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Original Article

Effects of enteral nutrition formulas with varying carbohydrate amounts on glycemic control in diabetic mice

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Abstract

Purpose: This study evaluated the effects of an 8-week liquid diets with different carbohydrate contents–64% energy in HINE E-Gel (ST) and 50% energy in HINE E-Gel LC (LC)–on glycemic control and nutritional status in a mouse model of type 2 diabetes mellitus (db/db mice). The objective was to determine whether reducing carbohydrate intake within the Dietary Reference Intakes for Japanese people improves glycemic control indices, addressing the evidence gap in regarding the long-term safety and efficacy of low-carbohydrate enteral nutrition in patients with diabetes.

Methods: db/db mice (n=10 per group) and non-diabetic db/m mice (n=4) as controls were fed ST, LC, or AIN-93G diets ad libitum for 8 weeks. The diets primarily differed in carbohydrate content (64% in ST vs. 50% in LC). Blood glucose and glycated hemoglobin (HbA1c), plasma glucose and glycoalbumin, organ weights, and renal function markers were measured weekly or at 4 and 8 weeks. Histopathological examinations of the liver and kidneys were performed at 8 weeks.

Results: At 8 weeks, the LC group showed significantly lower plasma glucose (P=0.0051) and glycoalbumin (P=0.0013) levels compared to the ST group, with a trend toward lower HbA1c (P=0.0514). Although body weight was significantly higher in the LC group (P=0.0038), there were no significant differences between the ST and LC groups in caloric intake, renal function, or histopathological findings.

Conclusion: Reducing carbohydrate intake to 50% of total energy within dietary guidelines may improve glycemic control in diabetic mice, suggesting the need for further long-term evaluation for clinical applications.

Keywords: Blood glucose; Glycated hemoglobin; Liquid diet; Low-carbohydrate diet; Recommended dietary allowances

Introduction

Background

In nutritional management utilizing enteral nutrition formulas, products with reduced carbohydrate content compared to the standard range (45%–60% energy) may be employed as needed to attenuate post-administration blood glucose spikes [1]. Enteral nutrition products with a lower carbohydrate content (32.4% energy) have been reported to mitigate blood glucose elevations and reduce insulin usage in critically ill patients receiving early enteral nutritional management [2], as well as improve glycemic control in perioperative enteral nutrition patients [3].

The Dietary Reference Intakes for Japanese 2020 were

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established for healthy individuals and populations, with a target carbohydrate level of 50%–65% energy [4]. Accordingly, standard enteral nutrition products are formulated to meet this target for long-term nutritional management. However, insufficient evidence supports the prognosis and safety of long-term use of enteral nutrition products containing carbohydrates below this target [5]. Moreover, a meta-analysis comparing low-carbohydrate and low-fat diets on cardiovascular risk factors found that low-carbohydrate diets resulted in weight loss and triglyceride reduction, while low-density lipoprotein cholesterol levels increased [6].

A consensus statement by the Japan Diabetes Society indicates that no definitive evidence exists for an optimal energy-producing nutrient ratio for the prevention and management of diabetes; thus, individualized and flexible approaches are recommended. As a general guideline, however, carbohydrate energy should comprise 50%–60% (150 g/day or more), protein should account for 20% or less, and the remaining energy should be derived from lipids. If the fat energy ratio exceeds 25%, it becomes crucial to consider the fatty acid composition and adjust by increasing the proportion of polyunsaturated fats [7].

Because prolonged hyperglycemia can lead to various forms of organ damage [8], effective glycemic control is paramount. Consequently, it is important to investigate the effects of long-term administration of standard enteral nutrition formulas in patients requiring stringent glycemic control.

Objectives

This study investigated the effects of an 8-week administration of enteral nutrition formulas, with varying carbohydrate amounts within the target range of the Dietary Reference Intakes for Japanese people, on glycemic control indices and nutritional status in a mouse model of type 2 diabetes mellitus. The test diets were HINE E-Gel (ST) and HINE E-Gel LC (LC), containing 64% and 50% carbohydrate energy, respectively, while being similar in overall composition. A preliminary study (unpublished) in normal animals indicated that a single oral administration of LC produced a significantly lower early-phase increase in blood glucose levels compared to ST.

Methods

Ethics statement

This study was conducted in accordance with the animal experiment guidelines of Otsuka Pharmaceutical Factory, Inc. and was approved by the Otsuka Pharmaceutical Factory Animal Experiment Committee (approval number: OPF-CAE-20-143).

Test diets

The test diets used were HINE E-Gel (ST) and HINE E-Gel LC (LC), which contain 64% and 50% carbohydrate energy, respectively, while being similar in overall composition except for carbohydrate content. Both ST and LC were lyophilized and provided to the animals as powdered diets. For comparison, a standard purified feed, AIN-93G (Oriental Yeast Co., Ltd.), was also used. AIN-93G has an energy ratio of 19.3% protein, 16.7% fat, and 64.0% carbohydrate [9]. In contrast, ST contained 16.0% protein, 19.8% fat, and 64.3% carbohydrate, while LC contained 16.0% protein, 34.0% fat, and 50.0% carbohydrate. The detailed nutrient composition of ST, LC, and AIN-93G [9] is presented in Table 1.

Experimental animals and study design

Six-week-old male BKS.Cg-+ Lepr ^{db}/+ Lepr ^{db}/Jcl mice (db/db mice) (CLEA Japan, Inc.) were used as experimental animals. db/db mice are commonly used as model animals for type 2 diabetes mellitus because they spontaneously develop symptoms of diabetes, such as obesity, overeating, and hyperinsulinemia [10]. BKS.Cg-m+/+Lepr ^{db}/Jcl mice (db/m mice) (CLEA Japan, Inc.), which are closely related to db/db mice but do not develop diabetes, were served as control animals. The mice were maintained at a temperature of 23±3 °C, 55%±15% humidity, and a 12-hour light-dark cycle (light period: 07:00–19:00). During the acclimation period, the mice were fed AIN-93G.

After 12 days of acclimation, the 8-week-old db/db mice were fasted overnight, and glucose was administered orally at 1.0 g/kg body weight. Tail-vein blood glucose levels were measured immediately before administration and at 15, 30, 60, and 90 minutes post-administration using a glucose analyzer (Glutest Mint, Sanwa Kagaku Kenkyusho Co., Ltd.). The area under the curve (AUC) for blood glucose levels was calculated with time on the horizontal axis and blood glucose on the vertical axis. Based on the AUC values, mice were assigned to three groups via stratified randomization using the equal-width method with the statistical analysis software EXSUS Ver. 10 (EP Croit Co., Ltd.): db/db mice fed AIN-93G (DM group, n=4), db/db mice fed the test diet ST (ST group, n=10), and db/db mice fed the test diet LC (LC group, n=10). The grouping results are presented in Tables 2 and 3.

There were no significant differences between groups, confirming that stratified randomization was effective. Similarly aged db/m mice were fed AIN-93G as the CT group (n=4). All animals were provided with the prescribed test diet and water ad libitum for 8 weeks. Food intake was measured before administration and at 4 and 8 weeks after administration. Boby weight and tail-vein blood glucose levels were

Table 1. Nutritional components of test foods

Component	Test food (per 100 kcal)		
	ST	LC	AIN-93G
Protein (g)	4.0	4.0	4.7
Lipid (g)	2.2	3.78	1.86
Saturated fatty acids (g)	0.85	1.33	0.29
MCT (g)	0.64	1.11	-
Monounsaturated fatty acids (g)	0.6	1.4	0.4
n-6 fatty acids (g)	0.39	0.53	-
n-3 fatty acids (g)	0.14	0.25	-
Carbohydrates (g)	16.76	13.25	17.09
Sugars (g)	15.38	11.75	-
Dietary fiber (g)	1.38	1.50	-
Vitamin			
Vitamin B_1 (mg)	0.225	0.225	0.133
Vitamin B_2 (mg)	0.238	0.238	0.159
Niacin (mgNE)	2.25	2.25	-
Vitamin B_6 (mg)	0.30	0.30	0.16
Folic acid (μg)	30	30	53
Vitamin B_{12} (μg)	0.30	0.50	0.66
Biotin (μg)	4.25	4.25	5.31
Pantothenic acid (mg)	1.25	1.25	-
Vitamin C (mg)	52.5	52.5	-
Vitamin A (μg RE)	67.5	67.5	-
Vitamin A (IU)	225	225	106
Vitamin E (mg)	2.38	2.38	1.99
Vitamin D (μg)	1.25	1.25	-
Vitamin D (IU)	50.0	50.0	26.6
Vitamin K (μg)	6.25	6.25	23.9
Mineral			
Sodium (mg)	166.3	166.3	27.6
Sodium (mEq)	7.2	7.2	
Salt equivalent (g)	0.422	0.422	0.070
Chloride (mg)	151.3	194.4	43.3
Chloride (mEq)	4.3	5.5	-
Potassium (mg)	156.3	156.3	95.6
Potassium (mEq)	4.0	4.0	-
Magnesium (mg)	22.5	22.5	13.6
Magnesium (mmol)	0.9	0.9	-
Calcium (mg)	58.8, 1.47	58.8, 1.47	132.8
Calcium (mmol)	1.47	1.47	-
Phosphorus (mg)	82.5	82.5	79.7
Phosphorus (mmol)	2.6	2.7	-
Chromium (μg)	2.88	2.88	26.55
Molybdenum (μg)	5.0	5.0	4.0
Manganese (mg)	0.325	0.325	0.266
Iron (mg)	0.588	0.588	1.195
Copper (mg)	0.080	0.080	0.159
Zinc (mg)	1.20	1.20	1.01
Selenium (μg)	3.25	3.25	4.78
Iodine (μg)	13.8	13.8	5.31
L-carnitine (mg)	-	25	-

ST, HINE E-Gel; LC, HINE E-Gel LC; MCT, medium-chain triglyceride.

measured weekly during the study period. Tail-vein blood and 24-hour urine samples were collected before administration and at 4 and 8 weeks after administration. Following the 8-week collection, the animals were sacrificed by exsanguination after blood collection from the posterior vena cava under isoflurane anesthesia, and necropsy was performed. Tissues including the liver, kidneys, lower limb skeletal muscles, and epididymal fat were collected.

Blood and urine analysis

At baseline, 4, and 8 weeks after administration, a small incision was made in the tail vein with a scalpel, and the exuded blood was used to determine blood glucose levels. The remaining blood was collected into heparin-filled microcentrifuge tubes for glycated hemoglobin (HbA1c) measurement (DCA Vantage, Siemens Healthcare Diagnostics Inc.). Plasma was separated from the necropsy blood. All plasma and urine samples were stored at –80 °C until analysis. Plasma glucose, glycoalbumin, urea nitrogen, total protein, albumin, total cholesterol, triglycerides, as well as urinary albumin and N-acetylglucosaminidase, were measured using an automatic analyzer 7180 (Hitachi High-Tech Corporation).

Histopathological examination

Tissue samples were weighed using a precision balance, and the liver and kidneys were fixed in a 10% neutral buffered formalin solution (pH 7.4). The hematoxylin- and eosin-stained liver and kidney specimens were subsequently examined pathologically. Note that one kidney specimen from the LC group was missing due to technical issues. Hepatocyte lipidosis, glomerulonephritis, and mesangial cell proliferation were graded as “very slight” when 0%–25% of the evaluation area was affected, and as “slight” when 25%–50% was affected.

Statistical analysis

Data are presented as the mean±standard deviation. Repeated measures analysis of variance was employed to compare blood glucose, HbA1c, and body weight at different time points between the ST and LC groups, with group and time point as the two factors. Fisher test was used to analyze histopathological differences in incidence, while the Wilcoxon rank-sum test (a non-parametric test) was used for other comparisons. The CT and DM groups were served as reference groups. A significance level of 5% was used. Statistical analyses were performed using EXSUS Ver. 10 (EP Croit Co., Ltd.) and Bell Curve Ver. 4.00 (Social Survey Research Information Co., Ltd.) for histopathological examinations.

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Table 2. Grouping based on AUC

Group[a]	AUC mean	SD	SE	Median
DM (mg·min/dL) (n=4)	52,170	4,887	2,444	53,505
ST (mg·min/dL) (n=10)	52,289	5,441	1,721	52,395
LC (mg·min/dL) (n=10)	52,099	6,036	1,909	52,384

AUC, area under the curve; SD, standard deviation; SE, standard error; DM group, db/db mice fed AIN-93G; ST group, db/db mice fed the test diet ST (HINE E-Gel); LC group, db/db mice fed the test diet LC (HINE E-Gel LC).
[a]Tolerance range: 2,691.

Table 3. Tukey-type multiple comparison test of AUC

Reference group[a]	Comparison group	t-statistic	P-value	Significance
DM	ST	0.0358	0.9993	NS
DM	LC	−0.0214	0.9997	NS
ST	LC	−0.0756	0.9969	NS

AUC, area under the curve; DM group, db/db mice fed AIN-93G; ST group, db/db mice fed the test diet ST (HINE E-Gel); LC group, db/db mice fed the test diet LC (HINE E-Gel LC); NS, no significant difference.
[a]Tolerance range: 2,691.

Table 4. Daily calorie intake

Measurement time	CT (n=4)	DM (n=4)	ST (n=10)	P-value	LC (n=10)
Before administration (kcal/day)	13.59±2.64	21.85±2.18	20.79±5.92	0.6484	18.96±8.27
4 wk administration (kcal/day)	15.20±1.91	23.85±1.50	17.55±3.50	0.6229	16.86±1.75
8 wk administration (kcal/day)	14.63±2.16	21.57±2.35	18.03±1.75	0.0533	16.25±2.15

Values are presented as mean±standard deviation.
CT group, control group; DM group, db/db mice fed AIN-93G; ST group, db/db mice fed the test diet ST (HINE E-Gel); LC group, db/db mice fed the test diet LC (HINE E-Gel LC).

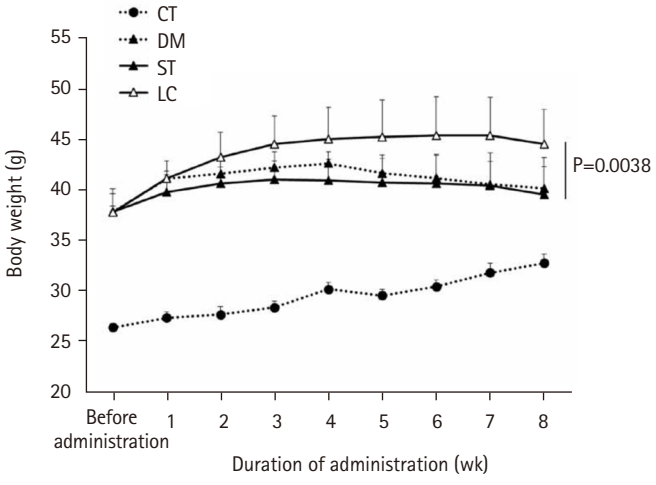

Fig. 1. Weight transition. Vales are presented as mean±standard deviation; ST versus LC (repeated measurement variance analysis). CT group, control group; DM group, db/db mice fed AIN-93G; ST group, db/db mice fed the test diet ST (HINE E-Gel); LC group, db/db mice fed the test diet LC (HINE E-Gel LC).

Results

Caloric intake, body weight, and organ weights

There were no significant differences in daily caloric intake between the ST and LC groups at baseline, 4 weeks, or 8 weeks after administration (Table 4). However, body weight was significantly higher in the LC group compared to the ST group (P=0.0038) (Fig. 1). When organ weights were normalized to body weight, differences were observed between the ST and LC groups in the liver and tibialis anterior muscles, but not in the epididymal fat, kidneys, gastrocnemius, or soleus muscles (Table 5).

Blood glucose control index

At 8 weeks, plasma glucose and glycoalbumin levels were significantly lower in the LC group than in the ST group (P=0.0051 and P=0.0013, respectively) (Table 6). Although HbA1c tended to be lower in the LC group than in the ST group (P=0.0514), no differences in overall blood glucose levels were observed between the two groups (Fig. 2).

Table 5. Tissue weights at 8 weeks after administration

	CT (n=4)	DM (n=4)	ST (n=10)	P-value	LC (n=10)
Tissue weights per body weight (g/kg)					
Epididymal adipose tissue	14.66±1.19	28.56±2.12	27.60±3.06	0.7337	27.40±3.87
Liver	33.19±3.43	49.01±2.80	70.32±4.52	0.0010	61.56±4.21
Kidney	6.72±0.26	6.51±0.58	5.37±0.64	0.9698	5.39±0.68
Gastrocnemius muscle	3.96±0.16	1.71±0.19	1.62±0.26	0.4274	1.49±0.13
Soleus muscle	0.16±0.03	0.12±0.03	0.11±0.02	0.4727	0.11±0.03
Tibialis anterior muscle	1.50±0.05	0.64±0.09	0.63±0.05	0.0091	0.56±0.04
Absolute tissue weights (g)					
Epididymal adipose tissue	0.465±0.036	1.102±0.055	1.077±0.159	0.0640	1.193±0.151
Liver	1.056±0.141	1.900±0.218	2.731±0.136	0.6232	2.693±0.307
Kidney	0.213±0.014	0.251±0.011	0.208±0.018	0.0140	0.235±0.025
Gastrocnemius muscle	0.126±0.003	0.066±0.009	0.063±0.009	0.6232	0.065±0.005
Soleus muscle	0.005±0.001	0.004±0.001	0.004±0.001	0.3840	0.005±0.001
Tibialis anterior muscle	0.047±0.003	0.025±0.002	0.025±0.002	0.7052	0.025±0.002

Values are presented as mean±standard deviation.
CT group, control group; DM group, db/db mice fed AIN-93G; ST group, db/db mice fed the test diet ST (HINE E-Gel); LC group, db/db mice fed the test diet LC (HINE E-Gel LC).
ST versus LC (Wilcoxon two-group comparison test).

Table 6. Blood glucose control indicators

	Measurement time	CT (n=4)	DM (n=4)	ST (n=10)	P-value	LC (n=10)
Plasma glucose (mg/dL)	8-wk administration	287.8±27.4	870.0±89.1	1,034±167.3	0.0051	857.8±69.0
Plasma glycoalbumin (%)	8-wk administration	3.2±0.3	11.6±0.9	12.1±1.5	0.0013	9.4±0.9
HbA1c (%)	Before administration	4.18±0.05	6.88±0.21	6.67±0.31	0.0514	6.60±0.34
	4-wk administration	4.33±0.10	13.48±0.56	12.72±0.95		12.13±1.47
	8-wk administration	4.25±0.13	13.53±0.66	13.06±0.83		11.83±1.08

Values are presented as mean±standard deviation.
CT group, control group; DM group, db/db mice fed AIN-93G; ST group, db/db mice fed the test diet ST (HINE E-Gel); LC group, db/db mice fed the test diet LC (HINE E-Gel LC); HbA1c, glycated hemoglobin.
ST versus LC (Wilcoxon two-group comparison test).

Renal function-related indices

Urinary albumin and N-acetylglucosaminidase levels showed no significant differences between the ST and LC groups at 8 weeks after administration (Table 7).

Blood biochemistry

At 8 weeks, no significant differences were observed between the ST and LC groups in total protein, albumin, or triglycerides. However, blood urea nitrogen was significantly higher in the ST group than in the LC group (P=0.0058), and total cholesterol was significantly higher in the LC group than in the ST group (P=0.0125) (Table 8).

Histopathological examination

In the liver, lobular-centered, very slight hepatocyte lipidosis was observed in all four mice in the DM group, in nine of 10 mice in the ST group, and in seven of 10 mice in the LC group; additionally, slight lipidosis was observed in three of 10 mice in the LC group. Very slight glomerulonephritis and mesangial cell proliferation in the kidneys were observed in two of four mice in the DM group, three of 10 mice in the ST group, and three of nine mice in the LC group. The incidences of hepatocyte lipidosis, as well as very slight glomerulonephritis and mesangial cell proliferation in the kidneys, did not differ significantly between the ST and LC groups.

Discussion

Interpretation/comparison with previous studies

The comparison between the CT and DM groups, established to confirm that the groups accurately represent the disease state of diabetes mellitus, demonstrated that the DM group consistently exhibited higher blood glucose and HbA1c levels than the CT group throughout the evaluation

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period, confirming the onset of diabetes mellitus. There was no significant difference in caloric intake between the ST and LC groups at any time point (before administration and at 4 and 8 weeks post-administration). Similarly, no significant differences in blood glucose levels were observed between the ST and LC groups at these time points; however, HbA1c tended to be lower in the LC group (P=0.0514). Glycoalbumin—a potentially more useful marker than HbA1c for assessing whether blood glucose levels remain within a target range, as it reflects variations in postprandial blood glucose in addition to mean levels [11]—was significantly lower in the LC group than in the ST group at 8 weeks post-administration. Plasma glucose was also significantly lower in the LC group. In our preliminary study comparing blood glucose levels in normal animals after a single oral administration of ST and LC, blood glucose levels were significantly lower in the LC group than in the ST group during the early phase of administration (unpublished). In this study, blood glucose levels were measured at fixed intervals after feeding, suggesting that variations in the degree of blood glucose elevation during feeding accumulated over time and were reflected in the differences observed in HbA1c and glycoalbumin levels.

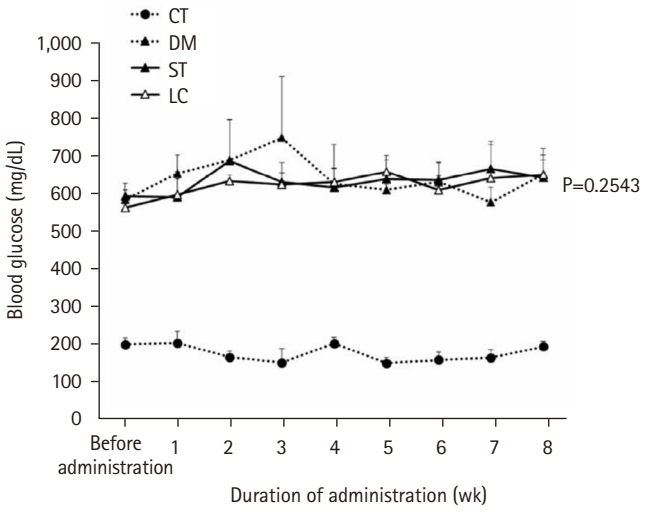

Fig. 2. Changes in blood sugar levels from time to time. Vales are presented as mean±standard deviation. CT group, control group; DM group, db/db mice fed AIN-93G; ST group, db/db mice fed the test diet ST (HINE E-Gel); LC group, db/db mice fed the test diet LC (HINE E-Gel LC).

A study using diabetic mice observed differences in HbA1c after feeding diabetic model mice (Akita mice) diets with widely varying carbohydrate contents (68% vs. 16% energy ratio) for 8 weeks [12]. Furthermore, STZ-induced diabetic model mice were fed diets with carbohydrate energy ratios of 75, 20, 15, and 1% for 14 weeks. Lower carbohydrate ener-

Table 7. Renal function tests

	Measurement point	CT (n=4)	DM (n=4)	ST (n=10)	P-value	LC (n=10)
U-ALB (μg/kg)	Before administration	922±279	12,640±3,763	13,650±6,413	0.6776	13,174±3,926
	4-wk administration	512±229	10,970±2,544	6,360±1,463	0.3447	7,142±1,697
	8-wk administration	458±210	13,908±4,173	6,934±2,087	0.7337	7,282±2,113
U-NAG (IU/kg)	Before administration	2.59±0.93	2.93±0.42	2.96±0.56	0.5205	2.78±0.68
	4-wk administration	3.05±0.47	2.63±0.50	1.07±0.25	0.1859	1.15±0.15
	8-wk administration	3.23±1.08	2.65±0.46	1.06±0.21	0.4727	1.15±0.26

Values are presented as mean±standard deviation.
CT group, control group; DM group, db/db mice fed AIN-93G; ST group, db/db mice fed the test diet ST (HINE E-Gel); LC group, db/db mice fed the test diet LC (HINE E-Gel LC); U-ALB, urinary albumin; U-NAG, urinary N-acetylglucosaminidase.
ST versus LC (Wilcoxon two-group comparison test).

Table 8. Blood biochemistry tests (after 8 weeks of administration)

Variable	CT (n=4)	DM (n=4)	ST (n=10)	P-value	LC (n=10)
Total protein (g/dL)	4.95±0.17	6.00±0.39	6.05±0.29	0.0848	6.29±0.27
Albumin (g/dL)	3.10±0.08	3.68±0.19	3.93±0.18	0.2769	3.91±0.29
Blood urea nitrogen (mg/dL)	18.0±2.2	23.4±2.3	29.2±6.2	0.0058	22.2±3.7
Total cholesterol (mg/dL)	107.5±13.1	146.0±11.5	129.1±16.3	0.0125	155.2±26.6
Triglycerides (mg/dL)	37.3±13.2	121.5±51.2	155.5±61.8	0.6232	148.0±71.7

Values are presented as mean±standard deviation.
CT group, control group; DM group, db/db mice fed AIN-93G; ST group, db/db mice fed the test diet ST (HINE E-Gel); LC group, db/db mice fed the test diet LC (HINE E-Gel LC).
ST versus LC (Wilcoxon two-group comparison test).

gy ratios were associated with reduced postprandial blood glucose levels, and the glucose AUC during the glucose tolerance test was also lower. Additionally, significant differences were noted between the groups with the lower carbohydrate energy ratios (20% and 15%) [13]. In a human study, it was reported that over 14 days, diets with widely different carbohydrate contents (approximately 10% vs. 75% energy) resulted in higher blood glucose levels and poorer glucose tolerance in the high-carbohydrate group [14]. Thus, the difference in carbohydrate energy ratio between ST and LC (64% vs. 50%) in this study may have affected glucose tolerance, leading to the observed differences in plasma glucose and glycoalbumin levels.

The present results suggest that even a modest difference in carbohydrate content in enteral nutrition formulas, such as a 64% versus 50% energy ratio, can produce measurable differences in plasma glucose and glycoalbumin, which are key indicators of glucose control, after a period of management. Recently, cases of ketoacidosis have been reported in patients who are on extremely low-carbohydrate diets and are taking SGLT2 inhibitors [15]. We hypothesize that reducing carbohydrate intake within the dietary reference intakes could be beneficial for preventing ketoacidosis and controlling blood glucose in these patients.

Long-term nutritional management requires attention not only to glycemic control indices but also to other nutritional parameters. Plasma total protein and albumin, which are standard nutritional indices, did not differ between the ST and LC groups, suggesting that whole-body protein metabolism was similar in both groups. The body weight of the LC group was significantly higher than that of the ST group, despite similar daily caloric intakes between the two groups at all time points (before, 4 weeks, and 8 weeks post-administration). When normalized to body weight, organ weights showed no differences in epididymal fat and lower limb skeletal muscle, except for the tibialis anterior muscle, between the ST and LC groups. Body weight is maintained when caloric intake equals caloric expenditure. Although the ST and LC groups had similar caloric intakes, carbohydrates require more energy for diet-induced thermogenesis compared to fats. This may explain why the LC group, with its lower carbohydrate intake, exhibited a higher body weight than the ST group. However, this difference in body weight may not have been sufficient to produce differences in organ weights normalized to body weight. Body weight and composition are as critical as blood glucose levels in managing diabetes mellitus; therefore, future studies should examine changes in these parameters in detail.

In this study, differences in carbohydrate content among the test diets were balanced by adjusting the fat content; thus, the effects of fat intake must be considered in long-term management. Liver histopathology revealed no evidence of accelerated hepatocyte lipogenesis in the LC group, suggesting that the impact of increased fat content on liver fat accumulation was minimal. Liver weight normalized to body weight was significantly higher in the ST group than in the LC group, likely attributable to weight loss in the ST group. Plasma total cholesterol levels were significantly higher in the LC group compared to the ST group; however, these levels were similar to those in the DM group, and no differences in triglyceride levels were observed between the two groups. In the LC group, the fat content was primarily composed of medium-chain fatty acids and monounsaturated fatty acids. Since monounsaturated fatty acids are known to improve lipid metabolism [16], the impact of fat content on these parameters is likely minor.

Since hyperglycemia can damage various organs [8], renal function was evaluated, and renal histopathological examinations were performed. The mouse models of diabetes mellitus used in this study exhibit pathologies similar to progressive diabetic kidney disease, including increased urinary albumin and mesangial substrate [10], paralleling human pathology, where urinary albumin excretion further increases as the disease progresses [17]. The DM group, serving as the reference, was used to evaluate both renal function and histopathological changes. As expected, the DM reference group exhibited significantly higher levels of urinary albumin and N-acetylglucosaminidase compared to the ST and LC groups. The low urinary albumin levels in the ST and LC groups suggest a milder disease progression. Because restricted feeding is known to improve renal parameters [18], the reduced caloric intake or variations in the composition of the test diets likely contributed to mitigating the symptoms. The LC group exhibited urinary albumin and N-acetylglucosaminidase levels comparable to those of the ST group. Moreover, no significant differences were observed in renal histopathology between the ST and LC groups, suggesting that the test diets exerted a similar impact on the kidneys. However, the long-term progression of diabetic nephropathy remains unclear and warrants further investigation. The protein sources for the test diets were soybean peptide and collagen peptide in the ST and LC groups, and casein (AIN-93G) in the DM group. Soybean peptide has been reported to improve glucose metabolism by increasing GLUT4 expression in skeletal muscle more effectively than casein [19]. Additionally, one report suggests that protein quality is more critical than quantity in preserving renal function, noting a reduced risk of end-stage renal failure when the protein source was switched

to soy [20]. Therefore, it is important to consider both carbohydrate content and protein source when evaluating the effects of diet on glycemic control and renal function in diabetes mellitus.

Limitations

The limitations of this study include the 8-week evaluation period and the potential effects of the test diets on caloric intake. For applications beyond the study period, careful administration is necessary, and a longer-term evaluation is required to clarify the effects on glycemic control, nutritional status, and renal disorders. Further studies involving both young and aged animals, which are not pathological models, are necessary for clinical translation. ST and LC are enteral nutrition formulas developed for humans; in this study, they were powdered and used as test foods. Consequently, it is possible that mice disliked the taste and odor of these test diets, which may have affected caloric intake during ad libitum feeding. Since no differences in caloric intake were observed between the ST and LC groups at any time point (before, 4 weeks, and 8 weeks post-administration), comparisons between the two groups are considered valid. However, for a more rigorous nutritional assessment, equalizing the caloric intake between the three groups (DM, ST, LC) is necessary in a mouse model of type 2 diabetes mellitus.

Conclusion

This study demonstrated that reducing carbohydrate intake within the range of the Dietary Reference Intakes for Japanese people may lower plasma glucose and glycoalbumin levels—key indices of glycemic control—in diabetic mice. Further long-term evaluations are needed.

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Authors' contribution

Conceptualization: KH. Methodology: YM, KH. Formal analysis/validation: YM, KH. Project administration: KH. Funding acquisition: Not applicable. Writing – original draft: YM. Writing – review and editing: YM, KH. All authors read and approved the final manuscript.

Conflict of interest

YM and KH are employees of Otsuka Pharmaceutical Factory, Inc. Except for that, no potential conflict of interest relevant to this article was reported.

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Data availability

Research data are available from the corresponding author upon reasonable request.

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Supplementary materials

None.

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