

ORIGINAL

Effectiveness of metformin and lifestyle interventions as an initial treatment in Japanese patients with newly diagnosed type 2 diabetes : a prospective observational study

Satoru Sumitani^{1,2}, Shinya Morita², Yoshihiko Utsu², Kosuke Mukai², Shunji Miki², Bunzo Sato², Hideji Nakamura¹, and Soji Kasayama²

¹Center for Preventive Medicine and ²Department of Medicine, Nissay Hospital, Osaka, Japan

Abstract : Although global guidelines recommend metformin and lifestyle interventions as an initial treatment in patients with newly diagnosed type 2 diabetes (T2DM), few reports exist about its effectiveness in Japanese patients. To examine its effectiveness, we performed a prospective observational study within a routine clinical setting. We provided metformin ($\geq 1,500$ mg/day) and lifestyle interventions to 23 patients with newly diagnosed T2DM (20 men and 3 women, mean age 53 years, mean body mass index [BMI] 25.7 kg/m²). After 16 weeks, HbA1c levels significantly decreased from $9.1 \pm 2.1\%$ (mean \pm SD) to $6.6 \pm 0.8\%$ ($p < 0.001$). Thirteen patients (56.5%) achieved a target HbA1c $< 6.5\%$. We did not find a significant correlation between baseline BMI and the changes in HbA1c (Δ HbA1c) ($r = -0.165$, $p = 0.451$). In contrast, we found a significant correlation between baseline fasting plasma glucose and Δ HbA1c ($r = -0.755$, $p < 0.001$). Body weight decreased from 73.3 ± 13.3 kg to 69.8 ± 11.6 kg ($p < 0.001$). Total cholesterol, low density lipoprotein-cholesterol, non-high density lipoprotein-cholesterol, and serum vitamin B-12 concentrations also significantly decreased. Adverse events included diarrhea (26.1%) and mild elevation of liver enzymes (8.7%). These results suggest that metformin and lifestyle interventions is effective and safe as an initial treatment in Japanese patients with newly diagnosed T2DM. *J. Med. Invest.* 59 : 166-173, February, 2012

Keywords : management, type 2 diabetes, metformin, lifestyle interventions, hypoglycemia

INTRODUCTION

Optimal control of hyperglycemia and associated risk factors reduces the risk of diabetes-related complications. Metformin is the most widely prescribed

biguanide, which lowers blood glucose level primarily through the inhibition of hepatic glucose production. The UK Prospective Diabetes study (UKPDS) showed that metformin reduces the risk of diabetes-related endpoints, myocardial infarction, diabetes-related death and all-cause mortality in overweight patients with newly diagnosed type 2 diabetes (1, 2). Moreover, recent observational studies reveal that body mass index (BMI) of patients is unlikely to influence the antihyperglycemic effect of metformin (3-5). Therefore, global guidelines now recommend

Received for publication November 15, 2011 ; accepted December 28, 2011.

Address correspondence and reprint requests to Satoru Sumitani, MD, PhD, Center for Preventive Medicine, Nissay Hospital, 6-3-8 Itachibori, Nishi-ku, Osaka, Osaka 550-0012, Japan and Fax : +81-6-6532-6402.

the use of metformin as an initial oral pharmacotherapy for both overweight and normal weight patients with type 2 diabetes (6, 7). The antihyperglycemic effect of metformin is dose-dependent, and the maximal effect is obtained at 2,000 mg/day in most patients (8). Therefore, the maximal recommended daily dose of metformin is 2,000 mg/day in the USA and 3,000 mg/day in Europe and in other regions. Importantly, the metabolic and clinical outcomes for metformin in the UKPDS were obtained using a median daily dose of 2,550 mg/day (1). The local regulation in Japan, however, had placed an upper limit of daily dose of metformin at 750 mg/day from 1977 to 2010. Therefore, we know few studies that examined the antihyperglycemic effect of metformin at doses greater than 750 mg/day in Japanese patients with newly diagnosed type 2 diabetes.

The American Diabetes Association (ADA) and the European Society for the Study of Diabetes (EASD) issued a consensus algorithm for the initial management of hyperglycemia in patients with type 2 diabetes in 2006 (9) and updated it in 2009 (10), where the standard dose of metformin ($\geq 1,500$ mg/day) (11) and lifestyle interventions is recommended as an initial treatment in patients with newly diagnosed type 2 diabetes. We are, however, aware of no published report that describes the effectiveness of this approach in Japanese patients with type 2 diabetes. Therefore, we performed a prospective observational study to examine the effectiveness of metformin and lifestyle interventions as an initial treatment in Japanese patients with newly diagnosed type 2 diabetes.

PATIENTS AND METHODS

Study design

We performed a prospective observational study within a routine clinical setting to examine the effectiveness of metformin and lifestyle interventions as an initial treatment in Japanese patients with newly diagnosed type 2 diabetes. The follow-up period of the study was 16 weeks. The study was conducted in an outpatient clinic in a community-based hospital located in Osaka, Japan. Between June 2010 and November 2010, 23 patients with newly diagnosed type 2 diabetes (20 men and 3 women) were consecutively enrolled in the study.

After diagnosis of type 2 diabetes, metformin and lifestyle interventions were provided at the same time. The patients were examined every two weeks

until the administered dose of metformin was fixed, and thereafter every four weeks to 16 weeks. Biochemical measurements and physical examinations, including taking vital signs and measuring body weight, were performed at every visit. At the baseline, anthropometric characteristics and demographic characteristics were also collected. The study was conducted in accordance with the Declaration of Helsinki and with the approval of the Ethics Committee of Nissay Hospital. Each patient provided written informed consent for study participation and the use of their data for research purposes. This study is registered with the University Hospital Medical Information Network (UMIN) clinical trial registry, number 000004193.

Patients

Eligible patients were newly diagnosed patients with type 2 diabetes aged 20-75 years with HbA1c levels $\geq 6.5\%$, and who had never taken an oral hypoglycemic agent. The diagnosis of type 2 diabetes was made on the WHO 1999 criteria (12). Patients were excluded from the study if they had fasting plasma glucose (FPG) ≥ 300 mg/dL, proliferative diabetic retinopathy, elevated serum creatinine (≥ 1.3 mg/dL in men and ≥ 1.2 mg/dL in women), elevated liver enzymes (aspartate transaminase [AST], alanine transaminase [ALT] $> 2.5 \times$ the upper limit of normal), or a history of lactic acidosis.

Treatments

Lifestyle interventions were composed of an individualized dietary counseling and an encouragement to increase daily physical activities. Each patient received an individualized dietary counseling from a registered dietitian, which was based on carbohydrate counting (13-16). Briefly, each patient was instructed to take a fixed amount of daily carbohydrate (180 g/day for men and 150 g/day for women). The distribution of the total amount of daily carbohydrate to each meal was negotiated with the patients. No instruction regarding the energy consumption and the amount of fat or protein was provided. Specific instructions, however, were provided to the patients about the type of fat to be consumed (recommendation of mainly monounsaturated fatty acids and polyunsaturated fatty acids with restriction of saturated fat). The patients were encouraged to increase their daily physical activities through, for example, a habitual walking. Adherence to lifestyle interventions was reinforced at every visit.

Metformin was started with 250 mg two times a day (500 mg/day) with meals (breakfast and dinner). Every two weeks, the dose was advanced to 500 mg two times a day (1,000 mg/day) with meals and finally to 500 mg three times a day (1,500 mg/day) with meals. When high plasma glucose level (FPG \geq 140 mg/dL) persisted with 1,500 mg/day, the dose was further advanced to 750 mg three times (2,250 mg/day) with meals. When the patients had gastrointestinal symptoms, we decreased the dose to the previous lower dose and later tried to advance the dose again.

Outcomes and adverse events

The primary outcomes of this study were the change in HbA1c levels from the baseline to 16 weeks and the proportions of patients achieving target HbA1c levels ($< 6.5\%$ and $< 7.0\%$) at 16 weeks. The secondary outcomes were the changes in body weight, BMI, FPG, glycated albumin (GA), total cholesterol, triglyceride, high density lipoprotein (HDL)-cholesterol, low density lipoprotein (LDL)-cholesterol, non-HDL-cholesterol, and serum vitamin B-12 during the follow-up period. Vitamin B-12 deficiency was defined as the serum level < 200 pg/mL (17). Occurrence of adverse events, including gastrointestinal adverse events and hypoglycemia was assessed by medical interviews at every visit. Hypoglycemia was defined as the presence of typical adrenergic or neuroglycopenic symptoms and signs. Gastrointestinal adverse events were defined as the events by which the patient was unable to advance the dose to 1,500 mg/day. They included diarrhea, abdominal discomfort, constipation, and nausea. All adverse events were recorded and judged for severity and possible relationship to the treatment.

Laboratory analysis

HbA1c levels were measured with HLC-723G8 (Tosoh, Tokyo, Japan) by high performance liquid chromatography (HPLC). The value for HbA1c (%) was shown as an National Glycohemoglobin Standardization Program (NGSP) equivalent value (%) calculated by the formula $\text{HbA1c (\%)} = \text{HbA1c (Japan Diabetes Society [JDS]) (\%)} + 0.4\%$, considering the relational expression of HbA1c (%) measured by the previous Japanese standard substance and measurement methods and HbA1c (NGSP) (18). The difference of HbA1c level between the baseline and 16 weeks (ΔHbA1c) was calculated by subtracting the value of HbA1c at the baseline from that of at 16

weeks. GA was measured by the latex agglutination method (Asahi Kasei Pharma, Tokyo, Japan). Serum C-peptide and vitamin B-12 concentrations were measured by chemiluminescent enzyme immunoassays (CLEIA). FPG, total cholesterol, triglyceride, HDL-cholesterol, were measured using standard laboratory methods. LDL-cholesterol was calculated by Friedwald equation (19). Non-HDL-cholesterol was calculated by subtracting the value of HDL-cholesterol from total cholesterol. The estimated glomerular filtration rates (eGFR) were estimated using serum creatinine values and the abbreviated Modification of Diet in Renal Disease (MDRD) study equation, modified with the Japanese coefficient (20). Assessment of diabetic retinopathy was performed by fundoscopic examinations by an ophthalmologist. Diabetic retinopathy was graded as follows: no diabetic retinopathy (NDR); non-proliferative diabetic retinopathy (NPDR); and proliferative diabetic retinopathy (PDR). Diabetic nephropathy was staged as follows: normoalbuminuria (urinary albumin excretion < 30 mg/g.creatinine[Cr]); microalbuminuria (urinary albumin excretion 30-299 mg/g.Cr); and macroalbuminuria (urinary albumin excretion > 300 mg/g.Cr). Diabetic neuropathy was diagnosed using the Michigan Neuropathy Screening Instrument (MNSI) (21).

Statistical analysis

All statistical analyses were performed using SPSS for Windows, Version 11.0 (SPSS, Chicago, IL, USA). Continuous variables are expressed as mean \pm SD or median [25%, 75%]. Categorical variables are expressed as number. Changes in HbA1c level during the follow-up period were analyzed with the Friedman test. The Wilcoxon signed-rank test with the Bonferroni correction was used for multiple comparisons. Spearman's correlation coefficients were used to determine the correlations between the ΔHbA1c and the baseline FPG or BMI. Differences of the clinical variables between the baseline and 16 weeks were analyzed with the Wilcoxon signed-rank test. All p values were two-sided. A p value < 0.05 was considered to be statistically significant.

RESULTS

Baseline and follow-up characteristics

Baseline characteristics of the study patients are described in Table 1. All variables in the baseline

Table 1 Baseline characteristics of patients

Variable	
Men/Women	20/3
Age (years)	53 ± 11
Body weight (kg)	73.3 ± 13.3
Body mass index (kg/m ²)	25.7 ± 4.5
Systolic blood pressure (mmHg)	114 ± 27
Diastolic blood pressure (mmHg)	74 ± 12
Fasting plasma glucose (mg/dL)	182 ± 59
HbA1c (%)	9.1 ± 2.1
Fasting C-peptide (ng/mL)	1.67 ± 1.04
eGFR (ml/min/1.73 m ²)	86.9 ± 18.5
Retinopathy (NDR/NPDR/PDR)	23/0/0
Nephropathy (norm/micro/macro)	21/1/1
Neuropathy (-/+)	22/1

Data are number or mean ± SD. eGFR: estimated glomerular filtration rate, NDR: no diabetic retinopathy, NPDR; non-proliferative diabetic retinopathy, PDR: proliferative diabetic retinopathy, norm: normoalbuminuria, micro: microalbuminuria, macro: macroalbuminuria.

characteristics were measured at the time of enrollment. The mean age was 53 years, the mean HbA1c level 9.1%, and the mean BMI 25.7 kg/m². The final dose of metformin at 16 weeks was 750 mg/day in three, 1,000 mg/day in three, 1,500 mg/day in fourteen, and 2,250 mg/day in three patients.

Primary outcomes

Time course of HbA1c level of each patient is depicted in Fig. 1A. The mean HbA1c significantly decreased at 4 weeks, and it progressively decreased from 9.1 ± 2.1% at baseline to 6.6 ± 0.8% after 16 weeks (p < 0.001, Fig. 1B). The mean ΔHbA1c was -2.41% (95% confidence interval [CI] -1.57--3.25). We did not find a significant correlation between baseline BMI and ΔHbA1c (r = -0.165, p = 0.451, Fig. 2A). In contrast, we found a significant correlation between baseline FPG and ΔHbA1c (r = -0.755, p < 0.001, Fig. 2B). Among the 23 patients, 13 (56.5%) achieved HbA1c < 6.5% and 16 (69.6%) achieved HbA1c < 7.0% at 16 weeks.

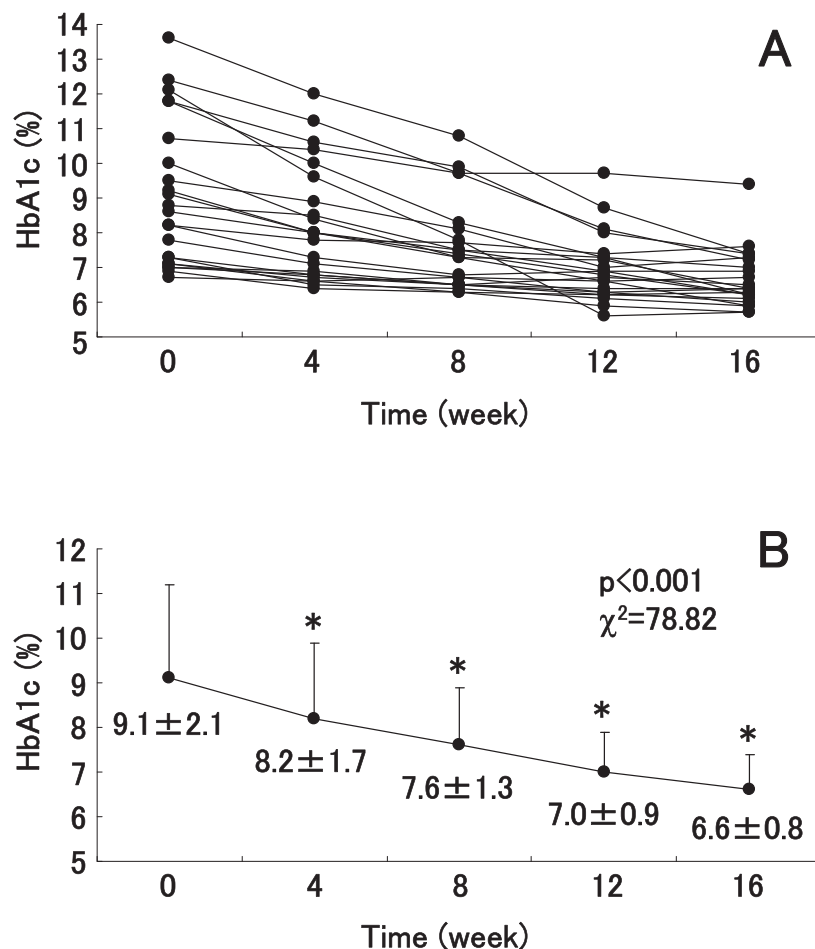


Fig. 1 Time course of HbA1c levels of each patient (A) and the change in the mean HbA1c levels (B) during the follow-up period. The p and χ^2 values were by the Friedman test. Asterisks denote p < 0.001 compared with the baseline HbA1c level by the Wilcoxon signed-rank test.

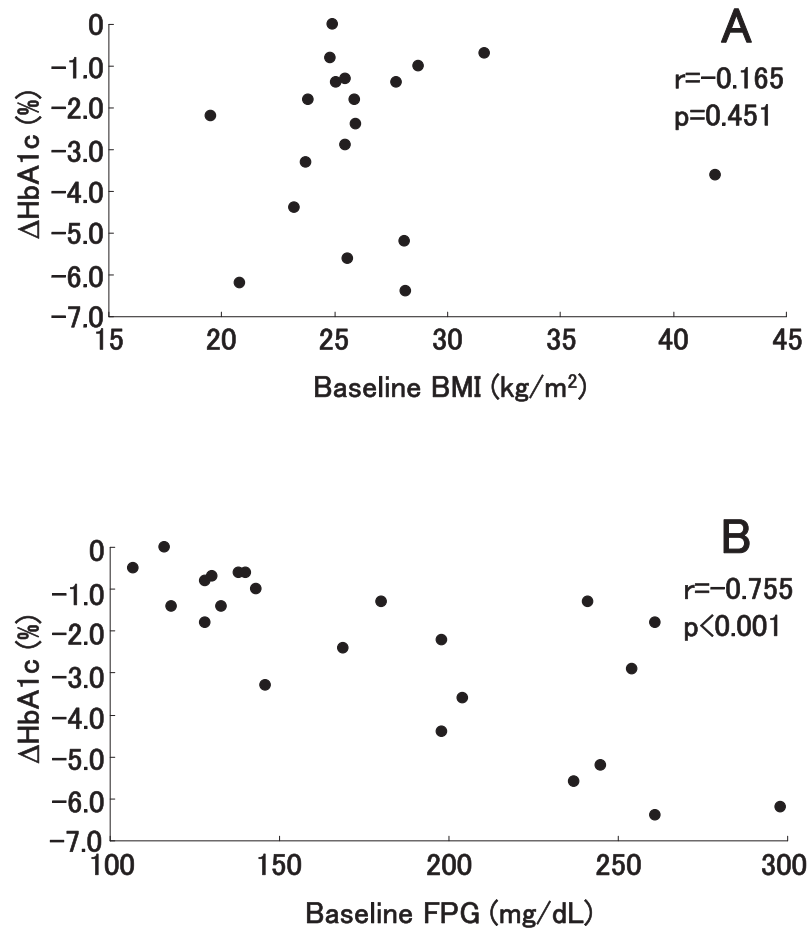


Fig. 2 Correlation between baseline body mass index (BMI) and Δ HbA1c (A), and between baseline fasting plasma glucose (FPG) and Δ HbA1c (B). Δ HbA1c was calculated by subtracting the HbA1c values at the baseline from those at 16 weeks. r is Spearman's rank correlation coefficient.

Secondary outcomes

The changes in various clinical variables over the study period are presented in Table 2. Body weight significantly decreased from 73.3 ± 13.3 kg at baseline to 69.8 ± 11.6 kg at 16 weeks ($p < 0.001$). FPG

significantly decreased from 182 ± 59 mg/dL to 116 ± 15 mg/dL ($p < 0.001$). Levels of total cholesterol, LDL-cholesterol, and non-HDL-cholesterol also significantly decreased. In contrast, levels of triglyceride and HDL-cholesterol did not statistically change. Serum vitamin B-12 levels also significantly

Table 2 Clinical variables at the baseline and after 16 weeks

Variable	Baseline	After 16 weeks	p
Body weight (kg)	73.3 ± 13.3	69.8 ± 11.6	< 0.001
Body mass index (kg/m^2)	25.7 ± 4.5	24.5 ± 4.0	< 0.001
Fasting plasma glucose (mg/dL)	182 ± 59	116 ± 15	< 0.001
Glycated albumin (%)	25.1 ± 8.5	15.9 ± 2.8	< 0.001
Total cholesterol (mg/dL)	214 ± 30	184 ± 24	< 0.001
Triglyceride (mg/dL)	132 [92, 214]	126 [79, 189]	0.200
HDL-cholesterol (mg/dL)	51 ± 11	51 ± 10	0.910
LDL-cholesterol (mg/dL)	126 ± 34	105 ± 23	0.010
Non-HDL-cholesterol (mg/dL)	163 ± 34	133 ± 25	0.001
Vitamin B-12 (pg/mL)	458 [277, 714]	353 [216, 577]	0.017

Data are mean \pm SD or median [25%, 75%]. HDL : high density lipoprotein, LDL : low density lipoprotein. Between group differences were examined with the Wilcoxon signed-rank test.

decreased. No patient, however, developed vitamin B-12 deficiency defined by the serum vitamin B-12 level.

Adverse events

No hypoglycemia was observed in any patients. Among the 23 patients, six patients (26.1%) were unable to advance to 1,500 mg/day because of diarrhea. Among them, however, no patient discontinued taking metformin. No other gastrointestinal adverse event, including abdominal discomfort, constipation and nausea was observed. Two patients (8.7%) showed slight elevation of ALT (less than $\times 1.5$ of the upper limit of normal). No patient had lactic acidosis.

DISCUSSION

We found that metformin and lifestyle interventions decreased HbA1c levels by 2.41% (95% CI 1.57-3.25) after 16 weeks. To our knowledge, this is the first report on the effectiveness of metformin ($\geq 1,500$ mg/day) and lifestyle interventions in Japanese patients with newly diagnosed type 2 diabetes within a routine clinical setting. In this regard, Saito *et al.* reported the efficacy of high-dose metformin (1,500 mg/day) in 12 patients with type 2 diabetes (22). All of their study patients, however, were inadequately controlled (HbA1c $> 7.4\%$) with metformin alone (750 mg/day) or metformin plus sulfonylureas at the baseline. Therefore, the efficacy of high-dose metformin as an initial treatment in patients with newly diagnosed type 2 diabetes was not evaluated in their study. In the previous placebo-controlled trials, metformin lowered HbA1c levels by about 1.5% (8, 23). The difference between the present results and the previous results may be owing to the different study designs. The previous studies included drug-naive patients who were inadequately controlled with lifestyle interventions to rigorously examine the efficacy of metformin. In contrast, our study did not aim to examine the efficacy of metformin *per se*, but the effectiveness of metformin and lifestyle interventions as an initial treatment in patients with type 2 diabetes in a routine clinical setting. Therefore, the reduction in HbA1c levels in the present study appears to be a composite effect of metformin and lifestyle interventions. This may be one of the reasons why the reduction of HbA1c in the present study is greater than that observed in the previous studies.

Because of the results of the UKPDS34 (1), metformin had been preferentially administered to overweight or obese type 2 diabetic patients. We did not find, however, a statistically significant correlation between baseline BMI and Δ HbA1c (Fig. 2A), indicating that BMI does not influence the antihyperglycemic effect of metformin and lifestyle interventions. This is consistent with the recent studies showing BMI does not influence the antihyperglycemic effect of metformin (3-5) and supports the notion that metformin may be effective as an initial pharmacotherapy irrespective of BMI of the patients. In contrast, we found a significant correlation between baseline FPG and Δ HbA1c (Fig. 2B). This indicates that antihyperglycemic effect of metformin is more potent in patients with poorer glycemic control. Progressive deterioration of glycemic control in type 2 diabetic patients correlates with the increased hepatic glucose production (24). Therefore, it is conceivable that antihyperglycemic effect of metformin, which is chiefly through inhibition of hepatic glucose production, is more potent in patients with poorer glycemic control. The proportion of patients who achieved a target HbA1c $< 6.5\%$ and $< 7.0\%$ was 56.5% and 69.6%, respectively, indicating that about 30% of patients remained inadequate glycemic control with metformin and lifestyle interventions. Although addition of a sulfonylurea or basal insulin to patients who fail to achieve a target HbA1c $< 7.0\%$ with metformin and lifestyle interventions is recommended in the ADA/EASD algorithm (10), further studies are needed to confirm that this approach is effective as a second treatment in Japanese patients with type 2 diabetes.

We found that metformin and lifestyle interventions induced significant weight loss after 16 weeks. Because weight gain is one of the unfavorable problems by treatment with thiazolidinediones or sulfonylureas (25), our finding further supports the notion that an initial treatment with metformin has a health benefit in overweight or obese patients. Moreover, we found that levels of total cholesterol, LDL-cholesterol, and non-HDL cholesterol decreased significantly during the follow-up period. In contrast, levels of triglyceride and HDL-cholesterol did not change significantly. This is consistent with the previous results obtained in randomized-controlled study (23). Because control of LDL-cholesterol is the primary focus of lipid management, the property of metformin to decrease LDL-cholesterol may add a further health benefit to patients with type 2 diabetes. A recent study showed that

long-term treatment with metformin in patients with type 2 diabetes has a risk of vitamin B-12 deficiency (26). Although we found a significant decrease in the level of vitamin-B12 during the follow-up period, no patients developed vitamin B-12 deficiency. A careful monitoring of vitamin B-12 levels may be warranted with long-term metformin treatment.

We observed no hypoglycemia in the study patients during the follow-up period. Because the patients did not have self-monitoring of blood glucose, we are formally unable to exclude the occurrence of asymptomatic hypoglycemia. Metformin is, however, an insulin sensitizer and does not stimulate insulin secretion (27). Therefore, metformin rarely induces hypoglycemia as a monotherapy. Because hypoglycemia is the greatest obstacle to achieve near normal glycemia, an initial treatment with metformin and lifestyle interventions appears to be a safe approach to achieve near normal glycemia.

Gastrointestinal adverse events, including diarrhea, constipation, nausea and other symptoms, have been shown common in patients taking metformin. Six patients (26.1%) were unable to advance to 1,500 mg/day because of diarrhea. The incidence of gastrointestinal adverse events is different among previous studies, ranging from 6% to 43% (28). This may be due to the different definition of gastrointestinal adverse events or the different doses of metformin.

The main limitation of the present study is its observational design without a parallel control group, which prevents us drawing conclusions about causality. Another limitation is that the small number of patients and short duration of the follow-up period prevent the precise estimate of the effectiveness of the treatment. Large-scale, long-term randomized-controlled studies are needed to confirm the findings of our study.

In conclusion, we found that metformin and lifestyle interventions reduced HbA1c levels by 2.41% after 16 weeks. Moreover, 56.5% of patients achieved a target HbA1c < 6.5% without hypoglycemia and body weight gain. These results suggest that metformin and lifestyle interventions may be effective and safe as an initial treatment in Japanese patients with newly diagnosed type 2 diabetes.

CONFLICT OF INTEREST

The authors declare no conflict of interest relevant to this manuscript

ACKNOWLEDGEMENTS

We thank Mizuho Yamahara R.D. and Rie Ikegami R.D. for their expertise in dietary counseling.

REFERENCES

1. UK Prospective Diabetes Study (UKPDS) Group : Effect of intensive blood glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS34). *Lancet* 352 : 854-865, 1998
2. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HW : 10-year follow-up of intensive glucose control in type 2 diabetes. *N Engl J Med* 359 : 1577-1589, 2008
3. Donnelly LA, Doney ASF, Hattersley AT, Morris AD, Pearson ER : The effect of obesity on glycaemic response to metformin or sulfonylureas in type 2 diabetes. *Diabetic Med* 23 : 128-133, 2006
4. Kaku K, Tajima N, Kawamori R : Melbin Observational Research (MORE) study of metformin therapy in patients with type 2 diabetes. *J Japan Diab Soc* 49 : 325-331, 2006 (in Japanese)
5. Hosokawa K, Meguro S, Funae O, Murata C, Katou K, Mokubo A, Suzuki Y, Anazawa S, Atsumi Y, Matsuoka K : Clinical effects of metformin in patients with nonobese type 2 diabetes. *J Japan Diab Soc* 52 : 1-6, 2009 (in Japanese)
6. IDF Clinical Guidelines Task Force : Global guideline for Type 2 diabetes. Brussels : International Diabetes Federation, 2005
7. Chan JCN, Deerochanawong C, Shera AS, Yoon KH, Adam JMF, Binh TV, Chan SP, Fernando RE, Horn LC, Khue NT, Litonjua AD, Soegondo S, Zimmet P : Role of metformin in the initiation of pharmacotherapy for type 2 diabetes : an Asian-Pacific perspective. *Diabetes Res Clin Pract* 75 : 255-266, 2007
8. Garber AJ, Duncan TG, Goodman AM, Mills DJ, Rohlf JL : Efficacy of metformin in Type II diabetes : Results of a double-blind, placebo-controlled, dose-response trial. *Am J Med* 102 : 491-497, 1997
9. Nathan DM, Buse JB, Davidson MB, Heine RJ, Holman RR, Sherwin R, Zinman B : Management of hyperglycemia in type 2 diabetes : a consensus algorithm for the initiation and

- adjustment of therapy. *Diabetes Care* 29 : 1963-1972, 2006
10. Nathan DM, Buse JB, Davidson MB, Ferrannini E, Holman RR, Sherwin R, Zinman B : Medical management of hyperglycemia in type 2 diabetes : a consensus algorithm for the initiation and adjustment of therapy. *Diabetes Care* 32 : 193-203, 2009
 11. Scarpello JH : Optimal dosing strategies for maximising the clinical response to metformin in type 2 diabetes. *Br J Diabetes Vasc Dis* 1 : 28-36, 2001
 12. World Health Organization : Definition, diagnosis and classification of diabetes mellitus and its complications. Report of a WHO consultation. Part 1 : Diagnosis and classification of diabetes mellitus. Geneva, World Health Organization, 1999
 13. Gillespie SJ, Kulkarni KD, Daly AE : Using carbohydrate counting in diabetes clinical practice. *J Am Diet Assoc* 98 : 897-905, 1998
 14. Franz MJ, Bantle JP, Beebe CA, Brunzell JD, Chiasson JL, Garg A, Holzmeister LA, Hoogwerf B, Mayer-Davis E, Mooradian AD, Purnell J, Wheeler M : Evidence-based nutrition principles and recommendations for the treatment and prevention of diabetes and related complications. *Diabetes Care* 25 : 148-198, 2002
 15. American Diabetes Association : Nutrition recommendations and interventions for diabetes : a position statement of the American Diabetes Association. *Diabetes Care* 30 : S48-S65, 2007
 16. Kirk JK, Graves DE, Craven TE, Lipkin EW, Austin M, Margolis KL : Restricted-carbohydrate diets in patients with type 2 diabetes : a meta-analysis. *J Am Diet Assoc* 108 : 91-100, 2008
 17. Dali-Youcef N, Andres E : An update on cobalamin deficiency in adults. *Q J Med* 102 : 17-28, 2009
 18. The Committee of Japan Diabetes Society on the diagnostic criteria of diabetes mellitus : Report of the Committee on the classification and diagnostic criteria of diabetes mellitus. *J Diabetes Invest* 1 : 212-228, 2010
 19. Friedwald WT, Levy RI, Friedrickson DS : Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 18 : 499-502, 1972
 20. Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, Yamagata H, Hishida A : Revised equations for estimated GFR from serum creatinine in Japan. *Am J Kid Dis* 53 : 982-992, 2009
 21. Feldman EL, Stevens MJ, Thomas PK, Brown MB, Canal N, Greene DA : A practical two-step quantitative clinical and electrophysiological assessment for the diagnosis and staging of diabetic neuropathy. *Diabetes Care* 17 : 1281-1289, 1994
 22. Saito R, Ishii H, Morioka K, Matsumoto M : Efficacy of high-dose metformin in type 2 diabetes. *J Japan Diab Soc* 46 : 399-402, 2003 (in Japanese)
 23. DeFronzo RA, Goodman AM, the Multicenter Metformin Study Group : Efficacy of metformin in patients with non-insulin-dependent diabetes mellitus. *N Engl J Med* 333 : 541-549, 1995
 24. DeFronzo RA, Ferrannini E, Simonson DC : Fasting hyperglycemia in non-insulin dependent diabetes mellitus : contributions of excessive hepatic glucose production and impaired tissue glucose uptake. *Metabolism* 38 : 387-395, 1989
 25. Inzucchi SE : Oral hypoglycemic therapy for type 2 diabetes. *JAMA* 287 : 360-372, 2002
 26. de Jager J, Kooy A, Lehert P, Wulffele MG, van del Kolk J, Bets D, Verburg J, Donker AJ, Stehouwer CD : Long term treatment with metformin in patients with type 2 diabetes and risk of vitamin B-12 deficiency : randomized placebo-controlled trial. *BMJ* 340 : c2181 doi : 10.1136/bmj.c2181, 2010
 27. Bailey CJ, Turner RC : Metformin. *N Engl J Med* 334 : 574-579, 1996
 28. Bailey CJ, Howlett HC : Tolerability of metformin. *In* : Bailey CJ, Campbell JW, Chan JCN, Davidson JA, Howlett HC, Ritz P, eds. *Metformin The gold standard*. John Wiley and Sons Ltd, Chichester, 2007, pp.173-178