Effect of a Novel Palatinose-Based Liquid Balanced Formula (MHN-01) on Glucose and Lipid Metabolism in Male Sprague-Dawley Rats After Short- and Long-Term Ingestion

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Postprandial hyperglycemia and hyperinsulinemia are often present in obese subjects with glucose intolerance in whom insufficient early phase insulin secretion and subsequent delayed hyperinsulin response are observed. To address this problem, a novel palatinose-based enteral formula designated as MHN-01 was developed for the prevention of postprandial hyperglycemia and hyperinsulinemia. The effects of MHN-01 on carbohydrate and lipid metabolism in Sprague-Dawley (SD) rats were compared with those of the standard balanced formula (SBF). After a bolus intragastric injection of each formula equivalent to 0.9g/kg carbohydrate, the peak levels of plasma glucose (PG) and insulin (IRI) in peripheral and portal veins of the MHN-01 group were significantly lower than those of the SBF group. The areas under the curve of PG and IRI in the MHN-01 group were 58.0% and 43.1% of those in the SBF group in the femoral vein and 65.0% and 69.3% in the portal vein, respectively. In the 2-month study, serum levels of IRI and triglyceride in peripheral blood in the MHN-01 group decreased and those in the SBF group increased compared with initial levels. Consequently, both levels in the MHN-01 group were significantly lower than those in the SBF group. In addition, the amount of accumulated fat in abdominal adipose tissue and liver tissue of the MHN-01 group was markedly reduced in comparison to that of the SBF group. Insulin sensitivity, evaluated as glucose infusion rate using the hyperinsulinemic euglycemic clamp technique, in the MHN-01 group was higher than that in the SBF group. Thus, in comparison to SBF, MHN-01 suppressed postprandial hyperglycemia and hyperinsulinemia, reduced visceral fat accumulation, and improved insulin sensitivity. Therefore, human study on the effects of MHN-01 on carbohydrate and lipid metabolism will be recommended to confirm whether MHN-01 may be a useful functional food for the treatment and prevention of insulin resistance.

THE WORLD TOTAL of people with diabetes mellitus is expected to increase from the current estimate of 150 million to 220 million in 2010 and 300 million in 2025.[1,2] Pronounced changes in the human environment, and in human behavior and lifestyle, have accompanied globalization and have resulted in escalating rates of both obesity and type 2 diabetes.

The insulin secretory pattern of type 2 diabetes involves impairment in the rapid, pulsatile secretion of insulin in response to an increase in blood glucose. Obese subjects with type 2 diabetes, especially soon after the onset of diabetes, usually exhibit postprandial hyperglycemia with delayed hyperinsulinemia. It is recognized that insulin resistance causes postprandial hyperglycemia[3,4]; however, it is also possible that impairment of early insulin secretion in response to an oral glucose load (OGL) is the reason why postprandial hyperglycemia occurs.[5] Insulin-resistant individuals with normal β-cell function have virtually the same glucose response as insulin-sensitive subjects. An important characteristic of insulin resistance is that it is, at least initially, somewhat reversible. An overnight infusion of insulin to lower blood glucose concentrations into the normal range is associated with an improvement of insulin secretion.[6] Similarly, patients with early type 2 diabetes who are placed on a hypocaloric diet show a marked improvement in early phase insulin secretion over a period of several months.[7,8]

Because even modest increases in postprandial glucose values can be a risk factor for cardiovascular disease,[9] patients with diabetes would probably benefit from early and effective mealtime treatment. The meal-induced activation of homeostasis in patients with type 2 diabetes can be reduced by decreasing postprandial hyperglycemia.[10] Therefore, strategies to reduce postprandial hyperglycemia and hyperinsulinemia represent an important approach to improving glycemic control in patients with type 2 diabetes mellitus and may even prevent the deterioration of glucose metabolism in impaired glucose tolerance and the subsequent progression to diabetes. Thus, there is a strong need for the development of an enteral feeding product that produces an attenuated postprandial glycemic increase.

Palatinose (isomaltulose), which is present in honey, has shown promise as a noncariogenic caloric sweetener.[11,12] A previous study clearly demonstrated that the increase in plasma glucose (PG) and insulin (IRI) after palatinose ingestion was significantly smaller than that after sucrose.[13] Furthermore, palatinose has been shown to be an insulin-sparing caloric sweeteer with a lower glycemic index than sucrose in type 2 diabetic patients and streptozotocin-diabetic animals.[14,15] The difference may be due to a difference in digestibility because palatinose is digested to glucose and fructose by the intestinal

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Table 1. Composition of the Administered Formulas and Standard Meal

	MHN-01	SBF	MF
Energy (kJ/mL)	4.19	4.19	
(kJ/g)	19.4	18.0	15.1
Energy balance			
Protein (energy %)	20.0	16.0	27.3
Milk protein (%)*	(100)	(100)	
Fat (energy %)	29.7	25.0	14.0
SFA (%)†	(10.9)	(9.0)	
MUFA (%)†	(72.3)	(45.0)	
PUFA (%)†	(15.1)	(40.0)	
Other fatty acid (%)†	(1.7)	(6.0)	
Carbohydrate (energy %)	50.3	59.0	58.7
Sucrose (%)‡	(0)	(2.8)	
Branched dextrin (%)‡	(23.9)	(0)	
Dextrin (%)‡	(0)	(97.2)	
Palatinose (%)‡	(55.7)	(0)	
Xylitol (%)‡	(5.3)	(0)	
Other carbohydrate (%)‡	(15.1)	(0)	

Abbreviations: SBF, standard balanced formula; MF, control diet for rats (protein: 246g/kg, fat: 56g/kg, carbohydrate: 528g/kg); SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; other carbohydrate is dietary fiber and mixed carbohydrates from raw material. Palatinose and xylitol have energy contents of 16.76kJ/g and 12.57 kJ/g, respectively.

*% of nutrient in protein;

†% of nutrient in fat;

‡% of nutrient in carbohydrate.

isomaltase and the hydrolysis of palatinose by a homogenate of human intestinal mucosa was one fourth that of sucrose.[16] However, palatinose was completely cleaved and absorbed.[17]

In this study, a palatinose-based formula (MHN-01) for humans was prepared, and the effects of bolus and 2-month administration of this diet on glucose and lipid metabolism in Sprague-Dawley (SD) rats were compared with those of a dextrin-based standard balanced formula (SBF) (MEIBALANCE 200; MEIJI MILK PRODUCTS) for humans and MF for rats.

MATERIALS AND METHODS

Preparation and Composition of the Palatinose-Based Enteral Formula

The novel enteral liquid formula designated as MHN-01 was prepared by the replacement of dextrin in SBF with 55.7% palatinose among carbohydrates (Table 1). MHN-01 contains palatinose, branched dextrin, xylitol, and other carbohydrates containing dietary fiber and mixed carbohydrates from raw material as the principal carbohydrates, and the percentages of protein, fat, and carbohydrates in the formula are 20%, 29.7%, and 50.3%, respectively. The commercially available conventional enteral formula (SBF) that was used for comparison contains dextrin and sucrose as the principal carbohydrates, and the percentages of protein, fat, and carbohydrates are 16%, 25%, and 59%, respectively. Spray-dried powder versions of MHN-01 and SBF were prepared for use in the 2-month study.

Animals

All rats were cared for in accordance with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals. Thirty male SD rats, 12 weeks of age (weight ~350 g) for experiment 1 (short-term: bolus intragastric injection) and 20 weeks of age (weight ~550 g) for experiment 2 (long-term: 2-month study), were purchased from Japan SLC (Hamamatsu, Japan) and used for the following experiments after being acclimated for 1 week with food and water available ad libitum.

Catheterization

Catheterization was performed as described previously.[18] In the nutrient loading study, under sodium pentobarbital anesthesia (50 mg/kg body weight [BW]), a silicon rubber catheter was inserted into the left femoral vein, and a polyethylene tube (sp 8; Natsume, Tokyo, Japan) was inserted into the portal vein through the splenic vein, and the line of the catheter was led out through subcutaneous tissue to an intravenous hyperalimentation (IVH) kit (Bio-Cannula; Bio-Medica, Osaka, Japan). In the long-term study, after 2 months of feeding the test formulas, silicon rubber catheters (FT-025, Bio-Medica) were inserted into the left femoral vein and into the left jugular vein, and the clamp study was conducted on the 5th day after catheterization. Catheterized rats were kept in a special IVH cage (BG-781, Bio-Medica) and were continuously infused with physiologic saline until the test.

Bolus Intragastric Administration of MHN-01, SBF, or D-Glucose

On the fifth day after catheterization, all tests were conducted after an overnight fast and while the animals were conscious. Nutrient bolus loading was performed on 30 rats, which had been randomly allocated into 3 groups of 10 rats each. MHN-01, SBF, or 15% D-glucose solution containing an equal amount of carbohydrate (0.9 g/kg) was administered orally, because the total amount of carbohydrate was important in determining the postprandial blood glucose increase. Actually, 7.16 mL/kg BW of MHN-01 (energy 30.0 KJ/kg, carbohydrates 0.90 g/kg BW, fat 0.24 g/kg, protein 0.36 g/kg), 6.11 mL/kg BW of SBF (energy 25.6 KJ/kg, carbohydrates 0.90 g/kg, fat 0.17 g/kg, protein 0.24 g/kg), and 6 mL/kg BW of 15% D-glucose solution (energy 15.1 KJ/kg, carbohydrates 0.90 g/kg) were administered. Blood samples were withdrawn from the catheter using a 1.0-mL syringe and collected into chilled tubes containing EDTA and dipeptidylpeptidase IV inhibitor (Linco Research, St Charles, MO) at 0, 15, 30, 60, 90, and 120 minutes for the determination of PG and IRI and at −10, 0, 15, 30, 45, and 60 minutes for the determination of truncated glucagon-like peptide-1 (tGLP-1), respectively.

Long-Term Feeding of MHN-01, SBF, or Control MF Diet

The 2-month study was performed using 30 rats (age, 20 weeks). BW and food intake were monitored daily during a 1-week acclimation period. The animals were divided into 3 groups of 10 rats each. One group received spray-dried MHN-01 powder, the second received spray-dried SBF powder, and the third received MF (Oriental Yeast, Tokyo, Japan) that is standard diet for rats. A paired feeding program, which supplies different formulas (16.4 g/d of MHN-01, 17.7 g/d of SBF, 21.2 g/d of MF) with equal energy amount to all 3 groups of rats was conducted to compare the effect of each diet (Table 2). The nutrient composition (carbohydrate, fat, and protein) of the diets actually consumed was 159.8 KJ/d, 94.3 KJ/d, and 63.5 KJ/d in MHN-01, 188.28 KJ/d, 79.78KJ/d, and 51.08 KJ/d in SBF and 187.88 KJ/d, 44.88 KJ/d, and 87.38 KJ/d in MF, respectively. After 8 weeks, hyperinsulinemic euglycemic clamp tests were performed. Diarrhea was not found in any rats throughout the study.

Measurement of In Vivo Glucose Disposal and Hepatic Glucose Uptake

Insulin-mediated whole-body glucose uptake was measured in conscious rats using the hyperinsulinemic euglycemic clamp technique as

Table 2. Food Intake, Body Weight, and Body Weight Gain of Rats Fed MHN-01, SBF, or MF

	MHN-01	SBF	MF
Food intake (kJ/d)	317.6 ± 3.8*	318.9 ± 1.8	319.9 ± 1.5
(g/d)	16.4 ± 0.2	17.7 ± 0.1	21.2 ± 0.1
Body weight (g)			
Day 0 (20 wk of age)	556 ± 12	554 ± 5	551 ± 5
Day 56	589 ± 13	621 ± 12	620 ± 10
Day of clamp	586 ± 9	617 ± 12	615 ± 7
Body weight gain (g/d)	0.6 ± 0.1	1.2 ± 0.2†	1.2 ± 0.1†

*Values are mean ± SEM, n = 10.
†$P < .05$ (*v* MHN-01).

reported previously.[19] After an overnight fast, each rat received an infusion of insulin (Eli Lilly, Kobe, Japan) at a rate of 60 pmol/kg/min for 3.5 hours and an infusion of 10% of glucose solution, which was started at time zero through the catheter in the jugular vein. The infusion rate was adjusted to clamp PG at approximately 5.0 mmol/L. Blood samples for determination of PG level were obtained from the catheter in the femoral vein at 3-minute intervals throughout the study. Data on total body glucose uptake were represented by the mean values for the glucose infusion rate (GIR) at 120 minutes during the final 20 minutes. After determination of baseline GIR during the clamp, glucose was orally administrated at a dose of 0.2 g/kg BW.[20] Thereafter, the clamp was continued and the extent of decrease of GIR was monitored for 1.5 hours to evaluate hepatic glucose uptake (HGU), which was used as an indicator of insulin sensitivity in the liver.

Preparation of Organs

On the fifth day after the clamp study, the rats were killed under overnight fasting, and the organs were quickly removed for the biochemical studies. The liver tissue of each rat was immediately frozen in liquid N_2 for measurement of triglyceride (TG) concentration in the liver tissue. The extraction of lipids from liver tissue was performed as described by Folch et al.[21]

Assays

PG was determined by the glucose oxidase method (Advantage II; Roche Diagnostics, Mainz-Hechtsheim, Germany), IRI was determined by a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Morinaga, Yokohama, Japan), and plasma tGLP-1 level was determined by a commercially available ELISA kit (Linco Research). TG levels in serum and liver tissue were determined by an enzymatic method (Wako, Osaka, Japan).

Calculation of HGU

After an OGL during hyperinsulinemic euglycemic clamp,[20] the glucose appearance rate (Ra (t)) is calculated by the formula:

$$\text{Ra (t)} = [\text{OGL (t)} - \text{HGU (t)}] + \text{GIR (t)}$$

where OGL (t), the rate of absorption of OGL by the intestine at time (t); HGU (t), the rate of HGU of OGL at time (t); and GIR (t), GIR at time (t). The glucose disappearance rate from the systemic circulation (RdT (t)) is calculated by the formula:

$$\text{RdT (t)} = \text{RdP (t)} + \text{RdS (t)}$$

where RdP (t) and RdS (t) are extrasplanchnic and splanchnic glucose disappearance rates from the systemic circulation at time (t), respectively. Under hyperinsulinemic euglycemic clamp conditions, Ra (t) is equal to RdT (t), therefore

$$\text{RdP (t)} + \text{RdS (t)} = [\text{OGL(t)} - \text{HGU (t)}] + \text{GIR (t)}$$

HGU can be calculated from the difference between the amount of ingested glucose and the summation of GIR decrements after glucose ingestion.

$\Sigma(\text{HGU (t)} + \text{RdS (t)}) = \Sigma\text{OGL (t)} - \Sigma(\text{RdP (t)} - \text{GIR (t)})$. Total HGU was expressed as a percentage of OGL.

Calculations and Statistical Analysis

All results are presented as the means ± SEM. The statistical significance of the difference was analyzed by analysis of variance (ANOVA), followed by Student's *t* test for individual comparison of mean values. The data for plasma TG levels at each time point were analyzed by the Wilcoxon single-ranks test. $P < .05$ was considered statistically significant.

RESULTS

Changes in PG and IRI Levels in Femoral and Portal Veins After Intragastric Administration of MHN-01, SBF, or Glucose

At 15 minutes after intragastric administration of MHN-01, SBF, or glucose, the peak levels of PG in the femoral vein were 5.5 ± 0.3, 8.2 ± 0.3, and 8.2 ± 0.4 mmol/L ($P < .01$: MHN-01 *v* SBF, $P < .05$: MHN-01 *v* glucose), respectively, and those in the portal vein were 7.6 ± 0.3, 10.6 ± 0.4, and 11.0 ± 0.5 mmol/L ($P < .001$: MHN-01 *v* SBF, MHN-01 *v* glucose), respectively (Fig 1A and C). The total incremental area (area under the curve [AUC]) of PG from the basal level for 120 minutes after MHN-01 ingestion was significantly smaller than that after SBF or glucose ingestion, and the values were 162.0 ± 14.2, 279.5 ± 28.5, and 283.7 ± 51.3 mmol · min/L ($P < .01$: MHN-01 *v* SBF, $P < .05$: MHN-01 *v* glucose), respectively, in the femoral vein, and 139.0 ± 15.5, 214.0 ± 25.0, and 264.0 ± 18.0 mmol · min/L ($P < .05$: MHN-01 *v* SBF, $P < .001$: MHN-01 *v* glucose), respectively, in the portal vein (Fig 2A).

The peak levels of IRI in the femoral vein at 15 minutes after ingestion of MHN-01, SBF, or glucose were 62.8 ± 0.1, 112 ± 11.7, and 110.5 ± 6.7 pmol/L ($P < .05$: MHN-01 *v* SBF, $P < .01$: MHN-01 *v* glucose), respectively, and those in the portal vein were 78.3 ± 3.2, 125.8 ± 8.3, and 111.3 ± 3.3 pmol/L ($P < .01$: MHN-01 *v* SBF, $P < .001$: MHN-01 *v* glucose), respectively (Figs 1B and D). The AUC of IRI in the femoral vein 120 minutes after MHN-01 ingestion was significantly smaller than that after SBF ingestion. The values were 1,392.0 ± 160.0, 3,230.0 ± 391.7, and 2,826.7 ± 173.3 pmol · min/L ($P < .001$: MHN-01 *v* SBF, MHN-01 *v* glucose), respectively. The AUCs of IRI in the portal vein were 2,389.0 ± 60.0, 3,445.0 ± 130.7, and 3,821.1 ± 118.3 pmol · min/L ($P < .001$: MHN-01 *v* SBF, MHN-01 *v* glucose), respectively (Fig 2B).

A significant response of tGLP-1 was detected at 45 minutes after administration of the nutrients, and the response curve for 60 minutes did not show any differences between the MHN-01 and SBF groups (data not shown). tGLP-1 was not measured in the glucose group.

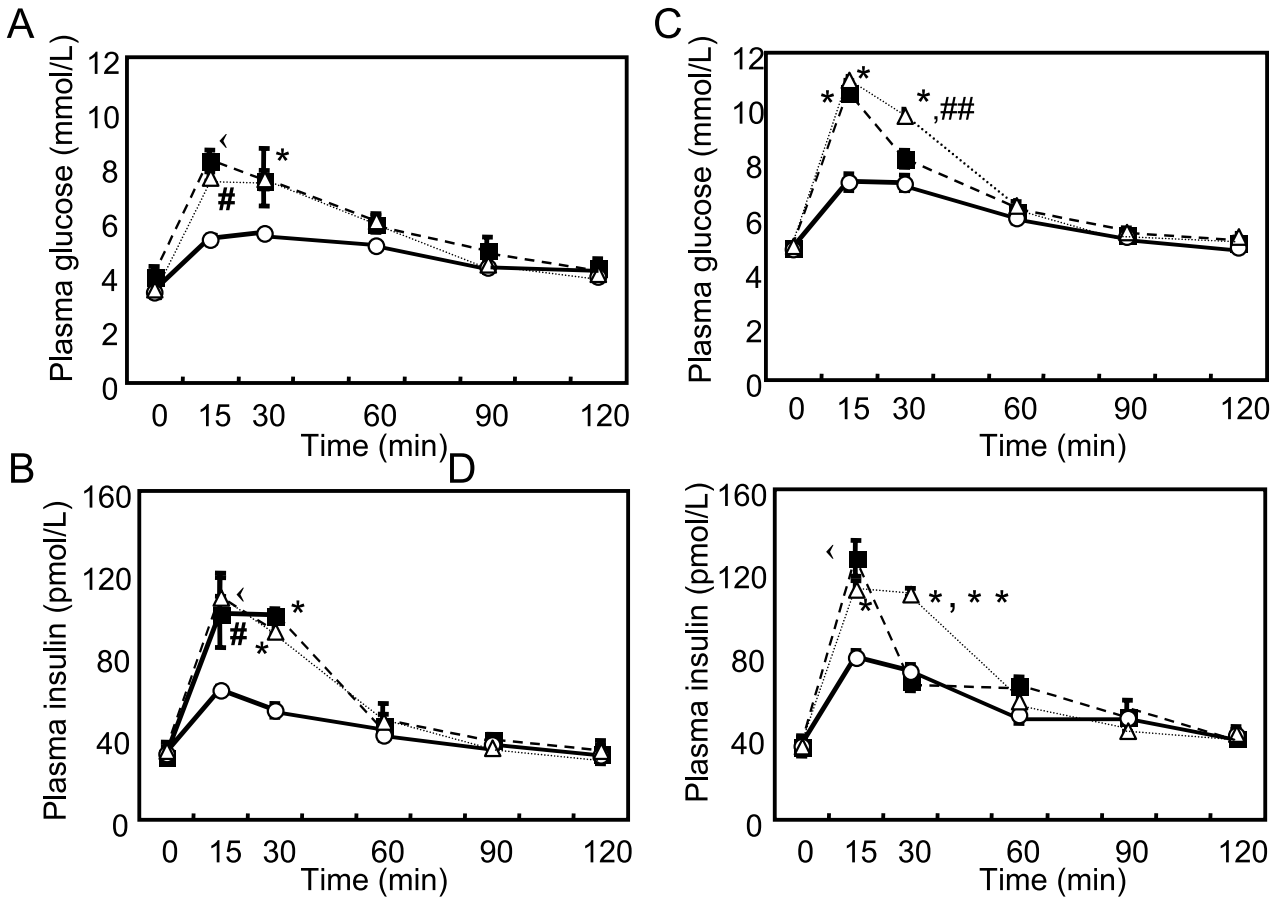

Fig 1. Changes in PG and IRI in the femoral vein and the portal vein after oral administration of MHN-01 (○), SBF (■), and glucose (△). (A) PG in femoral vein, (B) IRI in femoral vein, (C) PG in portal vein, (D) IRI in portal vein. Values are means ± SE for n = 10. #*P* < .05 (*v* MHN-01), †*P* < .01 (*v* MHN-01), **P* < .001 (*v* MHN-01), ##*P* < .05 (*v* SBF), ***P* < .001 (*v* SBF).

Glucose and Fat Metabolism After Oral Administration of MHN-01, SBF, or MF for Two Months

Food intake and BW. From 20 to 27 weeks of age, daily food intake and BW did not differ significantly among the MHN-01, SBF, and MF groups; however, BW gain in the MHN-01 group was lower than in other 2 groups (Table 2).

Fasting PG, IRI, and TG levels. After ingestion of MHN-01, SBF, or MF for 2 months, fasting PG levels were not different among the 3 groups, but the IRI level in the MHN-01 group was significantly lower than that in the SBF group ($P < .01$) (Table 3). After 2 months, the TG level markedly decreased by 34% (from 0.81 ± 0.09 mmol/L to 0.54 ± 0.04 mmol/L, $P < .05$) in the MHN-01 group and increased by 23% (from 0.92 ± 0.09 mmol/L to 1.13 ± 0.12 mmol/L, $P < .05$) in the SBF group; it did not change in the MF group. The TG level of the MHN-01 group was significantly lower than that of the SBF group ($P < .001$). The concentrations of serum free fatty acid (FFA) and total cholesterol did not differ among the 3 groups.

Insulin Sensitivity Evaluated by Hyperinsulinemic Euglycemic Clamp

Insulin sensitivity in the MHN-01, SBF, and MF groups was evaluated by the hyperinsulinemic euglycemic clamp test with OGL as described in Materials and Methods. The GIR, which reflected the insulin sensitivity in peripheral tissues, of the MHN-01 group was significantly higher than that of the SBF group (0.94 ± 0.03 *v* 0.76 ± 0.03 mmol/kg/min, respectively, $P < .05$); however, it was not different from that of the MF group (0.83 ± 0.11 mmol/kg/min) (Fig 3A). The rate of HGU, which might reflect insulin sensitivity in the liver, was significantly higher in the MHN-01 group than in the SBF group (57.4 ± 3.4 *v* 40.0% ± 1.1%, respectively, $P < .05$). It was not different from that of the MF group (52.1% ± 3.9%) (Fig 3B).

Weights of Adipose Tissues, Liver, and Pancreas

The weights of epididymal, mesenteric, and retroperitoneal adipose tissues were significantly lower in the MHN-01 group than in the SBF group ($P < .05$) (Table 4). The weights of liver and pancreas in the MHN-01 group were higher than in the SBF group ($P < .05$). The concentration of TG in the liver in the MHN-01 group was significantly lower than that in the SBF group ($P < .05$).

DISCUSSION

In subjects fed enteral feeding products and mixed meals, the total amount of carbohydrate is important in determining the

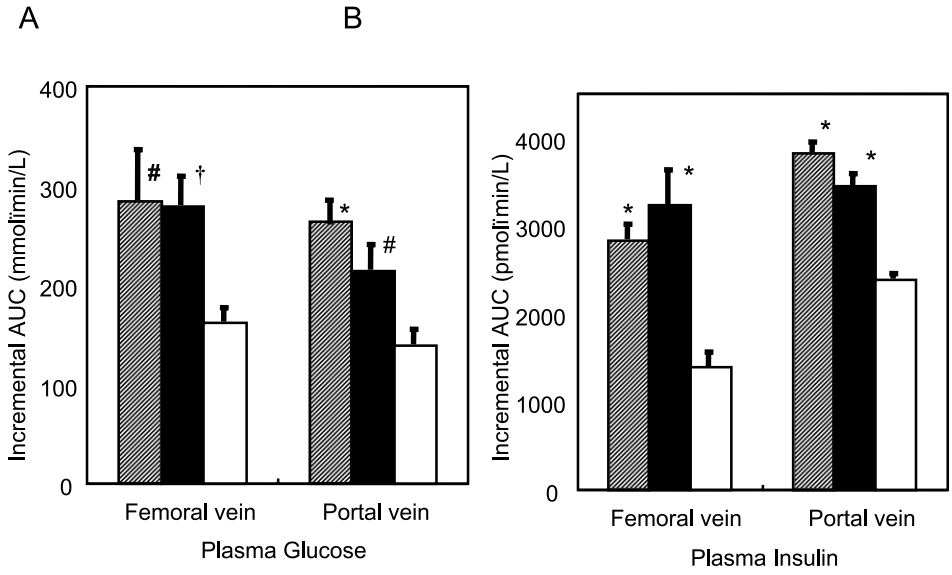

Fig 2. AUC after 120 minutes of PG and IRI in the femoral vein and the portal vein during oral administration of MHN-01 (white bar), SBF (black bar), or glucose (hatched bar). (A) AUC of glucose, (B) AUC of insulin. Values are means ± SE for n = 10. *$P < .001$ (*v* MHN-01), †$P < .01$ (*v* MHN-01), #$P < .05$ (*v* MHN-01).

postprandial blood glucose increase.[22-26] In contrast, abundant literature supports the idea that the source of carbohydrate affects postprandial glycemic response.[27-29] In an oral bolus loading of this study, PG levels in the femoral and portal veins at 15 and 30 minutes in the MHN-01 (a palatinose-based liquid balanced formula) group were significantly lower than those in the SBF and glucose groups. The peak values of PG at 15 and 30 minutes in the MHN-01 group were less than 5.5 mmol/L, although a pattern of insulin response at 15 minutes seemed to be evoked. The insulinogenic index (ΔIRI/ΔPG) at 15 minutes did not differ among the 3 groups. This indicates that MHN-01 did not affect early phase insulin response. In addition, the tGLP-1 response did not show any differences between MHN-01 and SBF. Therefore, it seems that MHN-01 may spare insulin by diminishing the elevation of PG after ingestion.

In the 2-month ingestion study, MHN-01 administration lowered serum TG and IRI levels and improved insulin sensitivity. This is likely due primarily to the low glycemic index of the palatinose in MHN-01. In addition, the stimulating effect on insulin secretion may differ between MHN-01 and SBF so that MHN-01 spares insulin secretion. Thus, MHN-01 could be used not only in the treatment of impaired glucose tolerance and diabetes, but also in the prophylaxis of them. Both metabolic and epidemiologic evidence suggest that replacing high-glycemic-index forms of carbohydrates with low-glycemic-index carbohydrates could reduce the risk of type 2 diabetes.[30] Differences in the pattern of postprandial glucose response offer a potential explanation for the conflicting results on insulin sensitivity, with the possibility that increases in insulin exposure may affect insulin sensitivity through downregulation of insulin action.[29] Therefore, it seems that MHN-01 belongs to a new category of functional foods.

Palatinose has the specific characteristic of delaying digestion and absorption, but it is completely absorbed.[16,17] The increase in BW was blunted and the accumulation of fat in abdominal adipose tissue and liver tissue was lessened in rats consuming MHN-01. These data interestingly indicate that postprandial glucose and insulin levels affect BW and body fat even though the intake was the same amount of energy. The

Table 3. Concentration of Serum Lipids, Plasma Glucose, and Plasma Insulin

	MHN-01	SBF	MF
Free fatty acid (mEq/L)	0.6 ± 0.06*	0.6 ± 0.02	0.62 ± 0.06
Cholesterol (mmol/L)	2.08 ± 0.16	2.08 ± 0.12	2.14 ± 0.14
Triglyceride (mmol/L)	0.54 ± 0.04	1.31 ± 0.12‡	0.89 ± 0.1†§
Glucose (mmol/L)	4.51 ± 0.26	4.9 ± 0.25	4.62 ± 0.39
Insulin (pmol/L)	50.2 ± 3.7	74.2 ± 2.0‡	52.8 ± 6.7‖

*Values are mean ± SEM, n = 10.
†$P < .01$ (*v* MHN-01);
‡$P < .001$ (*v* MHN-01),
§$P < .05$ (*v* SBF);
‖$P < .01$ (*v* SBF).

Table 4. Weight of Adipose Tissues, Liver, and Pancreas and Hepatic Content of Triglycerides

	MHN-01	SBF	MF
Abdominal fat			
Epididymal (g/kg BW)	14.5 ± 1.2*	23.8 ± 2.4‡	17.7 ± 1.2‖
Mesenteric (g/kg BW)	11.6 ± 0.6	22.0 ± 1.3§	15.8 ± 1.4†
Retroperitoneal (g/kg BW)	19.6 ± 1.2	29.7 ± 1.9§	21.2 ± 1.5¶
Liver (g)	22.7 ± 0.5	20.3 ± 0.8‡	22.3 ± 0.7
Pancreas (g)	2.1 ± 0.1	1.9 ± 0.2	1.9 ± 0.1
Triglyceride in liver (mmol/g tissue)	77.9 ± 10.1	136.7 ± 20.3†	103.9 ± 12.4

*Values are mean ± SEM, n = 10 in each group.
†$P < .05$ (*v* MHN-01);
‡$P < .01$ (*v* MHN-01);
§$P < .001$ (*v* MHN-01);
‖$P < .05$ (*v* SBF);
¶$P < .01$ (*v* SBF).

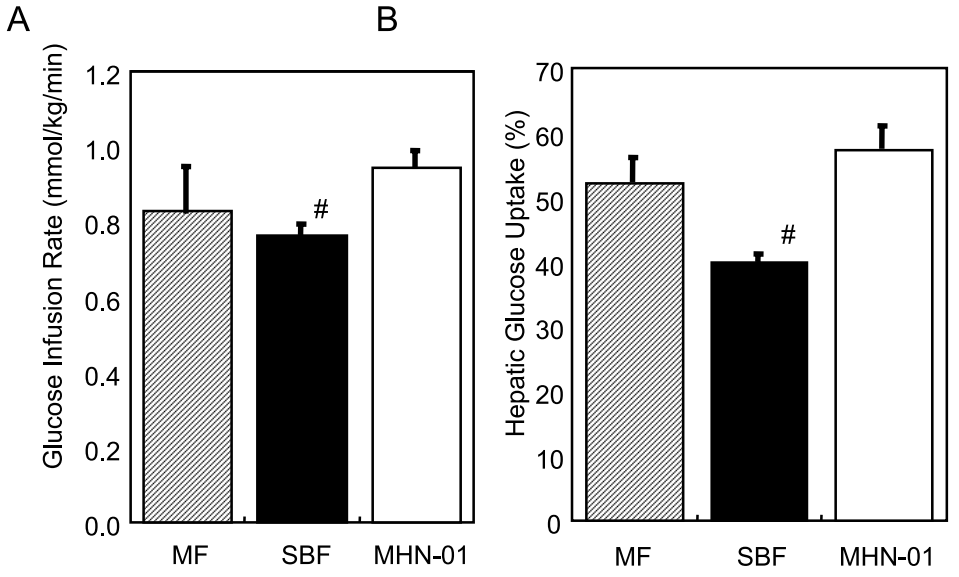

Fig 3. (A) GIR and (B) HGU during hyperinsulinemic euglycemic clamp with OGL in MHN-01 (white bar), SBF (black bar), and MF (hatched bar) groups. Values are means ± SE for n = 10. *$P < .05$ (*v* MHN-01).

effects of MHN-01 could be due to the suppression of the excess calories exposed in adipocytes, which were controlled by insulin. In fact, lean body mass among the 3 groups was not different.

An α-glucosidase inhibitor (α-GI) significantly decreased the BW, the postprandial increase in PG, and reduced resistance to insulin in patients with impaired glucose tolerance.[31-34] Because insulin resistance is an important factor in the development of type 2 diabetes, such an effect of α-GI could have a role in delaying the progression of impaired glucose tolerance to diabetes. It would also possibly explain why α-GI treatment was associated with an increased reversion to normal glucose tolerance.

Insulin resistance is characterized by the inability of insulin to increase glucose uptake in the normal manner in peripheral muscle and adipose tissues[35] and to suppress hepatic glucose output. Increased fatty acid levels are recognized as key components of the pathogenesis of insulin resistance.[36] High-fat feeding and elevation of circulating FFA are clearly sufficient to induce peripheral and hepatic insulin resistance.[37-40] Fasting TG levels after 2 months increased in the SBF group; in contrast, they decreased in the MHN-01 group. Therefore, the increased insulin resistance observed in the SBF group could be related to abnormalities in lipid metabolism. Furthermore, postprandial hyperglycemia with subsequent hyperinsulinemia was also observed in the SBF group; this deterioration was not observed in the MHN-01 group. Thus, the partial replacement of complex digestible carbohydrates with palatinose in an enteral formula can improve glycemic control in subjects with postprandial hyperglycemia and hyperinsulinemia induced by insulin resistance.

The SD rat is metabolically normal and is not analogous to humans with early type 2 diabetes and insulin resistance. The fact that an insulin sensitizing effect was detectable in these rats administered MHN-01 is encouraging, but should be followed up with study in an insulin-resistant rat model, of which there are several available. Further studies on the effects of other nutrients besides palatinose in MHN-01 on carbohydrate and lipid metabolism will be required in various models of hyperinsulinemia/insulin resistance, because insulin secretion can be more potently stimulated by oral loading of lipids or amino acids in addition to glucose compared with glucose alone.[41] However, the results observed in this study using mixed meals may encourage human study to confirm whether MHN-01 may be a useful functional food for the treatment and prevention of insulin resistance.

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