



# Prevalence of Vitamin B<sub>12</sub> Deficiency in Patients Treated With Metformin

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## Abstract

**Background/Aim:** Metformin has been associated with vitamin B<sub>12</sub> (cobalamin) deficiency, pushing scientific societies such as the American Diabetes Association and the European Association for the Study of Diabetes to emphasise the need for screening B<sub>12</sub> levels, without specific metformin doses or exposure durations triggering this screening. Robust data regarding the prevalence of B<sub>12</sub> deficiency in metformin-treated patients in Portugal are currently lacking. Aim of this study was to identify the prevalence of B<sub>12</sub> deficiency in a sample of diabetic patients taking metformin. Secondary objectives were determining the minimum dose and minimum and median time exposure time leading to this deficiency and identifying the average duration of metformin use in the patients with this deficiency.

**Methods:** Descriptive and cross-sectional observational study was performed on a sample of 79 users from a population of 714 diabetic patients on metformin. Inclusion criteria comprised individuals aged 18 or older, receiving metformin for at least 1 month and voluntarily participating in the study. Exclusion criteria included a history of gastrectomy or B<sub>12</sub> supplementation.

**Results:** A prevalence of 25.3 % of vitamin B<sub>12</sub> deficiency was identified in the study sample. Minimum doses of 500 mg of metformin per day and a minimum exposure period of 1 year were associated with B<sub>12</sub> deficiency. An average exposure time of 5.33 years was identified.

**Conclusion:** These results align with the prevalence described in the few international studies and should alert physicians to potential clinical manifestations of this deficiency, such as anaemia and neurological symptoms like neuropathy.

**Key words:** Metformin; Vitamin B<sub>12</sub>; Diabetes mellitus.

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## Introduction

Diabetes mellitus, a chronic metabolic disorder, has reached a prevalence of 537 million in adults aged between 20 and 79 years worldwide in 2021, a number which is expected to increase to 700 million by 2045.<sup>1</sup> Metformin is generally one of the first-line therapy options due to its favourable safety profile, glucose-lowering efficacy and potential cardiovascular benefits.<sup>2</sup> However,

a growing body of evidence has raised concerns about its association with vitamin B<sub>12</sub> (cobalamin) deficiency.

The American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) recommend periodic assessment of vitamin B<sub>12</sub> serum levels in patients on long-term

metformin, without specifying exposure times or metformin doses. On the other hand, the Endocrine Society (ES), American Association of Clinical Endocrinology (AACE) and American College of Endocrinology (ACE) advocate for the assessment of vitamin B<sub>12</sub> levels exclusively in individuals who manifest neuropathic symptoms.<sup>3-5</sup>

The International Society of Nephrology (ISN) recommends the evaluation of serum B<sub>12</sub> after 4 years of metformin or in those patients with increased risk of deficit (eg, patients with malabsorption syndrome or reduced dietary intake [vegans]). Furthermore, the Canadian Diabetes Association (CDA) advises a periodic measurement of Vitamin B<sub>12</sub> levels in people taking metformin or with signs or symptoms of deficiency (such as impaired proprioception or peripheral neuropathy).<sup>6</sup>

The prevalence of vitamin B<sub>12</sub> deficiency in diabetic patients undergoing metformin therapy constitutes a subject of escalating scientific interest and clinical significance due to its association with anaemia and peripheral nerve damage, potentially exacerbating symptoms related to diabetic peripheral neuropathy.<sup>7,8</sup> Understanding the interplay between metformin, diabetes and vitamin B<sub>12</sub> status is crucial for optimising patient care and mitigating the potential long-term consequences associated with B<sub>12</sub> deficiency.

The principal aim of this investigation was to evaluate the prevalence of vitamin B<sub>12</sub> deficiency among diabetic patients taking metformin. The secondary objectives of this study encompassed: appraising the minimal doses of metformin correlated with the occurrence of vitamin B<sub>12</sub> deficiency and scrutinising the minimal and median durations of exposure to metformin associated with the manifestation of vitamin B<sub>12</sub> deficiency.

## Methods

### Participants

The study adhered to the ethical principles outlined in the Declaration of Helsinki and obtained approval from the institutional ethical board at Regional Administration of Health of North of Portugal. All participants provided written informed consent.

In this prospective, on-site investigation, individuals diagnosed with type 2 diabetes, prescribed metformin and presenting for routine consultations with their family physicians during the period from May to August 2023, were invited to participate. The sampling method employed was convenience sampling, selected deliberately to minimise interference with the routine vigilance conducted by family physicians. In instances where patients required blood samples for diabetes monitoring or other purposes, family physicians included B<sub>12</sub> vitamin analysis in the requisition. The ensuing anonymised results were meticulously entered into a Microsoft Excel® spreadsheet by the family physicians for subsequent analysis by the researchers.

The sample size calculation was executed using the Raosoft® calculator, with a confidence level of 90 %, a margin of error of 10 %, a population size of 714 and a response distribution assumption of 50 %. Consequently, the calculated sample size was determined to be 62 subjects. Ultimately, 79 patients consented to participate in the study.

Inclusion criteria encompassed individuals aged ≥ 18 years, utilising metformin for a minimum of one month and expressing voluntary willingness to partake in the research. Conversely, exclusion criteria encompassed individuals with a history of gastrectomy or those currently supplementing with vitamin B<sub>12</sub>.

### Vitamin B<sub>12</sub> assessment

Serum levels of vitamin B<sub>12</sub> were quantified through blood analysis and vitamin B<sub>12</sub> deficiency was defined as follows: serum B<sub>12</sub> levels less than 300 pg/mL were considered deficient, levels between 200 and 300 pg/mL were categorised as low-normal and levels equal to or exceeding 300 pg/mL were deemed normal.

There is no universally accepted “gold standard” for the measurement of vitamin B<sub>12</sub> deficiency, as various methods exist for assessing serum vitamin B<sub>12</sub> levels, each associated with specific normal values and research findings. A study demonstrating reduced specificity reported that 60 % of patients exhibited symptoms of vitamin B<sub>12</sub> deficiency with B<sub>12</sub> levels inferior to 200 pg/mL and 90 % displayed symptoms when the level was below 100 pg/mL.<sup>9</sup> Generally, serum vitamin B<sub>12</sub> levels can be interpreted as follows: levels greater than 300 pg/mL suggest that B<sub>12</sub> deficiency is

unlikely (probability of 1 %–5 %); levels between 200 and 300 pg/mL indicate a possible B<sub>12</sub> deficiency (probability of 5 %–15 %) and levels below 200 pg/mL are consistent with B<sub>12</sub> deficiency (specificity of 90 %–100 %).<sup>9,10</sup>

## Results

The cohort comprised 79 patients with ages ranging from 38 to 92 years, presenting a mean age of 67 ± 8.26 years. Of the total, 51.9 % (41 patients) were male.

### Metformin doses and exposure time

The mean dose of metformin administered was 1642 ± 701 mg/day and the median dose was 2000 mg/day. The dosage range varied from a minimum of 500 mg/day to a maximum of 3000 mg/day. The doses were subsequently classified into four groups, with a predominant proportion of patients (50.6 %, n = 40) receiving metformin doses exceeding 2000 mg per day (Table 1).

Table 1: Metformin doses and exposure time

Metformin	Mean (SD)
Dose (mg/day)	1,642 (701)
< 1000	17.7 (14)
1000 – 1500	16.5 (13)
1500 – 2000	15.2 (12)
≥ 2000	50.6 (40)
Exposure time (years)	7.06 (1.18)
< 5	32.9 (26)
5-10	40.5 (32)
10-15	20.3 (16)
≥ 15	6.3 (4)

The mean duration of metformin use among the participants was 7.06 years ± 1.18 and a median duration of 7 years. The duration ranged from a minimum of 1 year to a maximum of 17 years. The study population was further stratified into four temporal categories: < 5 years: 32.9 % (n = 26); ≥ 5 and < 10 years: 40.5 % (n = 32); ≥ 10 and < 15 years: 20.3 % (n = 16); ≥ 15 years: 6.3 % (n = 4).

### Vitamin B<sub>12</sub> levels

The mean B<sub>12</sub> levels observed were 412 ± 162 pg/mL, ranging from a minimum of 166 pg/mL to a maximum of 962 pg/mL. Within the study cohort, 25.3 % (n = 20) of individuals exhibited B<sub>12</sub> deficiency (B<sub>12</sub> < 300 pg/mL) and this deficiency

group was further categorised into distinct sub-groups: B<sub>12</sub> levels ≥ 200 and < 300 pg/mL: 22.8 % and B<sub>12</sub> < 200 pg/mL: 2.5 %. In addition, the majority of patients (74.7 %) did not exhibit B<sub>12</sub> deficiency as anticipated. A sub-analysis was performed, comparing the B<sub>12</sub> deficiency group with the non-deficiency group.

#### a) Non-deficiency group

The group with normal B<sub>12</sub> levels was composed of 59 patients, representing 74.7 % of the total population. The mean age in this group was 67.12 ± 8.3 years, mirroring the B<sub>12</sub> deficiency group. The sex distribution was also similar, with females constituting 52.5 %. The mean B<sub>12</sub> level in this group was 466.61 ± 153.46 pg/mL, with a range from 303 to 962 pg/mL. This value denoted a mean difference of 212.06 pg/mL when compared to the deficiency group.

In terms of metformin dosage, the mean dose was 1643 ± 753 mg, closely resembling the deficit group (1655 mg). The majority of patients in this group received doses equal to or greater than 2000 mg (49.2 %), a very similar proportion to the deficiency group (55.0 %). Other patients were similarly distributed cross groups: < 1000 mg (18.6 %), ≥ 1000 mg and < 1500 mg (16.9 %) and ≥ 1500 mg and < 2000 mg (15.3 %). The minimum dose observed was 500 mg.

Table 2: Characteristics of the vitamin B<sub>12</sub> non-deficiency and deficiency groups

Parameter	Non-deficiency group	Deficiency group
N (%)	59 (74.7)	20 (25.3)
Age (years)	67.1 (8.3)	67.4 (9.5)
Sex		
Female	52.5 (31)	35.0 (7)
Male	47.5 (28)	65.0 (13)
Mean B <sub>12</sub> levels (pg/mL)	466.6 (153.5)	254.5 (38.8)
Mean daily metformin dose (mg)	25.3 (20)	1655 (604)
< 1000	18.6 (11)	15.0 (3)
1000 – 1500	16.9 (10)	15.0 (3)
1500 – 2000	15.3 (9)	15.0 (3)
≥ 2000	49.2 (29)	55.0 (11)
Mean metformin exposure time (years)	7.6 (4.6)	5.3 (3.5)*
Metformin exposure time groups (years)		
< 5	28.8 (17)	40.0 (8)
5-10	37.3 (22)	55.0 (11)
≥ 15	33.9 (20)	5.0 (1)

The results are presented as mean (standard deviation); \* T-test, p = 0.041;

Regarding the duration of metformin use, the mean time was  $7.65 \pm 4.56$  years. The predominant duration in this group was between  $\geq 5$  years and  $< 10$  years, accounting for 37.3 %, followed by the segment  $< 5$  years (28.8 %) and  $> 10$  years (33.9 %).

### b) Deficiency group

The deficiency group comprised 20 patients with a mean age of  $67.4 \pm 9.5$  years. Within this group, males constituted 65 % of the population. The mean  $B_{12}$  levels were  $254.55 \pm 8.8$  pg/mL, ranging from a minimum of 166 to a maximum of 297 pg/mL. The mean metformin dosage in this group was  $1655 \pm 604$  mg/day. Among them, the majority (55 %,  $n = 11$ ) were prescribed doses exceeding 2000 mg/day, with an even distribution (15 %) among the groups receiving less than 1000 mg, between 1000 and 1499 mg and between 1500 and 1999 mg. The minimum prescribed dose was 500 mg/day.

The mean duration of metformin use for the  $B_{12}$  deficiency group was  $5.33 \pm 3.45$  years, demonstrating statistical significance compared to the non-deficiency group (t-test,  $p = 0.041$ ). The temporal distribution within this group was as follows: 40 % were within the  $< 5$  years category, 55 % in the  $\geq 5$  to  $< 10$  years category and only 5 % exceeded 10 years. The duration range in the deficiency group spanned from a minimum of 1 year to a maximum of 13 years.

## Discussion

In presented study, a prevalence of vitamin  $B_{12}$  deficiency of 25.3 % among patients with type 2 diabetes receiving metformin therapy was identified. This high prevalence underscores the imperative need to promptly address this deficiency to alleviate potential symptoms and mitigate overall health repercussions. The observed prevalence aligns with findings from other studies, which report a range of  $B_{12}$  deficiency between 6 % and 30 %.

Kim et al identified a prevalence of 22.2 % in a study involving 1111 patients, while Aroda et al, in a prospective study with 1073 participants, reported a prevalence of 19.1 % after 5 years and 20.3 % after 13 years of metformin usage.<sup>10,11</sup> Ad-

ditionally, the National Health and Nutrition Examination Survey demonstrated that 41 % of  $B_{12}$  deficiency cases among individuals with diabetes were attributable to metformin use.<sup>12</sup> In a Korean study, the prevalence of vitamin  $B_{12}$  deficit was lower (9.5 %), emphasising the influence of population differences as a potential bias.

Regarding the duration of metformin use, some studies suggest a cutoff of 4 years for detecting  $B_{12}$  deficiency.<sup>13</sup> In presented study, patients with  $B_{12}$  deficiency were identified after just 1 year of metformin use. The mean duration under metformin for the  $B_{12}$  deficiency group was 5.33 years, consistent with previous data.

The dosage of metformin is also a significant consideration in various studies. For instance, Kim et al reported a decrease in vitamin  $B_{12}$  levels by 0.142 pg/mL with a 1 mg increase in metformin, while Beulens et al found a decrease of 0.042 pg/mL.<sup>10,14</sup> In presented study, doses as low as 500 mg per day were associated with  $B_{12}$  deficiency and the majority of the deficiency group (55 %,  $n = 11$ ) had prescribed doses exceeding 2000 mg/day, aligning with prior data.

In future studies, aim is to replicate these findings on a multicentre or national level to enhance the robustness of presented conclusions. Additional secondary outcomes, including folic acid levels, homocysteine levels, and methylmalonyl-CoA mutase, can also be explored to deepen understanding of  $B_{12}$  deficiency.

$B_{12}$  deficiency presents with varied symptoms that may mislead doctors and patients, as neurologic symptoms (characterised by decreased position and vibratory sensation in the extremities accompanied by mild to moderate weakness and hyporeflexia, that may develop in a stocking-glove distribution). It can mimic the diabetic foot symptoms, leading to unnecessary therapy and investigation. Other symptoms such as irritability, depression, weight loss and poorly localised abdominal pain may occur, leading to poor quality of life.<sup>15</sup>

Correcting this disorder is simple, as various  $B_{12}$  supplement formulations are available and haematologic abnormalities are usually corrected within 6 weeks. However, doctors should be aware that neurologic symptoms may take much longer and may even become irreversible if they persist for months or years.<sup>15</sup>

## Conclusion

The significance of presented results, revealing a 25.3 % prevalence of B<sub>12</sub> deficiency in patients under metformin, emphasises the importance of physician awareness and proactive management of this side effect to minimise possible symptoms of the patients that may diminish their quality of life.

## Ethics

The study was approved by the Ethic Committee of the *Administração Regional de Saúde do Norte* (Northern Regional Health Administration), decision No CE/2024/1, dated 4 January 2024.

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## Conflicts of interest

The authors declare that there is no conflict of interest.

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## Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

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