Long term treatment with metformin in patients with type 2 diabetes and risk of vitamin B-12 deficiency: randomised placebo controlled trial

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ABSTRACT

Objectives To study the effects of metformin on the incidence of vitamin B-12 deficiency (<150 pmol/l), low concentrations of vitamin B-12 (150-220 pmol/l), and folate and homocysteine concentrations in patients with type 2 diabetes receiving treatment with insulin.

Design Multicentre randomised placebo controlled trial.

Setting Outpatient clinics of three non-academic hospitals in the Netherlands.

Participants 390 patients with type 2 diabetes receiving treatment with insulin.

Intervention 850 mg metformin or placebo three times a day for 4.3 years.

Main outcome measures Percentage change in vitamin B-12, folate, and homocysteine concentrations from baseline at 4, 17, 30, 43, and 52 months.

Results Compared with placebo, metformin treatment was associated with a mean decrease in vitamin B-12 concentration of −19% (95% confidence interval −24% to −14%; P<0.001) and in folate concentration of −5% (95% CI −10% to −0.4%; P=0.033), and an increase in homocysteine concentration of 5% (95% CI −1% to 11%; P=0.091). After adjustment for body mass index and smoking, no significant effect of metformin on folate concentrations was found. The absolute risk of vitamin B-12 deficiency (<150 pmol/l) at study end was 7.2 percentage points higher in the metformin group than in the placebo group (95% CI 2.3 to 12.1; P=0.004), with a number needed to harm of 13.8 per 4.3 years (95% CI 43.5 to 8.3). The absolute risk of low vitamin B-12 concentration (150-220 pmol/l) at study end was 11.2 percentage points higher in the metformin group (95% CI 4.6 to 17.9; P=0.001), with a number needed to harm of 8.9 per 4.3 years (95% CI 21.7 to 5.6). Patients with vitamin B-12 deficiency at study end had a mean homocysteine level of 23.7 μmol/l (95% CI 18.8 to 30.0 μmol/l), compared with a mean homocysteine level of 18.1 μmol/l (95% CI 16.7 to 19.6 μmol/l; P=0.003) for patients with a low vitamin B-12 concentration and 14.9 μmol/l (95% CI 14.3 to 15.5 μmol/l; P<0.001 compared with vitamin B-12 deficiency; P=0.005 compared with low vitamin B-12) for patients with a normal vitamin B-12 concentration (>220 pmol/l).

Conclusions Long term treatment with metformin increases the risk of vitamin B-12 deficiency, which results in raised homocysteine concentrations. Vitamin B-12 deficiency is preventable; therefore, our findings suggest that regular measurement of vitamin B-12 concentrations during long term metformin treatment should be strongly considered.

Trial registration Clinicaltrials.gov NCT00375388.

INTRODUCTION

Metformin is considered a cornerstone in the treatment of diabetes and is the most frequently prescribed first line therapy for individuals with type 2 diabetes.[1] In addition, it is one of a few antihyperglycaemic agents associated with improvements in cardiovascular morbidity and mortality,[2,3] which is a major cause of death in patients with type 2 diabetes.[4]

There are few disadvantages to the use of metformin. Metformin does, however, induce vitamin B-12 malabsorption, which may increase the risk of developing vitamin B-12 deficiency[5-7]—a clinically important and treatable condition. In addition, metformin treatment has been reported to be associated with decreased folate concentration, although the mechanism of this effect has not been elucidated.[8] Finally, decreases in both folate and vitamin B-12 concentrations might, in turn, result in an increase in homocysteine concentrations (web figure A), an independent risk factor for cardiovascular disease, especially among individuals with type 2 diabetes.[9-11]

All current evidence on vitamin B-12 deficiency in metformin treatment comes from short term studies.[5-7 12-14] No long term, placebo controlled data on the effects of metformin on concentrations of vitamin B-12 in patients with type 2 diabetes have been reported. In addition, placebo controlled data on the effects of metformin on homocysteine concentrations in type 2 diabetes are sparse,[12 15] and again no long term data are available.
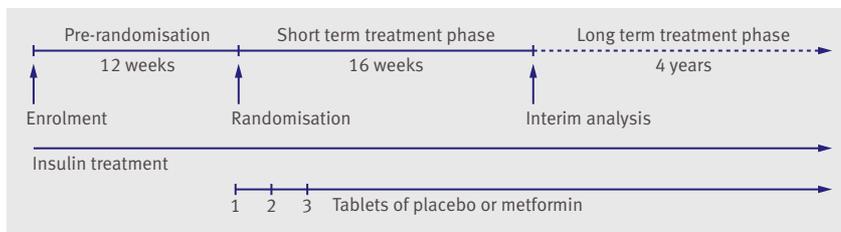
Fig 1 | HOME trial schedule

We studied the effects of metformin treatment on serum concentrations of vitamin B-12, folate, and homocysteine in patients with type 2 diabetes in a long term placebo controlled trial.

METHODS

Patients

This study was part of the Hyperinsulinaemia: the Outcome of its Metabolic Effects (HOME) randomised trial investigating the effects of metformin on metabolism and on microvascular and macrovascular disease in type 2 diabetes. The trial included 390 patients aged 30-80 years with type 2 diabetes who were receiving treatment with insulin, as previously described.[3 16]

Study design

The HOME trial was conducted in the outpatient clinics of three non-academic hospitals in the Netherlands: Bethesda General Hospital, Hoogeveen; Diaconessen Hospital, Meppel; and Aleida Kramer Hospital, Coevorden. Patients were randomly assigned by a computer program to receive either 850 mg of metformin three times a day or 850 mg of placebo thrice daily, which were provided in identical looking boxes.

The trial consisted of three phases: the 12 week pre-randomisation phase, in which patients were treated with insulin only and concomitant medication was discontinued; the 16 week short term treatment phase, at the beginning of which patients were randomised to receive either metformin or placebo in addition to insulin therapy; and the four year (48 month) long term treatment phase (fig 1). An interim analysis was conducted at the end of the short term treatment phase, during which the treatment codes were not disclosed to the investigators.[3 12 16]

Visits and data collection

Patients visited the clinics at the start of the pre-randomisation phase (three months before randomisation), at baseline (for randomisation to metformin or placebo), and one month after baseline (to check the tolerance of the drug titration), then subsequently every three months until the end of the trial. During these visits, a physical examination was carried out, a medical history was taken, and laboratory investigations were performed. At baseline, and after 10 and 52 months, dietary counselling was given to all patients.

Laboratory investigations

Blood samples for this study were drawn at baseline and after 4, 17, 30, 43, and 52 months, and stored at −80°C until analysis. Concentrations of vitamin B-12, folate, and homocysteine were measured in serum. Vitamin B-12 and folate concentrations were determined by an electrochemiluminescence immunoassay (ECLIA) using the competition principle. The mean intra-assay coefficients of variation for vitamin B-12 and folate were 2.3% and 3.5%, respectively. The mean inter-assay coefficients were 2.9% and 4.7%, respectively.

Total homocysteine concentration was measured using a kit from Chromsystems (Martinsried, Germany). The results were corrected against two types of "consensus plasma samples" (SKML, Nijmegen, the Netherlands) that had concentrations of 13 μmol/l and 55 μmol/l. The correction factor found was 0.90. The intra-assay coefficients of variation were 2.2% and 1.8% at 12.8 μmol/l and 72.2 μmol/l, respectively. The inter-assay coefficients of variation were 6.1% and 5.2% at 9.8 μmol/l and 21.1 μmol/l, respectively.

In the HOME trial, vitamin B-12, folate, and homocysteine concentrations had been measured previously in samples obtained at baseline and after 16 weeks of treatment.[12] To investigate the stability of the assay procedures, we compared the previously obtained values with values obtained for the present investigation. The correlation between old and new measurements of vitamin B-12 was 0.58 for baseline measurements and 0.91 for measurements taken after 16 weeks; for folate these values were 0.90 and 0.83, respectively, and for homocysteine 0.99 and 0.99, respectively. The relatively low correlation for vitamin B-12 values obtained at baseline was caused by five cases for which a large discrepancy existed between old and new values; these cases were subsequently excluded from analyses involving vitamin B-12.

Statistical analysis

Sample size calculations were based on expected differences in the occurrence of disease related end points, as described previously.[3] With the sample size obtained, however, a decrease in vitamin B-12 concentration of 5% in the metformin group compared with the placebo group according ANCOVA tests should be detectable at a two sided 95% confidence level, with a power of 0.82.

We log transformed data on vitamin B-12, folate, and homocysteine concentrations before analysis because their distribution was skewed. Data are given as geometric means with 95% confidence intervals. Given that log values are not directly interpretable, the antilogs are reported instead. These values are the geometric mean percentage change from baseline.

The end point of interest was the percentage change of each variable from baseline at 4, 17, 30, 43, and 52 months, which was calculated from baseline values and the summary mean. The differences between the metformin and the placebo group were tested by a central *t* test on log transformed values. We also calculated
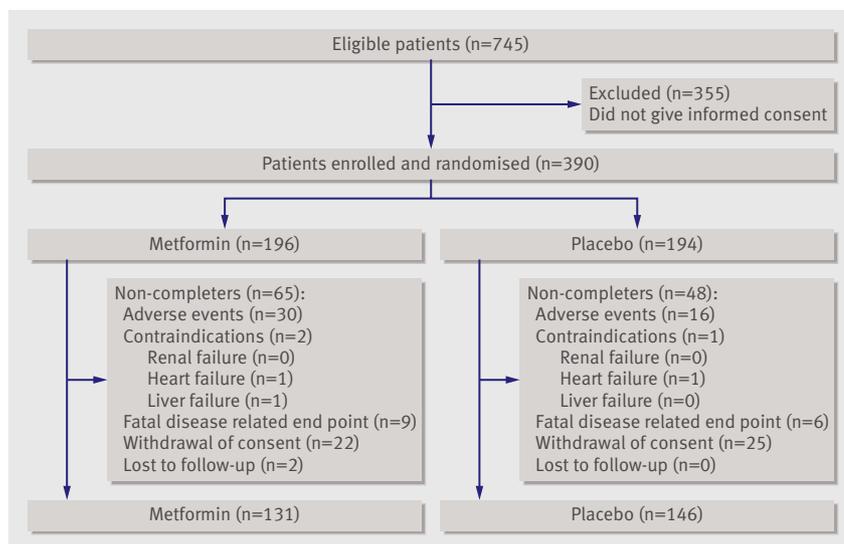
Fig 2 | HOME patient profile

the hazard ratio for developing vitamin B-12 deficiency, which was defined as a vitamin B-12 concentration below the value of 150 pmol/l, and of having low vitamin B-12 levels, which was defined as a vitamin B-12 concentration below 220 pmol/l but above 150 pmol/l.[17] All analyses were by intention to treat and used the last observation carried forward. To test whether results obtained were robust, we also used mixed models analysis to impute missing data. Patients with vitamin B-12 concentrations below 150 pmol/l at baseline, at the interim analysis, or at both time points were supplemented at 16 weeks (n=8) and, therefore, excluded from analyses after 16 weeks.

We used linear mixed models to explore the effects of metformin on concentrations of vitamin B-12, folate, and homocysteine. We also investigated whether metformin associated changes in homocysteine concentrations, if any, could be explained by changes in the concentrations of folate, vitamin B-12, or both, and, if so, whether the changes were independent of age, gender, duration of diabetes, smoking, body mass index, insulin dose, serum creatinine, high density lipoprotein cholesterol, or glycated haemoglobin. The goodness of fit between alternative models was compared using the maximum likelihood technique.

RESULTS
Patients
We screened the medical files of all three participating outpatient clinics and identified 745 eligible patients. All were approached to enrol into the trial and 390 individuals gave written informed consent. A total of 196 patients were randomised to receive metformin and 194 to receive placebo. Out of the 390 included patients, 277 individuals (72%) were still receiving metformin or placebo at the end of the trial (fig 2). A total of 46 patients (30 metformin, 16 placebo) discontinued because of adverse effects, which have been described

more extensively elsewhere.[3] Only two participants were lost to follow-up (at 33 and 26 months, respectively), both of whom were in the metformin group.

The actual mean dose in the metformin group was 2050 mg a day. At the final visit, laboratory samples were available for 256 patients (127 metformin, 129 placebo). The main outcomes of this trial have been reported previously.[3]

Table 1 shows baseline characteristics of all patients analysed. Five randomised patients were excluded from the analysis because of poor correlations between old and newly measured vitamin B-12 values (see Methods). Patients randomised to metformin were older than those randomised to placebo (64±10 years v 59±11 years), and were more likely to have a history of cardiovascular disease and less likely to be a smoker (30 (19%) v 59 (30%)). The other characteristics were comparable between the two treatment groups.

Vitamin B-12, folate, and homocysteine concentrations
During the 52 months of placebo treatment, vitamin B-12 concentration increased from baseline by 0.2 pmol/l (0% change, 95% confidence interval −3% to 4%), folate increased by 1.01 nmol/l (8%, 95% CI 4% to 12%), and homocysteine increased by 1.60 μmol/l (20%, 95% CI 16% to 25%; fig 3). During metformin treatment, vitamin B-12 decreased by 89.8 pmol/l (−19%, 95% CI −22% to −15%) from baseline, whereas folate concentration increased by 0.21 nmol/l (3%, 95% CI −1% to 6%) and homocysteine concentration increased by 3.26 μmol/l (26%, 95% CI 21% to 31%).

Compared with placebo, metformin treatment was associated with a 19% decrease in vitamin B-12 concentration (95% CI −24% to −14%; P<0.001) and a 5% decrease in folate concentration (95% CI −10% to −0.4%; P=0.033), and a 5% increase in homocysteine concentrations (95% CI −1% to 11%; P=0.091). The effects of metformin on concentrations of vitamin B-12, folate, and homocysteine were re-analysed following adjustment for age, previous metformin treatment, duration of diabetes, gender, insulin dose, and smoking habits. None of these variables materially changed the results for vitamin B-12 and homocysteine (data not shown), but they did have an effect on the results for folate. After adjustment for body mass index and smoking, no significant effect of metformin on folate concentration was found (change in concentration compared with placebo −0.1%; P=0.57).

At baseline, three patients (1.6%) in the metformin group and four (2.2%) in the placebo group had vitamin B-12 deficiency (vitamin B-12 concentration <150 pmol/l), whereas 14 patients (7.3%) and 14 patients (7.5%), respectively, had a low vitamin B-12 concentration (150-220 pmol/l). At the end of the study period, 19 patients (9.9%) in the metformin group and five (2.7%) in the placebo group had vitamin B-12 deficiency, whereas 35 patients (18.2%) and 13 patients (7.0%), respectively, had a low vitamin B-12 concentration.

The risk for vitamin B-12 deficiency at study end was 7.2 percentage points higher in the metformin group

Table 1 | Baseline characteristics of all patients analysed

	Metformin (n=194)	Placebo (n=191)
Demographics		
Men:women (n:n)	81:113	95:96
Age (years)	64 (10)	59 (11)
Currently smoking (n (%))	30 (19)	59 (30)
Duration of diabetes (years)	14 (9)	12 (8)
Insulin treatment (years)	7 (8)	6 (6)
Concomitant medication		
Lipid lowering drugs (n (%))	32 (17)	29 (15)
Blood pressure lowering drugs (n (%))	91(47)	74 (39)
Metabolic variables		
Weight (kg)	85(16)	87 (15)
Body mass index (kg/m^2)	30 (5)	30 (5)
Waist to hip ratio		
Men	1.02	1.02
Women	0.93	0.92
Plasma glycated haemoglobin (HbA$_{1c}$)	7.9 (1.2)	7.9 (1.2)
Daily dose of insulin (IU/day)	61	64
Systolic blood pressure (mm Hg)	160 (25)	160 (25)
Diastolic blood pressure (mm Hg)	86 (12)	86 (11)
Total cholesterol (mmol/l)	5.6 (1.3)	5.5 (1.2)
Low density lipoprotein cholesterol (mmol/l)	3.6 (1.1)	3.4 (1.0)
Triglycerides (mmol/l)	1.7 (1.2)	1.9 (1.5)
High density lipoprotein cholesterol (mmol/l)	1.3 (0.4)	1.3 (0.4)
Vitamin B-12 (pmol/l)	378 (130)	380 (135)
Folate (nmol/l)	18.7 (7.2)	18.7 (7.5)
Homocysteine (µmol/l)	14.4 (9.7)	14.6 (10.3)
Previous cardiovascular disease		
Myocardial infarction (n (%))	24 (12)	21 (11)
Cardiovascular intervention (n (%))	27 (14)	17 (9)
Stroke (n (%))	8 (4)	8 (4)
Non-traumatic amputation (n (%))	5 (3)	3 (2)

Values are mean (standard deviation) unless otherwise stated.

than in the placebo group (95% CI 2.3 to 12.1; P=0.004), with a number needed to harm of 13.8 per 4.3 years (95% CI 43.5 to 8.3). The risk difference at study end for a low vitamin B-12 concentration was 11.2 percentage points higher in the metformin group (95% CI 4.6 to 17.9; P=0.001), with a number needed to harm of 8.9 per 4.3 years (95% CI 21.7 to 5.6). The hazard ratio for developing vitamin B-12 deficiency when treated with metformin was 5.5 (95% CI 1.6 to 19.1; P=0.01), and the hazard ratio for a low vitamin B-12 concentration was 3.0 (95% CI 1.3 to 6.6; P=0.007).

Patients with a vitamin B-12 deficiency at the end of the study had a mean homocysteine level at study end of 23.7 µmol/l (95% CI 18.8 to 30.0 µmol/l), compared with 18.1 µmol/l (95% CI 16.7 to 19.6 µmol/l; P=0.003) for patients with a low vitamin B-12 concentration and 14.9 µmol/l (95% CI 14.3 to 15.5 µmol/l; P<0.001 compared with vitamin B-12 deficiency; P=0.005 compared with low vitamin B-12) for patients with a normal vitamin B-12 level (>220 pmol/l; fig 4). Homocysteine concentrations did not differ significantly between treatment groups when stratified for end of treatment vitamin B-12 concentration.

Linear mixed model

The interaction between treatment and time was a significant determinant of vitamin B-12 concentration (P=0.023)—that is, the lowering effect of metformin on vitamin B-12 concentrations increased with time. Body mass index and smoking were strong inverse determinants of folate concentration (P=0.003 and P<0.0001, respectively). There was no relation between time and folate concentration. After adjustment for body mass index and smoking, treatment with metformin was not a significant determinant of folate concentration, nor was the interaction between treatment and time (P=0.57 and P=0.23, respectively). Vitamin B-12 and folate levels were strong determinants of homocysteine concentration (P<0.0001). Homocysteine concentration increased with age at baseline (P<0.0001). There was no significant interaction between treatment and time for homocysteine concentrations (P=0.16).

Additional analysis

Per protocol analysis using only available data for those patients who remained in the trial until the final visit (n=256) yielded similar results to our original intention to treat analysis (data not shown). General mixed model analysis yielded similar results to analysis using last observation carried forward (data not shown).

DISCUSSION

Our study on the long term effects of metformin treatment on serum concentrations of vitamin B-12, folate, and homocysteine in patients with type 2 diabetes treated with insulin had three main findings. Firstly, metformin significantly reduced concentrations of vitamin B-12, in accordance with findings from previous studies.[13 18 19] Importantly, our study shows that this decrease is not a transitory phenomenon, but persists and grows over time. Secondly, a small, significant decrease in folate concentrations was found in the metformin group compared with the placebo group; however, this reduction was not statistically significant after adjustments for body mass index and smoking. Thirdly, the decrease in vitamin B-12 concentrations was associated with an increase in homocysteine levels, which was not statistically significant. Further analyses, however, showed that homocysteine concentrations did increase in individuals in whom vitamin B-12 levels decreased below the concentration generally considered to indicate clinical deficiency—that is, 150 pmol/l.

The finding of decreases in vitamin B-12 concentration during metformin treatment is not novel and has been reported before. A novel finding here, however, is that the decrease in vitamin B-12 levels is progressive. Furthermore, concentrations in some patients drop to the level at which most authorities agree vitamin substitution is required. This is also a novel finding, because although earlier trials in well fed, middle aged patients showed that metformin decreases vitamin B-12 concentrations, levels recorded remained within the normal range.[5 6 14]

Metformin is thought to induce malabsorption of vitamin B-12 and intrinsic factor in the ileum, an effect that can be reversed by increasing calcium intake.[6 18] The consequences of clinically important decreases in vitamin B-12 concentrations—such as macrocytic anaemia, neuropathy, and mental changes—can be profound. We note that there is no consensus on the issue of whether "asymptomatic" vitamin B-12 deficiency should be treated.[20] However, studies show that some symptoms of vitamin B-12 deficiency are difficult to diagnose and can be irreversible, and treatment of vitamin B-12 deficiency is relatively easy, cheap, safe, and effective,[21-24] in effect arguing in favour of treatment. In addition, although the necessity of treating "spontaneous" vitamin B-12 deficiency may be debated, one should be more easily inclined to treat drug induced vitamin B-12 deficiency, as a key principle of drug prescription is to do no harm. On the other hand, our study shows that it is reasonable to assume harm will eventually occur in some patients with metformin induced low concentrations of vitamin B-12.

Folate concentration increased in both the metformin group and the placebo group, possibly as a result of dietary counselling received by all patients throughout the trial. Our short term interim analysis showed a significantly larger increase in folate concentration in the placebo group,[12] a finding that was initially replicated in the current analysis but that disappeared after adjustment for body mass index and smoking.

Previous studies have shown either no or small effects of metformin treatment on concentrations of homocysteine.[13 14 25 26] We clearly show that homocysteine concentrations do increase with decreasing levels of vitamin B-12 (fig 4). The finding that metformin treatment significantly lowered concentrations of vitamin B-12 but did not significantly alter levels of homocysteine probably reflects the relatively low incidence of vitamin B-12 deficiency in the entire study population. As treatment with metformin continues, however, we expect that vitamin B-12 levels will continue to decrease, making increases in homocysteine concentrations inevitable in time.

Strengths and limitations of study

Strengths of our study include the randomised, placebo controlled, double blind design and its relatively long follow-up of 4.3 years, as well as frequent serum collection. Furthermore, the study was conducted in a non-academic setting and, therefore, the findings have high value in a community setting.

Another strength is that we used last observation carried forward in this analysis because this method is considered more conservative than general mixed model analysis, "freezing" any observed divergence between two groups by retaining the last observation made. In a mixed model analysis with missing data, estimations of future observations are made on the basis of observations made earlier in the trial, thereby reflecting a divergence more accurately but less conservatively.

Limitations of our study include the fact that we measured only total vitamin B-12 levels and not levels of
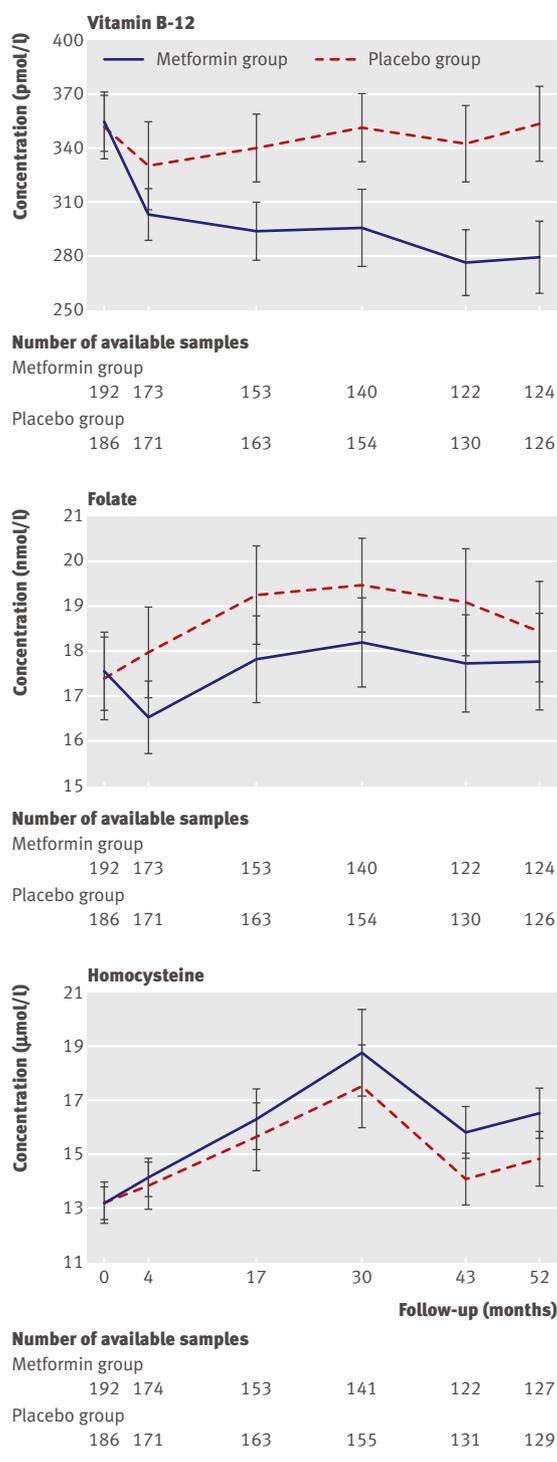

Fig 3 | Concentrations of vitamin B-12, folate, and homocysteine with 95% confidence intervals. Solid lines represent the metformin group, dotted lines the placebo group. Number of available samples for the metformin and placebo group is indicated. Five patients were excluded from the analysis because of poor correlations between old and newly measured vitamin B-12 values (see Methods). In addition, some patients had vitamin B-12 levels below 150 pmol/l at baseline, at interim analysis, or at both stages, and were supplemented; these individuals were also excluded.
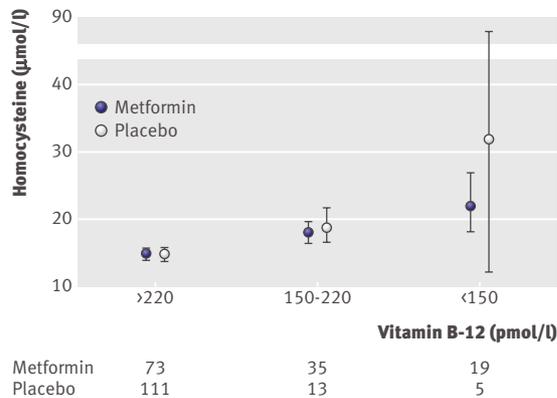
Fig 4 | Homocysteine concentrations with 95% confidence intervals for patients with a normal vitamin B-12 concentration (>220 pmol/l), a low vitamin B-12 concentration (150-220 pmol/l), and vitamin B-12 deficiency (<150 pmol/l) after 4.3 years. The number of patients in each treatment group is indicated

holotranscobalamin II or methylmalonic acid, which may have been more precise indicators of vitamin B-12 status. Finally, it is likely that, if anything, we underestimated the impact of metformin treatment on the risk of clinically important vitamin B-12 deficiency. We showed that metformin treatment was associated not only with a raised risk of developing vitamin B-12 concentrations below 150 pmol/l but also with an elevated risk of developing vitamin B-12 levels between 150 and 220 pmol/l, which is likely to represent clinically important vitamin B-12 deficiency in at least some individuals.[27] A further reason that we may have somewhat underestimated the adverse effects of metformin is that all participants in our trial received frequent dietary counselling, which may have attenuated the impact of metformin treatment on vitamin status and may not be available in routine clinical practice.

Conclusions and policy implications

In conclusion, we showed that in patients with type 2 diabetes being treated with insulin, those additionally treated with metformin had a seven percentage point greater absolute risk of vitamin B-12 deficiency than those treated with placebo during 4.3 years of follow-up. In addition, the reduction in vitamin B-12 concentration associated with metformin increased with time.

Current guidelines indicate that metformin is a cornerstone in the treatment of type 2 diabetes, but make no recommendations on the detection and prevention of vitamin B-12 deficiency during treatment. Our data provide a strong case for routine assessment of vitamin B-12 levels during long term treatment with metformin.

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WHAT IS ALREADY KNOWN ON THIS TOPIC

Metformin is considered a cornerstone in the treatment of type 2 diabetes and is frequently prescribed

Metformin is known to induce malabsorption of vitamin B-12 and may be associated with decreased folate concentrations, which might, in turn, result in an increase in homocysteine concentrations

Few and only short term data exist on the effect of metformin treatment on vitamin B-12, folate, and homocysteine

WHAT THIS STUDY ADDS

Long term treatment with metformin in patients with type 2 diabetes receiving insulin increases the risk of vitamin B-12 deficiency, which results in higher levels of homocysteine

The negative effect of metformin on vitamin B-12 concentrations increases over time

Our data provide a strong case for routine assessment of vitamin B-12 levels during long term treatment with metformin

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