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## ASSOCIATION BETWEEN VITAMIN B12 DEFICIENCY AND METFORMIN USE: A CROSS-SECTIONAL STUDY IN A TERTIARY HOSPITAL

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# ASSOCIATION BETWEEN VITAMIN B12 DEFICIENCY AND METFORMIN USE: A CROSS-SECTIONAL STUDY IN A TERTIARY HOSPITAL

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14 ABSTRACT

15 Background: Vitamin B12 deficiency is a common and neglected adverse effect  
16 experienced by type 2 diabetic patients treated with metformin. A lack of vitamin B12 may  
17 have severe consequences, such as fatigue, macrocytic anemia, and worsened peripheral  
18 diabetic neuropathy. This study aims to identify the rate of vitamin B12 deficiency and the  
19 associated factors in type 2 diabetic patients treated with metformin.

20 Materials and methods: This cross-sectional study was conducted with 145 type 2 diabetic  
21 patients treated with metformin. Levels of vitamin B12 and associated clinical and  
22 paraclinical parameters were measured and collected.

23 Results: The study population's mean age was 61.5, of which 51.7% were female. The  
24 median duration of metformin use was 10 years, and the median metformin dose was 1700  
25 mg. The vitamin B12 deficiency rate was 22.1%. HbA1c, duration of metformin use, and  
26 metformin dose were independent factors statistically associated with vitamin B12  
27 deficiency.

28 Conclusion: This study identified the rate of vitamin B12 deficiency and associated factors  
29 in type 2 diabetic patients treated with metformin. These findings can be helpful in  
30 screening patients and replacing vitamin B12 in high risk populations with vitamin B12  
31 deficiency.

32 Keywords: metformin; vitamin B12 deficiency; type 2 diabetes mellitus; Vietnam

## INTRODUCTION

Metformin belongs to the biguanide class and is synthesized from galegine, an isoprenyl derivative of guanidine found in the perennial herb French lilac (*Galega officinalis*)<sup>1</sup>. Metformin is recommended in most treatment guidelines as the first-line drug for the management of type 2 diabetes, based on its effectiveness in blood glucose control, low cost, safety, and cardiovascular benefits, as demonstrated by the UK Prospective Diabetes Study<sup>2,3</sup>.

The drug's effectiveness is dose-dependent, and the mechanism of action of metformin is not fully understood. Extensive evidence obtained in clinical studies and animal models suggests that the main function of metformin in regulating glucose homeostasis is to inhibit hepatic glucose production through the regulation of hepatic gluconeogenesis and glycogenolysis<sup>4,5</sup>. Metformin also improves cellular sensitivity to insulin, thereby enhancing peripheral glucose uptake, mainly in skeletal muscle, and significantly reducing fasting plasma insulin levels<sup>6</sup>. Improvements in insulin sensitivity with metformin may be due to its effects on insulin receptor expression and tyrosine kinase activity.

The most common adverse effects of metformin are gastrointestinal symptoms, such as diarrhea, nausea, and/or abdominal discomfort<sup>7</sup>. These symptoms are usually mild, transient, and dose-dependent. A rare but more concerning adverse effect of biguanides is lactic acidosis. Risk factors for metformin-induced lactic acidosis include conditions that increase lactate production, such as severe infections, severe liver or kidney disease, recent surgery, and any condition causing hypoxia or decreased tissue perfusion<sup>8,9</sup>.

In 1969, Berchtold et al. first reported vitamin B12 deficiency in patients using metformin, resulting from decreased gastrointestinal vitamin B12 absorption<sup>10</sup>. This effect can occur as early as the third month of using metformin. Several mechanisms have been proposed to explain the effect of metformin, including changes in small intestinal motility leading to overgrowth of intestinal bacteria, competitive inhibition and inactivation of vitamin B12 absorption capacity, alteration of intrinsic factor levels, and interaction with the

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intracellular cubilin receptor<sup>11,12</sup>. Of these, the most widely accepted mechanism is the inhibition of the calcium-dependent binding of the B12-intrinsic factor complex to the cubilin receptor in the ileum. The HOME study is one of the first clinical trials that confirmed the causative effect of vitamin B12 deficiency by metformin. The results showed that metformin usage at a dose of 850 mg three times daily reduces vitamin B12 levels by 19% compared to a placebo<sup>13</sup>.

Type 2 diabetes mellitus is a complicated, heterogeneous, and multifactorial disease of which treatment often requires the combination of metformin and other medications<sup>14–19</sup>, however, there are few studies on vitamin B12 deficiency related to metformin use in Vietnam. In 2023, Huynh et al. conducted a cross-sectional study on the prevalence of vitamin B12 deficiency in type 2 diabetic patients using metformin at the Vinmec Central Park Hospital, a private outpatient clinic in Ho Chi Minh City<sup>20</sup>. Of the 156 type 2 diabetic patients treated with metformin, the rate of vitamin B12 deficiency was 18.6%. Vitamin B12 deficiency may differ depending on region, diet, and studied population; this study set out to investigate the rate of vitamin B12 deficiency and its associated factors in type 2 diabetic patients treated with metformin in Cho Ray Hospital, one of the largest tertiary hospitals in Vietnam.

## MATERIALS AND METHODS

### 1. Participant recruitment

The protocol for this cross-sectional study was approved by the Ethical Committee of Cho Ray Hospital (approval number 1711/CN-HDDĐ). A sample size calculation was performed to estimate the rate of vitamin B12 deficiency in the target population using the formula below:

$$N = Z^2_{(1-\frac{\alpha}{2})} \frac{p(1-p)}{d^2}$$

In which,  $Z = 1.96$ ,  $p = 0.095$  (based on a similar study by Ko et al.<sup>21</sup>), and  $d = 0.05$ . The estimated patient number was 132. Recruitment took place from March to August 2024. The main inclusion criteria were type 2 diabetic patients treated with metformin for at least 3 months with unchanged dose in the last 3 months. Patients were excluded from the study if they had (i) a recent acute illness, such as severe infection, cancer, or acute coronary syndrome, within the past 3 months, or (ii) serious organ damage defined as cirrhosis, liver cell damage with aspartate aminotransferase or alanine aminotransferase increased  $\geq 3$  times the upper limit of normal, symptoms of heart failure, impaired renal function (serum creatinine levels  $>1.7$  mg/dL for men and  $>1.5$  mg/dL for women), estimated glomerular filtration rate (eGFR)  $< 30$  mL/min/1.73 m<sup>2</sup>, or (iii) a history of partial or total gastrectomy or pancreatectomy or colectomy. Patients were also excluded from the study if they were diagnosed with (i) gastrointestinal malabsorption disorders, such as chronic colitis or irritable bowel syndrome, or (ii) pernicious anemia or other hematological disorders. Finally, patients were excluded from the study if they were currently on vitamin B12 or multivitamin replacement, or they had been treated with proton-pump inhibitors or H2-receptor antagonists for at least 12 months consecutively, or they had followed a vegetarian/vegan diet for 3 years.

All the selected patients gave their informed written consent upon participating in the study. Baseline characteristics of the patients were recorded (age, gender, weight, height,



body mass index (BMI), blood pressure, waist and hip circumference, duration of type 2 diabetes, duration of metformin use, and metformin dose). Hypertension was identified based on the criteria of the American College of Cardiology/American Heart Association Task Force in 2017<sup>22</sup>, or if the patients were currently on antihypertensive drugs. Diabetic peripheral neuropathies were diagnosed based on patient history or the diagnostic criteria of the Toronto Diabetic Neuropathy Expert Group<sup>23</sup>. The metformin usage index (MUI) was calculated by multiplying the daily metformin dose (mg) by usage duration (year) and dividing by 1000<sup>24</sup>.

## 2. Paraclinical measurements

Fasting blood samples for all the participants were sent to the central laboratory of the Department of Biochemistry, Cho Ray Hospital. Vitamin B12 and folic acid levels were measured using an Architect i2000SR Immunoassay analyzer (Abbott Laboratories, Chicago, USA). Vitamin B12 deficiency was defined as a vitamin B12 level < 300pg/ml with a normal level of folic acid ( $\geq 4\text{ng/ml}$ ). An ADVIA 1800 Chemistry System (Siemens Healthineers, Erlangen, Germany) was used to measure other biochemical parameters, including fasting plasma glucose, creatinine, AST, ALT, and lipid profile. HbA1c was measured using a G8 HPLC analyzer (Tosoh Corporation, Tokyo, Japan). Total blood count was obtained using a DxH 600 Hematology Analyzer (Beckman Coulter).

## 3. Statistical analysis

Continuous variables were represented either as means  $\pm$  standard deviation (SD) or as medians with interquartile ranges (IQR), depending on their distribution. These variables were compared using either T-tests or Mann-Whitney U tests. Qualitative variables were characterized by frequencies and counts. Chi-square tests were used to compare categorical variables between groups. To investigate the associations between vitamin B12 deficiency and patients' characteristics, univariate and multivariate logistic regression was applied to calculate odds ratios (OR) and 95% confidence intervals (CI). A p-value of equal or less than 0.05 was considered statistically significant in all analyses. Data analysis was

132 performed using SPSS Statistics for Windows version 20.0 (IBM Corporation, Armonk,  
133 NY, USA)

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RESULTS

A total of 145 patients participated in this study. The baseline characteristics of participants are described in Table 1. Briefly, the mean age of the studied population was 61.5, with 51.7% females. The median duration of metformin use was 10 years, the median metformin dose was 1700 mg, and the mean level of HbA1c was 8.1%. The rate of vitamin B12 deficiency was 22.1% (32 out of 145 participants). We could not detect any cases of macrocytic anemia.

There were no differences between the groups with and without vitamin B12 deficiency in terms of age, gender, BMI, hemoglobin level, MCV, creatinine level, and lipid profile. Patients with vitamin B12 deficiency had significantly longer duration of type 2 diabetes, longer duration of metformin use, higher metformin dose, and lower HbA1c levels than patients without vitamin B12 deficiency.

Univariate logistic analyses also showed that hypertension, duration of metformin use, metformin dose, HbA1c level, and MUI were statistically associated with vitamin B12 deficiency in the studied population (Table 2). Notably, a duration of metformin use > median (10 years), metformin dose > median dose (1700 mg/day), and MUI ≥ 10 were strongly associated with vitamin B12 deficiency with OR (95% CI) = 8.539 (2.459–29.648), 4.942 (1.777–13.749), and 5.095 (1.459–17.788), respectively. After adjusting for covariates using continuous and binomial variable models, HbA1c levels, duration of metformin use, and metformin dose were still independently associated with vitamin B12 deficiency (Table 3).

## DISCUSSION

In our study, the vitamin B12 deficiency rate was 22.1% in type 2 diabetic Vietnamese patients treated with metformin for at least 3 months. This deficiency rate may vary between countries and ethnicities, and previous studies have reported a wide range of 4.3% to 30% for patients undergoing metformin treatment<sup>21,25–27</sup>. Our study showed similar results to the study by Kim et al. at Yongin Severance Hospital, South Korea, and Gao et al. at 12 tertiary hospitals in China<sup>28,29</sup>. This similarity may reflect consistency between the studies in average age, duration of type 2 diabetes, duration of metformin use, and metformin dosage. However, our results are higher than those of Ko et al. in South Korea, who found a vitamin B12 deficiency rate of 9.5% with a cut-off of vitamin B12 levels  $\leq 300$  pg/mL and no folate deficiency (folate  $> 4$  ng/mL)<sup>21</sup>. These differences in vitamin B12 deficiency rates between studies may come from differences in testing methods, patient characteristics, and the duration and dosage of metformin prescribed. Except for similar average ages, our patients' duration of type 2 diabetes, duration of metformin use, and average metformin dosage were all higher than those in Ko's study, which partly explains why the vitamin B12 deficiency rate in our study is higher. More importantly, the subjects in our study had a longer duration of type 2 diabetes, longer duration of metformin use, and a higher prescribed metformin dose compared to the patients in Ko's study<sup>21</sup>.

Compared with a domestic study in Vietnam by Huynh et al. conducted at Vinmec Central Park Hospital, Ho Chi Minh City in 2023, the vitamin B12 deficiency rate in our study is higher<sup>20</sup>. This difference can be explained by differences in the characteristics of the subjects and the research context. Huynh's study mainly focused on patients undergoing health checks at a private hospital. These are usually patients with better economic conditions, better access to high quality medical services, and who potentially pay more attention to their diet. Thus, the vitamin B12 deficiency rate in their study is lower. Moreover, the patients in Huynh's study were younger, had lower metformin doses, and a shorter duration of metformin treatment. In contrast, our study was conducted at Cho Ray Hospital, a large tertiary hospital that receives patients with complex and more severe

medical conditions. Therefore, the patients in our study had a longer disease duration, longer duration of metformin use, higher metformin dose, and a higher rate of vitamin B12 deficiency.

Our study found that metformin dose and duration of metformin use were statistically associated with vitamin B12 deficiency. These findings are similar to previous studies and emphasize again the important role of vitamin B12 screening in patients with long-term use and/or high doses of metformin<sup>28,30</sup>. In addition, MUI  $\geq 10$  was found to be an independent factor that is statistically associated with vitamin B12 deficiency, suggesting that MUI can be used as a reliable and alternative parameter to decide when to screen for vitamin B12 deficiency rather than using duration of metformin use or metformin dose alone. We could not use a cut-off value of 5 years for the duration of metformin use and 5 for MUI because the number of participants with the duration of metformin use of less than 5 years was small (only 21 out of 145 cases), leading to underpowered statistical analyses. Interestingly, lower HbA1c was statistically associated with vitamin B12 deficiency in this study. This outcome may result from the fact that patients achieving lower HbA1c were intensively treated with higher dose of metformin.

Our study has several limitations. First, we did not measure methylmalonic acid or homocysteine levels; these markers reflect early vitamin B12 deficiency in tissues. Second, data were collected in a single center and may not represent the landscape of type 2 diabetic patients in the southern region of Vietnam.

In conclusion, our study showed that the rate of vitamin B12 deficiency was up to 22.1% in type 2 diabetic patients with a median of 10 years of metformin use and 1700 mg of metformin dose. This rate presents a warning and requires routine screening and replacement of vitamin B12 in metformin-treated patients. Further multi-center studies are necessary to understand the landscape of vitamin B12 deficiency in patients treated with metformin in Vietnam.

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214 **Data availability statement:** The data that support the findings of this study are available  
215 within the article text and tables.

216 **Ethical approval:** This study was approved by the Ethics Committee for Medical Research  
217 of Cho Ray Hospital (approval number 1711/CN-HĐĐĐ).

218 **Conflict of interest:** The authors of this work have nothing to disclose.  
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314 Table 1. Baseline characteristics of participants.

Characteristics	Total (N=145)	No vitamin B12 deficiency (N=113)	Vitamin B12 deficiency (N=32)	p-value
Age (years) Mean $\pm$ SD	61.5 $\pm$ 11.0	60.6 $\pm$ 11.0	64.5 $\pm$ 10.7	0.075
Gender				0.844
Female, N (%)	75 (51.7)	59 (52.5)	16 (50)	
Male, N (%)	70 (48.3)	54 (47.8)	16 (50)	
BMI (kg/m <sup>2</sup> ) Mean $\pm$ SD	22.9 $\pm$ 1.9	22.9 $\pm$ 2.1	22.8 $\pm$ 1.5	0.755
Hypertension				<b>0.031</b>
Yes, N (%)	105 (61.9)	77 (68.1)	28 (87.5)	
No, N (%)	40 (38.1)	36 (31.9)	4 (12.5)	
Diabetic peripheral neuropathy				0.972
Yes, N (%)	54 (37.2)	42 (37.2)	12 (37.5)	
No, N (%)	91 (62.8)	71 (62.8)	20 (62.5)	
Diabetes duration (years) Median [Q1-Q3]	10.0 [6.0 – 10.0]	10.0 [5.5 – 15.0]	12.0 [10.0 – 15.0]	<b>0.029</b>
Metformin use duration (years) Median [Q1-Q3]	10.0 [6.0 – 10.0]	10.0 [5.5 – 15.0]	12.0 [10.0 – 15.0]	<b>0.044</b>
Metformin dose (mg) Median [Q1-Q3]	1700.0 [1000.0 – 1850.0]	1700.0 [1000.0 – 1700.0]	1700.0 [1700.0 – 2000.0]	<b>0.001</b>
Hb (g/dl) Mean $\pm$ SD	13.6 $\pm$ 1.6	13.6 $\pm$ 1.6	13.4 $\pm$ 1.5	0.674
MCV (fL) Mean $\pm$ SD	87.6 $\pm$ 6.6	87.1 $\pm$ 7.0	89.5 $\pm$ 5.1	0.071
Fasting plasma glucose (mg/dl) Mean $\pm$ SD	158.4 $\pm$ 72.1	162.4 $\pm$ 77.2	144.2 $\pm$ 48.2	0.208
HbA1c (%) Mean $\pm$ SD	8.1 $\pm$ 1.9	8.3 $\pm$ 2.0	7.4 $\pm$ 1.6	<b>0.021</b>

Creatinine (mg/dl) Median [Q1-Q3]	0.9 [0.7 – 1.0]	0.9 [0.7 – 1.0]	0.9 [0.8 – 1.0]	0.274
Total cholesterol (mmol/l) Median [Q1-Q3]	146.0 [114.0 – 180.0]	148.0 [114.5 – 181.5]	138.0 [107.3 – 164.5]	0.292
Triglyceride (mmol/l) Median [Q1-Q3]	163.0 [109.5 – 238.0]	163.0 [107.0 – 231.0]	162.0 [131.8 – 307.3]	0.166
HDL-C (mmol/l) Median [Q1-Q3]	44.0 [38.0 – 51.0]	44.0 [39.0 – 52.0]	44.0 [35.0 – 49.0]	0.425
LDL-C (mmol/l) Median [Q1-Q3]	75.0 [49.5 – 107.5]	77.0 [53.8 – 112.5]	64.2 [48.2 – 94.9]	0.242
Vitamin B12 level (pg/ml) Median [Q1-Q3]	478.0 [323.0 – 608.0]	514.0 [416.0 – 667.5]	233.0 [196.5 – 273.8]	< <b>0.001</b>
Folate level (µmol/mL) Mean ± SD	11.5 ± 3.5	11.6 ± 3.5	11.1 ± 3.5	0.457

BMI: body mass index, Hb: hemoglobin, MCV: mean cell volume, HDL-C: high-density lipoprotein-cholesterol, LDL-C: low-density lipoprotein-cholesterol.

Table 2. Univariate logistic regression analysis of factors associated with vitamin B12 deficiency.

Variables	OR (95% CI)	p-value
Age	1.034 (0.996 – 1.074)	0.077
Gender	1.093 (0.498 – 2.396)	0.825
BMI	0.967 (0.786 – 1.190)	0.753
Hypertension	3.268 (1.068 – 10.000)	<b>0.038</b>
Duration of diabetes	1.047 (0.989 – 1.108)	0.115
Duration of metformin use	1.043 (0.985 – 1.103)	0.147
Long-term use of metformin (Duration > median duration)	8.539 (2.459 – 29.648)	<b>&lt; 0.001</b>
Metformin dose	1.002 (1.001 – 1.003)	<b>0.002</b>
Metformin dose > median dose	4.942 (1.777 – 13.749)	<b>0.002</b>
Hb	0.949 (0.744 – 1.210)	0.671
MCV	1.068 (0.993 – 1.148)	0.075
Fasting plasma glucose	0.995 (0.988 – 1.003)	0.210
HbA1c	0.733 (0.557 – 0.964)	<b>0.026</b>
MUI	1.043 (1.009 – 1.078)	<b>0.014</b>
MUI ≥ 10	5.095 (1.459 – 17.788)	<b>0.011</b>

BMI: body mass index, Hb: hemoglobin, MCV: mean cell volume, MUI: metformin usage index.

Table 3. Multivariate logistic regression analysis of factors associated with vitamin B12 deficiency.

Model	Variables	OR (95% CI)	p-value
Model 1	HbA1c	0.624 (0.455 – 0.856)	0.003
	Duration of metformin use	1.076 (1.005 – 1.151)	0.037
	Metformin dose	1.002 (1.001 – 1.004)	0.002
Model 2	HbA1c	0.631 (0.460 – 0.865)	0.004
	Long-term use of metformin (Duration > median duration)	10.078 (2.729 – 37.219)	0.001
	Metformin dose > median dose	4.429 (1.475 – 13.303)	0.008
Model 3	HbA1c	0.647 (0.480 – 0.874)	0.005
	MUI ≥ 10	7.644 (2.109 – 27.707)	0.002

MUI: metformin usage index.

# BMJ Open

## ASSOCIATION BETWEEN VITAMIN B12 DEFICIENCY AND METFORMIN USE IN TYPE 2 DIABETIC PATIENTS: A CROSS-SECTIONAL STUDY IN A SOUTH VIETNAM TERTIARY HOSPITAL

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2 IN TYPE 2 DIABETIC PATIENTS: A CROSS-SECTIONAL STUDY IN A SOUTH  
3 VIETNAM TERTIARY HOSPITAL

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15 ABSTRACT

16 Objectives: Vitamin B12 deficiency is a common and neglected adverse effect experienced  
17 by type 2 diabetic patients treated with metformin. A lack of vitamin B12 may have severe  
18 consequences, such as fatigue, macrocytic anemia, and worsened peripheral diabetic  
19 neuropathy. This study aims to identify the rate of vitamin B12 deficiency and the  
20 associated factors in type 2 diabetic patients treated with metformin.

21 Design: cross-sectional study.

22 Setting and participants: This study involved 145 type 2 diabetic patients treated with  
23 metformin in Cho Ray hospital, a tertiary healthcare center in the South Vietnam. The  
24 levels of vitamin B12 and associated clinical and paraclinical parameters were measured  
25 and collected.

26 Results: The study population's mean age was 61.5, of which 51.7% were female. The  
27 median duration of metformin use was 10 years, and the median metformin dose was 1700  
28 mg. The vitamin B12 deficiency rate was 22.1%. HbA1c, duration of metformin use, and  
29 metformin dose were independent factors statistically associated with vitamin B12  
30 deficiency.

31 Conclusions: This study identified the rate of vitamin B12 deficiency and associated factors  
32 in type 2 diabetic patients treated with metformin. These findings can be helpful in  
33 screening patients and replacing vitamin B12 in high risk populations with vitamin B12  
34 deficiency.

35 Keywords: metformin; vitamin B12 deficiency; type 2 diabetes mellitus; Vietnam

36 STRENGTHS AND LIMITATIONS OF THIS STUDY:

- 37 - This study investigated the vitamin B12 deficiency rate and its associated factors in type
- 38 2 diabetic Vietnamese patients treated with metformin, a population with very limited data.
- 39 - The levels of methylmalonic acid and homocysteine were not measured in this study,
- 40 these markers reflect early vitamin B12 deficiency in tissues.

41 - This is a single center, cross-sectional study, therefore, the results may not represent the  
42 prevalence of vitamin B12 deficiency in type 2 diabetic patients treated with metformin in  
43 the southern region of Vietnam.

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INTRODUCTION

Metformin belongs to the biguanide class and is synthesized from galegine, an isoprenyl derivative of guanidine found in the perennial herb French lilac (*Galega officinalis*)<sup>1</sup>. Metformin is recommended in most treatment guidelines as the first-line drug for the management of type 2 diabetes, based on its effectiveness in blood glucose control, low cost, safety, and cardiovascular benefits, as demonstrated by the UK Prospective Diabetes Study<sup>2,3</sup>.

The drug's effectiveness is dose-dependent, and the mechanism of action of metformin is not fully understood. Extensive evidence obtained in clinical studies and animal models suggests that the main function of metformin in regulating glucose homeostasis is to inhibit hepatic glucose production through the regulation of hepatic gluconeogenesis and glycogenolysis<sup>4,5</sup>. Metformin also improves cellular sensitivity to insulin, thereby enhancing peripheral glucose uptake, mainly in skeletal muscle, and significantly reducing fasting plasma insulin levels<sup>6</sup>. Improvements in insulin sensitivity with metformin may be due to its effects on insulin receptor expression and tyrosine kinase activity.

The most common adverse effects of metformin are gastrointestinal symptoms, such as diarrhea, nausea, and/or abdominal discomfort<sup>7</sup>. These symptoms are usually mild, transient, and dose-dependent. A rare but more concerning adverse effect of biguanides is lactic acidosis. Risk factors for metformin-induced lactic acidosis include conditions that increase lactate production, such as severe infections, severe liver or kidney disease, recent surgery, and any condition causing hypoxia or decreased tissue perfusion<sup>8,9</sup>.

In 1969, Berchtold et al. first reported vitamin B12 deficiency in patients using metformin, resulting from decreased gastrointestinal vitamin B12 absorption<sup>10</sup>. This effect can occur as early as the third month of using metformin. Several mechanisms have been proposed to explain the effect of metformin, including changes in small intestinal motility leading to overgrowth of intestinal bacteria, competitive inhibition and inactivation of vitamin B12 absorption capacity, alteration of intrinsic factor levels, and interaction with the

intracellular cubilin receptor<sup>11,12</sup>. Of these, the most widely accepted mechanism is the inhibition of the calcium-dependent binding of the B12-intrinsic factor complex to the cubilin receptor in the ileum. The HOME study is one of the first clinical trials that confirmed the causative effect of vitamin B12 deficiency by metformin. The results showed that metformin usage at a dose of 850 mg three times daily reduces vitamin B12 levels by 19% compared to a placebo<sup>13</sup>.

Type 2 diabetes mellitus is a complicated, heterogeneous, and multifactorial disease of which treatment often requires the combination of metformin and other medications<sup>14–19</sup>, however, there are few studies on vitamin B12 deficiency related to metformin use in Vietnam. In 2023, Huynh et al. conducted a cross-sectional study on the prevalence of vitamin B12 deficiency in type 2 diabetic patients using metformin at the Vinmec Central Park Hospital, a private outpatient clinic in Ho Chi Minh City<sup>20</sup>. Of the 156 type 2 diabetic patients treated with metformin, the rate of vitamin B12 deficiency was 18.6%. Vitamin B12 deficiency may differ depending on region, diet, and studied population; this study set out to investigate the rate of vitamin B12 deficiency and its associated factors in type 2 diabetic patients treated with metformin in Cho Ray Hospital, one of the largest tertiary hospitals in Vietnam.

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MATERIALS AND METHODS

1. Participant recruitment

The protocol for this cross-sectional study was approved by the Ethical Committee of Cho Ray Hospital (approval number 1711/CN-HDDĐ). A sample size calculation was performed to estimate the rate of vitamin B12 deficiency in the target population using the formula below:

$$N = Z^2_{(1-\frac{\alpha}{2})} \frac{p(1-p)}{d^2}$$

In which,  $Z = 1.96$ ,  $p = 0.095$  (based on a similar study by Ko et al.<sup>21</sup>), and  $d = 0.05$ . The estimated patient number was 132. Recruitment took place from March to August 2024. The main inclusion criteria were type 2 diabetic patients treated with metformin for at least 3 months with unchanged dose in the last 3 months. Patients were excluded from the study if they had (i) a recent acute illness, such as severe infection, cancer, or acute coronary syndrome, within the past 3 months, or (ii) serious organ damage defined as cirrhosis, liver cell damage with aspartate aminotransferase or alanine aminotransferase increased  $\geq 3$  times the upper limit of normal, symptoms of heart failure, impaired renal function (serum creatinine levels  $>1.7$  mg/dL for men and  $>1.5$  mg/dL for women), estimated glomerular filtration rate (eGFR)  $< 30$  mL/min/1.73 m<sup>2</sup>, or (iii) a history of partial or total gastrectomy or pancreatectomy or colectomy. Patients were also excluded from the study if they were diagnosed with (i) gastrointestinal malabsorption disorders, such as chronic colitis or irritable bowel syndrome, or (ii) pernicious anemia or other hematological disorders. Finally, patients were excluded from the study if they were currently on vitamin B12 or multivitamin replacement, or they had been treated with proton-pump inhibitors or H2-receptor antagonists for at least 12 months consecutively, or they had followed a vegetarian/vegan diet for 3 years.

All the selected patients gave their informed written consent upon participating in the study. Baseline characteristics of the patients were recorded (age, gender, weight, height,

body mass index (BMI), blood pressure, waist and hip circumference, duration of type 2 diabetes, duration of metformin use, and metformin dose). Hypertension was identified based on the criteria of the American College of Cardiology/American Heart Association Task Force in 2017<sup>22</sup>, or if the patients were currently on antihypertensive drugs. Diabetic peripheral neuropathies were diagnosed based on patient history or the diagnostic criteria of the Toronto Diabetic Neuropathy Expert Group<sup>23</sup>. The metformin usage index (MUI) was calculated by multiplying the daily metformin dose (mg) by usage duration (year) and dividing by 1000<sup>24</sup>.

All the patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research

## 2. Paraclinical measurements

Fasting blood samples for all the participants were sent to the central laboratory of the Department of Biochemistry, Cho Ray Hospital. Vitamin B12 and folic acid levels were measured using an Architect i2000SR Immunoassay analyzer (Abbott Laboratories, Chicago, USA). Vitamin B12 deficiency was defined as a vitamin B12 level < 300pg/ml with a normal level of folic acid ( $\geq 4\text{ng/ml}$ ). An ADVIA 1800 Chemistry System (Siemens Healthineers, Erlangen, Germany) was used to measure other biochemical parameters, including fasting plasma glucose, creatinine, AST, ALT, and lipid profile. HbA1c was measured using a G8 HPLC analyzer (Tosoh Corporation, Tokyo, Japan). Total blood count was obtained using a DxH 600 Hematology Analyzer (Beckman Coulter).

## 3. Statistical analysis

Continuous variables were represented either as means  $\pm$  standard deviation (SD) or as medians with interquartile ranges (IQR), depending on their distribution. These variables were compared using either T-tests or Mann-Whitney U tests. Qualitative variables were characterized by frequencies and counts. Chi-square tests were used to compare categorical variables between groups. To investigate the associations between vitamin B12 deficiency and patients' characteristics, univariate and multivariate logistic regression was applied to

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143     calculate odds ratios (OR) and 95% confidence intervals (CI). A p-value of equal or less  
144     than 0.05 was considered statistically significant in all analyses. Data analysis was  
145     performed using SPSS Statistics for Windows version 20.0 (IBM Corporation, Armonk,  
146     NY, USA)  
147

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## RESULTS

A total of 145 patients participated in this study. The baseline characteristics of participants are described in Table 1. Briefly, the mean age of the studied population was 61.5, with 51.7% females. The median duration of metformin use was 10 years, the median metformin dose was 1700 mg, and the mean level of HbA1c was 8.1%. The rate of vitamin B12 deficiency was 22.1% (32 out of 145 participants). We could not detect any cases of macrocytic anemia.

There were no differences between the groups with and without vitamin B12 deficiency in terms of age, gender, BMI, hemoglobin level, MCV, creatinine level, and lipid profile. Patients with vitamin B12 deficiency had significantly longer duration of type 2 diabetes, longer duration of metformin use, higher metformin dose, and lower HbA1c levels than patients without vitamin B12 deficiency. The medications used for type 2 diabetes treatment including insulin, sulfonyl urea, dipeptidyl peptidase 4 inhibitor (DDP4i), and sodium-glucose cotransporter 2 inhibitor (SGLT2i) were not statistically different between the groups with and without vitamin B12 deficiency.

Univariate logistic analyses also showed that hypertension, duration of metformin use, metformin dose, HbA1c level, and MUI were statistically associated with vitamin B12 deficiency in the studied population (Table 2). Notably, a duration of metformin use > median (10 years), metformin dose > median dose (1700 mg/day), and MUI  $\geq 10$  were strongly associated with vitamin B12 deficiency with OR (95% CI) = 8.539 (2.459–29.648), 4.942 (1.777–13.749), and 5.095 (1.459–17.788), respectively. After adjusting for covariates using continuous and binomial variable models, HbA1c levels, duration of metformin use, and metformin dose were still independently associated with vitamin B12 deficiency (Table 3).



## 174 DISCUSSION

175 In our study, the vitamin B12 deficiency rate was 22.1% in type 2 diabetic Vietnamese  
176 patients treated with metformin for at least 3 months. This deficiency rate may vary  
177 between countries and ethnicities, and previous studies have reported a wide range of 4.3%  
178 to 30% for patients undergoing metformin treatment<sup>21,25–27</sup>. Our study showed similar  
179 results to the study by Kim et al. at Yongin Severance Hospital, South Korea, and Gao et  
180 al. at 12 tertiary hospitals in China<sup>28,29</sup>. This similarity may reflect consistency between the  
181 studies in average age, duration of type 2 diabetes, duration of metformin use, and  
182 metformin dosage. However, our results are higher than those of Ko et al. in South Korea,  
183 who found a vitamin B12 deficiency rate of 9.5% with a cut-off of vitamin B12 levels  $\leq$   
184 300 pg/mL and no folate deficiency (folate  $> 4$  ng/mL)<sup>21</sup>. These differences in vitamin B12  
185 deficiency rates between studies may come from differences in testing methods, patient  
186 characteristics, and the duration and dosage of metformin prescribed. Except for similar  
187 average ages, our patients' duration of type 2 diabetes, duration of metformin use, and  
188 average metformin dosage were all higher than those in Ko's study, which partly explains  
189 why the vitamin B12 deficiency rate in our study is higher. More importantly, the subjects  
190 in our study had a longer duration of type 2 diabetes, longer duration of metformin use,  
191 and a higher prescribed metformin dose compared to the patients in Ko's study<sup>21</sup>.

192 Compared with a domestic study in Vietnam by Huynh et al. conducted at Vinmec Central  
193 Park Hospital, Ho Chi Minh City in 2023, the vitamin B12 deficiency rate in our study is  
194 higher<sup>20</sup>. This difference can be explained by differences in the characteristics of the  
195 subjects and the research context. Huynh's study mainly focused on patients undergoing  
196 health checks at a private hospital. These are usually patients with better economic  
197 conditions, better access to high quality medical services, and who potentially pay more  
198 attention to their diet. Thus, the vitamin B12 deficiency rate in their study is lower.  
199 Moreover, the patients in Huynh's study were younger, had lower metformin doses, and a  
200 shorter duration of metformin treatment. In contrast, our study was conducted at Cho Ray  
201 Hospital, a large tertiary hospital that receives patients with complex and more severe

medical conditions. Therefore, the patients in our study had a longer disease duration, longer duration of metformin use, higher metformin dose, and a higher rate of vitamin B12 deficiency.

Our study found that metformin dose and duration of metformin use were statistically associated with vitamin B12 deficiency. These findings are similar to previous studies and emphasize again the important role of vitamin B12 screening in patients with long-term use and/or high doses of metformin<sup>28,30</sup>. In addition, MUI  $\geq 10$  was found to be an independent factor that is statistically associated with vitamin B12 deficiency, suggesting that MUI can be used as a reliable and alternative parameter to decide when to screen for vitamin B12 deficiency rather than using duration of metformin use or metformin dose alone. We could not use a cut-off value of 5 years for the duration of metformin use and 5 for MUI because the number of participants with the duration of metformin use of less than 5 years was small (only 21 out of 145 cases), leading to underpowered statistical analyses. Interestingly, lower HbA1c was statistically associated with vitamin B12 deficiency in this study. This outcome may result from the fact that patients achieving lower HbA1c were intensively treated with higher dose of metformin.

Our study has several limitations. First, we did not measure methylmalonic acid or homocysteine levels; these markers reflect early vitamin B12 deficiency in tissues. Second, data were collected in a single center and may not represent the landscape of type 2 diabetic patients in the southern region of Vietnam.

In conclusion, our study showed that the rate of vitamin B12 deficiency was up to 22.1% in type 2 diabetic patients with a median of 10 years of metformin use and 1700 mg of metformin dose. This rate presents a warning and requires routine screening and replacement of vitamin B12 in metformin-treated patients. Further multi-center studies are necessary to understand the landscape of vitamin B12 deficiency in patients treated with metformin in Vietnam.

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**Author Contributions:** HP, AN, MD designed the research study. AN recruited the participants for the study. HP and MD analyzed the data. HP, AN, and MD wrote the manuscript. MD is the guarantor.

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**Data availability statement:** Data supporting the findings of this study are available from the corresponding author [MD] on request. Raw data were generated at Cho Ray hospital, Ho Chi Minh City, Vietnam.

**Ethical approval:** This study was approved by the Ethics Committee for Medical Research of Cho Ray Hospital (approval number 1711/CN-HĐĐĐ).

**Conflict of interest:** The authors of this work have nothing to disclose.

**Patient and public involvement:** Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

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336 Table 1. Baseline characteristics of participants.

Characteristics	Total (N=145)	No vitamin B12 deficiency (N=113)	Vitamin B12 deficiency (N=32)	p-value
Age (years) Mean ± SD	61.5 ± 11.0	60.6 ± 11.0	64.5 ± 10.7	0.075
Gender				0.844
Female, N (%)	75 (51.7)	59 (52.5)	16 (50)	
Male, N (%)	70 (48.3)	54 (47.8)	16 (50)	
BMI (kg/m <sup>2</sup> ) Mean ± SD	22.9 ± 1.9	22.9 ± 2.1	22.8 ± 1.5	0.755
Hypertension				<b>0.031</b>
Yes, N (%)	105 (61.9)	77 (68.1)	28 (87.5)	
No, N (%)	40 (38.1)	36 (31.9)	4 (12.5)	
Diabetic peripheral neuropathy				0.972
Yes, N (%)	54 (37.2)	42 (37.2)	12 (37.5)	
No, N (%)	91 (62.8)	71 (62.8)	20 (62.5)	
Diabetes duration (years) Median [Q1-Q3]	10.0 [6.0 – 10.0]	10.0 [5.5 – 15.0]	12.0 [10.0 – 15.0]	<b>0.029</b>
Metformin use duration (years) Median [Q1-Q3]	10.0 [6.0 – 10.0]	10.0 [5.5 – 15.0]	12.0 [10.0 – 15.0]	<b>0.044</b>
Metformin dose (mg) Median [Q1-Q3]	1700.0 [1000.0 – 1850.0]	1700.0 [1000.0 – 1700.0]	1700.0 [1700.0 – 2000.0]	<b>0.001</b>
Hb (g/dl) Mean ± SD	13.6 ± 1.6	13.6 ± 1.6	13.4 ± 1.5	0.674
MCV (fL) Mean ± SD	87.6 ± 6.6	87.1 ± 7.0	89.5 ± 5.1	0.071
Fasting plasma glucose (mg/dl) Mean ± SD	158.4 ± 72.1	162.4 ± 77.2	144.2 ± 48.2	0.208
HbA1c (%) Mean ± SD	8.1 ± 1.9	8.3 ± 2.0	7.4 ± 1.6	<b>0.021</b>

Creatinine (mg/dl) Median [Q1-Q3]	0.9 [0.7 – 1.0]	0.9 [0.7 – 1.0]	0.9 [0.8 – 1.0]	0.274
Total cholesterol (mmol/l) Median [Q1-Q3]	146.0 [114.0 – 180.0]	148.0 [114.5 – 181.5]	138.0 [107.3 – 164.5]	0.292
Triglyceride (mmol/l) Median [Q1-Q3]	163.0 [109.5 – 238.0]	163.0 [107.0 – 231.0]	162.0 [131.8 – 307.3]	0.166
HDL-C (mmol/l) Median [Q1-Q3]	44.0 [38.0 – 51.0]	44.0 [39.0 – 52.0]	44.0 [35.0 – 49.0]	0.425
LDL-C (mmol/l) Median [Q1-Q3]	75.0 [49.5 – 107.5]	77.0 [53.8 – 112.5]	64.2 [48.2 – 94.9]	0.242
Vitamin B12 level (pg/ml) Median [Q1-Q3]	478.0 [323.0 – 608.0]	514.0 [416.0 – 667.5]	233.0 [196.5 – 273.8]	< <b>0.001</b>
Folate level (µmol/mL) Mean ± SD	11.5 ± 3.5	11.6 ± 3.5	11.1 ± 3.5	0.457
Insulin treatment Yes, N (%) No, N (%)	50 (34.5) 95 (65.5)	38 (33.6) 75 (66.4)	12 (37.5) 20 (62.5)	0.684
Sulfonyl urea treatment Yes, N (%) No, N (%)	45 (31.0) 100 (69.0)	33 (29.2) 80 (70.8)	12 (37.5) 20 (62.5)	0.370
DPP4i treatment Yes, N (%) No, N (%)	116 (80.0) 29 (20.0)	87 (77.0) 26 (23.0)	29 (90.6) 3 (9.4)	0.089
SGLT2i treatment Yes, N (%) No, N (%)	88 (60.7) 57 (39.3)	72 (63.7) 41 (36.3)	16 (50.0) 16 (50.0)	0.161

BMI: body mass index, DPP4i: dipeptidyl peptidase 4 inhibitor, Hb: hemoglobin, MCV: mean cell volume, HDL-C: high-density lipoprotein-cholesterol, LDL-C: low-density lipoprotein-cholesterol, SGLT2i: sodium-glucose cotransporter 2 inhibitor.



Table 2. Univariate logistic regression analysis of factors associated with vitamin B12 deficiency.

Variables	OR (95% CI)	p-value
Age	1.034 (0.996 – 1.074)	0.077
Gender	1.093 (0.498 – 2.396)	0.825
BMI	0.967 (0.786 – 1.190)	0.753
Hypertension	3.268 (1.068 – 10.000)	<b>0.038</b>
Duration of diabetes	1.047 (0.989 – 1.108)	0.115
Duration of metformin use	1.043 (0.985 – 1.103)	0.147
Long-term use of metformin (Duration > median duration)	8.539 (2.459 – 29.648)	<b>&lt; 0.001</b>
Metformin dose	1.002 (1.001 – 1.003)	<b>0.002</b>
Metformin dose > median dose	4.942 (1.777 – 13.749)	<b>0.002</b>
Hb	0.949 (0.744 – 1.210)	0.671
MCV	1.068 (0.993 – 1.148)	0.075
Fasting plasma glucose	0.995 (0.988 – 1.003)	0.210
HbA1c	0.733 (0.557 – 0.964)	<b>0.026</b>
MUI	1.043 (1.009 – 1.078)	<b>0.014</b>
MUI ≥ 10	5.095 (1.459 – 17.788)	<b>0.011</b>

BMI: body mass index, Hb: hemoglobin, MCV: mean cell volume, MUI: metformin usage index.

Table 3. Multivariate logistic regression analysis of factors associated with vitamin B12 deficiency.

Model	Variables	OR (95% CI)	p-value
Model 1	HbA1c	0.624 (0.455 – 0.856)	0.003
	Duration of metformin use	1.076 (1.005 – 1.151)	0.037
	Metformin dose	1.002 (1.001 – 1.004)	0.002
Model 2	HbA1c	0.631 (0.460 – 0.865)	0.004
	Long-term use of metformin (Duration > median duration)	10.078 (2.729 – 37.219)	0.001
	Metformin dose > median dose	4.429 (1.475 – 13.303)	0.008
Model 3	HbA1c	0.647 (0.480 – 0.874)	0.005
	MUI $\geq$ 10	7.644 (2.109 – 27.707)	0.002

MUI: metformin usage index.

# BMJ Open

## ASSOCIATION BETWEEN VITAMIN B12 DEFICIENCY AND METFORMIN USE IN TYPE 2 DIABETIC PATIENTS: A CROSS-SECTIONAL STUDY IN A SOUTH VIETNAM TERTIARY HOSPITAL

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2 IN TYPE 2 DIABETIC PATIENTS: A CROSS-SECTIONAL STUDY IN A SOUTH  
3 VIETNAM TERTIARY HOSPITAL

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15 ABSTRACT

16 Objectives: Vitamin B12 deficiency is a common and neglected adverse effect experienced  
17 by type 2 diabetic patients treated with metformin. A lack of vitamin B12 may have severe  
18 consequences, such as fatigue, macrocytic anemia, and worsened peripheral diabetic  
19 neuropathy. This study aims to identify the rate of vitamin B12 deficiency and the  
20 associated factors in type 2 diabetic patients treated with metformin.

21 Design: cross-sectional study.

22 Setting and participants: This study involved 145 type 2 diabetic patients treated with  
23 metformin in Cho Ray hospital, a tertiary healthcare center in the South Vietnam. The  
24 levels of vitamin B12 and associated clinical and paraclinical parameters were measured  
25 and collected.

26 Results: The study population's mean age was 61.5, of which 51.7% were female. The  
27 median duration of metformin use was 10 years, and the median metformin dose was 1700  
28 mg. The vitamin B12 deficiency rate was 22.1%. HbA1c, duration of metformin use, and  
29 metformin dose were independent factors statistically associated with vitamin B12  
30 deficiency.

31 Conclusions: This study identified the rate of vitamin B12 deficiency and associated factors  
32 in type 2 diabetic patients treated with metformin. These findings can be helpful in  
33 screening patients and replacing vitamin B12 in high risk populations with vitamin B12  
34 deficiency.

35 Keywords: metformin; vitamin B12 deficiency; type 2 diabetes mellitus; Vietnam

36 STRENGTHS AND LIMITATIONS OF THIS STUDY:

- 37 - This study investigated the vitamin B12 deficiency rate and its associated factors in type
- 38 2 diabetic Vietnamese patients treated with metformin, a population with very limited data.
- 39 - The levels of methylmalonic acid and homocysteine were not measured in this study,
- 40 these markers reflect early vitamin B12 deficiency in tissues.

41 - This is a single center, cross-sectional study, therefore, the results may not represent the  
42 prevalence of vitamin B12 deficiency in type 2 diabetic patients treated with metformin in  
43 the southern region of Vietnam.

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45 INTRODUCTION

46 Metformin belongs to the biguanide class and is synthesized from galegine, an isoprenyl  
47 derivative of guanidine found in the perennial herb French lilac (*Galega officinalis*)<sup>1</sup>.  
48 Metformin is recommended in most treatment guidelines as the first-line drug for the  
49 management of type 2 diabetes, based on its effectiveness in blood glucose control, low  
50 cost, safety, and cardiovascular benefits, as demonstrated by the UK Prospective Diabetes  
51 Study<sup>2,3</sup>.

52 The drug's effectiveness is dose-dependent, and the mechanism of action of metformin is  
53 not fully understood. Extensive evidence obtained in clinical studies and animal models  
54 suggests that the main function of metformin in regulating glucose homeostasis is to inhibit  
55 hepatic glucose production through the regulation of hepatic gluconeogenesis and  
56 glycogenolysis<sup>4,5</sup>. Metformin also improves cellular sensitivity to insulin, thereby  
57 enhancing peripheral glucose uptake, mainly in skeletal muscle, and significantly reducing  
58 fasting plasma insulin levels<sup>6</sup>. Improvements in insulin sensitivity with metformin may be  
59 due to its effects on insulin receptor expression and tyrosine kinase activity.

60 The most common adverse effects of metformin are gastrointestinal symptoms, such as  
61 diarrhea, nausea, and/or abdominal discomfort<sup>7</sup>. These symptoms are usually mild,  
62 transient, and dose-dependent. A rare but more concerning adverse effect of biguanides is  
63 lactic acidosis. Risk factors for metformin-induced lactic acidosis include conditions that  
64 increase lactate production, such as severe infections, severe liver or kidney disease, recent  
65 surgery, and any condition causing hypoxia or decreased tissue perfusion<sup>8,9</sup>.

66 In 1969, Berchtold et al. first reported vitamin B12 deficiency in patients using metformin,  
67 resulting from decreased gastrointestinal vitamin B12 absorption<sup>10</sup>. This effect can occur  
68 as early as the third month of using metformin. Several mechanisms have been proposed  
69 to explain the effect of metformin, including changes in small intestinal motility leading to  
70 overgrowth of intestinal bacteria, competitive inhibition and inactivation of vitamin B12  
71 absorption capacity, alteration of intrinsic factor levels, and interaction with the

intracellular cubilin receptor<sup>11,12</sup>. Of these, the most widely accepted mechanism is the inhibition of the calcium-dependent binding of the B12-intrinsic factor complex to the cubilin receptor in the ileum. The HOME study is one of the first clinical trials that confirmed the causative effect of vitamin B12 deficiency by metformin. The results showed that metformin usage at a dose of 850 mg three times daily reduces vitamin B12 levels by 19% compared to a placebo<sup>13</sup>.

Type 2 diabetes mellitus is a complicated, heterogeneous, and multifactorial disease of which treatment often requires the combination of metformin and other medications<sup>14–19</sup>, however, there are few studies on vitamin B12 deficiency related to metformin use in Vietnam. In 2023, Huynh et al. conducted a cross-sectional study on the prevalence of vitamin B12 deficiency in type 2 diabetic patients using metformin at the Vinmec Central Park Hospital, a private outpatient clinic in Ho Chi Minh City<sup>20</sup>. Of the 156 type 2 diabetic patients treated with metformin, the rate of vitamin B12 deficiency was 18.6%. Vitamin B12 deficiency may differ depending on region, diet, and studied population; this study set out to investigate the rate of vitamin B12 deficiency and its associated factors in type 2 diabetic patients treated with metformin in Cho Ray Hospital, one of the largest tertiary hospitals in Vietnam.

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MATERIALS AND METHODS

1. Participant recruitment

The protocol for this cross-sectional study was approved by the Ethical Committee of Cho Ray Hospital (approval number 1711/CN-HDDĐ). A sample size calculation was performed to estimate the rate of vitamin B12 deficiency in the target population using the formula below:

$$N = Z^2_{(1-\frac{\alpha}{2})} \frac{p(1-p)}{d^2}$$

In which,  $Z = 1.96$ ,  $p = 0.095$  (based on a similar study by Ko et al.<sup>21</sup>), and  $d = 0.05$ . The estimated patient number was 132. Recruitment took place from March to August 2024. The main inclusion criteria were type 2 diabetic patients treated with metformin for at least 3 months with unchanged dose in the last 3 months. Patients were excluded from the study if they had (i) a recent acute illness, such as severe infection, cancer, or acute coronary syndrome, within the past 3 months, or (ii) serious organ damage defined as cirrhosis, liver cell damage with aspartate aminotransferase or alanine aminotransferase increased  $\geq 3$  times the upper limit of normal, symptoms of heart failure, impaired renal function (serum creatinine levels  $>1.7$  mg/dL for men and  $>1.5$  mg/dL for women), estimated glomerular filtration rate (eGFR)  $< 30$  mL/min/1.73 m<sup>2</sup>, or (iii) a history of partial or total gastrectomy or pancreatectomy or colectomy. Patients were also excluded from the study if they were diagnosed with (i) gastrointestinal malabsorption disorders, such as chronic colitis or irritable bowel syndrome, or (ii) pernicious anemia or other hematological disorders. Finally, patients were excluded from the study if they were currently on vitamin B12 or multivitamin replacement, or they had been treated with proton-pump inhibitors or H2-receptor antagonists for at least 12 months consecutively, or they had followed a vegetarian/vegan diet for 3 years.

All the selected patients gave their informed written consent upon participating in the study. Baseline characteristics of the patients were recorded (age, gender, weight, height,

body mass index (BMI), blood pressure, waist and hip circumference, duration of type 2 diabetes, duration of metformin use, and metformin dose). Hypertension was identified based on the criteria of the American College of Cardiology/American Heart Association Task Force in 2017<sup>22</sup>, or if the patients were currently on antihypertensive drugs. Diabetic peripheral neuropathies were diagnosed based on patient history or the diagnostic criteria of the Toronto Diabetic Neuropathy Expert Group<sup>23</sup>. The metformin usage index (MUI) was calculated by multiplying the daily metformin dose (mg) by usage duration (year) and dividing by 1000<sup>24</sup>.

All the patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research

## 2. Paraclinical measurements

Fasting blood samples for all the participants were sent to the central laboratory of the Department of Biochemistry, Cho Ray Hospital. Vitamin B12 and folic acid levels were measured using an Architect i2000SR Immunoassay analyzer (Abbott Laboratories, Chicago, USA). Vitamin B12 deficiency was defined as a vitamin B12 level < 300pg/ml with a normal level of folic acid ( $\geq 4\text{ng/ml}$ ). An ADVIA 1800 Chemistry System (Siemens Healthineers, Erlangen, Germany) was used to measure other biochemical parameters, including fasting plasma glucose, creatinine, AST, ALT, and lipid profile. HbA1c was measured using a G8 HPLC analyzer (Tosoh Corporation, Tokyo, Japan). Total blood count was obtained using a DxH 600 Hematology Analyzer (Beckman Coulter).

## 3. Statistical analysis

Continuous variables were represented either as means  $\pm$  standard deviation (SD) or as medians with interquartile ranges (IQR), depending on their distribution. These variables were compared using either T-tests or Mann-Whitney U tests. Qualitative variables were characterized by frequencies and counts. Chi-square tests were used to compare categorical variables between groups. To investigate the associations between vitamin B12 deficiency and patients' characteristics, univariate and multivariate logistic regression was applied to

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143 calculate odds ratios (OR) and 95% confidence intervals (CI). A p-value of equal or less  
144 than 0.05 was considered statistically significant in all analyses. Data analysis was  
145 performed using SPSS Statistics for Windows version 20.0 (IBM Corporation, Armonk,  
146 NY, USA)  
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## RESULTS

A total of 145 patients participated in this study. The baseline characteristics of participants are described in Table 1. Briefly, the mean age of the studied population was 61.5, with 51.7% females. The median duration of metformin use was 10 years, the median metformin dose was 1700 mg, and the mean level of HbA1c was 8.1%. The rate of vitamin B12 deficiency was 22.1% (32 out of 145 participants). We could not detect any cases of macrocytic anemia.

There were no differences between the groups with and without vitamin B12 deficiency in terms of age, gender, BMI, hemoglobin level, MCV, creatinine level, and lipid profile. Patients with vitamin B12 deficiency had significantly longer duration of type 2 diabetes, longer duration of metformin use, higher metformin dose, and lower HbA1c levels than patients without vitamin B12 deficiency. The medications used for type 2 diabetes treatment including insulin, sulfonyl urea, dipeptidyl peptidase 4 inhibitor (DDP4i), and sodium-glucose cotransporter 2 inhibitor (SGLT2i) were not statistically different between the groups with and without vitamin B12 deficiency.

Univariate logistic analyses also showed that hypertension, duration of metformin use, metformin dose, HbA1c level, and MUI were statistically associated with vitamin B12 deficiency in the studied population (Table 2). Notably, a duration of metformin use > median (10 years), metformin dose > median dose (1700 mg/day), and MUI  $\geq 10$  were strongly associated with vitamin B12 deficiency with OR (95% CI) = 8.539 (2.459–29.648), 4.942 (1.777–13.749), and 5.095 (1.459–17.788), respectively. After adjusting for covariates using continuous and binomial variable models, HbA1c levels, duration of metformin use, and metformin dose were still independently associated with vitamin B12 deficiency (Table 3).



## 174 DISCUSSION

175 In our study, the vitamin B12 deficiency rate was 22.1% in type 2 diabetic Vietnamese  
176 patients treated with metformin for at least 3 months. This deficiency rate may vary  
177 between countries and ethnicities, and previous studies have reported a wide range of 4.3%  
178 to 30% for patients undergoing metformin treatment<sup>21,25-27</sup>. Our study showed similar  
179 results to the study by Kim et al. at Yongin Severance Hospital, South Korea, and Gao et  
180 al. at 12 tertiary hospitals in China<sup>28,29</sup>. This similarity may reflect consistency between the  
181 studies in average age, duration of type 2 diabetes, duration of metformin use, and  
182 metformin dosage. However, our results are higher than those of Ko et al. in South Korea,  
183 who found a vitamin B12 deficiency rate of 9.5% with a cut-off of vitamin B12 levels  $\leq$   
184 300 pg/mL and no folate deficiency (folate  $> 4$  ng/mL)<sup>21</sup>. These differences in vitamin B12  
185 deficiency rates between studies may come from differences in testing methods, patient  
186 characteristics, and the duration and dosage of metformin prescribed. Except for similar  
187 average ages, our patients' duration of type 2 diabetes, duration of metformin use, and  
188 average metformin dosage were all higher than those in Ko's study, which partly explains  
189 why the vitamin B12 deficiency rate in our study is higher. More importantly, the subjects  
190 in our study had a longer duration of type 2 diabetes, longer duration of metformin use,  
191 and a higher prescribed metformin dose compared to the patients in Ko's study<sup>21</sup>. There  
192 were several reasons why vitamin B12 deficiency rate was 22.1% but did not occur in all  
193 the participants. First, not all the participants were treated with metformin for four  
194 consecutive years and/or with high metformin dose. Second, the exact mechanisms of  
195 vitamin B12 deficiency in patients treated with metformin remained elusive, and who will  
196 absolutely develop vitamin B12 deficiency is still an unanswered question. Therefore,  
197 current American Diabetes Association guidelines recommend screening for high-risk  
198 patients and treating if they have vitamin B12 deficiency rather than treating all patients  
199 with metformin use for four consecutive years and more<sup>30</sup>.

200 Compared with a domestic study in Vietnam by Huynh et al. conducted at Vinmec Central  
201 Park Hospital, Ho Chi Minh City in 2023, the vitamin B12 deficiency rate in our study is



higher<sup>20</sup>. This difference can be explained by differences in the characteristics of the subjects and the research context. Huynh's study mainly focused on patients undergoing health checks at a private hospital. These are usually patients with better economic conditions, better access to high quality medical services, and who potentially pay more attention to their diet. Thus, the vitamin B12 deficiency rate in their study is lower. Moreover, the patients in Huynh's study were younger, had lower metformin doses, and a shorter duration of metformin treatment. In contrast, our study was conducted at Cho Ray Hospital, a large tertiary hospital that receives patients with complex and more severe medical conditions. Therefore, the patients in our study had a longer disease duration, longer duration of metformin use, higher metformin dose, and a higher rate of vitamin B12 deficiency.

Our study found that metformin dose and duration of metformin use were statistically associated with vitamin B12 deficiency. These findings are similar to previous studies and emphasize again the important role of vitamin B12 screening in patients with long-term use and/or high doses of metformin<sup>28,31</sup>. In addition, MUI  $\geq 10$  was found to be an independent factor that is statistically associated with vitamin B12 deficiency, suggesting that MUI can be used as a reliable and alternative parameter to decide when to screen for vitamin B12 deficiency rather than using duration of metformin use or metformin dose alone. We could not use a cut-off value of 5 years for the duration of metformin use and 5 for MUI because the number of participants with the duration of metformin use of less than 5 years was small (only 21 out of 145 cases), leading to underpowered statistical analyses. Interestingly, lower HbA1c was statistically associated with vitamin B12 deficiency in this study. This outcome may result from the fact that patients achieving lower HbA1c were intensively treated with higher dose of metformin.

Our study has several limitations. First, we did not measure methylmalonic acid or homocysteine levels; these markers reflect early vitamin B12 deficiency in tissues. Second, the sample size was small, and all the data were collected in a single center, therefore, the

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229 reported results may not represent the landscape of type 2 diabetic patients in the southern  
230 region of Vietnam.

231 In conclusion, our study showed that the rate of vitamin B12 deficiency was up to 22.1%  
232 in type 2 diabetic patients with a median of 10 years of metformin use and 1700 mg of  
233 metformin dose. This rate presents a warning and requires routine screening and  
234 replacement of vitamin B12 in metformin-treated patients. Further multi-center studies are  
235 necessary to understand the landscape of vitamin B12 deficiency in patients treated with  
236 metformin in Vietnam.

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**Author Contributions:** HP, AN, MD designed the research study. AN recruited the participants for the study. HP and MD analyzed the data. HP, AN, and MD wrote the manuscript. MD is the guarantor.

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**Data availability statement:** Data supporting the findings of this study are available from the corresponding author [MD] on request. Raw data were generated at Cho Ray hospital, Ho Chi Minh City, Vietnam.

**Ethical approval:** This study was approved by the Ethics Committee for Medical Research of Cho Ray Hospital (approval number 1711/CN-HĐĐĐ).

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348 Table 1. Baseline characteristics of participants.

Characteristics	Total (N=145)	No vitamin B12 deficiency (N=113)	Vitamin B12 deficiency (N=32)	p-value
Age (years) Mean $\pm$ SD	61.5 $\pm$ 11.0	60.6 $\pm$ 11.0	64.5 $\pm$ 10.7	0.075
Gender				0.844
Female, N (%)	75 (51.7)	59 (52.5)	16 (50)	
Male, N (%)	70 (48.3)	54 (47.8)	16 (50)	
BMI (kg/m <sup>2</sup> ) Mean $\pm$ SD	22.9 $\pm$ 1.9	22.9 $\pm$ 2.1	22.8 $\pm$ 1.5	0.755
Hypertension				<b>0.031</b>
Yes, N (%)	105 (61.9)	77 (68.1)	28 (87.5)	
No, N (%)	40 (38.1)	36 (31.9)	4 (12.5)	
Diabetic peripheral neuropathy				0.972
Yes, N (%)	54 (37.2)	42 (37.2)	12 (37.5)	
No, N (%)	91 (62.8)	71 (62.8)	20 (62.5)	
Diabetes duration (years) Median [Q1-Q3]	10.0 [6.0 – 10.0]	10.0 [5.5 – 15.0]	12.0 [10.0 – 15.0]	<b>0.029</b>
Metformin use duration (years) Median [Q1-Q3]	10.0 [6.0 – 10.0]	10.0 [5.5 – 15.0]	12.0 [10.0 – 15.0]	<b>0.044</b>
Metformin dose (mg) Median [Q1-Q3]	1700.0 [1000.0 – 1850.0]	1700.0 [1000.0 – 1700.0]	1700.0 [1700.0 – 2000.0]	<b>0.001</b>
Hb (g/dl) Mean $\pm$ SD	13.6 $\pm$ 1.6	13.6 $\pm$ 1.6	13.4 $\pm$ 1.5	0.674
MCV (fL) Mean $\pm$ SD	87.6 $\pm$ 6.6	87.1 $\pm$ 7.0	89.5 $\pm$ 5.1	0.071
Fasting plasma glucose (mg/dl) Mean $\pm$ SD	158.4 $\pm$ 72.1	162.4 $\pm$ 77.2	144.2 $\pm$ 48.2	0.208
HbA1c (%) Mean $\pm$ SD	8.1 $\pm$ 1.9	8.3 $\pm$ 2.0	7.4 $\pm$ 1.6	<b>0.021</b>



Creatinine (mg/dl) Median [Q1-Q3]	0.9 [0.7 – 1.0]	0.9 [0.7 – 1.0]	0.9 [0.8 – 1.0]	0.274
Total cholesterol (mmol/l) Median [Q1-Q3]	146.0 [114.0 – 180.0]	148.0 [114.5 – 181.5]	138.0 [107.3 – 164.5]	0.292
Triglyceride (mmol/l) Median [Q1-Q3]	163.0 [109.5 – 238.0]	163.0 [107.0 – 231.0]	162.0 [131.8 – 307.3]	0.166
HDL-C (mmol/l) Median [Q1-Q3]	44.0 [38.0 – 51.0]	44.0 [39.0 – 52.0]	44.0 [35.0 – 49.0]	0.425
LDL-C (mmol/l) Median [Q1-Q3]	75.0 [49.5 – 107.5]	77.0 [53.8 – 112.5]	64.2 [48.2 – 94.9]	0.242
Vitamin B12 level (pg/ml) Median [Q1-Q3]	478.0 [323.0 – 608.0]	514.0 [416.0 – 667.5]	233.0 [196.5 – 273.8]	< 0.001
Folate level (µmol/mL) Mean ± SD	11.5 ± 3.5	11.6 ± 3.5	11.1 ± 3.5	0.457
Insulin treatment Yes, N (%) No, N (%)	50 (34.5) 95 (65.5)	38 (33.6) 75 (66.4)	12 (37.5) 20 (62.5)	0.684
Sulfonyl urea treatment Yes, N (%) No, N (%)	45 (31.0) 100 (69.0)	33 (29.2) 80 (70.8)	12 (37.5) 20 (62.5)	0.370
DPP4i treatment Yes, N (%) No, N (%)	116 (80.0) 29 (20.0)	87 (77.0) 26 (23.0)	29 (90.6) 3 (9.4)	0.089
SGLT2i treatment Yes, N (%) No, N (%)	88 (60.7) 57 (39.3)	72 (63.7) 41 (36.3)	16 (50.0) 16 (50.0)	0.161

BMI: body mass index, DPP4i: dipeptidyl peptidase 4 inhibitor, Hb: hemoglobin, MCV: mean cell volume, HDL-C: high-density lipoprotein-cholesterol, LDL-C: low-density lipoprotein-cholesterol, SGLT2i: sodium-glucose cotransporter 2 inhibitor.

Table 2. Univariate logistic regression analysis of factors associated with vitamin B12 deficiency.

Variables	OR (95% CI)	p-value
Age	1.034 (0.996 – 1.074)	0.077
Gender	1.093 (0.498 – 2.396)	0.825
BMI	0.967 (0.786 – 1.190)	0.753
Hypertension	3.268 (1.068 – 10.000)	<b>0.038</b>
Duration of diabetes	1.047 (0.989 – 1.108)	0.115
Duration of metformin use	1.043 (0.985 – 1.103)	0.147
Long-term use of metformin (Duration > median duration)	8.539 (2.459 – 29.648)	<b>&lt; 0.001</b>
Metformin dose	1.002 (1.001 – 1.003)	<b>0.002</b>
Metformin dose > median dose	4.942 (1.777 – 13.749)	<b>0.002</b>
Hb	0.949 (0.744 – 1.210)	0.671
MCV	1.068 (0.993 – 1.148)	0.075
Fasting plasma glucose	0.995 (0.988 – 1.003)	0.210
HbA1c	0.733 (0.557 – 0.964)	<b>0.026</b>
MUI	1.043 (1.009 – 1.078)	<b>0.014</b>
MUI ≥ 10	5.095 (1.459 – 17.788)	<b>0.011</b>

BMI: body mass index, Hb: hemoglobin, MCV: mean cell volume, MUI: metformin usage index.

Table 3. Multivariate logistic regression analysis of factors associated with vitamin B12 deficiency.

Model	Variables	OR (95% CI)	p-value
Model 1	HbA1c	0.624 (0.455 – 0.856)	0.003
	Duration of metformin use	1.076 (1.005 – 1.151)	0.037
	Metformin dose	1.002 (1.001 – 1.004)	0.002
Model 2	HbA1c	0.631 (0.460 – 0.865)	0.004
	Long-term use of metformin (Duration > median duration)	10.078 (2.729 – 37.219)	0.001
	Metformin dose > median dose	4.429 (1.475 – 13.303)	0.008
Model 3	HbA1c	0.647 (0.480 – 0.874)	0.005
	MUI ≥ 10	7.644 (2.109 – 27.707)	0.002

MUI: metformin usage index.