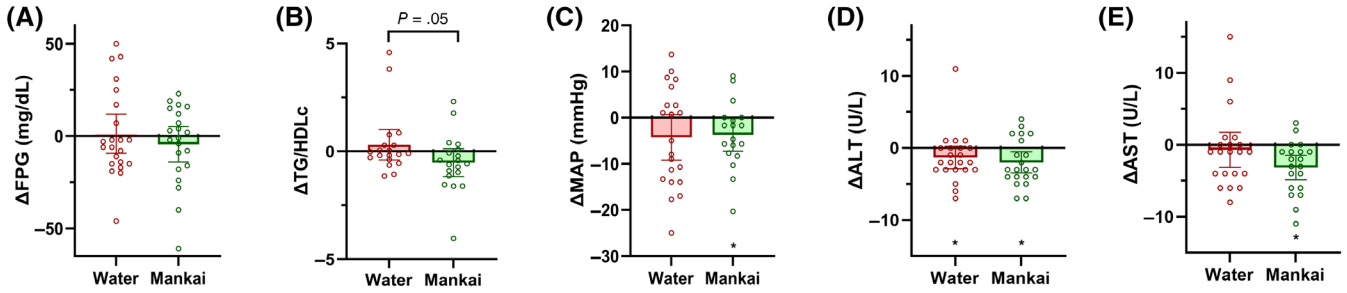

FIGURE 3 Key metabolic changes after 2 weeks of intervention. Red = water, green = Mankai. Significance was tested for within-group changes across water and Mankai interventions during the first 2 weeks of intervention. *Denotes within-group significance ($P < .05$). ALT, alanine transaminase; AST, aspartate transaminase; FPG, fasting plasma glucose; HDLc, high-density lipoprotein cholesterol; MAP, mean arterial pressure; TG, triglycerides.

(∼50 units/day), but a gradual decrease in basal insulin requirements during the Mankai intervention phase (from 50 to 37 units/day by the end of the study). One of the two other participants under insulin therapy included in the final CGM analysis reported no use of basal insulin during the study. The other, who started with the water intervention, reported stable insulin requirements during the water intervention (∼35 units/day), but a trend towards lower requirements of insulin use during the Mankai intervention where basal insulin requirements were dramatically reduced in nine out of 14 days of intervention (20 units/day during five intervention days and no basal insulin was required during four intervention days).

3.6 | Safety and satiety

During the intervention, there were no reported side effects related to the intervention by any of the participants. No participant withdrew from the study as a result of intolerance to interventions or a perceived inability to consume Mankai. Participants reported high personally perceived satiety after dinner, which did not differ during the Mankai or water interventions (rating of 9 [IQR: 8,10], $P = .78$). Overall daily satiety (the rounded mean satiety rankings of breakfast, lunch and dinner) was also similar across interventions (rating of 9 [IQR: 8,10] for both interventions, $P = .75$).

4 | DISCUSSION

In this randomized crossover study among 45 patients with stable T2D, we found that a 300-mL Mankai drink after dinner resulted in a significantly better ∼20% postprandial glucose response, as measured with a CGM device. The results were consistent across several measures of postprandial glucose outcomes, including glucose rise and fall rate, peak glucose levels, net-AUC and mean glucose values between 30 and 105 minutes postprandially. Interestingly, the postprandial glucose levels were consistently lower after Mankai consumption for 5 hours. To the best of our knowledge, this is one of the first studies to show that adding a specific food may mitigate postprandial glucose levels.

This study revealed a potential benefit of postprandial Mankai in improving postprandial glycaemic control among patients with T2D. These results augment previous evidence that showed a beneficial effect of Mankai compared with yogurt on postprandial glucose excursions among individuals with prediabetes.[24] Of note, only two-thirds of the individuals responded beneficially to Mankai, while the others showed neutral responses. A similar proportion of positive responses to Mankai has been reported among non-diabetic patients,[23] suggesting individual responses that should be further explored and possibly mediated by personal microbiome chains.[20] Taken together, the findings suggest that Mankai might be a unique potential nutritional product to aid in treatment and prevention across the spectrum of insulin resistance. Long-term Mankai consumption, as part of a green Mediterranean diet, was shown to promote weight loss, regression in visceral and hepatic fats, and regression of proximal aortic stiffness.[21,22,27,28] Thus, it is plausible that the beneficial effect of Mankai on postprandial glucose metabolism might be yet another mechanism by which Mankai consumption promotes cardiometabolic health and lowers cardiovascular risk.

Interestingly, we observed varying postprandial glucose metabolism responses across antidiabetic pharmacotherapy exposure groups. Patients on antihyperglycaemic medications exhibited a more pronounced rise and fall in postprandial glucose levels after dinner, whereas those not receiving pharmaceutical treatment showed a more gradual postprandial glucose response. These differences are probably a result of varying degrees of insulin resistance severity. Another source of variability was observed in the allocation sequence response to Mankai, probably reflecting interparticipant glucose variability. Participants randomized to start with the Mankai intervention experienced a steeper postpeak glucose decline compared with those who started with the water intervention. However, across both the antidiabetic pharmacotherapy exposure groups and intervention sequence subgroups, the effect of Mankai was consistent, with no statistically significant interaction effect. These findings are consistent with a previous study that examined the effect of Mankai compared

with yogurt in patients with prediabetes, where a variable interpersonal response was also evident.[24]

Mankai, rich in fibre and polyphenols and containing bioavailable protein and vitamin B12,[14,17] was linked with an increase in fasting ghrelin levels, which promoted recovery of sensitivity to insulin among patients with obesity with or without diabetes.[18] Mankai altered gut microbiome composition and function in the same population, mainly affecting microbial glucose metabolism, which correlated with alterations in host glucose metabolism.[19,20] Also, dietary fibre by itself can potentially reduce glucose absorption from the gut.[29] Importantly, in the current study, most of the impact related to Mankai consumption was observed in the early postprandial phase, 30-105 minutes after dinner, and impacted the glucose upslope rate and time to glucose peak. These findings may be attributed partly to delayed carbohydrates and macronutrient absorption from the gut by impacting gastric emptying time and intraluminal chyme transition or by directly impacting macronutrient absorption.[30] Hence, the mechanisms by which Mankai promotes postprandial glycaemic control may be diverse, including gut intraluminal pathways and gut- and non-gut–related endocrine and metabolic pathways.

The Mankai drink provided in the study contained erythritol, a fermentable sugar alcohol chosen for its low glycaemic and insulinogenic indices.[31] To avoid potential side effects, the amount of erythritol used was approximately 0.3 g/kg, well within the recommended dose range of 0.66 g/kg for men and 0.8 g/kg for women.[23] While some rodent studies suggest erythritol may attenuate glucose absorption in the gut, possibly through the inhibition of alpha-glucosidase,[32,33] human studies have not shown significant postprandial glycaemic or insulinaemic effects of erythritol.[34–36] Notably, placebo-controlled studies in humans are still lacking. In a recent placebo-controlled, randomized study, chronic erythritol consumption did not improve the glycaemic response to an oral glucose tolerance test.[37] Therefore, while we cannot entirely rule out some influence of erythritol on postprandial glycaemic response, any effects are probably minimal. Additionally, because the intervention was administered after the meal, it did not impact prandial satiety or energy intake.

This study has several limitations. First, participants were not on an identical diet or provided with a specific menu to standardize their carbohydrate intake. However, all the participants were instructed to maintain consistent dietary behaviour during the study; moreover, the trial's crossover design thus ensures that each participant served as a valid control for themselves during the study. Also, participants recorded their diet daily, enabling us to recognize any deviations from the protocol. Second, Mankai was provided as an additional product to the meal, and we did not provide any calorie equivalent substitute during the water intervention. While this small (40 kcal) imbalance might have contributed to a higher energetic and glycaemic load in the Mankai intervention arm, it was not reflected in glucose excursions following Mankai consumption, which were lower than those observed in the water intervention phase. Third, this pilot study was small with a short follow-up duration. The postprandial response does not reflect the long-term impact of regular postprandial Mankai consumption on glycaemic control or HbA1c. However, the trend towards a reduction in the TG/HDL-c ratio with Mankai, which should be interpreted with caution given a numerical difference in baseline values, may suggest an impact on systemic insulin resistance, which is fundamental in improving long-term glycaemic control. Fourth, the duration of the beneficial effects of Mankai consumption is unknown, which might have impacted the postprandial response during the water intervention phase among patients who began with the Mankai intervention first. However, the CGM sensors might be a powerful intervention that was consistently applied to all participants and thus cannot affect the observed differences between interventions. Fifth, in our study, nearly one-third of the participants did not show a beneficial response to Mankai. While we could not identify specific characteristics unique to these patients, further mechanistic studies focusing on genetic, microbial and metabolic attributes may shed light on the factors contributing to an attenuated response to the Mankai intervention. Such research could help improve patient screening for suitability for this intervention. However, given the safety of the Mankai intervention, it may be worthwhile for individuals to test their personal response through a short-term follow-up. Sixth, no standardized dinner was provided during the study, and self-reporting of dietary intake may introduce inaccuracies and biases. However, the crossover design was implemented to minimize interpersonal dietary variations, as each participant served as their own control, contributing observations to both study arms. The use of dietary logs ensured that dietary habits and portion sizes were generally maintained at the participant level throughout the study. We believe that conducting the study in 'non-sterile' real-world settings, while maintaining similar (although not identical) dietary and lifestyle habits, strengthens the validity and consistency of the results. Seventh, as with any randomized clinical trial, the findings of this study are representative of the specific population in which it was conducted. While the study was designed to align with common life routines and minimize the influence of 'laboratory settings', the short intervention period and the unique characteristics of the study population might limit the generalizability of the findings. A larger-scale and longer-duration study may be warranted to enhance the generalizability of our results.

Our study shows a consistent ~20% reduction in key postprandial glycaemic markers (net-AUC and personal mean glucose peaks) under the Mankai intervention. This observed glucose reduction is comparable with that reported after 4 days of treatment with dapagliflozin or canagliflozin and with that reported after treatment with sulphonylurea medications.[38–40] Other studies testing the effects of different antidiabetic medications, such as glucagon-like peptide-1 agonists and metformin, on postprandial glucose excursions in animal models, showed comparable overall glucose reductions ranging from 10% to 40%.[41,42] Thus, it might be speculated that regular long-term Mankai consumption can promote glycaemic control. This hypothesis, of course, along with further mechanistic studies examining different intervention timing and postprandial hormonal responses, should be tested in dedicated studies.

AUTHOR CONTRIBUTIONS

GT, GAH, IS and AT conceived and planned the trial and drafted the manuscript. GG, GAH, SS, HZ, AYM, DP, DTG, OK and LA conducted the trial. GT and IS analysed the data. MJS, DDW, LQ, MB, MS and FH critically reviewed the trial plan and results. All the authors have critically reviewed the manuscript.

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CONFLICT OF INTEREST STATEMENT

All authors have no conflicts of interest to disclose.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ORCID

Gal Tsaban https://orcid.org/0000-0003-4234-6504
Lu Qi https://orcid.org/0000-0002-8041-7791
Matthias Blüher https://orcid.org/0000-0003-0208-2065

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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