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ORIGINAL ARTICLE

Relationship between metformin use and vitamin B_{12} status in patients with type 2 diabetes in Japan

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ABSTRACT

Aims/Introduction: Metformin therapy has been associated with vitamin B_{12} (VB12) deficiency, but information regarding this adverse effect in Asian populations is limited. We have now examined the relationship between metformin use and VB12 status in individuals with type 2 diabetes mellitus in Japan.

Materials and Methods: This cross-sectional study was carried out with type 2 diabetes mellitus patients treated (Met group, $n = 122$) or not treated (control group, $n = 63$) with metformin. The primary end-point was the difference in the serum concentration of homocysteine, a marker of VB12 activity, between the two groups. The serum concentrations of VB12, blood hemoglobin level and mean corpuscular volume were also compared between the groups. Subset analysis was carried out with individuals aged ≥70 years. The potential correlation between the daily dose or duration of metformin treatment and the other measured parameters was also examined.

Results: The level of homocysteine, as well as the VB12 level, hemoglobin concentration and mean corpuscular volume, did not differ significantly between the control and treated with metformin groups. The level of homocysteine was positively and that of VB12 negatively correlated with the daily dose of metformin. Among elderly individuals, the hemoglobin level was significantly lower in the treated with metformin group than in the control group, although the mean corpuscular volume was similar in the two groups.

Conclusions: The risk of VB12 deficiency during metformin treatment appears to be low in Japanese type 2 diabetes mellitus patients. However, high doses of metformin might result in a moderate decrease in the circulating VB12 level, as well as in anemia in elderly individuals.

INTRODUCTION

Although the efficacy and safety of metformin for the treatment of type 2 diabetes mellitus have been well established by its long-term clinical use, the administration of this drug is sometimes associated with adverse events. Gastrointestinal adverse events including diarrhea, anorexia and dyspepsia are common during treatment with metformin, but are not serious in most cases. In contrast, lactic acidosis, which occurs infrequently during metformin treatment, sometimes progresses to a serious condition[1].

Some studies have also found that the administration of metformin is associated with deficiency of vitamin B_{12} (VB12)[2–7], whereas others have not detected such an association[8–11]. Homocysteine, which is converted to methionine in the presence of VB12, is a marker for the activity of the vitamin[12]. Some studies have found that the serum level of homocysteine was unaltered, whereas that of VB12 was reduced in patients treated with metformin[13–17], suggesting that the depletion of the vitamin was not sufficient to affect its biological actions in the body. The relationship between metformin use and both VB12 deficiency and the risk of associated health problems thus remains unclear.

It is possible that the apparent inconsistency in previous studies of VB12 status in individuals who take metformin is

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attributable to differences in the background of study participants, including parameters such as age, ethnicity and eating behavior. Evidence suggests that elderly patients are at higher risk of VB12 deficiency than are younger individuals as a result of inadequate intake and absorption of this vitamin[18–21]. The prevalence of VB12 deficiency has also been found to vary among countries[22]. Differences in age and ethnicity should therefore be taken into account in studies of the relationship between metformin and VB12 levels.

In Japan, the maximum approved dose of metformin was limited to a level (~750 mg/day) much lower than that in many other countries until 2010, when the dose was increased to a level similar to that in other countries (2250 mg/day). After this increase in the maximum dose, only one study of the relationship between metformin administration and VB12 status in Japanese individuals has been presented, with the mean duration of metformin administration in that study being 2.8 years[13]. Given that the effect of metformin on VB12 levels also appears to be influenced by the duration of its administration[23], it is important to investigate the effect of longer-term use of the drug in order to fully understand its relationship to VB12 status. In the present study, we determined the circulating levels of both VB12 and homocysteine in 122 Japanese users of metformin, for whom the mean duration of drug administration was 6.6 years.

METHODS

Study design

This single-center, cross-sectional study was approved by the institutional review board of Kobe University Hospital (approval no. 160082), and was carried out in accordance with the Declaration of Helsinki and its amendments. Written informed consent was obtained from all study participants. The study was also registered with the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR) as UMIN000019126.

Individuals who visited Kobe University Hospital, Kobe, Japan, as outpatients between May 2017 and April 2018, and who met the following inclusion and exclusion criteria were invited to enroll in the study. Inclusion criteria included a diagnosis of type 2 diabetes mellitus, an age of at least 20 years and either use of metformin for at least 6 months or no use of the drug for at least 1 year. Exclusion criteria included iron deficiency with a transferrin saturation (calculated by dividing the serum iron level by total iron binding capacity) of <20%, gastrectomy, gastric bypass surgery, a malabsorption syndrome, intake of VB12, a serum creatinine concentration of >1.3 mg/dL, a diagnosis of cancer, pregnancy or diagnosed VB12 deficiency. A total of 185 patients who met the study stipulations – 122 taking metformin for at least 6 months (Met group), and 63 who had not taken the drug for at least 12 months (control group) – agreed to participate in the study.

Serum VB12 and homocysteine concentrations were measured by LSI Medience Corporation (Tokyo, Japan) with the use of a chemiluminescence-based immunoassay. If the serum level of VB12 exceeded the upper limit of the assay (1500 pg/mL), the value was considered to be 1500 pg/mL.

Outcomes

The primary end point of the study was the difference in serum homocysteine level between the control and Met groups. Secondary end points included the differences in serum VB12 concentration, blood hemoglobin level, and mean corpuscular volume (MCV), as well as the correlation between the measured parameters and either metformin dose or the duration of metformin treatment. Similar analysis was also performed with the subset of patients aged 70 years or older.

Statistical analysis

Intergroup differences were tested for significance with the unpaired Student's *t*-test. Correlation analysis was based on Pearson's correlation coefficient. A *P*-value of <0.05 was considered statistically significant. All statistical analysis was carried out with EZR software (The R Foundation for Statistical Computing, Vienna, Austria)[24].

RESULTS

Patient characteristics

A total of 122 type 2 diabetes mellitus patients who took metformin (Met group) and 63 type 2 diabetes mellitus patients

Table 1 | Baseline clinical characteristics of the control group and the group that received metformin treatment

Characteristic	Control	Met	*P*
n	63	122	
≥70 years-of-age (%)	36 (57.1)	54 (44.3)	0.13
Men (%)	43 (68.3)	72 (59.0)	0.29
Age (years)	70.4 ± 9.6	67.9 ± 7.6	0.05
Body mass index (kg/m^2)	23.3 ± 3.7	24.4 ± 4.0	0.06
Diabetes duration (years)	13.7 ± 13.9	16.7 ± 12.6	0.13
HbA_{1c} (%)	7.0 ± 1.0	7.0 ± 0.7	0.85
Duration of metformin use (years)		6.6 ± 3.7	
Daily dose of metformin (mg)		979.5 ± 491.2	
Concomitant antidiabetic medications (%)			
Sulfonylurea/glinide	15.9	21.3	0.49
DPP-4 inhibitor	60.3	68.9	0.32
SGLT-2 inhibitor	7.9	10.7	0.74
α-Glucosidase inhibitor	27.0	22.1	0.58
Thiazolidinedione	3.2	5.7	0.68
Insulin	22.2	32.0	0.25
GLP-1 receptor agonist	4.8	15.6	0.05

Data are *n* values or mean ± standard deviation. *P*-values were determined with the unpaired Student's *t*-test. Control, the group that did nor receive metformin treatment; DPP-4, dipeptidyl peptidase-4; GLP-1, glucagon-like peptide-1; HbA_{1c}, hemoglobin A_{1c}; Met, the group that received metformin treatment; SGLT-2, sodium–glucose cotransporter 2.

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who did not take the drug (control group) were investigated in the present study. The baseline characteristics of these participants are shown in Table 1. A total of 36 (57%) and 54 (44%) patients were aged at least 70 years in the control and Met groups, respectively, with this difference not being statistically significant. Age, body mass index, duration of diabetes and hemoglobin A_{1c} level also did not differ significantly between the two groups. The average duration and daily dose of metformin administration were 6.6 ± 3.7 years and 979.5 ± 491.2 mg, respectively.

Table 2 | Serum levels of homocysteine and vitamin B_{12}, blood hemoglobin concentration and mean corpuscular volume for the control group and the group that received metformin treatment

Parameter	Control	Met	*P*
Homocysteine (nmol/mL)	14.3 ± 4.8	14.7 ± 6.9	0.67
VB12 (pg/mL)	521.8 ± 285.6	518.4 ± 293.6	0.94
Hemoglobin (g/dL)	13.9 ± 1.6	13.5 ± 1.3	0.07
MCV (fL)	93.0 ± 3.7	92.5 ± 4.8	0.49

Data are means ± standard deviation. *P* values were determined with the unpaired Student's *t*-test. MCV, mean corpuscular volume; Met, the group that received metformin treatment; VB12, vitamin B_{12}.

Serum VB12 and homocysteine concentrations, as well as blood hemoglobin level and MCV, in the Met and control groups

The serum concentrations of homocysteine and VB12 did not differ significantly between the control and Met groups (Table 2). The blood hemoglobin level and MCV were also similar in the two groups.

Correlation between measured parameters and either the daily dose or duration of metformin treatment

Within the Met group, serum homocysteine and VB12 levels were positively and negatively correlated with the daily dose of metformin, respectively (Figure 1a). Neither blood hemoglobin level nor MCV was correlated with the daily dose of metformin. The duration of metformin administration was not significantly correlated with any of the parameters tested (Figure 1b).

Subset analysis of elderly participants

Subset analysis with elderly participants (aged at least 70 years) showed that the serum concentrations of homocysteine and VB12 did not differ significantly between the control and Met groups (Table 3). The blood hemoglobin level was significantly

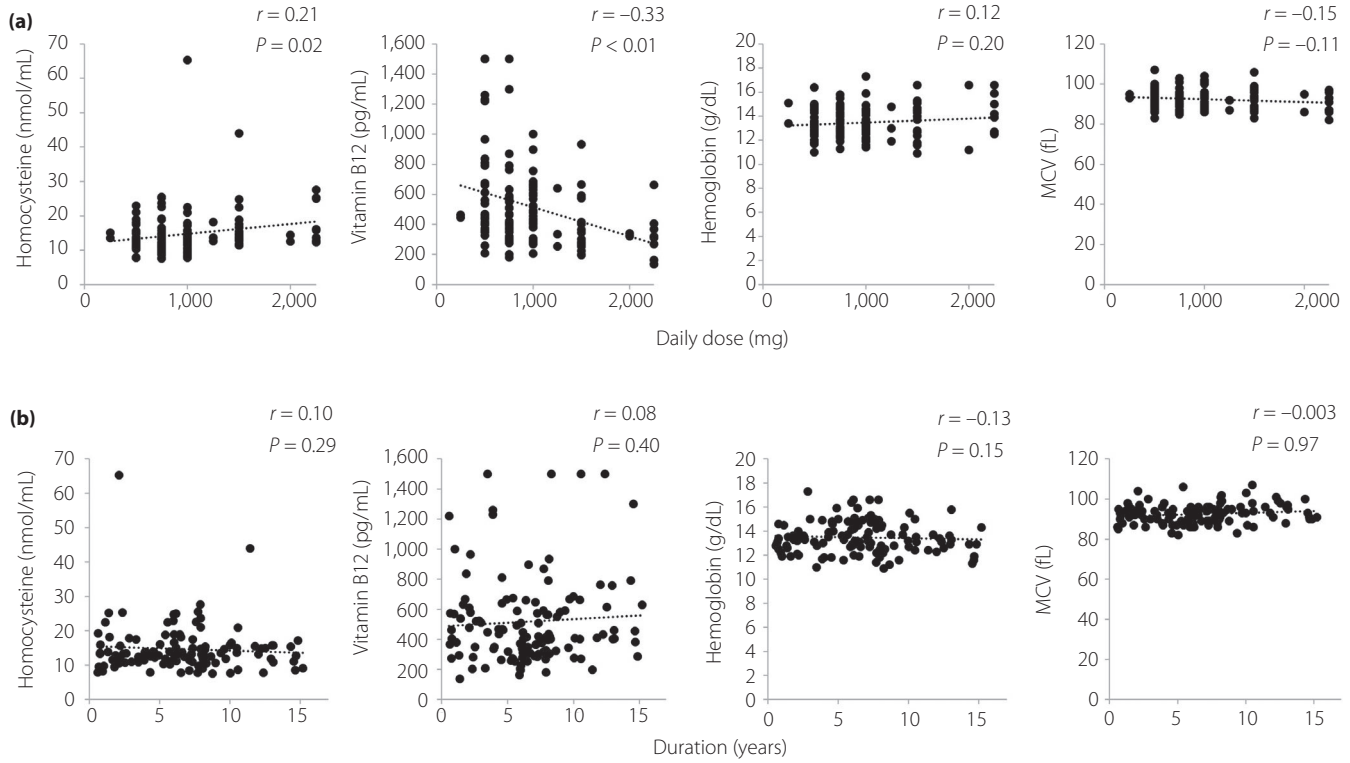

Figure 1 | Correlation analysis for all study participants in the group that received metformin treatment. The correlation between (a) the daily dose of metformin or (b) the duration of metformin administration and either serum homocysteine or vitamin B_{12} levels, blood hemoglobin level or mean corpuscular volume (MCV) was analyzed. Dashed lines are regression lines. Pearson's correlation coefficient (*r*) and *P*-values are shown.

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Table 3 | Serum levels of homocysteine and vitamin B_{12}, blood hemoglobin concentration and mean corpuscular volume for elderly participants in the control group and the group that received metformin treatment

Parameter	Control	Met	*P*
Homocysteine (nmol/mL)	13.9 ± 4.3	14.3 ± 4.6	0.70
VB12 (pg/mL)	550.1 ± 303.9	541.1 ± 330.0	0.90
Hemoglobin (g/dL)	13.6 ± 1.4	13.0 ± 1.1	<0.05
MCV (fL)	92.8 ± 3.3	93.0 ± 5.1	0.85

Data are means ± standard deviation. *P*-values were determined with the unpaired Student's *t*-test. MCV, mean corpuscular volume; Met, the group that received metformin treatment; VB12, vitamin B_{12}.

lower in the Met group than in the control group, whereas MCV was similar in the two groups.

In correlation analysis with elderly participants in the Met group, the serum concentration of VB12 was negatively correlated with the daily dose of metformin (Figure 2a). The duration of metformin administration was not significantly correlated with any of the parameters tested (Figure 2b).

DISCUSSION

Here, we have measured the serum concentration of homocysteine, a marker for the activity of VB12, in Japanese individuals with type 2 diabetes mellitus taking metformin or not. We found that the serum homocysteine level did not differ significantly between the two groups of participants, consistent with the results of a previous study carried out in Japan[13].

Several studies carried out in countries other than Japan have shown that treatment with metformin was associated with a decrease in VB12 and an increase in homocysteine levels[2–7]. The reason for this apparent inconsistency between these previous and our present findings is unclear, but it might be due, at least in part, to differences in the dose of metformin. The average daily dose of metformin was available in three of the previous studies, and was 1,700[2], 2,200[5] and 2,550[4] mg, whereas that in the present study was 979.5 mg. Given that VB12 levels in the body are dependent on food intake, the risk of VB12 deficiency during metformin administration might also be influenced by diet and nutritional status. The dietary source of VB12 in Japan is mostly fish and shellfish[25,26]. Serum VB12 levels were found to be significantly greater in individuals eating a fish-based diet than in those eating a meat diet[27], with fish and shellfish being important contributors to human VB12

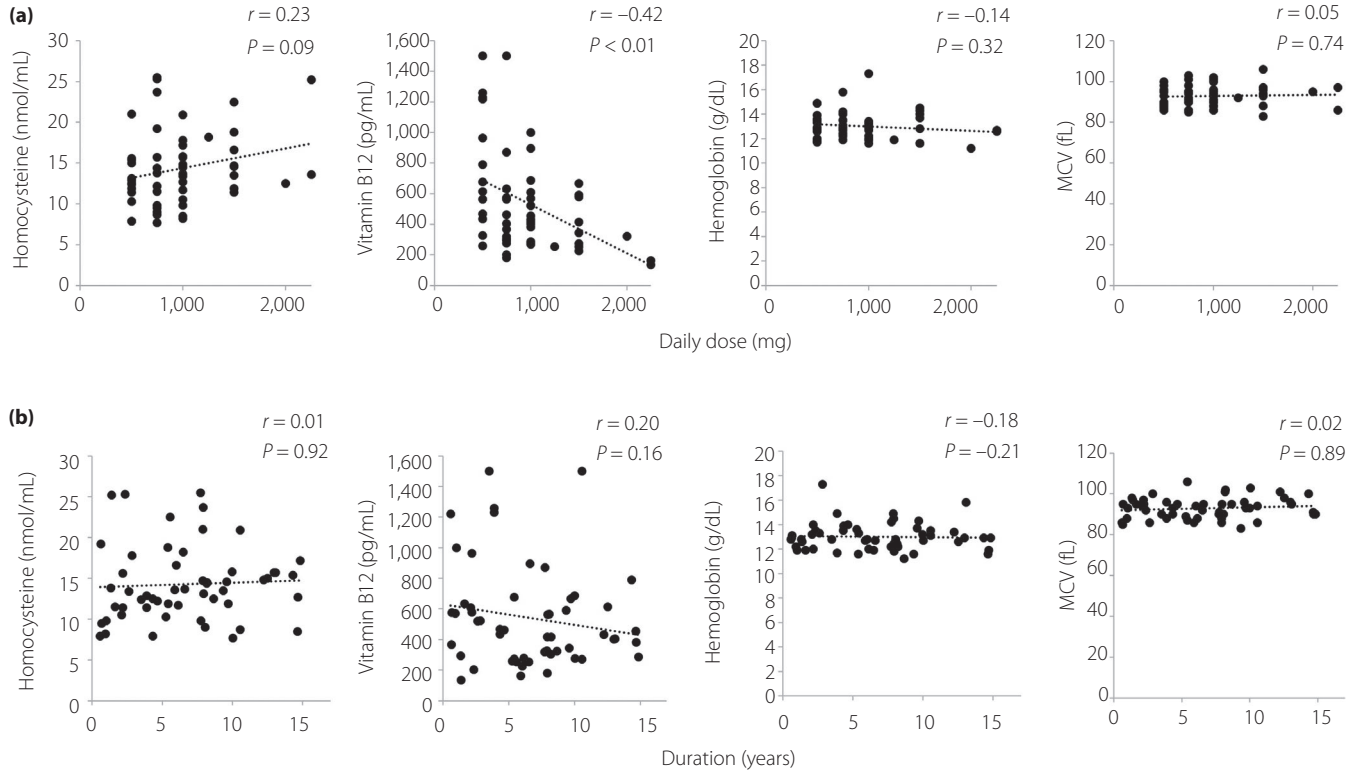

Figure 2 | Correlation analysis for elderly participants in the group that received metformin treatment. The correlation between (a) the daily dose of metformin or (b) the duration of metformin administration and either serum homocysteine or vitamin B_{12} levels, blood hemoglobin level or mean corpuscular volume (MCV) was analyzed. Dashed lines are regression lines. Pearson's correlation coefficient (*r*) and *P*-values are shown.

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status[28,29]. It is thus possible that populations that preferentially consume fish and shellfish, such as the Japanese population, are at low risk of VB12 deficiency.

We found that the dose, but not the duration, of metformin treatment was significantly correlated with the serum levels of both homocysteine and VB12, indicating that not only the circulating level, but also the biological activity of VB12, was affected by high doses of the drug. However, neither blood hemoglobin concentration nor MCV was correlated with metformin dose. It is thus possible that, whereas high doses of metformin reduce VB12 levels, the extent of this effect is not sufficient to impair hematopoiesis in Japanese individuals with type 2 diabetes mellitus.

Elderly people are at a higher risk of VB12 deficiency[21]. We have shown here, however, that the serum level of VB12, as well as that of homocysteine, was not significantly altered by metformin administration in the elderly subset of our study participants. In contrast, the blood hemoglobin concentration was significantly lower in the Met group than in the control group of elderly patients. In general, anemia triggered by VB12 deficiency is associated with an increase in MCV. Given that MCV was similar in the two groups, the decrease in hemoglobin level in the elderly metformin users might have been attributable not solely to VB12 deficiency, but also to other unidentified factors.

There were several limitations to the present study. The patient number was thus relatively small, and the retrospective nature of the study did not allow definitive conclusions to be drawn regarding the relationship between metformin use and VB12 deficiency. The average dose of metformin in the present study was 979.5 mg/day, which is much smaller than that in previous studies. Whereas the mean duration of metformin use in the present study (6.6 years) was longer than that 2.8 years in a previous study carried out in Japan[13], and we did not detect a correlation between the duration of metformin administration and VB12-related parameters, whether longer use of metformin might influence VB12 status in the body remains unknown. Given that just 9 years have elapsed since the maximum dose of metformin in Japan was increased to a level similar to that in many other countries (2,250 mg/day), further studies will be required to clarify the effects of long-term use of this antidiabetic drug.

In conclusion, the present findings suggest that the risk of VB12 deficiency during treatment with metformin is relatively low in Japanese individuals with type 2 diabetes mellitus. However, high doses of metformin might be a risk factor not only for VB12 deficiency, but also anemia, especially in the elderly population. Measurement of VB12 levels, as well as erythrocyte-related parameters, is thus advisable during the administration of metformin.

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DISCLOSURE

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