Journal of Libyan Academy Bani Walid 2026
مجلة الأكاديمية الليبية بني وليد
e-ISSN: 3104-3860
Volume 2, Issue 1, 2026, Pages: 145-160
Website: https://journals.labjournal.ly/index.php/Jlabw/index

A study to investigate the effect of vitamin B12 levels in type 2 diabetic patients on body mass and blood count at the Diabetes Control and Treatment Center in Al-Khums

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دراسة لتقصي تأثير مستويات فيتامين ب 12 عند مرضى السكري النوع الثاني على كثلة الجسم ومعدل الدم بمركز مكافحة وعلاج داء السكري بالخمس.

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Received: 28-11-2025	**Accepted:** 02-01-2026	**Published**: 20-01-2026

Abstract

Metformin is the most commonly prescribed oral anti-diabetic drug and the firstline treatment for Type II Diabetes Mellitus (T2DM). Despite its established efficacy, clinical evidence suggests a potential association between its long-term use and Vitamin B12 deficiency, a condition that may lead to severe neurological and hematological complications. Comparative cross-sectional study, conducted in Libya at the Diabetes Control and Treatment Center in Al-Khums city, aimed to investigate the prevalence of Vitamin B12 deficiency among diabetic patients, assess the relationship between vitamin levels and both the daily metformin dose and Body Mass Index (BMI), in addition to measuring patients' level of awareness regarding this clinical association. The study included a sample of 226 flow of participants divided into three groups (metformin group, insulin group, and a healthy control group). Data were collected using a structured questionnaire and laboratory tests during the period from June 1, 2024, to February 28, 2025. Statistical analysis revealed a statistically significant relationship between long-term metformin use and decreased Vitamin B12 levels ($P < 0.05$). Furthermore, an elevated BMI was found to be an independent risk factor that increases the severity of the deficiency. Regarding health awareness, the study revealed a substantial knowledge gap, as 83.2% of the participants were unaware of the association between metformin use and Vitamin B12 deficiency. The study concludes that metformin negatively impacts Vitamin B12 levels in a dose-dependent manner, and that obesity or high BMI exacerbate this effect. It strongly

recommends the routine inclusion of Vitamin B12 screening in the follow-up protocols for T2DM patients treated with metformin, particularly those receiving high doses or with an elevated BMI, and emphasizes the administration of appropriate drug supplements and adherence to necessary dietary intake, alongside activating health education programs to bridge the existing awareness gap.

Keywords: Type II Diabetes Mellitus, Metformin, Body Mass Index (BMI), Vitamin B12 Deficiency.

الملخص

يُعدّ الميتفورمين أكثر أدوية السكري الفموية شيوعًا، وهو العلاج الأولي لداء السكري من النوع الثاني. على الرغم من فعاليته المؤكدة، تشير الأدلة السريرية إلى وجود ارتباط محتمل بين استخدامه طويل الأمد ونقص فيتامين ب12، وهي حالة قد تؤدي إلى مضاعفات عصبية ودموية خطيرة. هدفت دراسة مقطعية مقارنة، أُجريت في ليبيا بمركز مكافحة وعلاج السكري في مدينة الخمس، إلى التحقق من مدى انتشار نقص فيتامين ب12 بين مرضى السكري، وتقييم العلاقة بين مستويات الفيتامين وكل من جرعة الميتفورمين اليومية ومؤشر كتلة الجسم، بالإضافة إلى قياس مستوى وعي المرضى بهذا الارتباط السريري.

شملت الدراسة عينة من 226 مشاركًا تم تقسيمهم إلى ثلاث مجموعات (مجموعة الميتفورمين، ومجموعة الأنسولين، ومجموعة ضابطة من الأصحاء). جُمعت البيانات باستخدام استبيان مُنظّم وفحوصات مخبرية خلال الفترة من 1 يونيو 2024 إلى 28 فبراير 2025. وكشف التحليل الإحصائي عن وجود علاقة ذات دلالة إحصائية بين استخدام الميتفورمين طويل الأمد وانخفاض مستويات فيتامين ب12 ($P < 0.05$). علاوة على ذلك، وُجد أن ارتفاع مؤشر كتلة الجسم يُعد عامل خطر مستقلًا يزيد من حدة النقص. وفيما يتعلق بالوعي الصحي، كشفت الدراسة عن فجوة معرفية كبيرة، حيث لم يكن 83.2% من المشاركين على دراية بالارتباط بين استخدام الميتفورمين ونقص فيتامين ب12. وتخلص الدراسة إلى أن الميتفورمين يؤثر سلبًا على مستويات فيتامين ب12 بطريقة تعتمد على الجرعة، وأن السمنة أو ارتفاع مؤشر كتلة الجسم يُفاقمان هذا التأثير. توصي هذه الدراسة بشدة بإدراج فحص فيتامين ب12 بشكل روتيني ضمن بروتوكولات المتابعة لمرضى السكري من النوع الثاني الذين يتناولون الميتفورمين، وخاصةً أولئك الذين يتلقون جرعات عالية أو لديهم مؤشر كتلة جسم مرتفع، وتؤكد على ضرورة تناول المكملات الدوائية المناسبة والالتزام بالنظام الغذائي اللازم، إلى جانب تفعيل برامج التثقيف الصحي لسدّ فجوة الوعي الحالية.

الكلمات المفتاحية: داء السكري من النوع الثاني، الميتفورمين، مؤشر كتلة الجسم، نقص فيتامين ب12.

1. Introduction

Diabetes is one of the human diseases that have been known since ancient times, and it was described by ancient Egyptian scientists **(Professor Belo's., 2013)**.

Since 2000 BC, they described the appearance of diabetes in the urine, and ancient Chinese scientists described the symptoms of diabetes as increased urination, thirst and hunger. **(Ritz & Zeng., 2011).**

In the first century AD, the Greek Aretus described diabetes as the melting of the flesh of the body and limbs and then its exit through the urine, and gave it its current name Diabetes, from the Greek word for "siphon". which means running water **(Ahmed., 2019).**

Scientific research and discoveries have continued during the 18, 19, and 20 centuries in the field of diabetes in an unprecedented manner until scientists reached the most accurate details about this disease, and research is still ongoing **(Hasson and Zoudi 2022).** The history of diabetes in modern times coincides with the advent of experimental medicine. An important

milestone in the history of diabetes is the identification of the liver's role in glycogen formation and the concept that diabetes is due to excess glucose production by Claude Bernard Merging and Minkowski **(Obeid 2019)** discovered the role of the pancreas in the pathogenesis of diabetes. Later, this discovery formed the basis for the isolation and clinical use of insulin by Banting and Best **(Ahmed., 2016).**

Type I Diabetes accounts for only about 5-10% of all cases of diabetes; however, its incidence continues to increase worldwide and it has serious short-term and long-term implications. Type I indicates the process of beta-cell destruction in the pancreas that may ultimately lead to diabetes mellitus in which "insulin is required for survival" to prevent the development of ketoacidosis, coma and death (Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications **Bauman *et al.*, 2000.**

Type II Diabetes (NIDD) T2DM, one of the most prevalent metabolic illnesses, is brought on by a combination of two main factors: impaired insulin production by pancreatic cells and improper insulin response in insulin sensitive tissues. Because the processes of insulin re lease and activity are crucial for maintaining glucose homeostasis **(Galicia Garcia *et al.*, 2020).**

Metformin a biguanide derivate, is currently the most widely prescribed drug to treat hyperglycemia in individuals with T2D and is recommended, in conjunction with lifestyle modification (diet, weight control and physical activity), as a first line oral therapy along in the recent guidelines of the

Potential Pathophysiological Mechanisms of Metformin-Induced Vitamin B_{12} Deficiency**:**

The exact mechanisms underlying metformin-induced vitamin B_{12} deficiency are still not fully understood **(Mazokopakis& Starakis.,2012)**. However, the mechanisms are thought to cause vitamin B_{12} deficiency primarily through altered vitamin B_{12} absorption and metabolism **(Obeid *et al*.,2019& Greibe *et al*.,2013)** Proposed mechanisms responsible for metformin-induced vitamin B_{12} deficiency include:(1) interference with calcium-dependent binding of the IF-vitamin B_{12} complex to the cubilin receptor on enterocytes at the ideal level and/or interaction with the endogenous cubilin receptor; (2) alteration in small intestinal motility, leading to bacterial overgrowth in the small intestine and subsequent inhibition of IF-vitamin B_{12} complex absorption in the distal ileum; (3) alteration in bile acid metabolism and reabsorption, leading to impaired enterohepatic circulation of vitamin B_{12}; (4) increased accumulation of vitamin B_{12} in the liver, leading to altered tissue distribution and metabolism of vitamin B_{12}; (5) decreased secretion of IF by gastric parietal cells**(Bauman *et al*.,2000)**

Aim of Research

1. Investigate the influence of body mass index (BMI) on the prevalence of vitamin B_{12} deficiency in patients treated with metformin.
2. To assess the effect of vitamin B_{12} deficiency on hemoglobin levels in patients with type II diabetes receiving metformin.
3. To evaluate patient awareness regarding the relationship between metformin use and vitamin B_{12} deficiency.

Literature Review:

(Ahmed *et al.*, 2016) proved an association between long-term metformin use and low vitamin B_{12} levels. The potential of the deficiency to cause or worsen peripheral neuropathy in type II diabetes mellitus (T2DM) patients has been investigated with conflicting results.

In a large study of type II diabetes patients by **(Arodda *et al.*, 2016),** researchers found no significant association between BMI and the presence of vitamin B_{12} deficiency in patients treated with metformin.

A study in Pakistan by **(Ahmed *et al.*, 2019)** showed that obese patients had higher rates of vitamin B_{12} deficiency when taking metformin, with a clear association between higher BMI and vitamin deficiency.

large-scale studies finding that men are more likely to be deficient Khan and

Jameel *et al.* (2020): A study in Iraq showed that a higher body mass index (BMI) is associated with an increased risk of vitamin B_{12} deficiency.

Atalay *et al*. (2020): A study in Jordan found no statistically significant relationship between vitamin B_{12} levels and body mass index.

Also **(Mohtar *et al.*, 2020)** Pakistani study found that the deficiency rate was 3.9% among metformin users versus 2.1% among non-users, a relatively small difference.

A study by **(Kuwabara *et al.*, 2019)** in Japan did not show an association between vitamin B_{12} levels and BMI in type II diabetes patients taking metformin, suggesting that other factors may be at play.

(Hassan, F., & Al-Zoubi, A. (2022). conducted a study that included 248 participants (response rate 95.4%), of whom 26 (10.5%) had vitamin B_{12} deficiency, and 53 (21.4%) had severe deficiency. The mean daily metformin dose was highest in the vitamin B_{12} deficiency group (1981 ± 222 mg; P = 0.004).

Also **(Shahwan *et al*., 2020)** designed a study: To evaluate vitamin B_{12} deficiency and its associated risk factors among type II diabetes patients in Palestine, this study included a total sample size of 400 patients, and the results showed that (39%) of the participants had low blood vitamin B_{12} levels. The majority of the participants who had low blood vitamin B_{12} levels (38.4%) were using metformin followed by (29.5%) who were using insulin. There was a negative (P=0.198) between low blood vitamin B_{12} levels and metformin treatment, while a significant association was observed with insulin use (P=0.039). These results could be attributed to different factors. It also showed a negative effect on the prevalence of low blood B_{12} levels in patients with diabetic complications

Some studies have also found that metformin has a role in affecting vitamin B_{12} levels. In a study conducted by **(Fitouri and others**., **2024)** the results showed a relatively strong relationship between the use of metformin and a decrease in vitamin B_{12} in the serum, which showed an inverse relationship between the dose and duration of metformin use and cobalamin concentrations in the serum.

(Atkinson *et al*., 2024) – Mini-Systematic Review, a mini-systematic review analyzed 21 studies to assess the association between metformin use and vitamin B_{12} deficiency. The review concluded that higher doses and longer durations of metformin therapy are linked to increased risk of B_{12} deficiency, recommending routine screening for at-risk patients.

2 -Materials and Laboratory Equipment:

This section provides a detailed description of the laboratory materials, equipment, and instruments utilized throughout the study. All procedures were conducted in accordance with standard laboratory protocols to ensure reliability, accuracy, and reproducibility of results**.**

2.1 -Blood Collection Tubes (Vacutainer Tubes)

Red Top Tube , Purple (Lavender) Top Tube , Gray Top Tube and Centrifuge

3.2Features of Vitamin B12 Testing on the Cobas e 411

1- High sensitivity and accuracy: ECLIA technology enables detection of very low concentrations of Vitamin B_{12} with excellent precision and reliability.
2- Fully automated operation: The device performs all key steps (dispensing, mixing, and measuring) automatically, minimizing human error.
3- Short analysis time: The entire test is completed in approximately 27 minutes.
4- Low sample volume: Only about 15 µL of sample is required.
5- STAT capability: The device can prioritize and rapidly process emergency (STAT) samples.

Figure **1 :** Cobas e 114.

3.3 The System XP-300:

The Sysmex XP-300, manufactured by Sysmex Corporation (Japan), is an automated hematology analyzer designed to perform a Complete Blood Count (CBC) and classify white blood cells into two main populations: Lymphocytes and a Mixed-Cell group (2part differential)

3.4 Technical Features:

1- Utilizes Direct Current (DC) detection technology for precise cell counting.
2- Employs a non-cyanide method for hemoglobin measurement, ensuring safety and environmental compliance.
3- Designed for ease of use, making it suitable for small and medium-sized laboratories.
4- Requires minimal maintenance due to its reliable and compact design.

3.5Study Design, Location, and Duration:

Comparative cross-sectional study was conducted between June 1, 2024, and February 28, 2026, at the Al-Khums Diabetes Control and Treatment Center, located in the city of AlKhums, Libya. Al-Khums is situated on the northwestern coast of Libya (latitude 32.6 north and longitude 14.26 east). The study was designed to monitor changes in Vitamin B_{12} levels and their association with metformin dosage and BMI over time among patients diagnosed with T2DM.

3.6 Study Participants and Sampling:

A total of 226 cases were initially considered for inclusion. Participants were selected using a purposive sampling method from individuals attending the Al-Khums Diabetes Control

and Treatment Center and affiliated clinics. Participants were divided into three main categories: Patients with T2DM treated with metformin, patients with T2DM treated with insulin, and a healthy control group.

3.7**Laboratory Measurements**:

Laboratory analyses were performed using standardized procedures. Vitamin B_{12} was measured using ECLIA on a Roche Cobas e411 analyzer (with ELISA validation). HbA1c was determined using HPLC (with an immunoassay confirmatory method). BMI was calculated using the standard formula:

$$\text{BMI} = \frac{\text{Weight in kilograms}}{(\text{Height in meters})^2}$$

3.8 **Statistical Analysis**:

The statistical software SPSS was used for data analysis. Differences between groups were tested with a 95% confidence interval, and results were presented as arithmetic means ± standard deviations. Statistical tests included Chi-Square (χ^2) analysis, the Kolmogorov-Smirnov test for normality, and Analysis of Covariance (ANCOVA). The level of significance was set at $p < 0.05$. ANCOVA was specifically applied to compare Vitamin B_{12} mean levels among the three groups while adjusting for potential confounding variables such as age and duration of diabetes.

4. Results:

This section presents the study findings, focusing on the demographic and personal characteristics of participants across the three groups (metformin, insulin, and healthy control), and the statistical analyses conducted to assess group equivalence. Data are summarized in tables and described narratively to highlight key similarities and differences.

4.1. Chi-Square Test for Conformity (Chi-Square)

Table (1) shows the results of the Chi-Square test for the demographic and personal variables.

			Group				Chi ² Value	P-Value	Chi -Square)	Verification result
			A	B	C	Total				
Age	From 20Y to 30 Y	Count	16	4	0	20	5.13	.163		Equality
		% of Total	7.1%	1.8%	0.0%	8.8%				
	From 31Y to 40 Y	Count	18	9	1	28				
		% of Total	8.0%	4.0%	0.4%	12.4%				
	From 41Y to 50 Y	Count	21	10	17	48				
		% of Total	9.3%	4.4%	7.5%	21.2%				
		Count	6	20	29	55				

	From 51Y to 60 Y	% of Total	2.7%	8.8%	12.8 %	24.3%				
	From 61Y to 70 Y	Count	2	16	33	51				
		% of Total	0.9%	7.1%	14.6 %	22.6%				
	More than 70 Y	Count	0	8	16	24				
		% of Total	0.0%	3.5%	7.1%	10.6%				
	Total	Count	63	67	96	226				
		% of Total	27.9%	29.6 %	42.5 %	100.0 %				

			Group				Chi ^{2} Value	P-Value Chi -Square) (	Verification result
			A	**B**	**C**	**Total**			
Sex	Male	Count	18	33	41	92	110.36	.000	Inequality
		% of Total	8.0%	14.6%	18.1%	40.7%			
	Female	Count	45	34	55	134			
		% of Total	19.9%	15.0%	24.3%	59.3%			
	Total	Count	63	67	96	226			
		% of Total	27.9%	29.6%	42.5%	100.0%			
Education Level	Low	Count	6	31	54	91	6.02	.050	Equality
		% of Total	2.7%	13.7%	23.9%	40.3%			
	Medium	Count	19	20	32	71			
		% of Total	8.4%	8.8%	14.2%	31.4%			
	High	Count	38	16	10	64			
		% of Total	16.8%	7.1%	4.4%	28.3%			
	Total	Count	63	67	96	226			
		% of Total	27.9%	29.6%	42.5%	100.0%			
	Housewife	Count	0	26	0	26	2.66	.617	

Occupation		% of Total	0.0%	11.5%	0.0%	11.5%			Equality
	Unemployed	Count	0	7	1	8			
		% of Total	0.0%	3.1%	0.4%	3.5%			
	Employee	Count	63	33	93	189			
		% of Total	27.9%	14.6%	41.2%	83.6%			
	Retired	Count	0	1	2	3			
		% of Total	0.0%	0.4%	0.9%	1.3%			
	Total	Count	63	67	96	226			
		% of Total	27.9%	29.6%	42.5%	100%			

			Group				Chi ^{2} Value	P-Value Chi -Square) (	Verification result
			A	B	C	Total			
Economic situation	Low	Count	1	5	13	19	10.03	Chi.055	Equality
		% of Total	0.4%	2.2%	5.8%	8.4%			
	Medium	Count	57	57	81	195			
		% of Total	25.2%	25.2%	35.8%	86.3%			
	High	Count	5	5	2	12			
		% of Total	2.2%	2.2%	0.9%	5.3%			
	Total	Count	63	67	96	226			
		% of Total	27.9%	29.6%	42.5%	100.0%			
	Unhealthy	Count	34	47	60	141	3.62	.163	

Type of food	Healthy	% of Total	15.0%	20.8%	26.5%	62.4%			Equality
		Count	29	20	36	85			
		% of Total	12.8%	8.8%	15.9%	37.6%			
	Total	Count	63	67	96	226			
		% of Total	27.9%	29.6%	42.5%	100.0%			
exercise	No	Count	48	44	58	150	4.56	.119	Equality
		% of Total	21.2%	19.5%	25.7%	66.4%			
	Yes	Count	15	23	38	76			
		% of Total	6.6%	10.2%	16.8%	33.6%			
	Total	Count	63	67	96	226			
		% of Total	27.9%	29.6%	42.5%	100.0%			

Table (1) shows the results of the Chi-Square test for the demographic and personal variables.

I. Age Group

Age Group	A	B	C	Total	χ^2	Sig.
From 20 Y to 30 Y (Count)	16	4	0	20	5.13	.163
% of Total	7.1%	1.8%	0.0%	8.8%		
From 31 Y to 40 Y (Count)	18	9	1	28		
% of Total	8.0%	4.0%	0.4%	12.4%		
From 41 Y to 50 Y (Count)	21	10	17	48		
% of Total	9.3%	4.4%	7.5%	21.2%		
From 51 Y to 60 Y (Count)	6	20	29	55		
% of Total	2.7%	8.8%	12.8%	24.3%		
From 61 Y to 70 Y (Count)	2	16	33	51		
% of Total	0.9%	7.1%	14.6%	22.6%		
More than 70 Y (Count)	0	8	16	24		
% of Total	0.0%	3.5%	7.1%	10.6%		
Total (Count)	**63**	**67**	**96**	**226**		
% of Total	**27.9%**	**29.6%**	**42.5%**	**100.0%**		

II. Gender

Gender	A	B	C	Total	χ^2	Sig.
Male (Count)	18	33	41	92	110.36	.000
% of Total	8.0%	14.6%	18.1%	40.7%		
Female (Count)	45	34	55	134		
% of Total	19.9%	15.0%	24.3%	59.3%		
Total	**63**	**67**	**96**	**226**		

III. Education Level

Level	A	B	C	Total	χ^2	Sig.
Low (Count)	6	31	54	91	6.02	.050
% of Total	2.7%	13.7%	23.9%	40.3%		
Medium (Count)	19	20	32	71		
% of Total	8.4%	8.8%	14.2%	31.4%		
High (Count)	38	16	10	64		
% of Total	16.8%	7.1%	4.4%	28.3%		
Total	**63**	**67**	**96**	**226**		

IV. Occupation

Occupation	A	B	C	Total	χ^2	Sig.
Housewife (Count)	0	26	0	26	2.66	.617
% of Total	0.0%	11.5%	0.0%	11.5%		
Unemployed (Count)	0	7	1	8		
% of Total	0.0%	3.1%	0.4%	3.5%		
Employee (Count)	63	33	93	189		
% of Total	27.9%	14.6%	41.2%	83.6%		
Retired (Count)	0	1	2	3		
% of Total	0.0%	0.4%	0.9%	1.3%		
Total	**63**	**67**	**96**	**226**		

V. BMI Category

Category	A	B	C	Total	χ^2	Sig.
Low (Count)	1	5	13	19	10.03	.055
% of Total	0.4%	2.2%	5.8%	8.4%		
Medium (Count)	57	57	81	195		
% of Total	25.2%	25.2%	35.8%	86.3%		
High (Count)	5	5	2	12		
% of Total	2.2%	2.2%	0.9%	5.3%		
Total	**63**	**67**	**96**	**226**		

VI. Lifestyle

Lifestyle	A	B	C	Total	χ^2	Sig.
Unhealthy (Count)	34	47	60	141	3.62	.163
% of Total	15.0%	20.8%	26.5%	62.4%		
Healthy (Count)	29	20	36	85		
% of Total	12.8%	8.8%	15.9%	37.6%		
Total	**63**	**67**	**96**	**226**		

VII. Complications

Complications	A	B	C	Total	χ^2	Sig.
No (Count)	48	44	58	150	4.56	.119
% of Total	21.2%	19.5%	25.7%	66.4%		
Yes (Count)	15	23	38	76		
% of Total	6.6%	10.2%	16.8%	33.6%		
Total	**63**	**67**	**96**	**226**		

From the table above, we find that the P-Value (Chi-Square) value for all demographic and personal variables is greater than the significance value of 0.05,

except for the gender variable, for which the P-Value (Chi-Square) value was less than the significance value of 0.05. Thus, all demographic and personal variables are equivalent. Even if the statistical significance is weak, it doesn't mean there's no effect; it does have an impact, but it's not apparent because it's small and under specific conditions, requiring future studies.As for the gender variable, it is not equivalent between the three groups, so it is necessary to know the extent of its effect when studying the effect of the independent variables, and work to address this effect.

4.2. Statistical description of the research sample based on participants' general information

4.2.1 Body Mass Index (BMI)

Table No. (2) Shows the distribution of the research sample according to BMI

BMI	Frequency	Percent %
Normal Weight	90	39.8
Overweight	89	39.4
Moderate Obesity	29	12.8
Severe Obesity	10	4.4
Very Severe Obesity	8	3.5
Total	226	100%

From Table No. (2), we find that the highest percentage was (Normal Weight), which reached 39.8%.

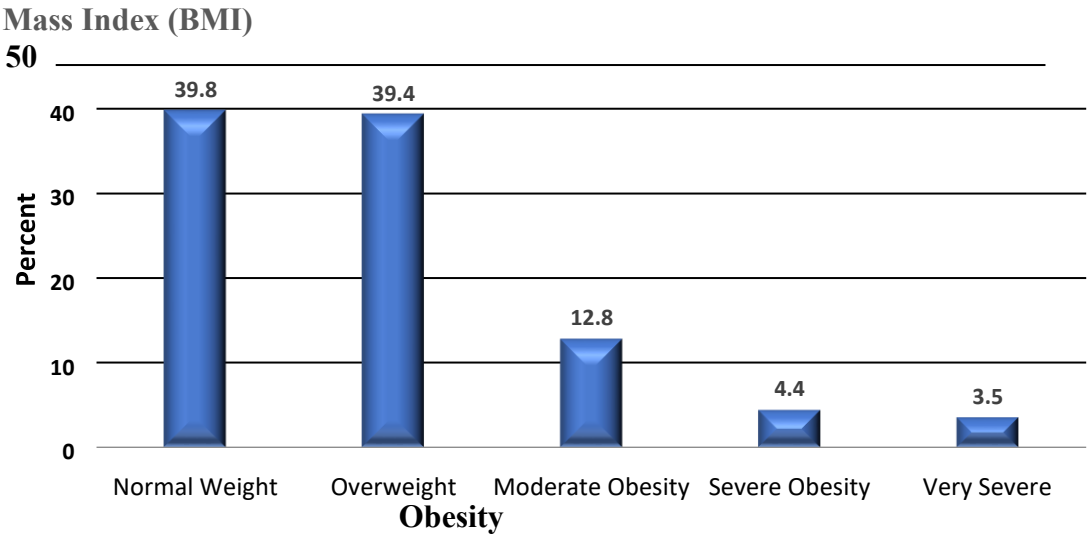

Figure 2: *Shows the distribution of the research sample according to BMI.*

Results of testing the third hypothesis:

The third hypothesis states, "There are statistically significant differences in hemoglobin concentrations between type II diabetes patients taking metformin and the healthy group who do not suffer from diabetes and therefore do not take medication for it."

To test this hypothesis, the analysis of common variance (ANCOVA) was used to eliminate the effect of gender and to determine the presence of differences between the pre- and post-test hemoglobin analyses.

Table (3) : shows the results of the analysis of common variance (ANCOVA) to test the third hypothesis.

Test	The group	Number of sample members	arithmetic mean	standard deviation	Corrected arithmetic mean*	Corrected standard deviation**	F-value	(P-Value) for F
Dimensional analysis of hemoglobin	control A	63.00	13.64	1.79	13.72	0.19	0.90	.409
Dimensional analysis of hemoglobin	experimental C	96.00	13.40	1.89	13.61	149.00		

1- The corrected arithmetic mean is the mean of the post-analysis of hemoglobin after eliminating the effect of the gender variable and the differences in the preanalysis results of hemoglobin between groups.

2- Corrected standard deviation is the standard deviation of the post-test hemoglobin analysis after eliminating the effect of gender and differences in the pre-test hemoglobin results between groups.

Looking at Table No. (1), we find that the (P-Value) for the F test is greater than the significance level of 0.05, which means that there are no statistically significant differences at the (0.05) level between the results of the post-hemoglobin analysis for the control group A and the results of the post-hemoglobin analysis for the experimental group C, which means that there is no effect of metformin treatment on blood hemoglobin in the study samples.

4.3.4 Results of testing the fourth hypothesis:

The fourth hypothesis states "Type II diabetes patients who take metformin and have a high BMI have a greater average decrease in vitamin B_{12} than type II diabetes patients who take metformin and have a low BMI."

To test this hypothesis, an ANCOVA test was used to eliminate the effect of gender and to determine the presence of differences between the pre- and post-analysis of B_{12}. .

Table (4) shows the results of the ANCOVA test for testing the fourth hypothesis

The group	Variable	Number of sample members	arithmetic mean	standard deviation	Corrected arithmetic mean*	Corrected standard deviation **	F-value	(P-value) for F
experimental C	BMI – Normal Weight	90	-10.24	18.54	-14.24	14.54		
experimental C	BMI – Overweight	89	-30.68	54.49	-36.68	48.49		
experimental C	BMI – Moderate Obesity	29	-68.578	82.147	-81.08	80.15		
experimental C	BMI – Severe Obesity	10	-48.699	107.737	-51.20	100.24	**1.90**	**.001**
experimental C	BMI – extreme obesity	8	-145.798	141.895	-157.15	134.03		
	TOTAL	**226**						

1- The corrected arithmetic mean is the mean of the post-test vitamin B_{12} deficiency after eliminating the effect of gender and differences in pre-test vitamin B_{12} scores between groups.

2- The corrected standard deviation is the deviation of the post-test vitamin B_{12} deficiency after eliminating the effect of gender and differences in pre-test vitamin B_{12} scores between groups.

Looking at Table No. (4), we find that the (P-Value) for the F test is less than the significance level of 0.05, which means that there are statistically significant differences at the (0.05) level for the rate of decline in the analysis of (B_{12}), between the classifications of body mass. When looking at the corrected arithmetic mean for the rate of decline, we find that it increases as the body mass increases, The decline in vitamin B12 levels increases with increasing BMI

Discussion

This discrepancy in results is also supported by a number of other studies that have indicated that women are more likely to suffer from vitamin B_{12} deficiency in areas such as Mecca and Jordan **(Khalifa and Jaber. 2024),** as well as data from the National Health and Nutrition Examination Survey (NHANES) in the United States (CDC). However, there are studies that do not support this relationship, with large-scale studies finding that men are more likely to be deficient **(Khan and Sharma. 2025; Margalit *et al.*, 2018),** or showing no statistically significant association between gender and vitamin levels, reinforcing the discrepancy observed in the scientific literature.

Therefore, it is recommended to consider the importance of equal gender distribution when selecting samples for future studies, as imbalances in this variable could potentially affect the interpretation of results, especially in research related to vitamin deficiencies and their associated biological factors.

Table (1) shows the distribution of the study sample by body mass index (BMI). The data indicate that the sample was fairly balanced, with the highest percentage of participants falling within the normal weight category (39.8%), closely followed by the overweight category (39.4%). Obesity categories were less represented, with moderate obesity comprising 12.8%, morbid obesity 4.4%, and severe obesity 3.5% of the total sample. This distribution reflects the demographic characteristics of the study sample and indicates that the study included a diverse group of individuals with respect to body weight, was observed alongside the reliability of the results.

Was observed alongside .

In addition to describing the sample characteristics, the main findings of the study show a statistically significant association between BMI and vitamin B_{12} deficiency ($F < 0.05$). This finding confirms the presence of significant differences in the rates of vitamin B_{12} deficiency across different BMI categories.

These findings are largely consistent with numerous previous studies that have examined the relationship between obesity and vitamin B_{12} deficiency, particularly in the context of metformin use. For example, a study by **(Kibirige *et al.*, 2014)** in Uganda found that diabetic patients with a higher BMI were more likely to develop vitamin B_{12} deficiency when using metformin. This convergence supports the idea that obesity may be a contributing factor that exacerbates (is associated with the negative effect of metformin on vitamin B_{12} levels.

Conclusions:

1- The results showed a highly statistically significant association between body mass index (BMI) and the severity of vitamin B_{12} deficiency.

2- The incidence of vitamin B_{12} deficiency was directly proportional to the is associated with increase in BMI, suggesting that obesity may be a compounding factor that increases the negative effects of metformin , is associated with

3- Despite the significant decrease in vitamin B_{12}, no statistically significant effect on hemoglobin levels was observed in the study sample. This suggests that the deficiency may be gradual, allowing the body to compensate for the deficiency for a period of time, but this does not reduce the risk of potential long-term complications.

4- The study revealed a disturbing finding: the vast majority of participants (83.2%) were unaware of the effect of metformin on vitamin B_{12}. This reflects a clear deficiency in health education and communication between healthcare providers and patients.

Recommendations

1-. Symptom Recognition and Adherence: Train patients to recognize early symptoms of deficiency (e.g., fatigue, numbness, tingling) and encourage timely medical reporting. This is critical for promoting self-monitoring and enhancing patient adherence to chronic disease self-management strategies.

2- Nutritional and Supplement Counseling: Counsel high-risk patients on increasing intake of B_{12}rich foods (e.g., meat, fish, dairy) and ensure strict adherence to prescribed supplementation protocols.

Future Research DirectionsThis section highlights critical knowledge gaps requiring further investigation to refine clinical guidelines.

3-Mechanistic Research and Dose Optimization: Investigate the molecular pathways by which elevated (BMI) influences Vitamin B_{12} absorption and metabolism in Metformin-

treated patients. Additionally, conduct Randomized Controlled Trials (RCTs) to rigorously determine the most effective dose and frequency for
prophylactic B_{12} supplementation.

4- Long-term Outcome Assessment: Implement long-term interventional studies assessing the impact of supplementation on B_{12} levels, neurological outcomes, hematological parameters (including hemoglobin status), and overall metabolic control.

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Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.