Malabsorption of Vitamin B₁₂ in Diabetic Patients Treated with Phenformin: A Comparison with Metformin*

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British Medical Journal, 1973, 3, 673-675

Summary

An investigation into B₁₂ absorption in diabetic patients on long-term phenformin therapy was undertaken and the results were compared to a similar investigation previously reported in diabetics on long-term metformin. Forty-six per cent of patients were found to have B₁₂ malabsorption as shown by abnormal results of Schilling tests.

The mechanism of B₁₂ malabsorption is unknown but it is suggested that all patients on long-term phenformin therapy should, like the patients on metformin, have annual serum B₁₂ estimations until the results of a longer follow-up series are known.

Introduction

The biguanide hypoglycaemic agents metformin and phenformin are known to interfere with absorption (Czyzyk *et al.*, 1968). Carbohydrate malabsorption is probably beneficial to maturity onset diabetes in obese subjects and the hypoglycaemic effect of the drugs may be in part related to their effect on absorption. A detrimental effect of metformin was the finding of vitamin B₁₂ malabsorption in 30% of patients on long-term metformin therapy (Tomkin *et al.*, 1971). Phenformin has not been shown to cause B₁₂ malabsorption in acute short-term experiments (Willms and Creutzfeldt, 1970) but it seemed important to verify this in patients on long-term therapy.

This paper reports an investigation of B₁₂ absorption in diabetic patients on long-term phenformin therapy and compares the results with the previously reported study of patients on long-term metformin therapy (Tomkin *et al.*, 1971).

Subjects and Methods

Twenty-four unselected patients with maturity onset diabetes who had been on phenformin for at least three years and were attending the Cincinnati General Hospital Diabetic Clinic were investigated. The mean age of these patients was 54 years (S.E. of mean $\pm$ 2.1). They had been on 102.4 $\pm$ 6.4 mg of phenformin for 4.8 $\pm$ 0.5 years (mean $\pm$ S.E. of mean). Eleven subjects who did not have diabetes or malabsorption, and who had normal renal function, acted as controls. A Schilling test was performed on all subjects using ⁵⁸Co B₁₂ with intrinsic factor. Urine was collected for 48 hours in two 24-hour aliquots. Serum B₁₂ and folic acid estimations were carried out in Belfast as previously described (Tomkin *et al.*, 1971) in order to compare the results with our previous study.

*Part of this work was presented at the Spring Meeting of the British Diabetic Association, 1973.

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Results

The results of the Schilling tests are shown in fig. 1. In 13 patients 24-hour Schilling tests showed normal results ($>10\%$). The mean urine excretion ($\pm$ S.E. of mean) in this group (group 1) was 15.2 $\pm$ 1.3%. This level was not significantly different from the mean excretion of the controls (group 3) which was 14.1 $\pm$ 1.1% ($P > 0.05$). Eleven patients on phenformin had abnormally low results of 24-hour Schilling tests (group 2). Their mean excretion was 5.9 $\pm$ 0.6%. This was significantly less than group 3 ($t = 6.54$, $P < 0.001$). The second 24-hour mean excretion of group 2 was 3.9 $\pm$ 0.6%, which was not significantly lower when compared to the second 24-hour excretion of group 3 (6.5 $\pm$ 2.2%, $P > 0.05$). Repeat tests were carried out on five patients in group 2 (fig. 2). One result had increased to borderline normal, the others remained abnormally low. Four patients from group 2 had their tests repeated after stopping phenformin for two weeks. All the repeat tests had become normal (fig. 3).

FIG. 1—Results of Schilling tests.

FIG. 2—Repeat Schilling test results in patients from group 2.

TABLE I—Comparison of Group 1 with Group 2

	Age (Mean $\pm$ S.E. of Mean)	Phenformin Dose (mg/day) (Mean $\pm$ S.E. of Mean)	Duration of Therapy (Years)	Haemoglobin (g/100 ml)	Serum B ₁₂ (pg/ml)	Serum Folic Acid (ng/ml)	Blood Urea Nitrogen (mg/100 ml)
Group 1	54.1 $\pm$ 2.9	104.5 $\pm$ 10.6	4.6 $\pm$ 0.5	13.4 $\pm$ 0.37	655.3 $\pm$ 82.3	6.6 $\pm$ 1.4	16.1 $\pm$ 2.3
Group 2	55.7 $\pm$ 2.6	100.0 $\pm$ 7.4	5.3 $\pm$ 0.8	13.6 $\pm$ 0.26	640.1 $\pm$ 24.9	5.1 $\pm$ 1.0	17.8 $\pm$ 2.6
Significance	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05

FIG. 3—Repeat Schilling test results in patients from group 2 after cessation of phenformin.

COMPARISON OF GROUP 1 WITH GROUP 2

The mean age of group 1 was 54.1 ± 2.9 years (table I). This was not significantly different from group 2 whose mean age was 55.7 ± 2.6 ($P > 0.05$). The mean dose of phenformin in group 1 was 104.5 ± 10.6 mg/day and in group 2 was 100.0 ± 7.4 mg/day ($P > 0.05$). There was no difference between the duration of therapy in the two groups (group 1 = 4.6 ± 0.5 years, group 2 = 5.3 ± 0.8 years; $P > 0.05$). The mean haemoglobin level in group 1 was 13.4 ± 0.37 g/100 ml. This was not significantly different from group 2 (13.6 ± 0.26 g/100 ml, $P > 0.05$). None of the patients were anaemic (Hb < 13.5 g/100 ml in men and < 11.5 g/100 ml in women). The mean serum B₁₂ level in group 1 was 655.3 ± 82.3 pg/ml, and in group 2 was 640.1 ± 24.9 pg/ml ($P > 0.05$). Serum folic acid in group 1 was 6.6 ± 1.4 ng/ml, and in group 2 was 5.1 ± 1.0 ng/ml. Again this difference was not significant ($P > 0.05$). Blood urea nitrogen (normal 10–20 mg/100 ml) was 16.1 ± 2.3 mg/100 ml in group 1 and 17.8 ± 2.6 mg/100 ml in group 2. The difference was not significant ($P > 0.05$).

COMPARISON WITH 1971 STUDY

The mean age of the patients on metformin was 62.2 ± 1.2 years (table II). They were significantly older than the patients on phenformin whose mean age was 53.9 ± 2.1 years ($t = 3.1$, $P < 0.01$). The incidence of low results of Schilling tests in this study was 46% as compared to 30% in the metformin study. This difference was not significant ($\chi^2 = 1.46$, $P > 0.05$). The mean duration of therapy of the phenformin patients was 4.8 ± 0.5 years as compared to a mean of 4.7 ± 0.4 years for the metformin patients. The mean 24-hour B₁₂ excretion during the Schilling test was $4.8 \pm 0.5\%$ in the metformin patients with abnormal results. This was very similar to the mean of group 2 patients on phenformin. The mean B₁₂ level in all the

TABLE II—Comparison of Patients on Phenformin (Groups 1 and 2) with Patients on Metformin (1971 Study)

	Age (Mean $\pm$ S.E. of Mean)	Incidence of Low Schilling Test Results	Duration of Therapy (Years)	Serum B ₁₂ (pg/ml)	Serum Folic Acid (ng/ml)
Metformin patients	62.2 $\pm$ 1.2	30%	4.7 $\pm$ 0.4	495.7 $\pm$ 28.9	7.9 $\pm$ 0.6
Phenformin patients	53.9 $\pm$ 2.1	46%	4.8 $\pm$ 0.5	636.0 $\pm$ 39.25	6.8 $\pm$ 0.72
		$\chi^2 = 1.46$; $P > 0.05$			

patients investigated on metformin was 495.7 ± 28.9 pg/ml, which was lower than the mean B₁₂ level of groups 1 and 2 on phenformin, which was 636.0 ± 39.25 pg/ml. The mean serum B₁₂ in the metformin patients with B₁₂ malabsorption was 381.2 ± 57.5 pg/ml. This was much lower than the mean level in group 2 on phenformin which was 640.1 ± 24.9 pg/ml.

Mean serum folic acid in all the patients investigated on metformin was 7.9 ± 0.6 ng/ml. This was similar to the mean level of groups 1 and 2 (mean 6.8 ± 0.7 ng/ml). The mean serum folic acid was 9.6 ± 1.6 ng/ml in the metformin patients with B₁₂ malabsorption. This was higher than the level found in group 2, which was 5.1 ± 1.0 ng/ml.

Discussion

The mode of action of the biguanides in lowering the blood sugar in mild diabetic patients is uncertain but it has been shown that these drugs inhibit the absorption of glucose (Czyzyk *et al.*, 1968; Hollobaugh *et al.*, 1970) and in vitro experiments have shown the impairment of the active absorption of glucose (Kruger *et al.*, 1970). Vitamin B₁₂ is another substance that is absorbed by active transport from the small bowel (distal ileum), and it has been shown that metformin interferes with the absorption of B₁₂ (Berchtold *et al.*, 1969; Willms and Creutzfeldt, 1970; Tomkin *et al.*, 1971). The effect of phenformin on B₁₂ absorption has been studied only in patients taking the drug for short periods (Willms and Creutzfeldt, 1970), when no abnormality of B₁₂ absorption was found. This study has shown that almost half (46%) of the patients examined had B₁₂ malabsorption as shown by pathologically low excretion of ⁵⁸Co B₁₂ in the urine. The Schilling test depends on the absorption of the labelled vitamin from the distal ileum and the renal clearance of the vitamin. Delay in the absorption of the vitamin during the test might be due to delay in gastric emptying which is known to occur in a proportion of diabetics (Tomkin, 1973), but if this was so it would be expected that the second 24-hour urine collection would contain a higher percentage of the vitamin than the controls. This was not found. The second 24-hour excretion in group 2 was 3.9% as compared with 6.5% in group 3. A second possibility is that the renal clearance of the labelled vitamin might have been impaired in group 2. This is also unlikely as the blood urea nitrogen levels were normal and not significantly different from group 1 ($P > 0.05$). If there was a delay in renal clearance then the second 24-hour value might be expected to be greater than that of group 3, but this was not the case. Finally, in our previous study a control group of 19 diabetics on long-term chlorpropamide therapy had normal

results of Schilling tests, which suggests that it is rare for a diabetic with a normal blood urea to have an abnormal test result unless they are on metformin or phenformin therapy.

The abnormality in the result of the Schilling test appears reproducible as only one out of five patients retested had improved the percentage $^{58}\text{Co B}_{12}$ excretion in the urine, and even then the result was only borderline normal (fig. 2). That the phenformin was responsible for the abnormal results is suggested by the normal repeat tests in the four patients from group 2 after the drug was discontinued (fig. 3). The group of patients on metformin previously studied were on average eight years older but the duration of therapy was similar as was the depression in B_{12} excretion. The effect of malabsorption of B_{12} in group 2 was not apparent as no patient was found to be anaemic or B_{12} deficient and B_{12} levels were not significantly different from group 1 ($P > 0.05$). As only 24 patients on phenformin were examined this is not significantly different from the findings in the metformin study. Only four patients out of 71 were discovered with evidence of B_{12} deficiency in the metformin study ($P = 0.57$). Twenty-one of these patients had low Schilling test results and the four patients discovered with low B_{12} levels all had low results. Only 11 patients in group 2 had low results and again the finding of no abnormal B_{12} level in this small sample was not significantly different from the findings in the metformin study ($P = 0.29$).

The higher mean B_{12} levels in groups 1 and 2 when compared to the mean level of the metformin patients (table II) suggests that the inhibition of B_{12} absorption may be less severe in patients on phenformin. This is further supported by the lower serum folic acid levels in group 2 when compared to the patients with B_{12} malabsorption on metformin, because some of the highest levels of serum folic acid are found in patients with pernicious anaemia (Herbert *et al.*, 1960; Waters and Mollin, 1961). Therefore, an inverse relation between serum B_{12} and folic acid might be expected.

The higher mean serum B_{12} in group 2 may be explained by the concentration of biguanide in the intestinal lumen, as the therapeutic dose of metformin is about 10 times that of phenformin. Alternatively, the difference may be due to the difference in the metabolism of the two drugs, metformin being excreted unchanged in the urine (Beckmann, 1969), whereas about one-third of phenformin is metabolized to hydroxy-

phenethyl biguanide (Beckmann, 1968). Clarification of the mechanism of B_{12} malabsorption may come when it is known whether the effect is a local one on mucosa of the distal ileum or whether prior absorption of the drug is necessary. It seems unlikely that a genetic difference in the handling of the biguanides could account for the high incidence of B_{12} malabsorption, as a unimodal distribution of results of 24-hour Schilling tests was found in the combined metformin and phenformin patients.

Partial gastric resection is known to cause B_{12} malabsorption but the discovery of vitamin B_{12} deficiency may take from 12 to 15 years (Deller and Witts, 1962; Weir *et al.*, 1963). It is therefore concluded that though no patient in this study was shown to have B_{12} deficiency, in view of the high proportion of abnormal results of Schilling tests, serum B_{12} levels should be estimated annually in all patients on long-term phenformin or metformin therapy.

I wish to thank Professor Alastair Connell for his helpful advice and encouragement during the tenure of my Fellowship in his department.

Professor Harvey Knowles, jun., and Dr. Kenneth Kreines kindly allowed me to study patients under their care.

Dr. Edward Silberstein suggested the 48-hour Schilling tests, which were carried out in his department. His helpful comments are gratefully acknowledged.

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