Does metformin increase the serum total homocysteine level in non-insulin-dependent diabetes mellitus?

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Abstract. Hoogeveen EK, Kostense PJ, Jakobs C, Bouter LM, Heine RJ, Stehouwer CDA (Vrije Universiteit, Amsterdam, the Netherlands). Does metformin increase the serum total homocysteine level in non-insulin-dependent diabetes mellitus? *J Intern Med* 1997; **242**: 389–94.

Objective. The aim of this study was to estimate the effect of metformin on the serum total homocysteine level in non-insulin-dependent diabetes mellitus (NIDDM) patients. An elevated serum total homocysteine level is a risk factor for atherosclerosis. Metformin decreases serum vitamin B_{12}, and may thereby indirectly increase the serum total homocysteine level.

Design. A cross-sectional study in a primary care setting.

Subjects, main outcome measures. Fasting serum total homocysteine level was measured in 40 NIDDM patients who had received treatment with metformin (500–2550 mg per day) for at least six months, and in 71 NIDDM patients not treated with metformin and matched for sex, age ($\pm$ 5 years), serum creatinine ($\pm$ 5 μmol L^{-1}) and current smoking habits. 'Exposed' patients were matched with 'nonexposed' patients. A two-way analysis of variance was performed.

Results. The mean serum total homocysteine level was 11.5 μmol L^{-1} in the metformin-exposed patients and 10.6 μmol L^{-1} in the nonexposed patients. Thus, the metformin-exposed patients had slightly higher serum total homocysteine levels (difference 0.8 μmol L^{-1}, 95% confidence interval (-0.4–2.0 μmol L^{-1}). Results were similar in men and women. Finally, no dose–response relationship between cumulative exposure to metformin (dose $\times$ duration of treatment) and the serum total homocysteine level could be demonstrated.

Conclusion. We conclude that the effect of metformin on serum total homocysteine level in NIDDM patients, if any, is likely to be small.

Keywords: atherosclerosis, homocysteine, metformin, vitamin B_{12}.

Introduction

Non-insulin-dependent diabetes mellitus (NIDDM) is accompanied by accelerated atherosclerosis which can be explained, in part, by concomitant hypertension and dyslipidaemia. There is an approximately three-fold increased risk of cardiovascular disease in NIDDM patients [1].

The most effective treatment for NIDDM is currently under debate. The University Group Diabetes Program reported that sulphonylureas [2] and a biguanide (phenformin) [3] appeared to increase cardiovascular mortality significantly as compared with diet alone. In addition, it is unknown whether intensive insulin treatment of NIDDM decreases the risk for cardiovascular complications by lowering glucose levels, or increases the risk by postulated direct atherogenic effects [4]. Finally, the United Kingdom Prospective Diabetes Study has not shown any specific therapy (sulphonylurea, insulin or metformin) to be advantageous or disadvantageous as compared with diet alone with respect to the incidence of major cardiovascular complications [5], although metformin favourably modifies secondary clinical alterations due to insulin resistance, such as arterial hypertension, obesity and hyperlipidaemia.

A possible explanation for this apparent discrepancy could be a deleterious effect of metformin on serum total homocysteine levels.

An elevated serum total homocysteine level is a recently recognized risk factor for atherosclerosis that is independent of 'classical' risk factors [6]. It has not been extensively investigated whether an association exists between non-insulin-dependent diabetes mellitus and serum total homocysteine level [7,8].

Treatment with metformin may increase serum total homocysteine levels in NIDDM. Metformin decreases serum vitamin B_{12} levels by up to 30% by inducing vitamin B_{12} malabsorption [9,10]. Low serum vitamin B_{12} may then increase the serum total homocysteine level [11] (Fig. 1). Metformin may induce malabsorption of vitamin B_{12} by two different mechanisms. First, metformin can bind free calcium, which is required for the uptake of the vitamin B_{12}-intrinsic factor-complex in the ileum by its receptor [12]. The second mechanism is permanent and mediated by depression of intrinsic factor secretion [13]. In addition, subclinical vitamin B_{12} deficiency is common amongst the elderly [14–16] and it is therefore important to know whether the use of metformin aggravates the metabolic consequences of low vitamin B_{12} levels amongst elderly NIDDM patients. There are no data, however, on whether the use of metformin by NIDDM patients is associated with an increase in serum total homocysteine levels. Therefore, in order to estimate the effect of metformin on the serum total homocysteine level in NIDDM patients, we compared serum total homocysteine levels in a group of metformin-treated NIDDM patients to those in a matched group of patients not treated with metformin.
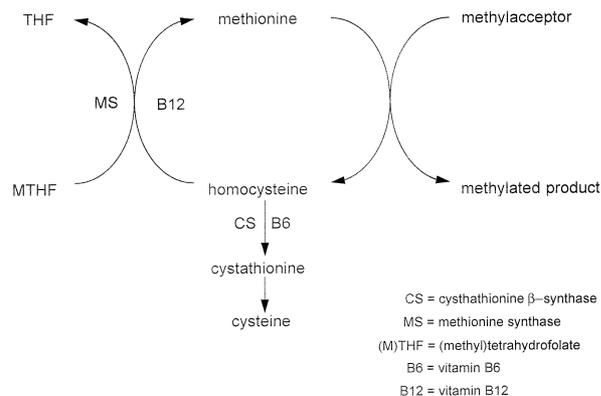

Fig. 1 Methionine–homocysteine metabolism.

Patients and methods

From 1992 to 1995 a cohort study investigating the influence of glycaemic control on well-being in primary care NIDDM patients was carried out in Hoorn, the Netherlands [17]. Twenty-seven of 31 general practitioners agreed to take part in this study, which was approved by the ethical review committee of the University Hospital Vrije Universiteit, Amsterdam. Informed consent was obtained from all participants. All participating general practitioners were encouraged to make treatment decisions according to a standardized step-up regimen based on the standard of the Netherlands College of General Practitioners. The main indication for treatment with metformin was NIDDM with a BMI > 27 kg m^{-2} if prior dietary measures ($\pm$ sulphonylureas) did not normalize fasting blood glucose levels. Contraindications for metformin use were impairments in renal and/or liver function, or heart failure.

For the present investigation we selected Caucasian patients between 40 and 75 years of age who had used metformin for at least six months. Forty NIDDM patients who had received treatment with metformin (500–2550 mg per day) for at least six months were matched with 71 NIDDM patients never treated with metformin. Matching was performed for the following determinants of the serum total homocysteine level: sex, age ($\pm$ 5 years), serum creatinine ($\pm$ 5 μmol L^{-1}) and current smoking habits. Each 'exposed' patient was matched with two 'nonexposed' patients, except in nine individuals who could be matched with only one nonexposed patient. We excluded patients using vitamin B_{12} or folic acid supplements, or medications known to interfere with folic acid metabolism; we also excluded patients with renal impairment (serum creatinine > 120 μmol L^{-1}), previous gastric surgery, a past or current history of hypothyroidism, malignant disease, psychiatric disease, or treatment with antibiotics at the time of investigation. For the metformin users we collected data (see below) at the time of their longest metformin use, which could be precisely reconstructed from individual patients' records. For the matched patients, we collected data at the time that the variables for which matching was performed showed the smallest difference with the corresponding variables in the exposed group. We thus recorded age, sex, body mass index BMI, diabetes

duration, and current smoking habits (yes or no). Blood pressure was defined as the average of two readings recorded on the right arm of seated patients after at least five minutes of rest with a random zero mercury sphygmomanometer (Hawksley–Gelman, Lancing, Sussex, UK). Hypertension was defined as blood pressure 1 $\geq$ 160 mmHg systolic and/or $\geq$ 95 mmHg diastolic and/or the current use of antihypertensive medication. We also assessed the previous cardiovascular history. A history of myocardial infarction, angina pectoris, stroke, transient ischaemic attack or intermittent claudication was considered present if corroborated by written information from the patients' physician(s). Finally, a blood sample was taken in the fasted state.

We measured glycated haemoglobin (by HPLC; Bio-Rad, Veenendaal NL, the Netherlands; reference range, 4.3–6.1%), serum creatinine (Jaffé method (kinetic) without deproteinization), total cholesterol (enzymatic techniques; Boehringer–Mannheim, Germany) and serum total homocysteine. Fasting blood samples were centrifuged within one hour following collection. Fasting blood samples were stored a comparable time at -20 °C; the median storage periods for the nonexposed patients and exposed

patients were 1.9 and 1.5 years, respectively ($P = 0.3$). There is good evidence that serum total homocysteine levels are stable in serum for 10 years or more [14,18]. To minimize the imprecision of the assay, all samples were analysed in the same run in December 1995. Serum total (free plus protein bound) homocysteine level was measured by using tri-n-butylphosphine as the reducing agent and ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulphonate as the thiol-specific fluorochromophore, followed by HPLC with fluorescence detection [19]. The intra- and interassay coefficients of variation are 2.1% and 5.1%, respectively. Reference values are 8–18 μmol L^{-1} for men, 6–15 μmol L^{-1} for premenopausal women and 6–19 μmol L^{-1} for postmenopausal women.

Statistical methods

The patients were categorized by exposure to metformin. Descriptive data are given as mean (SD), median (interquartile range) or number (percentage of the total). Differences in their baseline characteristics were then evaluated by means of a Mann–Whitney test for numerical variables, and a chi-squared test for categorical variables.

Table 1 Patient characteristics by exposure to metformin

	Non-exposed	Exposed	P-value
n (% men)	71 (52)	40 (53)	
Age, years	63 (9)	64 (9)	
NIDDM duration, years	5 (3–9)	6 (4–10)	0.3
Current smokers (%)	13 (18)	8 (20)	
BMI (kg m^{-2})	28.4 (4.0)	29.6 (4.6)	0.2
Diabetes therapy			
Metformin mg per day, current		1000 (500–2550)*	
Metformin use, years		1 (0.5–2.0)*	
Sulphonylurea (%)	54 (76)	28 (70)	0.4
Insulin (%)	16 (23)	1 (3)	0.006
Diet only (%)	1 (1)		
History of cardiovascular events (%)	17 (24)	9 (23)	0.8
Angina pectoris (%)	8 (11)	2 (5)	
Myocardial infarction (%)	8 (11)	4 (10)	
Transient ischaemic attack (%)	1 (3)		
Stroke (%)	1 (3)		
Intermittent claudication (%)	2 (3)	3 (8)	
Blood pressure, mmHg	148/84 (24/14)	145/82 (21/14)	0.2/0.4
Hypertension, %	45	55	0.2
HbA1c (%)	7.4 (1.6)	7.4 (1.3)	0.8
Serum total cholesterol, mmol L^{-1}	6.1 (1.2)	6.0 (1.0)	0.7
Serum creatinine, μmol L^{-1}	81 (11)	81 (11)	
Serum homocysteine, μmol L^{-1}	10.6 (3.3)	11.5 (3.0)	0.1

Data are means (SD), n (%), or median (interquartile range or *range); P-values are reported for the nonmatched variables only.

A two-way analysis of variance was performed in order to estimate the effect of use of metformin on serum total homocysteine levels, with the matching structure of the study taken into account. The resulting 95% confidence interval for this effect is presented. This analysis was repeated for men and women separately; the resulting effect estimates were compared, so as to exclude the possibility of effect modification by sex.

In order to investigate the possible dose–response relationship between exposure to metformin and serum total homocysteine level, we performed a linear regression analysis, with cumulative exposure to metformin, defined as prescribed dose $\times$ duration, as the independent variate. As the dependent variate we chose the difference between the serum total homocysteine level of an exposed patient and the mean of the serum total homocysteine levels of the corresponding matched nonexposed patients. We made this a weighted regression analysis, with lower weights allocated to matched pairs than to triplets.

Statistical analyses were performed with SPSS for Windows version 6.1. Two-sided P-values < 0.05 were considered statistically significant.

Results

Table 1 shows the clinical and laboratory data. The mean serum total homocysteine level in the exposed
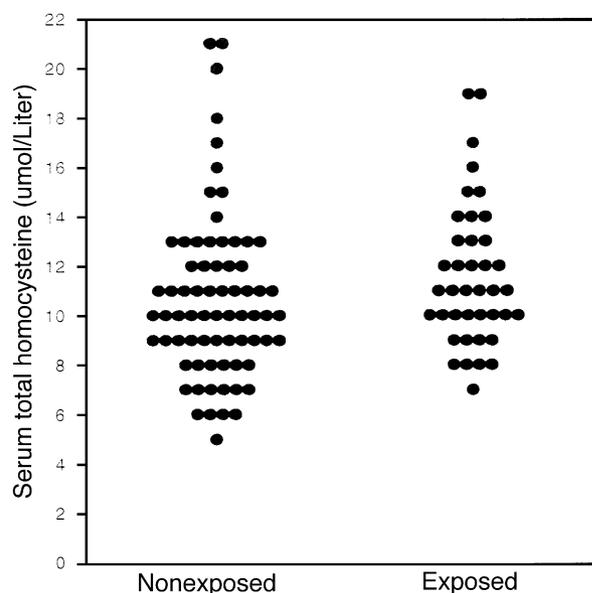

Fig. 2 Serum total homocysteine levels in 71 NIDDM patients not exposed to metformin and in 40 patients exposed to metformin. Values shown have been rounded.

patients was 11.5 μmol L^{-1} (range, 7.0–19.2), and in the nonexposed patients was 10.6 μmol L^{-1} (range, 4.9–20.8). The serum total homocysteine levels of exposed and nonexposed patients are shown in Fig. 2. The metformin-exposed patients had slightly higher serum total homocysteine levels (difference 0.8 μmol L^{-1}, 95% CI: -0.4–2.0). The difference was similar in men (0.4 μmol L^{-1}, 95% CI: -1.5–2.3) and women (1.2 μmol L^{-1}, 95% CI: -0.3–2.6). Furthermore, a dose–response relationship between cumulative exposure to metformin and the serum total homocysteine level could not be demonstrated.

Discussion

We investigated the effect of metformin on the serum total homocysteine level in NIDDM patients and found that this effect, if any, appeared to be small.

High concentrations of metformin accumulate in the wall of the gastrointestinal tract and may induce vitamin B$_{12}$ malabsorption [20]. Evaluating the status of vitamin B$_{12}$ by measuring serum concentrations or haemoglobin level and erythrocyte mean corpuscular volume can be misleading [14,18,21,22]. Therefore, even though there are, during metformin therapy, no changes in haemoglobin level or haematocrit [9], this does not indicate that the intracellular vitamin B$_{12}$ concentration is normal [18]. However, vitamin B$_{12}$ is necessary for the metabolism of homocysteine. Thus, an elevated concentration of serum total homocysteine level has proven to be a highly sensitive indicator of tissue deficiency of vitamin B$_{12}$ [15,23]. Therefore, and because an elevated serum total homocysteine level is a cardiovascular risk factor [6,7], we chose to measure serum total homocysteine level in order to estimate intracellular vitamin B$_{12}$ deficiency. Moreover, circulating vitamin B$_{12}$ does not necessarily mirror intracellular vitamin B$_{12}$. It has been estimated that 5–10% of all patients with clinical vitamin B$_{12}$ deficiency have normal to high serum levels of vitamin B$_{12}$. [23]. This finding may be even more frequent in elderly patients [16]. In addition, it has been observed that 25–50% of patients with low serum vitamin B$_{12}$ do not have evidence for tissue or clinical vitamin B$_{12}$ deficiency. An explanation for this discrepancy may be the distribution of binding of vitamin B$_{12}$ to transcobalamin (TC) I and II. A minor fraction of serum vitamin B$_{12}$, 10–20%, is bound to TCII, which plays an important role in cellular deliv-

ery of vitamin B_{12}. Therefore, a decrease in TCII-bound vitamin B_{12} may impair cellular vitamin B_{12} delivery without an important decrease in serum vitamin B_{12} levels [24].

The prescription of metformin is related to the BMI, which, however, shows no association with the total homocysteine level [25]. Therefore, it is unlikely that the prescription practice confounded the results. We matched for important determinants of the serum total homocysteine level, i.e. sex, age, serum creatinine and current smoking habits [21, 26–33]. Finally, we decided to restrict this study to a Caucasian population, because there is evidence for differences in homocysteine metabolism amongst races. For example, blacks may metabolize homocysteine more effectively than whites [34].

The difference of the serum total homocysteine level between exposed and nonexposed patients, 0.8 μmol L^{-1}, was small and not statistically significant. Possibly the mean duration (one year) and the mean dose (1000 mg metformin day per day) were too short and too small, respectively, to induce vitamin B_{12} deficiency. The total-body vitamin B_{12} pool has been estimated to be about 2–3 mg. Daily losses of vitamin B_{12} are approximately 0.1% of the body pool, resulting in a long half-life of vitamin B_{12} (480–1360 days) [35]. The vitamin B_{12} pool is partly supplied by food and partly by reabsorption of vitamin B_{12} excreted into the bile. Therefore, malabsorption can induce vitamin B_{12} deficiency in a relatively short time (1–3 years) [24]. It is reasonable to hypothesize that one year of metformin-induced malabsorption of vitamin B_{12} might increase serum total homocysteine levels. In addition, DeFronzo *et al.* have shown that even after six months of treatment with 2550 mg metformin per day a decrease of serum vitamin B_{12} levels by up to 30% can be induced [9]. Moreover, malabsorption of vitamin B_{12} can already be induced after 10 days of treatment with 3 g of metformin per day [10].

Another explanation for the small rise of the serum total homocysteine level could be the fact that homocysteine levels do not differ substantially between individuals with moderate and high serum vitamin B_{12} levels. If plasma vitamin B_{12} levels drop below 300 pg mL^{-1}, however, homocysteine levels rise dramatically [36]. Thus, if the included patients had moderate to high serum vitamin B_{12} levels prior to their metformin treatment, a decline of 30% of the serum vitamin B_{12} would not have raised the serum total homocysteine level by more than 1–2 μmol L^{-1} [37]. Serum storage conditions did not allow assessment of vitamin B_{12} levels; therefore, we could not analyse this issue in our study. Finally, a randomized placebo-controlled trial would have allowed more definite conclusions.

We conclude that treatment with 1000 mg of metformin per day during one year in Caucasian NIDDM patients is likely to have, if any, a small effect on serum total homocysteine level. The significance of small rises of serum total homocysteine level in diabetic patients is unknown, however, and requires further study. To put this into perspective, it has been estimated that a 1 μmol L^{-1} increment in the serum total homocysteine level elevates the risk of coronary artery disease by a factor of approximately 1.1 [6]. Finally, we cannot exclude that more prolonged use of metformin does lead to an important increase of the serum total homocysteine level.

Acknowledgements

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