Table 3. Genes related to circadian rhythm.

Gene Symbol	Probe Name	P value
PER1	A_23_P89589	0.0011
ERBB3	A_23_P349416	0.0050
CLOCK	A_23_P419038	0.0120
PROK2	A_23_P97342	0.0230
CRY2	A_23_P127394	0.0489
CRY2	A_23_P388027	0.0565
CYP7B1	A_23_P169092	0.0669
CRY2	A_23_P158587	0.0699
AANAT	A_23_P152527	0.0704
CRY1	A_23_P36665	0.0864
PRF1	A_23_P1473	0.0939
HEBP1	A_23_P117082	0.0956
PHLPP1	A_23_P89762	0.1440
KCNMA1	A_23_P61150	0.1618
TIMELESS	A_23_P53276	0.2195
PER2	A_23_P411162	0.2494
PER2	A_23_P209320	0.3684
CRY1	A_24_P407235	0.4662
ATOH7	A_23_P378514	0.4755
ARNTL	A_24_P162037	0.5197
MAT2A	A_23_P401568	0.5893
NR1D1	A_23_P250227	0.7034
JUN	A_23_P420873	0.7405
HTR7	A_23_P500381	0.7585
MAT2A	A_32_P87703	0.9325
PROKR2	A_23_P412603	0.9702

PER1 : period homolog 1, ERBB3 : v-erb-b2 erythroblastic leukemia viral oncogene homolog 3, CLOCK : clock homolog, PROK2 : prokineticin 2, CRY2 : cryptochrome 2, CYP7B1 : cytochrome P450, family 7, subfamily B, polypeptide 1, ANNAT : arylalkylamine N-acetyltransferase, CRY1 : cryptochrome 1, PRF1 : perforin 1, HEBP1 : heme binding protein 1, PHPP1 : PH domain and leucine rich repeat protein phosphatase 1, KCNMA1 : potassium large conductance calcium-activated channel, subfamily M, alpha member 1, TIMELESS : timeless homolog, PER2 : Period homolog 2, ATOH7 : atonal homolog 7, ARNTL : aryl hydrocarbon receptor nuclear translocator-like, MAT2A : methionine adenosyltransferase II, alpha, NR1D1 : nuclear receptor subfamily 1, group D, member 1, JUN : jun oncogene, HTR7 : 5-hydroxytryptamine receptor 7, PROKR2 : prokineticin receptor 2.

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was a significant determinant of PER1 mRNA level in peripheral blood cells.

Chronic low-grade inflammation is closely associated with the metabolic syndrome. Immune cell infiltration and production of reactive oxygen species (ROS) are increased in obese adipose tissue and such changes can cause adipocyte dysfunction. The latter can cause disorders of circulating fatty acids, ROS, and adipocytokines, which are located upstream in the development of metabolic syndrome and atherosclerosis [5–8,15–17]. As shown in Table 2, several genes related to inflammation and ROS were associated with visceral fat adiposity, suggesting that inflammation of the adipose tissue may reflect on the expression of genes in peripheral blood cells. Interestingly, lymphocyte, monocyte, and neutrophil counts correlated positively with eVFA. The present data are in agreement with the reported increase in monocytes in obese subjects [18]. Such change in leukocyte subsets in visceral fat adiposity may be initiated by adipose local inflammation. Alternatively, it is also possible that the increase in the number of peripheral lymphocytes, monocytes, and neutrophils, which are somehow activated in bone marrow in visceral fat obesity, could results in the induction of local and/or systemic inflammation, with subsequent development of the metabolic syndrome. It is possible that some leukocyte subsets may affect the expression profile of certain genes, especially the mRNA level of PER1 in peripheral blood cells. PER1 mRNA level might be high in CD4-positive T cell rather than the other cells such as neutrophil, monocyte, CD8-positive T cell, and B cell, by analyzing microarray database (GSE22886)(data not shown), but further studies are needed to determine the exact leukocyte subtype(s) that influence peripheral blood PER1 mRNA level. In addition, target blood cell population of visceral fat should be identified in future.

Accumulating evidence indicates a close interrelationship between the circadian clock oscillator and metabolic syndrome [12–14]. Several genetic models of circadian disruption also exhibited metabolic disorders and vascular dysfunction [19]. One recent study highlighted the role of mouse Per genes in the development of obesity [20]. Furthermore, experimental evidence suggests that high-fat diet can alter the amplitude of peripheral circadian clock genes in mouse adipose tissue and liver [21]. In the present study, 18.5% of circadian genes in peripheral blood cells correlated significantly with eVFA (Figure 1A) and a significant correlation between PER1 mRNA level and eVFA was observed (Figure 1B). Other reports investigated circadian clock genes in human peripheral blood cells. In healthy male subjects, no distinct circadian changes were observed in the mRNA levels of PER2 and aryl hydrocarbon receptor nuclear translocator-like (ARNTL/BMAL1), whereas PER1 mRNA levels exhibited a clear oscillation during the 24-hour period with a peak expression level at 8 am [22]. We also obtained the preliminary data that the peripheral blood PER1 mRNA levels were oscillated with a peak expression level at 7:30 am (data not shown). These data support the present findings that peripheral blood PER1 mRNA level was reduced in visceral fat accumulation since the blood samples were collected exactly at 7:30 am in the present study. Circadian changes in Per1 mRNA were also reported in the mouse white adipose tissue [23] and disturbances of its expression were also reported in obese mice [24]. However, there is still a gap in our understanding of the circadian oscillation in mouse Per1 mRNA. Furthermore, the regulatory mechanism that control human PER1 expression in peripheral blood cells also remains uncertain. Haimovich et al [25] recently showed that a bolus administration of endotoxin resulted in down-regulation of PER1 mRNA in peripheral blood cells following a rise in plasma IL-6 and TNF-α levels but had no effect on melatonin secretory rhythm in human subjects [25]. Interestingly, our data (Table 4) showed that CRP was correlated with peripheral blood PER1 mRNA level. Considered collectively, it is possible that chronic low-grade inflammation could cause impairment of circadian oscillation of PER1 mRNA in peripheral blood cells with visceral fat accumulation. Alternatively, peripheral blood leukocytes with low PER1 mRNA level may have pro-inflammatory properties capable of initiating local inflammation in the adipose tissue. Further prospective studies are needed to examine whether dysregulation of circadian genes in peripheral blood cells can induce a vicious cycle, leading to the development of metabolic syndrome and cardiovascular events.

The present study has several limitations. Diabetes mellitus, dyslipidemia, and hypertension were common in the study population, since all subjects were inpatients. These metabolic diseases and medications could modulate the expression levels of various genes in peripheral blood cells directly or indirectly. The

Table 4. Correlation between PER1 and metabolic parameters.

	Univariate (non-adjusted)		Univariate (age,sex-adjusted)		Multivariate	
Parameter	**r**	**p value**	**R**	**p value**	**p value**	**F value**
Age	−0.28	0.031	–	–		
Sex	0.22	0.095	–	–		
BMI	−0.20	0.132	−0.27	0.047	–	–
Waist circumference (WC)	−0.26	0.044	−0.23	0.080		
Log-eVFA	−0.28	0.036	−0.29	0.023	0.005	8.969
Systolic blood pressure	−0.03	0.787	−0.03	0.786		
Diastolic blood pressure	0.05	0.705	−0.17	0.274		
Fasting glucose	−0.10	0.426	−0.12	0.354		
Hemoglobin A1c (JDS)	−0.17	0.206	−0.19	0.142		
HOMA-IR	−0.36	0.019	−0.42	0.013	0.090	3.074
AST	−0.13	0.329	−0.14	0.283		
ALT	−0.02	0.854	−0.10	0.439		
γ-GTP	−0.07	0.602	−0.08	0.551		
Total cholesterol	0.24	0.065	0.21	0.111		
LDL-C	0.19	0.149	0.17	0.202		
Triglyceride	0.10	0.456	−0.01	0.938		
HDL-C	0.11	0.389	0.20	0.128		
Creatinine	0.17	0.191	0.29	0.073		
Log adiponectin	−0.03	0.809	0.12	0.393		
WBC	−0.11	0.395	−0.37	0.011	0.087	3.128
CRP	−0.36	0.006	−0.37	0.003	0.096	2.968
Complication of DM		0.056	0.22	0.099		
Complication of HT		0.169	0.13	0.331		
Complication of DLP		0.788	0.02	0.885		
Mean IMT	0.02	0.886	0.10	0.551		

Data are mean ± SD. BMI; body mass index, eVFA; estimated visceral fat area, LDL-C; low density lipoprotein-cholesterol, HDL-C; high density lipoprotein-cholesterol, HOMA-IR; homeostasis model assessment of insulin resistance, DM; diabetes mellitus, HT; hypertension, DLP; dyslipidemia, IMT; imtima-media thickness.
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correlation between PER1 expression level and medication was also examined (data not shown), but there were no significant correlations in present study. Further studies will be needed in future to understand what kind of medications influence on peripheral blood cell mRNA expressions. In addition, the study participants were obese Japanese subjects (BMI $\geq$25 kg/m^2) and visceral fat area was measured by BIA, not CT or MRI. Future studies are needed to analyze the gene expression profile in peripheral blood cells from not only obese subjects but also non-obese healthy (low VFA) subjects, although we obtained the preliminary data that peripheral blood PER1 mRNA levels were significantly higher in non-obese healthy volunteers than in the current study population (data not shown). The effects of diet- and exercise-induced visceral fat reduction on gene expression profile in peripheral blood cells should be investigated in future.

In perspective, gene expression profiling in peripheral blood cells may be applied to detect the function and condition of visceral fat tissues in human, although further studies are needed in future. These analyses may provide the new knowledge of metabolic syndrome and will achieve the novel diagnostic and therapeutic approaches for metabolic syndrome.

Supporting Information

Figure S1 Correlation between estimated visceral fat area (eVFA) and various blood parameters. The homeostasis model−assessment of insulin resistance (HOMA-IR) was calculated as follows: HOMA-IR = fasting insulin (μU/mL)×fasting glucose (mg/dL)/405.
(TIFF)

Figure S2 Correlations between estimated visceral fat area (eVFA) and peripheral blood cell count.
(TIFF)

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Author Contributions

Conceived and designed the experiments: NM YM KM TF IS. Performed the experiments: MY NM SN SK YN AHS KO AI. Analyzed the data: MY NM SN. Contributed reagents/materials/analysis tools: SN. Wrote the paper: NM MY SN.

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INTERNAL MEDICINE

□ CASE REPORT □

Successful Treatment of Reactive Hypoglycemia Secondary to Late Dumping Syndrome Using Miglitol

Yukari Fujita[1], Daisuke Tamada[1], Junji Kozawa[1,2], Yoko Kobayashi[1], Shugo Sasaki[1], Tetsuhiro Kitamura[1], Tetsuyuki Yasuda[1], Norikazu Maeda[1], Michio Otsuki[1], Kohei Okita[1], Hiromi Iwahashi[1], Hideaki Kaneto[1], Tohru Funahashi[1,3], Akihisa Imagawa[1] and Iichiro Shimomura[1]

Abstract

We herein describe a 59-year-old woman who had undergone a total gastrectomy for gastric carcinoma and suffered from postprandial hypoglycemia characterized by a loss of consciousness and spasms. She was diagnosed with reactive hypoglycemia and treated with nutrition therapy, but the frequency and severity of the hypoglycemic episodes did not decrease. She was subsequently treated successfully with miglitol, an alpha-glucosidase inhibitor (α-GI) taken twice a day; other α-GIs (acarbose and voglibose) were not effective. In conclusion, the administration of miglitol was effective for preventing reactive hypoglycemia secondary to late dumping syndrome.

Key words: reactive hypoglycemia, dumping syndrome, alpha-glucosidase inhibitor

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Introduction

Reactive hypoglycemia is defined as a clinical disorder in which hypoglycemic symptoms occur postprandially. Serious and life-threatening hypoglycemia can occur without appropriate treatment (1, 2). Reactive hypoglycemia can be caused by fructose intolerance, galactosemia, drugs, and late dumping syndrome (3, 4). Late dumping syndrome is seen in 10-40% of patients after gastric surgery (5) and in more than 50% of patients after esophagectomy (6). In Japan, gastric cancer is one of the leading causes of cancer deaths (7), and gastrectomy is the mainstay of curative treatment (8).

Patients with reactive hypoglycemia secondary to late dumping syndrome are treated via dietary modifications, wherein meals are eaten five or six times a day, and the carbohydrate intake is reduced. However, this nutrition therapy is not always successful at preventing the development of hypoglycemia.

Alpha-glucosidase inhibitors (α-GIs), which are oral antidiabetic agents, work primarily in the small intestine. Because they reduce carbohydrate metabolism and carbohydrate absorption, they modulate the postprandial increase in the plasma glucose and insulin levels. Acarbose, an α-GI, has been reported to be effective in idiopathic reactive hypoglycemia (9, 10) and in late dumping syndrome (11, 12).

We herein report the case of a woman who suffered from severe reactive hypoglycemia secondary to late dumping syndrome and was successfully treated with miglitol twice a day, but for whom acarbose and voglibose were ineffective.

Case Report

A 59-year-old woman was admitted to Osaka University Hospital in September 2011 for the assessment and treatment of postprandial hypoglycemia. She had undergone total gastrectomy for gastric carcinoma in 2003. At that time, she was instructed to eat 6 divided small meals in a day, but she

[1]Department of Metabolic Medicine, Graduate School of Medicine, Osaka University, Japan, [2]Department of Community Medicine, Graduate School of Medicine, Osaka University, Japan and [3]Department of Metabolism and Atherosclerosis, Graduate School of Medicine, Osaka University, Japan

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Correspondence to Dr. Junji Kozawa, kjunji@endmet.med.osaka-u.ac.jp

Table. Laboratory Data on Admission

	Patient		Reference	
WBC	3820	/μL	3300 - 9400	/μL
RBC	374 × 10^4	/μL	390 - 510 × 10^4	/μL
Hb	12.3	g/dL	12.0 - 15.0	g/dL
Plt	27.9 × 10^4	/μL	13 - 32 × 10^4	/μL
Na	141	mEq/L	139 - 146	mEq/L
K	4.2	mEq/L	3.6 - 4.8	mEq/L
Cl	105	mEq/L	100 - 108	mEq/L
AST	18	U/L	< 40	U/L
ALT	17	U/L	< 40	U/L
γGTP	28	U/L	12 - 69	U/L
LDH	168	U/L	103 - 229	U/L
ALP	258	U/L	134 - 359	U/L
UN	16	mg/dL	7 - 22	mg/dL
Cr	0.79	mg/dL	0.5 - 0.9	mg/dL
TP	6.3	g/dL	6.4 - 8.1	g/dL
Alb	3.6	g/dL	3.6 - 4.7	g/dL
T-Chol	195	mg/dL	150 - 220	mg/dL
TG	80	mg/dL	30 - 80	mg/dL
FPG	89	mg/dL	70 - 110	mg/dL
F-IRI	1.9	μU/mL	0 - 12	μU/mL
F-CPR	1.2	ng/mL	1.0 - 2.0	ng/mL
HbA1c (NGSP)	6.0	%	4.7 - 6.2	%
GAD Ab	2.8	U/mL	< 1.5	IU/L
Insulin Ab	< 0.4	%	< 0.4	%
TSH	5.74	μU/mL	0.40 – 3.80	μU/mL
FT4	0.8	ng/dL	0.9 – 1.6	ng/dL
FT3	2.4	pg/mL	2.0 – 3.4	pg/mL
TgAb	53.7	IU/mL	< 40	IU/mL
Cortisol	13.3	μg/dL	4.5 - 24.5	μg/dL
ACTH	23	pg/ml	< 60	pg/ml
GH	0.23	ng/mL	< 2.7	ng/mL
IGF-1	83.7	ng/ml	71 - 203	ng/ml
CEA	4	ng/mL	< 5	ng/mL
CA 19-9	12	U/mL	< 37	U/mL

WBC: white blood cells, RBC: red blood cells, Hb: hemoglobin, Plt: platelets, Na: sodium, K: potassium, Cl chlorine, AST: aspartate aminotransferase, ALT: alanine aminotransferase, γGTP: glutamyl transpeptidase, LDH, lactate dehydrogenase, ALP: alkaline phosphatase, UN, urea nitrogen, Cr: creatinine, TP: total protein, Alb: albumin, T Chol: total cholesterol, TG, triglyceride, FPG, fasting plasma glucose, F-IRI: fasting immunoreactive insulin, F-CPR: fasting C-peptide immunoreactivity, HbA1c, hemoglobin A1c, GAD Ab: glutamic acid decarboxylase antibody, Insulin Ab: insulin antibody, TSH: thyroid stimulating hormone, FT3: free triiodothyronine, FT4: free thyroxine, TgAb: thyroglobulin antibody, ACTH: adrenocorticotropic hormone, GH: growth hormone, IGF-1: insulin-like growth factor-1, CEA: carcinoembryonic antigen

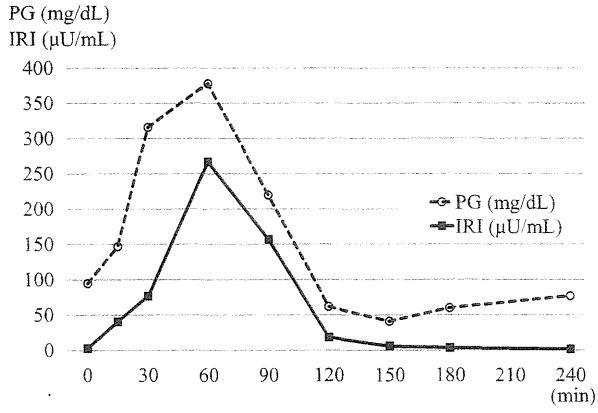

Figure 1. The results of the oral glucose tolerance test (75 g). The plasma glucose (○) and serum insulin (■) levels were measured up to 240 min after the glucose load.

frequently suffered from hypoglycemic episodes characterized by a loss of consciousness and spasms that occurred a few hours after meals. On two occasions, she was taken to an emergency room for hypoglycemic episodes; her plasma glucose level was 60 mg/dL on the first visit, in 2005, and 20 mg/dL on the second, in 2009. She had had no episodes of fasting hypoglycemia.

On admission in 2011, her height and weight were 152.4 cm and 45.9 kg (body mass index 19.9). Laboratory tests revealed mild glucose intolerance. The hemoglobin A1c (HbA1c) was estimated using the National Glycohemoglobin Standardization Program (NGSP) equivalent value (%) and calculated by the formula HbA1c (%) = HbA1c (Japan Diabetes Society [JDS], %) + 0.4%. Her HbA1c was 6.0%, and her fasting plasma glucose (FPG) level was 89 mg/dL. Her fasting immunoreactive insulin (F-IRI) and C-peptide immunoreactivity (F-CPR) were 1.9 μU/mL and 1.2 ng/mL, respectively. She was positive for anti-glutamic acid decarboxylase (GAD) antibodies. Although the anti-thyroglobulin antibody (TgAb) titer was also positive, her thyroid function was almost normal. Her adrenal function was also normal (Table).

An abdominal computed tomography (CT) scan detected no abnormalities in the pancreas. In a 75 g oral glucose tolerance test (OGTT), the baseline glucose and IRI levels were 95 mg/dL and 2.9 μU/mL, respectively; they rapidly increased to 378 mg/dL and 267.2 μU/mL after 60 minutes and decreased to 41 mg/dL and 5.9 μU/mL after 150 minutes (Fig. 1). Continuous glucose monitoring (CGMS-Gold™, Medtronic Minimed, Northridge, CA) under her regular diet revealed the mean blood glucose level to be 108 ± 52 mg/dL, and the blood glucose levels rapidly increased postprandially and then decreased to hypoglycemic levels after breakfast (Fig. 2A).

On the basis of these results, her hypoglycemia was diagnosed as reactive hypoglycemia secondary to late dumping syndrome. Every meal was divided into two, and the second meal was eaten 2 hours after the first. The total energy of the meals was 1,520 kcal/day. With the smaller, more frequent meals, the daily fluctuations in the blood glucose levels were reduced, and the mean blood glucose level was 108 ± 35 mg/dL. However, the reactive hypoglycemia following breakfast could not be prevented by dietary modifications alone (Fig. 2B). She was therefore given an additional α-GI orally, in combination with the dietary modifications. Because the postprandial hyperglycemia and hypoglycemia were most pronounced after breakfast in the hospital, an α-GI was administered once in the morning before the first

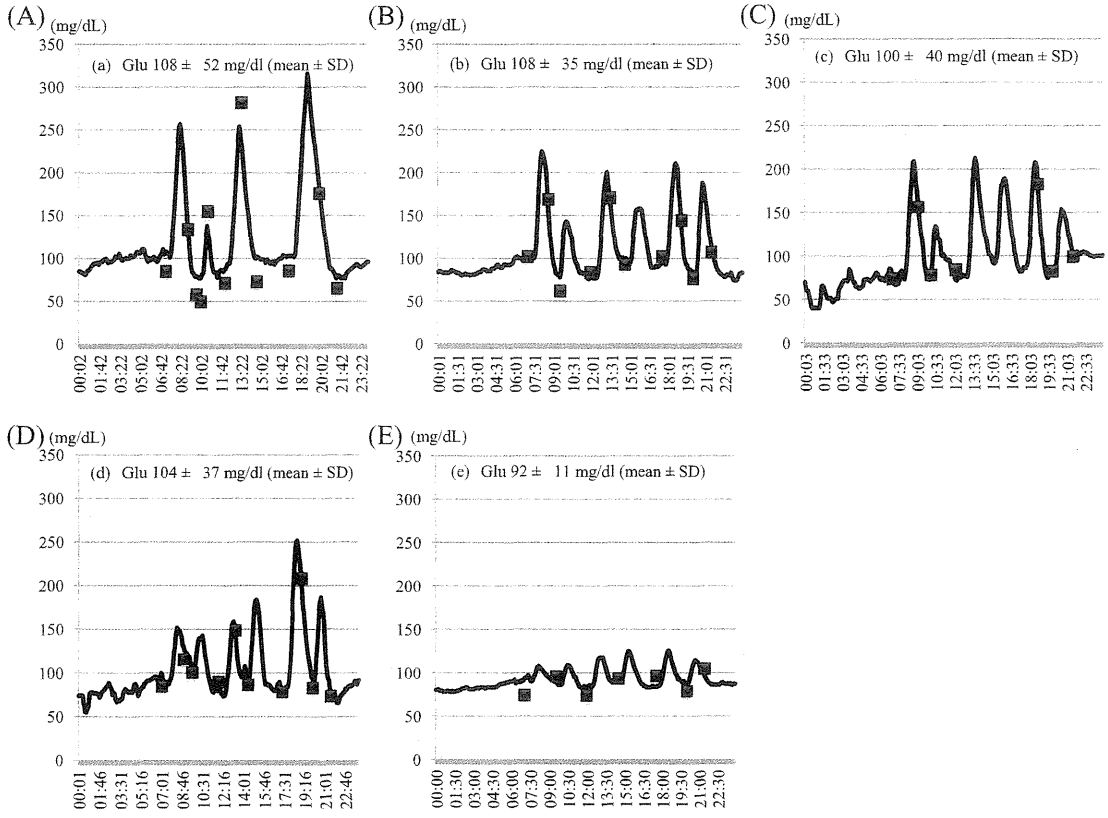

Figure 2. The daily profiles of the blood glucose levels according to CGMS under various conditions: (A) no medication, (B) divided meals, (C) after the addition of 0.3 mg of voglibose, (D) after the addition of 100 mg of acarbose and (E) after the addition of 50 mg of miglitol. Square symbols represent the self-monitored blood glucose levels.

meal. With the smaller, divided meals in combination with 0.3 mg of voglibose or 100 mg of acarbose, the mean blood glucose levels were 100 ± 40 mg/dL and 104 ± 37 mg/dL, respectively. Hence, these α-GIs were not effective for reducing the fluctuations of the blood glucose levels (Fig. 2C, D). However, after administration of 50 mg of miglitol, the mean blood glucose level was 92 ± 11 mg/dL, and the fluctuations throughout the day were markedly reduced (Fig. 2E).

The profiles of the blood glucose and IRI levels after breakfast (Fig. 3A, B) and after lunch (Fig. 3C, D) with the dietary modifications showed postprandial hyperglycemia and hyperinsulinemia, especially after breakfast. The administration of 0.3 mg of voglibose slightly reduced postprandial hyperinsulinemia following breakfast, but had no effect on the postprandial hyperglycemia after either breakfast or lunch. However, the administration of 50 mg of miglitol ameliorated both the postprandial hyperglycemia and the hyperinsulinemia, and the blood glucose level was elevated 120 minutes after breakfast, when reactive hypoglycemia often occurred.

Under a regimen of 50 mg of miglitol once a day, the peak plasma concentration (C_{max}) of miglitol was 2.2 μg/mL, the peak time (t_{max}) was 2.0 hours, and the half-life ($t_{1/2}$) was 2.8 hours. The amount of miglitol excreted in the urine was 28.5 mg/day, corresponding to 57% of the daily dose.

Under a regimen of 50 mg of miglitol once in the morning, the patient had infrequent hypoglycemic episodes after dinner in the hospital. Therefore, she was given an additional 50 mg of miglitol in the evening. After discharge, she was often constipated and was admitted to another hospital for a bowel obstruction, which was managed conservatively. Because her hypoglycemia is life-threatening, she has been carefully treated with the same dose of miglitol in conjunction with a laxative, and during the more than 6 months of follow-up, has not experienced any hypoglycemic episodes.

Discussion

We determined that miglitol, administered at 50 mg twice a day, was effective for preventing reactive hypoglycemia secondary to late dumping syndrome, and the efficacy of this agent was superior to that of two other α-GIs, voglibose and acarbose.

The differences in efficacy between these α-GIs might be attributable to their respective pharmacodynamics and pharmacokinetic properties. First, miglitol inhibits α-glucosidase in the upper section of the small intestine, which is the main site of intestinal absorption of glucose; almost all the miglitol is absorbed at this site (13, 14). In contrast, voglibose

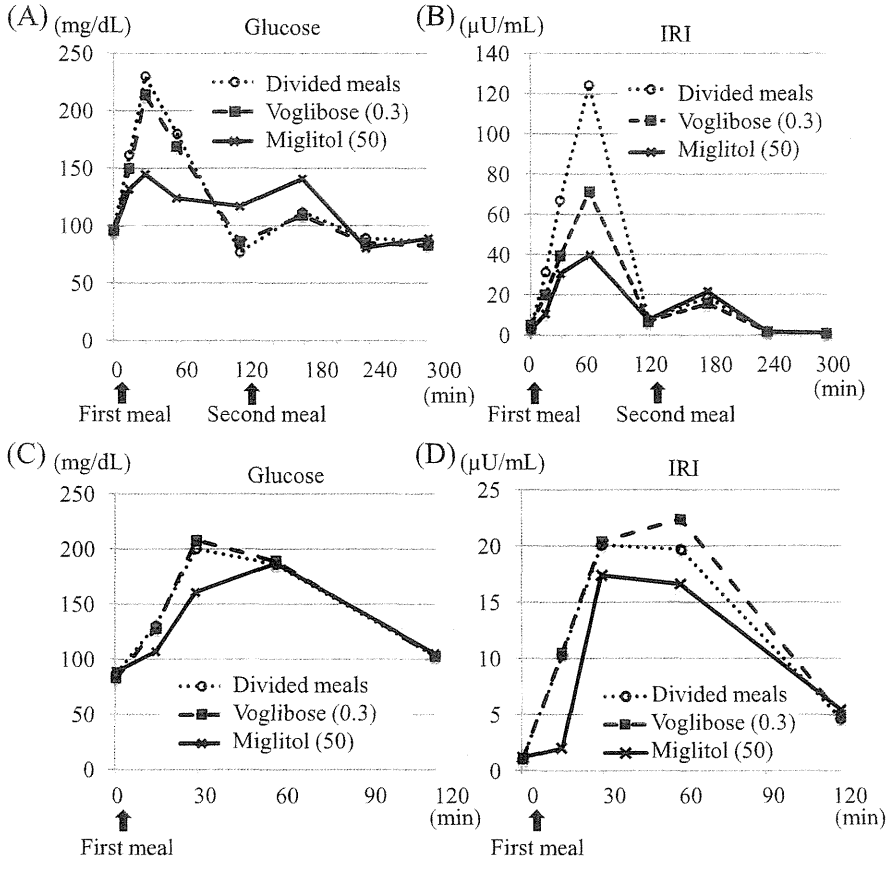

Figure 3. The profiles of the blood glucose and IRI levels (A, B) after breakfast and (C, D) after lunch with divided meals (○), after the addition of 0.3 mg of voglibose (■) or after the addition of 50 mg of miglitol (X).

and acarbose are not absorbed, and thus inhibit α-glucosidase throughout the small intestine. Second, miglitol has different specificities and affinities for the various metabolic enzymes compared with voglibose and acarbose (15). While acarbose is a complex oligosaccharide, miglitol is structurally similar to glucose; this similarity is suggested to be related to its broader specificity of inhibition against α-glucosidases (13). The inhibition of α-glucosidase primarily in the upper section of the small intestine and the broader specificity may lead to a significant advantage for miglitol in reducing the rapid postprandial increase of blood glucose and IRI levels compared with other α-GIs in patients with reactive hypoglycemia secondary to dumping syndrome, as has been reported in patients with type 2 diabetes (16-18).

The saturated absorption of miglitol in the upper section of the small intestine may be one mechanism through which the twice-daily administration of 50 mg of miglitol could prevent postprandial hyperglycemia and the reactive hypoglycemia throughout the day. At this site, the absorption of miglitol is already saturated, as has been reported for Caucasians, within the therapeutic dose range (with doses ≥50 mg or ≥0.7 mg/kg), and miglitol is not readily absorbed in the ileum or colon (14). For our patient, the administration 50 mg of miglitol corresponded to 1.1 mg/kg of miglitol. The unabsorbed miglitol may inhibit α-glucosidase for a longer period of time in the lower sections of the small intestine or colon. Indeed, when she was administered 50 mg of miglitol once a day, the daily amount of excretion of miglitol via urine corresponded to approximately 60% of the daily dose, thus suggesting that 40% could not be absorbed.

Another possible mechanism may be related to a change in incretin secretion. Miglitol enhances postprandial secretion of glucagon-like peptide-1 (GLP-1) (19-24), a gut peptide secreted by intestinal L cells in response to nutrient ingestion. In addition, the administration of miglitol once in the morning induced a prolonged increase of GLP-1 secretion not only after breakfast, but also after lunch, in non-diabetic men (19) and in patients treated with sitagliptin for type 2 diabetes (20). GLP-1 has a physiological effect of inhibiting gastrointestinal motility, including the slowing of gastric emptying (25, 26). An increased release of GLP-1 following the administration of miglitol may cause a decrease in gastrointestinal motility. In our patient who had undergone a total gastrectomy, the decreased gastrointestinal motility other than gastric emptying might have led to a reduction in the rapid postprandial increase in the glucose and insulin levels, which are the main causes of reactive hypoglycemia secondary to late dumping syndrome. In this case, the C_{max} of miglitol was much higher, and the $t_{1/2}$ was slightly longer than that in healthy volunteers who were ad-

ministered 1.4 mg/kg of miglitol (C_{max}; 1.13 μg/mL, $t_{1/2}$; 2.35 hours), while the duration of time at the t_{max} was not longer (2.0 hours in this case v.s. 2.3 hours in healthy volunteers) (14). It has been speculated that gastric surgery may induce augmented GLP-1 secretion and retention of miglitol in the upper section of the small intestine, while the absence of the stomach may allow miglitol to more rapidly reach the upper section of small intestine, thus leading to almost the same t_{max}. Therefore, the increased and prolonged release of GLP-1 resulting from twice-daily administration of miglitol may have contributed to the prevention of reactive hypoglycemia throughout the day. However, the secretion of miglitol-induced incretin, in addition to abdominal surgery, may reduce gastrointestinal motility and thus lead to bowel obstruction, as occurred in this case. The blood incretin concentrations and the rate of gastrointestinal motility were not examined in this patient. Therefore, further studies are needed to investigate this hypothesis.

In conclusion, the administration of miglitol, but not other α-GIs, twice a day was effective for preventing reactive hypoglycemia secondary to late dumping syndrome in our patient.

The authors state that they have no Conflict of Interest (COI).

Acknowledgement

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Yukari Fujita and Daisuke Tamada contributed equally to this work.

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Efficacy of liraglutide, a glucagon-like peptide-1 (GLP-1) analogue, on body weight, eating behavior, and glycemic control, in Japanese obese type 2 diabetes

Yuya Fujishima, Norikazu Maeda*, Kana Inoue, Susumu Kashine, Hitoshi Nishizawa, Ayumu Hirata, Junji Kozawa, Tetsuyuki Yasuda, Kohei Okita, Akihisa Imagawa, Tohru Funahashi and Iichiro Shimomura

Abstract

Background: We recently reported that short-term treatment with liraglutide (20.0 ± 6.4 days) reduced body weight and improved some scales of eating behavior in Japanese type 2 diabetes inpatients. However, it remained uncertain whether such liraglutide-induced improvement is maintained after discharge from the hospital. The aim of the present study was to determine the long-term effects of liraglutide on body weight, glycemic control, and eating behavior in Japanese obese type 2 diabetics.

Methods: Patients with obesity (body mass index (BMI) >25 kg/m^2) and type 2 diabetes were hospitalized at Osaka University Hospital between November 2010 and December 2011. BMI and glycated hemoglobin (HbA1c) were examined on admission, at discharge and at 1, 3, and 6 months after discharge. For the liraglutide group (BMI; 31.3 ± 5.3 kg/m^2, n = 29), patients were introduced to liraglutide after correction of hyperglycemic by insulin or oral glucose-lowering drugs and maintained on liraglutide after discharge. Eating behavior was assessed in patients treated with liraglutide using The Guideline For Obesity questionnaire issued by the Japan Society for the Study of Obesity, at admission, discharge, 3 and 6 months after discharge. For the insulin group (BMI; 29.1 ± 3.0 kg/m^2, n = 28), each patient was treated with insulin during hospitalization and glycemic control maintained by insulin after discharge.

Results: Liraglutide induced significant and persistent weight loss from admission up to 6 months after discharge, while no change in body weight after discharge was noted in the insulin group. Liraglutide produced significant improvements in all major scores of eating behavior questionnaire items and such effect was maintained at 6 months after discharge. Weight loss correlated significantly with the decrease in scores for recognition of weight and constitution, sense of hunger, and eating style.

Conclusion: Liraglutide produced meaningful long-term weight loss and significantly improved eating behavior in obese Japanese patients with type 2 diabetes.

Keywords: Liraglutide, Glucagon-like peptide-1 (GLP-1), Obesity, Eating behavior, Diabetes, Incretin

* Correspondence: norikazu_maeda@endmet.med.osaka-u.ac.jp
Department of Metabolic Medicine, Graduate School of Medicine, Osaka University, 2-2-B5, Yamada-oka, Suita, Osaka 565-0871, Japan

Introduction

The worldwide epidemic of type 2 diabetes has been fueled by the increase in the number of obese and overweight people. Similar to Western countries, in East Asian countries, the risk of death from cancer and cardiovascular diseases has increased in parallel with the increase in body mass index (BMI) [1]. The World Health Organization (WHO) estimates that 2.3 billion adults will be overweight and >700 million will be obese by 2015 [2]. Effective therapeutic strategy for obese type 2 diabetes should be developed without delay, but it is often difficult to control appetite and to maintain body weight in obese type 2 diabetes patients. Intensive insulin therapy may result in fine glycemic control and prevent microvascular complications, but such treatment usually increases body weight [3]. In addition, the oral glucose-lowering agents sulfonylurea (SU) and thiazolidinedione also increase body weight by enhancing glucose uptake into adipocytes.

Liraglutide, a glucagon-like peptide (GLP-1) analogue, is a member of new classes of anti-diabetic agents and is characterized by induction of insulin secretion only during hyperglycemia as an incretin effect. The Liraglutide Effect and Action in Diabetes (LEAD) studies have demonstrated a significant weight reduction by liraglutide [4], but such liraglutide-mediated weight reduction has not been observed in Japanese type 2 diabetes patients [5-7]. Subjects of the LEAD trial were obese type 2 diabetic patients, while the Japanese subjects enrolled in the liraglutide trial were non-obese type 2 diabetes patients (BMI; 23.5-25 kg/m^2). In a pilot study, we recently reported that short-term liraglutide treatment reduced BMI, waist circumference, and visceral fat area, and reduced the scale for eating behavior in Japanese type 2 diabetes inpatients (age; 61.2 ± 14.0 years, BMI; 28.3 ± 5.2 kg/m^2, duration of diabetes; 16.9 ± 6.6 years) [8]. However, this short-term study was performed only during hospitalization and thus it remains uncertain whether these effects of liraglutide are maintained after discharge. In the present study, we investigated the effect of liraglutide on body weight, glycemic control, and eating behavior until 6 months after discharge in Japanese obese patients (BMI >25 kg/m^2) with type 2 diabetes.

Materials and methods

Subjects and clinical examination

The study subjects were selected among patients hospitalized at the Division of Endocrinology and Metabolism of Osaka University Hospital between November 2010 and December 2011. All patients were hospitalized to undergo medical treatment for diabetes. The inclusion criteria were as follows: (1) type 2 diabetes with obesity (BMI >25 kg/m^2) at admission; (2) patients continued to visit Osaka University Hospital for treatment after discharge; (3) patients were treated with liraglutide or insulin at discharge and continued to be treated with the same regimen after discharge. Physical examination and various metabolic parameters were measured on admission. Body weight and HbA1c were examined at 1, 3, and 6 months after discharge. The selection of the glucose-lowering agent for treatment was left to the attending physician. In the liraglutide group, each patient was treated with insulin or oral glucose-lowering drugs under diet therapy after admission. After achieving the target levels of glycemic control [fasting plasma glucose (FPG) <150 mg/dL and postprandial 2-h plasma glucose <200 mg/dL], treatment with insulin or oral glucose-lowering agents was replaced with liraglutide at 0.3 mg/day, which was increased by 0.3 mg/day every one week to a final dose of 0.9 mg/day, representing the maximum dose used in Japan. The introduction of liraglutide and increase in liraglutide dosage was decided by the attending physician. In the insulin group, each patient was treated with insulin during hospitalization and glycemic control was maintained by insulin treatment after discharge.

Written consent was obtained from each subject after explaining the purpose and possible complications of the study. The study protocol was approved by the human ethics committee of Osaka University and was registered with the University hospital Medical Information Network (Number: UMIN 000004192).

Questionnaire for eating behavior

Eating behavior was assessed in patients on liraglutide treatment by using the questionnaire of The Guideline For Obesity issued by the Japan Society for the Study of Obesity, at admission, discharge, 3 and 6 months after discharge. As reported previously [8], this questionnaire consists of 55-item questions of seven major scales as follows: 1) Recognition for weight and constitution (e.g., 'Do you think it is easier for you to gain weight than others?'), 2) External eating behavior (e.g., 'If food smells and looks good, do you eat more than usual?'), 3) Emotional eating behavior (e.g., 'Do you have the desire to eat when you are irritated?'), 4) Sense of hunger (e.g. 'Do you get irritated when you feel hungry?'), 5) Eating style (e.g., 'Do you eat fast?'), 6) Food preference (e.g., 'Do you like meat?'), 7) Regularity of eating habits (e.g., 'Is your dinner time too late at night?'). All items were rated on a four-point scale ranging from 1 (seldom) to 4 (very often).

Statistical analysis

The Student's t-test and χ^2 test were used to compare baseline characteristics of the liraglutide and insulin groups. Variables with skewed distribution were analyzed by the Mann–Whitney *U*-test. The Student's t-test was

used for comparison of results obtained at admission, discharge, and post-discharge. In all cases, P values <0.05 were considered statistically significant. All analyses were performed using the JMP software (JMP 8.0; SAS Institute Inc., Cary, NC).

Results

Characteristics of participants

Table 1 shows the baseline characteristics of 29 subjects on liraglutide and 28 subjects on insulin treatment. For the liraglutide group, the mean BMI was 31.3 kg/m^2, glycated hemoglobin (HbA1c) was 8.5%, and fasting C-peptide was 2.09 ng/mL. Among the patients of the liraglutide group, 55% were treated with insulin at admission. The baseline characteristics of patients of the insulin group were similar to those of the liraglutide group, but HbA1c levels and frequencies of insulin treatment before admission were higher in the insulin group compared to the liraglutide group. The duration of diabetes and fasting C-peptide level were similar in both groups. Thus, the enrolled patients were obese Japanese subjects, had relatively long duration of diabetes, and most of them were treated with insulin, but had preserved insulin secretion capacity.

The number of patients treated at discharge with liraglutide at 0.3, 0.6, and 0.9 mg/day was 3, 11, and 15 patients, respectively. The total daily dose (TDD) of insulin was 0.59 unit/kg/day at discharge in the insulin group. In the liraglutide group, the duration of hospitalization was 30.8 ± 9.3 days and the time from liraglutide induction to discharge was 20.0 ± 6.9 days. In the insulin group, the duration of hospitalization was 29.1 ± 8.4 days, indicating similar duration of hospitalization in the two groups. At 6 month after discharge, the number of patients treated with liraglutide at 0.3, 0.6, and 0.9 mg/day was 3, 7, and 19 patients, and SU and biguanide was used in 10 and 15 patients of the liraglutide group, respectively. In the insulin group, SU, biguanide, and alpha-glucosidase inhibitor was used by 1, 9, and 3 patients at 6 month after discharge, respectively. There were no differences in the frequency of hypoglycemic episode between the liraglutide and insulin groups.

Changes in body weight and HbA1c

Body weight and HbA1c were measured during hospitalization and after discharge. In the liraglutide group, BMI at admission, discharge, 1, 3, and 6 months after discharge were 31.3 ± 5.3, 29.3 ± 4.8, 28.8 ± 4.6,

Table 1 Baseline characteristics

	Liraglutide (n = 29)	Insulin (n = 28)	P value
Males/females	12/17	14/14	0.514
Age (years)	58.1 ± 12.1	63.9 ± 12.2	0.080
Duration of diabetes (years)	16.0 ± 8.3	18.5 ± 10.0	0.309
Body weight (kg)	82.0 ± 16.2	76.6 ± 15.5	0.122
Body mass index (kg/m^2)	31.3 ± 5.3	29.1 ± 3.0	0.102
HbA1c (%)	8.5 ± 1.4	9.4 ± 1.6	0.023
Fasting plasma glucose (mg/dL)	159.0 ± 49.7	156.4 ± 59.2	0.655
Fasting C-peptide (mg/dL)	2.09 ± 0.97	1.97 ± 0.24	0.267
Low-density lipoprotein-cholesterol (mg/dL)	113.1 ± 36.5	116.6 ± 28.2	0.689
High-density lipoprotein-cholesterol (mg/dL)	44.2 ± 8.1	46.1 ± 13.8	0.534
Triglycerides (mg/dL)	149.4 ± 55.2	201.2 ± 145.0	0.432
Hypertension (%)	83	86	0.760
Dyslipidemia (%)	90	86	0.650
Previous treatment			
Biguanide (%)	38	18	0.092
Sulfonylurea (%)	31	21	0.410
Alpha-glucosidase inhibitor (%)	17	29	0.308
Thiazolidinedione (%)	21	14	0.525
DPP-IV inhibitor (%)	10	7	0.669
Glinide (%)	3	0	0.322
Insulin (%)	55	82	0.029

Data are mean ± SD or number of subjects.

28.5 ± 4.4, and 28.2 ± 4.3 kg/m^2, respectively. Liraglutide treatment significantly decreased BMI even after 6 months from discharge compared to admission ($P < 0.001$). Figure 1A shows changes in body weight from admission in both groups. In the insulin group, body weight was significantly reduced from admission to discharge while there were no changes in body weight from discharge to 6 months (Figure 1A). Changes in body weight at 1, 3, and 6 months after discharge were significantly larger in the liraglutide group than in the insulin group after adjustment for age, sex, BMI, HbA1c, and insulin treatment at admission ($P < 0.001$). Among the subjects treated with liraglutide, the change in body weight at 6 months after discharge correlated significantly with BMI at admission ($P = 0.002$).

HbA1c was significantly reduced at 1 month from discharge but tended to increase at 6 months after discharge in both groups (Figure 1B). Liraglutide treatment significantly reduced the mean HbA1c by −1.1% at 6 months after discharge compared to admission. Insulin administration also significantly decreased HbA1c, and the reduction in HbA1c was similar in both liraglutide and insulin group. After adjustment for age, sex, BMI, HbA1c, and insulin treatment at admission, the decrease in HbA1c was significant only at 1 month after discharge in the liraglutide group compared with the insulin group ($P < 0.05$).

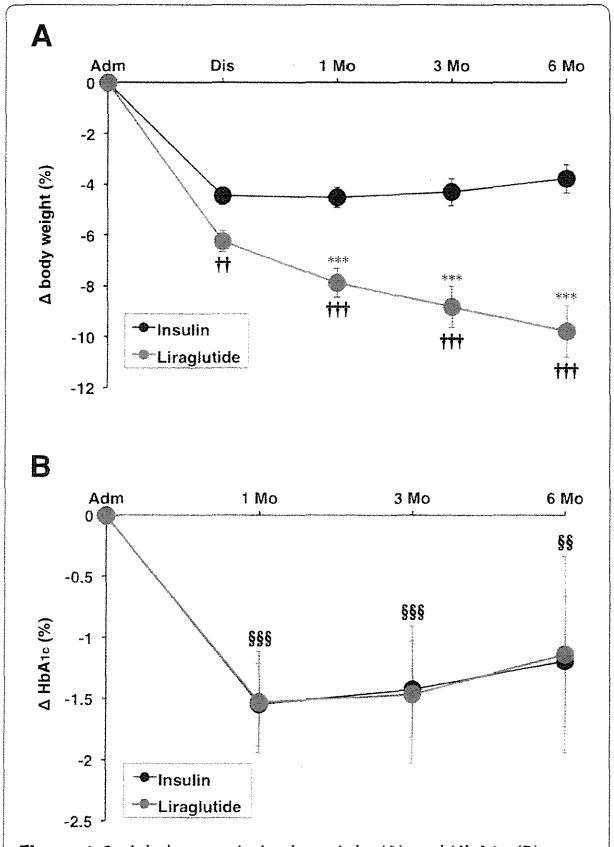

Figure 1 Serial changes in body weight (A) and HbA1c (B) induced by insulin and liraglutide. Data are mean ± SEM. Adm, admission; Dis, discharge; Mo, month. ***$P < 0.001$, compared with the values at discharge. †† $P < 0.01$, ††† $P < 0.001$, compared with the values of the insulin group. §§ $P < 0.01$, §§§ $P < 0.001$, compared with the values at admission.

Assessment of eating behavior

Next, the effect of liraglutide on eating behavior was evaluated in 16 subjects (8 males and 8 females) until 6 months after discharge. Figure 2 depicts the scores of the questionnaire for eating behavior at admission, discharge, 3 months, and 6 months after discharge. The scores for recognition of weight and constitution (Figure 2A) and external eating behavior (Figure 2B) were not changed at discharge, but were significantly decreased at 3 and 6 months after discharge. Emotional eating behavior was reduced at 3 month after discharge but tended to deteriorate at 6 month after discharge (Figure 2C). Importantly, liraglutide treatment promptly reduced the score for sense of hunger and markedly suppressed the sense of hunger (Figure 2D). The score for eating style decreased gradually and was significantly reduced at 3 and 6 months after discharge (Figure 2E). Interestingly, the score for food preference (Figure 2F), especially requirement for fat (Figure 2G), was significantly decreased by liraglutide at 3 and 6 months after discharge. Liraglutide treatment reasonably reduced the score for regularity of eating habit at discharge and maintained the same reduction until 6 months after discharge (Figure 2H).

Next, we examined the correlations between changes in body weight and eating behavior from admission to 6 months after discharge. The decrease in total score of eating behavior correlated significantly with the extent of weight loss ($P = 0.029$, R = 0.48). Interestingly, weight loss correlated significantly with the decrease in the scores for recognition of weight and constitution ($P = 0.007$, R = 0.57), sense of hunger ($P = 0.021$, R = 0.50), and eating style ($P = 0.037$, R = 0.46). The decrease in the score for external eating behavior tended to be associated with weight reduction ($P = 0.061$, R = 0.42). The scales for other parameters of eating behavior did not correlate significantly with weight reduction.

Figure 3 shows a radar chart of the eating behavior. The black dotted line represents the scores of non-obese healthy subjects. All scales for eating behavior were disordered at admission (red line), but these parameters tended to show improvement at discharge (yellow line). Furthermore, all scales showed further improvement and the scores of eating behavior moved towards those of the non-obese healthy subjects at 3 (green line) and 6 (blue line) months after discharge.

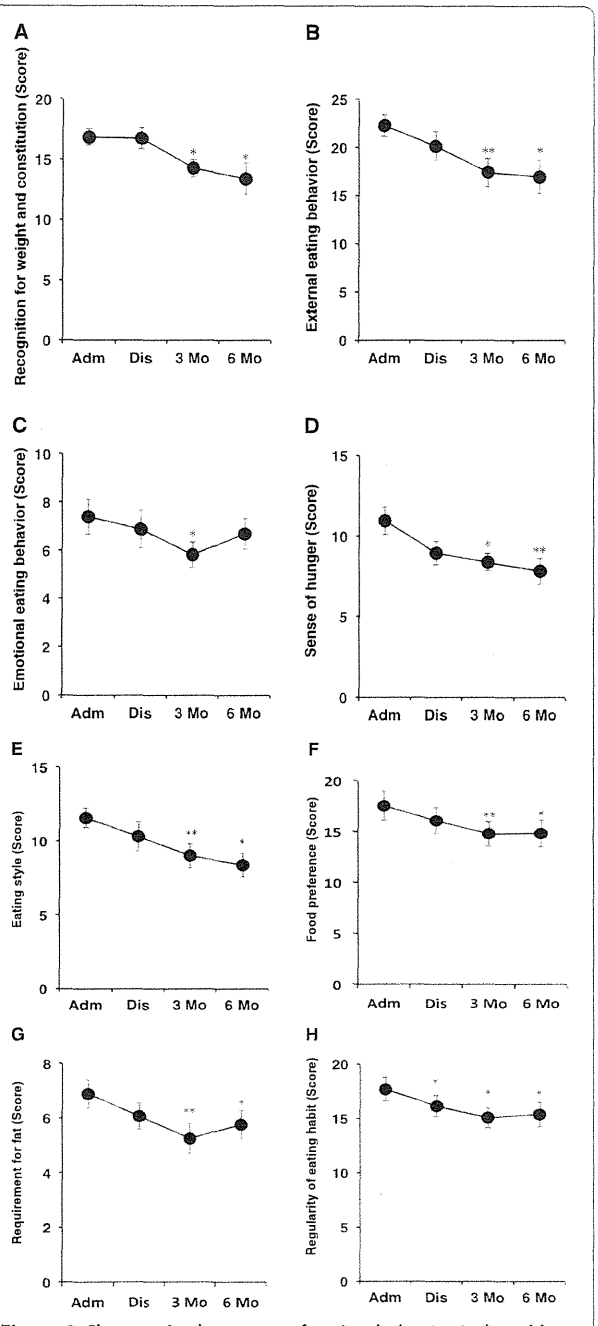

Figure 2 Changes in the scores of eating behavior induced by liraglutide. Eating behavior was assessed in 16 patients (8 males and 8 females) using a questionnaire as described in the Methods section. Data are mean ± SEM. *$P < 0.05$, **$P < 0.01$, compared with the values at admission.

Discussion

The major findings of the present study were: 1) liraglutide treatment effectively reduced body weight up to 6 months after discharge compared to conventional insulin therapy in Japanese obese patients with type 2 diabetes; 2) liraglutide-induced reduction in HbA1c was similar to that observed with insulin treatment; 3) maintained improvement in eating behavior up to 6 months after discharge in patients on liraglutide treatment with various scales gradually moving to those recorded in non-obese normal subjects.

The LEAD-5 study demonstrated that liraglutide decreased HbA1c similar to ultra-long acting insulin analogue, glargine, and also significantly reduced body weight compared with glargine [9]. In the present retrospective study, we compared the effects of liraglutide and insulin on body weight and HbA1c. Almost all patients of the insulin group received intensive insulin therapy at discharge, and the TDD of insulin was relatively high (0.59 unit/kg/day). Significant body weight reduction was observed in both liraglutide and insulin groups during hospitalization and this was due to the in-hospital diet therapy. However, weight reduction was persistently observed after discharge only in the liraglutide group. Liraglutide treatment may be distinguished from the intensive insulin therapy by effective reductions in body weight and HbA1c. Further studies are needed to determine the differences in the therapeutic effects of liraglutide and intensive insulin therapy. The combination treatment of GLP-1 receptor agonist and basal insulin has been also approved in the USA and EU [10], but is yet to be approved in Japan. The effect of such combination therapy on body weight needs to be examined in the future.

GLP-1 promotes satiety and reduces food intake [11,12], but the effect of GLP-1 treatment on eating behavior has not been fully examined in human subjects with type 2 diabetes. We reported recently that short-term treatment with liraglutide did not only reduce food intake and visceral fat adiposity but also decreased the scores of certain parameters of eating behavior during hospitalization [8]. However, we did not provide evidence for the long-term effects of liraglutide on eating behavior. Interestingly, the scores of obese type 2 diabetic patients at admission were higher in all major scales than non-obese subjects (Figure 3). Liraglutide administration decreased these scores and maintained such improvement in eating behavior, with the exception of the scale of emotional eating behavior, until 6 months after discharge, and the total score of eating behavior of some liraglutide-treated patients moved closer to that of non-obese healthy subjects. GLP-1 delays gastric emptying and induces satiety, which is probably related to the combined effect of GLP-1 on the gastrointestinal tract and the brain, leading to decreased energy intake and weight reduction [13,14]. Furthermore, the improvement in the scale for eating style by liraglutide may be partly accounted for by GLP-1-induced suppression of gastrointestinal peristalsis. The extent of weight reduction at

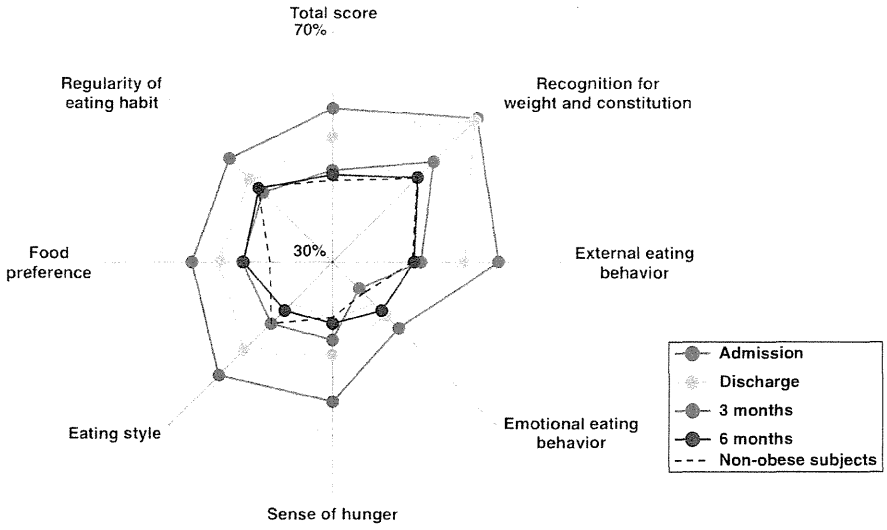

Figure 3 Liraglutide-induced improvement in eating behavior. Changes in the scores of eating behavior were expressed as a radar chart.

6 months after discharge correlated significantly with the reduction in the scores for sense of hunger and eating style, suggesting that the liraglutide-induced weight reduction is mediated partly through improvement in eating behavior.

A recent meta-analysis of weight reduction by GLP-1 receptor agonists showed that administration of these agents resulted in 3% weight reduction at 6 months [15]. The LEAD-5 trial showed 2.1% weight reduction under treatment with liraglutide at 1.8 mg/day [9]. Further, another study, which used liraglutide at up to 3.0 mg/day for 20 weeks for treatment of obesity (BMI; 30–40 kg/m^2), reported 7.4% weight loss [16]. In present study, liraglutide administered at the maximum dose of 0.9 mg/day achieved about 10% body weight reduction at 6 months after discharge. This result far exceeds those of previous studies [9,15,16]. What are the reasons for the excellent results noted in the present study? 1) Patients treated with liraglutide were also placed on strict diet therapy and received a special educational program for weight reduction during hospitalization over 30.8 ± 9.3 days. The importance of these two factors is evident from the rate of weight reduction in the liraglutide group achieved in the present study; weight reduction of −6.5% during hospitalization and only −3.5% between discharge and 6 months after discharge. 2) It is possible that GLP-1 analogue exhibited its utmost effectiveness on weight reduction, because glucose toxicity was corrected mainly by insulin therapy before induction of liraglutide. A poor response to GLP-1 has been described in patients with poorly controlled diabetes but this is reversed following normalization of glycemic control [17,18]. High levels of serum GLP-1 were also reported in patients with the metabolic syndrome [19], suggesting the existence of "GLP-1 resistance". Our group has also demonstrated low expression levels of GLP-1 receptor mRNA in the islets of obese mice, which were significantly reversed after glucose-lowering medications [20].

GLP-1 receptor agonists do not only have glucose-lowering effect but also improve other metabolic parameters such as lipid profile and blood pressure following 5-10% weight reduction [21]. Long-term treatment with exenatide, a GLP-1 receptor agonist, significantly improved cardiovascular risk factors, such as LDL-C, HDL-C, and triglyceride, and biomarkers of liver function [22]. Furthermore, treatment with GLP-1 improved postprandial hyperlipidemia, suggesting that GLP-1 could reduce cardiovascular disease risk in type 2 diabetes [23]. In present study, liraglutide significantly decreased LDL-C at 6 months after discharge (admission: 113.1 ± 36.5, at 6 months after discharge: 95.3 ± 22.9 mg/dL, P =0.03) although 66% of the patients were treated with statins at admission. As shown in Figure 2G, the score for requirement for fat decreased after liraglutide, suggesting that reduction of dietary fat intake partly contributed to the observed decrease in LDL-C. However, the questionnaire did not contain questions about the types of fat consumed, e.g. saturated fat or non-saturated fat, and daily frequency or quantity of fat intake. Questions for food preference would be further improved in the future. Several studies have described the extrapancreatic actions of GLP-1, especially its beneficial effects on the cardiovascular system [2]. Treatment with GLP-1 improved endothelial dysfunction in type 2 diabetics with ischemic heart diseases [24]. We recently showed that short-term liraglutide treatment decreased serum high-sensitivity C-reactive

protein (hsCRP) and soluble intracellular adhesion molecule-1 (sICAM-1) levels [8]. Long-term prospective studies are needed to determine whether the observed changes in obesity, eating behavior, and various metabolic parameters induced by treatment with liraglutide translate into protection against cardiovascular events in obese type 2 diabetics.

The present study has several limitations. The study was not a randomized clinical trial (RCT) and not prospective in design. The baseline characteristics of the patients of the liraglutide and insulin groups were not identical. In addition, the present study included a relatively small population. Furthermore, assessment of eating behavior could not been performed in the insulin group.

In summary, treatment with liraglutide effectively reduced body weight and improved eating behavior in obese Japanese patients with type 2 diabetes for up to 6 months. Liraglutide is potentially useful for the treatment of obese patients with type 2 diabetes. The weight-lowering effects of liraglutide should be examined in more detail in the future in a double-blind placebo-controlled clinical trial.

Competing interests

The authors declare no competing interests.

Authors' contributions

YF acquired and analyzed data, and wrote the manuscript. NM conceived study, analyzed data, and wrote the manuscript. KI, SK, HN, AM, JK, TY, KO, and AI acquired and researched data. TF and IS reviewed the manuscript. All authors read and approved the final manuscript.

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