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Diabetes & Metabolism 42 (2016) 316–327

Review

Association between metformin and vitamin B_{12} deficiency in patients with type 2 diabetes: A systematic review and meta-analysis

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Received 18 November 2015; received in revised form 22 March 2016; accepted 27 March 2016
Available online 26 April 2016

Abstract

Aim. – Metformin is the most widely used oral hypoglycaemic drug, but it may lower B_{12} status, which could have important clinical implications. We undertook a systematic review and meta-analysis of the relationship between metformin use and vitamin B_{12} deficiency in persons with type 2 diabetes.

Methods. – Electronic database searches were undertaken (1st January 1957–1st July 2013) using the Cochrane library, Scopus, CINAHL, Grey literature databases, Pub Med Central, NICE Clinical Guidelines UK, and ongoing clinical trials. Included studies were of any study design, with data from patients with type 2 diabetes of any age or gender, taking any dose or duration of metformin. Planned primary outcomes were serum vitamin B_{12} levels, % prevalence or incidence of vitamin B_{12} deficiency and risk of vitamin B_{12} deficiency.

Results. – Twenty-six papers were included in the review. Ten out of 17 observational studies showed statistically significantly lower levels of vitamin B_{12} in patients on metformin than not on metformin. Meta-analysis performed on four trials demonstrated a statistically significant overall mean B_{12} reducing effect of metformin of 57 pmol/L [WMD (fixed) = –0.57 (95% CI: –35 to –79 pmol/L)] after 6 weeks to 3 months of use.

Conclusion. – The evidence from this review demonstrates an association between metformin usage and lower levels of vitamin B_{12} by 57 pmol/L, which leads to frank deficiency or borderline status in some patients with type 2 diabetes. This suggests that it is prudent to monitor B_{12} levels in these patients who are at increased risk of deficiency.

Keywords: Diabetes mellitus; Meta-analysis; Metabolic adverse effects; Oral hypoglycaemic agents; Primary care; Vitamin deficiency

1. Introduction

The worldwide prevalence of diabetes mellitus is rising. For western countries, it is currently projected that there will be a 17% increase in persons with diabetes in France and Belgium by 2035 [1], with a 22% increase in both the USA and United Kingdom, a 31% increase in Canada, and a 3% to 37% increase in other European Union countries [1].

The most widely used class of drugs in persons with type 2 diabetes are the biguanides (e.g. metformin), which increase insulin sensitivity, and contribute to weight loss [2]. However, one of the side effects of metformin is to reduce vitamin B_{12} status. Vitamin B_{12} deficiency is under diagnosed and under-treated [3,4]. Severe deficiency (e.g. pernicious anaemia) can result in macrocytic anaemia, peripheral neuropathy and mental-psychiatric changes. Also, milder symptoms, such as weakness, tiredness, and memory loss can occur before frank anaemia [5–7].

Metformin-related vitamin B_{12} deficiency has been known for over 40 years. However, a systematic analysis of the data concerning B_{12} status and metformin usage has not been carried out to date specifically in patients with type 2 diabetes. Two recent meta-analyses [8,9] have shown a reduction in B_{12} levels in populations with metformin use but both used a mixed population of patient types (e.g. diabetes, polycystic ovary syndrome,

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http://dx.doi.org/10.1016/j.diabet.2016.03.008
1262-3636/

hyperlipidaemia). Therefore, their results may differ from that of patients specifically with a diagnosis of diabetes mellitus and a systematic review and meta-analysis is now required in this specific patient group.

Our objective was to perform the first systematic review and meta-analysis of the published literature (observational and intervention studies) on the association between metformin use and vitamin B_{12} deficiency (as measured by serum vitamin B_{12}) in patients with diabetes mellitus and to consider the potential implications for practice.

2. Method

2.1. Literature search

As recommended by the Cochrane Database of Systematic Reviews (http://www.cochranelibrary.com/); electronic, hand, web and reference list searches were made of the literature to date. Core health bibliographic databases were searched from 1st January 1957 (year of the first metformin clinical trial) to 1st July 2013. These databases included the Cochrane library, national, regional and subject specific databases, Scopus, CINAHL, Index to theses, Grey literature databases, Conference abstract databases, Pub Med Central, Zetoc, NICE Clinical Guidelines UK, ongoing trials and other electronic databases (e.g. Nexis). No ethical approval was required for this review as only analysis of already published data was used.

The full search strategy for each database is available in the online supplementary material (eSearches). A combination of Medical Subject Heading (MeSH) and free-text terms were used. The exact search phrases were: "(diabetics OR diabetes OR type 1 OR type 2 OR insulin dependent diabetics OR non-insulin dependent diabetics OR insulin dependent Diabetes OR non-insulin dependent Diabetes OR non-insulin dependent diabetics OR non-insulin dependent diabetes OR IDDM OR NIDDM) AND (metformin OR metformin hydrochloride OR biguanides OR biguanide) AND (B_{12} OR B_{12} deficiency OR Vitamin B_{12} OR Vitamin B_{12} deficiency OR cobalamin OR cyanocobalamin OR cobalamin deficiency OR cyanocobalamin deficiency OR hydroxycobalamin OR hydroxocobalamin OR hydroxycobalamin deficiency OR hydroxocobalamin deficiency)". Reference lists from the search results were checked for relevant papers and experts in the field and study authors were contacted to enquire about additional published or unpublished studies, ongoing trials and background data.

2.2. Eligibility criteria for inclusion and data extraction

All human studies of cross-sectional, cohort or intervention (metformin vs. placebo or other control) design published in the English language from the 1st January 1957 to 1st July 2013 were assessed. Studies reporting data from all patients of any age or gender with diabetes mellitus and taking metformin were considered for inclusion. No papers were found assessing patients with type 1 diabetes, so the review subsequently focussed only on patients with type 2 diabetes. Intervention studies were included in the systematic review and meta-analysis if they assessed oral metformin in patients with diabetes vs. placebo (or no intervention, i.e. comparison with background data for the population). Due to the anticipated small number of intervention studies in this subject area, we did not plan to carry out any subgroup analyses. Data presented in conference abstract form was not included in the analysis.

2.3. Primary and secondary outcomes

The primary outcomes were serum vitamin B_{12} levels, % prevalence or incidence of vitamin B_{12} deficiency and odds or risk of vitamin B_{12} deficiency. Diagnosis of vitamin B_{12} deficiency was based upon a serum vitamin B_{12} level below 150 pmol/L, with borderline deficiency between 150 and 220 pmol/L. In this paper, all B_{12} measurements reported are that of serum vitamin B_{12} (pmol/L), unless otherwise stated. Secondary outcomes eligible for inclusion included reported symptoms, active B_{12} (holotranscobalamin II; HoloTC), homocysteine (Hcy), methylmalonic acid (MMA), haemoglobin (Hb), mean cell volume (MCV), estimates of economic costs, and quality of life data. Due to lack of data on these secondary outcomes, only the primary outcomes were included in the text of the systematic review and in the meta-analysis.

2.4. Statistical analysis

For cross-sectional surveys, mean ± standard deviation (SD) and correlation coefficients (r) were obtained where available. Relative risk (RR), odds ratios (OR) or hazard ratios (HR) were extracted for case-control studies, and mean and standard deviations extracted for intervention studies. The number of participants (n) as well as P values and 95% confidence intervals for effect estimates were extracted for all study types. Multivariate adjusted analyses were used where possible to reduce the effects of confounding.

Review manager (RevMan5.2, Cochrane Collaboration) [10] was used to perform the meta-analysis and for observational studies multivariate adjusted data used where possible to reduce the effects of confounding. All estimates are presented as mean ± standard deviation unless otherwise stated. The Jadad scale and CONSORT statement were used to assess the quality of the design and conduct of the randomised controlled trials (RCT's) [11–13]. The international GRADE working group grading system was used to link the quality of the evidence into recommendations [14,15].

3. Results

The 26 databases identified 7257 records from which 25 studies were included in this review. Fig. 1, based on the PRISMA statement (http://www.prisma-statement.org/), illustrates the results and the process of screening and selection. The online supplement contains full results of searches (Appendix A; Table A.1–A.3; see supplementary material associated with this article online), with details of reasons for exclusions of

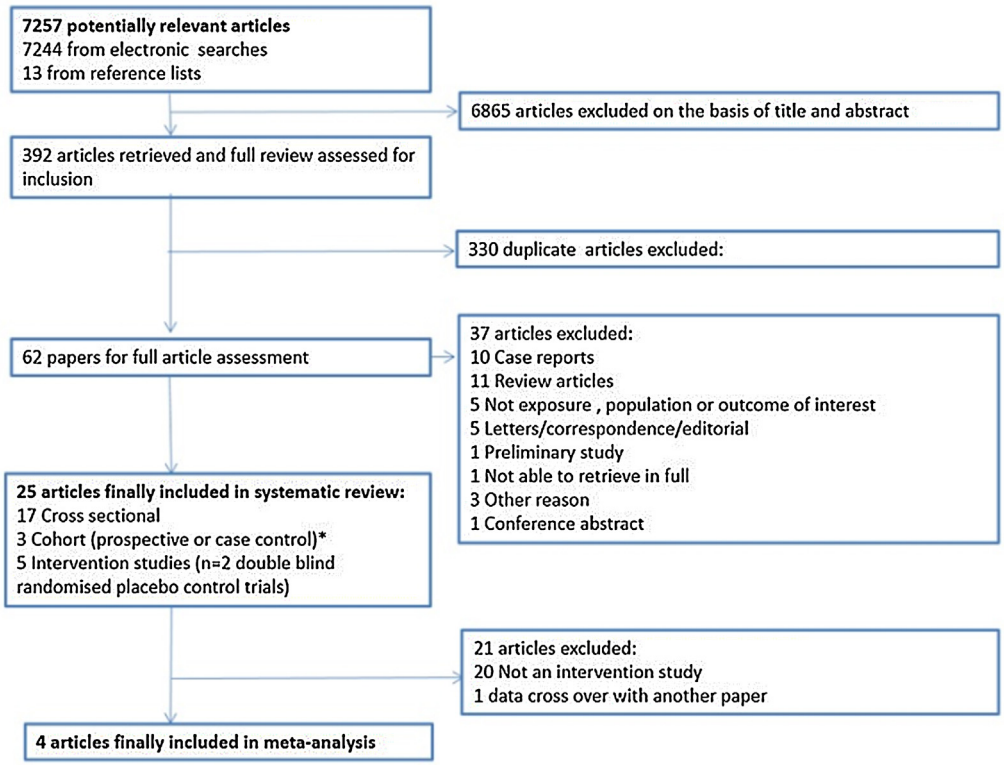

Fig. 1. Study flow diagram illustrating the results of the search and the process of screening and the selection of the studies for inclusion in the review. Legend: for the purposes of this flow chart, Hermann et al., 2004 [17] is classified under cross-sectional studies.

specific papers (Appendix B: Table B.1–B.2; see supplementary material associated with this article online).

3.1. Systematic review

Of the 25 papers included in the systematic review, the majority showed significantly lower vitamin B_{12} levels in those patients on metformin. There was only a small amount of data on clinical outcomes (e.g. neurological impairment [16], megaloblastic anaemia, homocysteine) and functional markers of B_{12} status. Indeed, most clinical outcomes were featured in case reports only (not included in this analysis due to poor generalizability of data for $n = 1$ case studies).

For observational studies, the lack of study data, between study differences and concerns over small sample sizes prevented pooling of data to assess overall effect size. One study had both cross-sectional and cohort data [17], so is mentioned twice.

The 17 cross-sectional studies (Table 1) indicated either an association of metformin use with a lower B_{12} concentration (10 studies [17–26]) or showed no association (7 studies [27–33]). In terms of cohort-based or retrospective case-control studies, 4 studies were included in the review (Table 2) showing a reduction in HoloTC and total vitamin B_{12} [17] or increased odds of B_{12} deficiency after 3 years [adjusted OR = 2.39 (95% CI 1.46 to 3.91)] [34] in metformin users, compared with non-users. A nested case-control study [35] identified a statistically significant reduction in vitamin B_{12} deficiency in patients with type 2 diabetes with peripheral neuropathy after 6 months of metformin usage. However, one retrospective case-control study of patient notes [36] found no statistically significant difference between the incidences of vitamin B_{12} deficiency in patients with diabetes who were using metformin compared with those not using metformin [36].

Characteristics of the intervention studies, including Jadad scores are shown in Table 3. Five intervention studies were identified [37–41] of which only two were double-blinded, randomised and placebo-controlled [37,40]. The Hyperinsulinemia: the Outcome of its Metabolic Effects (HOME) trial was a prospective multicentred RCT [37,40]. This trial had a three-phase process including a pre-randomisation measurement in the first 12 weeks, a post-randomisation measurement up to 16 weeks and a long-term post-randomisation phase measurement up to 4 years. At the 16-week phase, there was a statistically significant fall in vitamin B_{12} levels of –14% (95% CI –4.2% vs. –24%) in the metformin group relative to placebo ($P < 0.0001$) [37], which dropped further to –19% (95% CI –24% to –14%) ($P < 0.001$) by the end of the trial at 4 years [40]. There was a 7.2% (95% CI 2.3 to 12.1) higher absolute risk of B_{12} deficiency (< 150 pmol/L) in the metformin group compared to placebo ($P = 0.004$) and the corresponding number needed to harm (NNH) was 13.8 per 4.3 years (95% CI 43.5 to 8.3) [40]. Importantly, this study reported a treatment by time interaction, with vitamin B_{12} concentrations being more extensively lowered with a longer duration of metformin use [40].

Table 1
Characteristics of $n = 17$ included cross-sectional studies.

Study	Age	General study population (n)	Metformin population (n)	Dose	Duration	Primary outcome[a]; n for B_{12} deficiency	Secondary study outcomes[b]	Deficiency prevalence Study population (%)	Deficiency prevalence metformin population (%)	B_{12} level metformin	B_{12} level non-metformin	P value or 95% CI
Adetunji et al., 2009 [27]	Mean/SD 59 ± 14 Range 56–61	520	279	NS[b]	Mean/SD 11 ± 7 years	0 (units not specified)	B_{12} levels (if Hb <11 g/dL) BMI HbA_{1c} MCV	0	0	363 ± 149	416 ± 106	$P = 0.31$
Al-Gadeer et al., 2007 [28]	NS	134	NS	NS	NS	(Units and reference range not specified)	HCy (units and reference range not specified)	NS	NS	NS	NS	NS
Carpentier et al., 1976 [18]	Mean/SD 61.9 ± 2.3 years	70	30	NS	Mean/SEM 46.5 ± 7.9 months	5 (< 270 pg/mL)	None	7	12	Mean/SEM 454.9 ± 36.6	Mean/SEM 760.9 ± 59.9 751.6 ± 118	$P < 0.001$ $P < 0.005$
Chen et al., 2012 [19]	65.5 ± 10.6 (Met) 68.6 ± 12.1 (non-Met)	202	152	1500 ± 578 (mg/d)	8.5 ± 5.4 years	0 (< 130 ng/L)	HoloTC (> 35 pmol/L) MMA (< 0.42 μmol/L) Folate (2.1–20 μg/L) Ferritin (15–300 μg/L) Neuropathy – vibration perception threshold (< 25 V), s-LANSS (< 12), NTSS-6 (< 6)	0	0	219 ± 105.4	281.4 ± 95.1	$P < 0.001$
Filioussi et al., 2003 [29]	61.8/7.8 (M) 63.1/7.2 (F) Range 42–87	600	574	NS	11.8 ± 3.6 years	54 (< 200 pg/mL)	B_{12} levels (< 200 pg/mL)	9	9.4	NS	NS	95% CI: 6.8–11.6%
Hermann et al., 2004 [17]	Mean 61.5 Range 37–81	84	53	Mean 200 (mg/d) 2200	Mean 5.2 years	4 (< 150 pmol/L)	B_{12} (150–700 pmol/L) Folate (6–35 nmol/L) HCy (< 15 μmol/L) MMA (< 0.28 μmol/L) HoloTC (37–171 pmol/L)	5	8	289 ± 137	395 ± 162	$P < 0.0039$
Jones, 2012 [30]	Mean 63.5 Range 36–91	127	127	NS	1–25 years	32 (< 180 pg/mL)	None	25	25	NS	NS	NS
Kos et al., 2012 [20]	63 ± 13 Range 20–93	706	297	Mean (mg/d) 1500	NS	NS (< 220 pg/mL)	BMI Osteoporosis Osteoopenia	NS	NS	496 ± 282	637 ± 352	$P < 0.0001$
Obeid et al., 2013 [21]	67 (control) 64 (DM) Range 52–82	164 (B_{12} measured in 118)	49	NS	NS	NS (pmol/L) (reference range not specified)	RBC-B_{12} (pmol/L) tHCy (μmol/L) MMA (nmol/L) HoloTC (pmol/L) SAM (nmol/L) SAH (nmol/L)	NS	NS	Median (10th–90th percentiles): 256 (139–373)	Median (10th–90th percentiles) 305 (191–475)	$P < 0.006$

Table 1 (*Continued*)

Study	Age	General study population (*n*)	Metformin population (*n*)	Dose	Duration	Primary outcome[a]; *n* for B_{12} deficiency	Secondary study outcomes[b]	Deficiency prevalence Study population (%)	Deficiency prevalence metformin population (%)	B_{12} level metformin	B_{12} level non-metformin	*P* value or 95% CI
Pierce et al., 2012 [31]	59 ± 9.2	235	235	Mean (mg/d) 2050	Mean 5.2 years	6 (< 157 pg/mL)	Complications of deficiency	2.5	2.5	NS	NS	NS
Pflipsen et al., 2009 [22]	61.5 ± 96	195	133	< 1000–≥ 2000 (mg/d)	NS	1 (< 100 pg/mL)	B_{12} levels (100–350 pg/mL) Elevated MMA (> 243 nmol/L) HCy (> 11.9 nnmol/)	0.5	0.75	425.99	527.49	*P* < 0.012
Pongchaidecha et al., 2004 [23]	35–65 years (range)	152	88	NS	≥ 6 months	NS (pg/mL) (reference range not specified)	HCy Folic acid	NS	NS	318 ± 192.2	434.3 ± 300.7	*P* = 0.011
Qureshi et al., 2011 [32]	NS	283	283	≥ 2000 mg/d	≥ 4 years	23 (< 150 pg/mL)	Neuropathy Folate	33	33	NS	NS	NS
Reinstatler et al., 2006 [24]	63.4 ± 0.5 (Met) 66.4 ± 0.5 (non-Met)	8488	575	NS	5 years (median) < 1 to > 10 year	NS (< 148 pmol/L)	B_{12} levels (< 148–221 pmol/L) – borderline deficiency	2.2 (non-Met) 3.3 (non-DM)	5.8	317.5	386.7	*P* = 0.0116
Sorich et al., 2008 [33]	Median/range 73 (31–86)	60	60	500–3000 mg/d	Median/range 6.5 (2–26)	8 (< 150 pmol/L)	Dose and duration of metformin, age, calcium supplement, proton pump inhibitors	15	15	Median/range: 245 (83–807)	N/A	*P* = 0.10
Tal et al., 2010 [25]	81 ± 7.2 (M) 82 ± 6.9 (F)	1570	255	NS	NS	NS (< 200 pg/mL)	Age, medication, mortality	15	24	NS	NS	*P* < 0.001
Tomkin et al., 1971 [26]	Mean/SEM 62.5 ± 1.66	71	71	1500–2000 mg/d	4.2 years	3 (< 150 pg/mL)	Hb (g/100 mL) B_{12} (pg/mL)	4	4	NS	NS	*P* < 0.02

All data are Mean ± SD unless otherwise stated. N/A: not applicable; NS: not specified; Met: metformin; non-Met: non-metformin; DM: diabetes mellitus; HoloTC: holotranscobalamin; MMA: methylmalonic acid; Methylation markers: SAM: *S*-adenosylmethionine; SAH: *S*-adenosylhomocysteine; NTSS-6: Neuropathy Total Symptom Score-6 questionnaire; s-LANSS: the Self-administered Leeds Assessment of Neuropathic Symptoms and Signs questionnaire; Hb: haemoglobin; HbA_{1c}: glycosylated haemoglobin.

[a] Primary outcome, outcome of primary interest for the cross-sectional study.

[b] Secondary outcomes, outcomes of secondary interest for the cross-sectional study.

Table 2
Characteristics of $n = 4$ included cohort and case-control studies.

Study	Setting	Participants	n and exclusion criteria	Allocation	Primary study outcome[a]	Secondary study outcomes[b]	Review outcome (B_{12} levels)[c]	% Incidence B_{12} deficiency or odds ratio	P value	Comments
Long et al., 2012 [36]	US Medical Centre Electronic Medical Records Study period; not specified	Diabetics (type 2) 589 (96%) men/25 (4%) women Age (mean/SD) 65.08/9.23 Not specified how long patients had been on metformin	614 Exclusions: over 60 years, vegetarian, pernicious anaemia, pancreatic insufficiency, gastrectomy, bowel resection, calcium supplementation, H2 blockers in the last 3 months	Retrospective 146 (24%) metformin 123 (20%) metformin + PPI 129 (21%) PPI 216 (35%) control	B_{12} levels (< 300 pg/mL)	Effect of proton pump inhibitors (PPI's)	B_{12} levels (< 300 pg/mL)	32 (21.91%) metformin 42 (34.15%) metformin + PPI 33 (25.58%) PPI 48 (22%) control	$P = 0.9454$ metformin $P = 0.00096$ metformin + PPI $P = 0.9454$ PPI	No statistically significant difference between control group and those on metformin or PPI alone A statistically significant difference between control group and those on metformin + PPI
Hermann et al., 2004 [17]	Sweden Diabetic outpatient clinic Study period; May 2002–May 2003	Type 2 diabetes 50 (60%) men/34 (40%) women Age (mean) 61.5 Metformin for at least 1 year	84 Exclusions: metformin treatment < 1 year, elevated creatinine, B_{12} deficiency, pregnancy, thyroid/inflammatory bowel disease, acute diseases, cancer, psoriasis, relevant medication	Retrospective Non-randomised 53 (63%) metformin 31 (37%) non-metformin diabetics (controls)	B_{12} status (vitamin B_{12}) HoloTC, Hcy MMA	Vitamin B_{12} (150–700 pmol/L) Folate (6–35 nmol/L) Hcy (< 15 μmol/L) MMA (< 0.28 μmol/L) HoloTC (37–171 pmol/L)	B_{12} levels (vitamin B_{12}) (< 150 pmol/L) 289 ± 137 (Met) 395 ± 162 (non-Met)	4 (8%) prevalence of B_{12} deficiency in patients with diabetes on metformin (26.7% lower vitamin B_{12} levels in exposed group compared to non-exposed)	B_{12} $P < 0.0039$ HoloTC $P = 0.065$ Hcy $P = 0.0484$	Vitamin B_{12} ($P < 0.01$), Hcy ($P < 0.05$) HoloTC ($P < 0.05$) Were all statistically significantly lower in the metformin group 4 (8%) had vitamin B_{12} and 8 (16%) ($P < 0.05$) had HoloTC levels below the reference ranges
Ting et al., 2006 [34]	Hong Kong Pathology laboratory database Study period; Jan 2003–Nov 2005	Type 2 DM 57 (37%) men/98 (63%) women (cases) 127 (41%) men/183 (59%) women (controls) Age: 72.5 ± 9.3 (cases) vs. 71.4 ± 11.2 (controls) Median duration metformin (years); 4 (cases)/2 (controls) Dosage; 2 g (cases)/1.4 g (controls)	3987 Exclusions: diabetics not on metformin, less than 1 year of computerised medical records, pernicious anaemia, pancreatic exocrine insufficiency, gastrectomy/small bowel resection, vitamin B_{12} supplementation within 3 months prior to study	Nested 3632 (91%) diabetics on metformin with no B_{12} deficiency 355 (9%) diabetics on metformin with B_{12} deficiency After exclusions; 155 cases/310 matched controls	B_{12} levels (< 150 pmol/L) (203.3 pg/mL)	HbA_{1c}	B_{12} levels (<150 pmol/L) (203.3 pg/mL) Mean; 148.6 (cases)/466.1 (controls) Metformin-related B_{12} deficiency after 3 years	8.91 (95% CI, 0.91–73.9) – 2.37 (95% CI, 1.60–3.52)	$P = 0.06$ $P < 0.001$ $P < 0.001$	Cases more likely to be vegetarians ($P = 0.04$) Prevalence 2.6% (cases) vs. 0.3% (controls) Significant increased risk with dose and duration ($P < 0.001$). Each 1 g dose leads to over double the risk of B_{12} deficiency

Table 2 (*Continued*)

Study	Setting	Participants	*n* and exclusion criteria	Allocation	Primary study outcome[a]	Secondary study outcomes[b]	Review outcome (B_{12} levels)[c]	% Incidence B_{12} deficiency or odds ratio	*P* value	Comments
Wile and Tooth, 2010, [53]	Canada NM Clinic Study period; Dec 2002–May 2007	Diabetics (type 2) with Neuropathy 35 (59%) men/24 (41%) women (cases) 34 (54%) men/29 (46%) women (controls) Age (Mean/SD) 66.6 (11.9)/(cases) 64.8 (12.0)/(controls) Metformin for at least 6 months	122 Exclusions: non-DM potential causes for peripheral neuropathy, Cbl deficiency, discontinued metformin or had received < 6 months of metformin, impaired glucose tolerance, juvenile diabetes onset, insulin, refused testing	Prospective 59 (48%) metformin 63 (52%) non-metformin diabetics (matched controls)	Vitamin B_{12} (210 pmol/L) (285 pg/mL)	Vitamin B_{12} (pmol/L) Hcy (μmol/L) MMA (μmol/L) Neuropathy (nerve conduction studies/TCSS/NIS)	Vitamin B_{12} (210 pmol/L) (285 pg/mL) Median levels; 231 (cases)/486 (controls) Deficiency; 18 (31%) (cases) vs. 2 (3%) (controls) Cumulative dose: inversely correlated with B_{12} levels	NS	$P < 0.001$ $P < 0.001$ $P = 0.001$	Neuropathy: median TCSS and NIS score higher in cases ($P < 0.001$), and correlated to cumulative dose ($P < 0.001$) Nerve conduction studies were not significantly correlated

NS: not stated; Hcy: homocysteine; HoloTC: holotranscobalamin II; MMA: methylmalonic acid; TCSS: Toronto Clinical Scoring System; NIS: Neuropathy Impairment Score; NM: neuromuscular.

[a] Primary outcome, outcome of primary interest for the studies.

[b] Secondary outcomes, outcomes of secondary interest for the studies.

[c] Review outcome, outcome relevant for inclusion within the systematic review – serum B_{12} levels.

Table 3
Characteristics of $n = 5$ included clinical trials.

Study	Setting/study period	Participants male (%)/female (%)	Age	Inclusion/ exclusions	Intervention	Study design	Primary study outcome	Secondary study outcomes	Statistical analysis cases	Statistical analysis controls	Jadad/ GRADE	Comment
Bauman et al., 2000 [39]	US/4 months	21 21 (100%)	48.6 ± 10.1 cases 54 ± 4.9 control	I (30–60 years)/E[a]	850 mg 2 or 3 times daily	Controlled not randomised	B_{12} levels (< 220 pg/mL)	Hydrogen breath test for bacterial overgrowth	Baseline: 400 ± 119 3 months: 282 ± 24 4 months: 277 ± 22	Baseline: 335 ± 120 3 months: 364 ± 51 4 months: 375 ± 90	0 2C	Statistical significant difference at 3 months ($P < 0.001$), 3 to 4 months ($P < 0.005$) in cases
Leung et al., 2010 [41]	Canada/3 months	20 10 (50%)/10 (50%)	NS	I (67–91 years)/E (NS)	NS	Controlled not randomised	Vitamin B_{12} levels	HbA_{1c} (%) MMA (pm/L)	Baseline: 400 3 months: 290 ($P = 0.04$)	Baseline: 465 3 months: 439 ($P = 0.75$)	0 2C	Reduction in levels after 3 months in cases (24%)
Sahin et al., 2007 [38]	Turkey/6 weeks	165 66 (40%)/99 (60%)	NS	165 I (36–82 years)/E[b]	850 mg 3 times daily or placebo	RCT	B_{12} levels (pmol/L)	Hcy (μmol/L) Folate (nmol/L)	Baseline: 319.3 ± 149.6 6 weeks: 290.22 ± 140.5 ($P = 0.119$)	Baseline: 265.89 ± 398 6 weeks: 262.14 ± 44.4 ($P = 0.631$)	0/1 2B/2C	No statistical difference between cases and controls
de Jager et al., 2010 [40]	Holland/4.3 years	390	NS	I (30–80 years)/E (NS)	Insulin 850 mg od	Multicentred DBRPCT	B_{12} levels (< 150 pmol/L)	B_{12} levels (< 150–220 pmol/L) Folate (nmol/L) Hcy (μmol/L)	Baseline to 4.3 years: 89.8 pmol/L drop (−19% change) (95% CI −22% to −15%)	Baseline to 4.3 years: 0.2 pmol/L drop (0% change) (95% CI −3% to 4%)	5 1A	Reduction in levels by 19% (95% CI −24% to −14%) ($P < 0.001$)
Wulffele et al., 2003 [37]	Holland[c]	390	NS	I (30–80 years)/E (NS)	Metformin 850 mg once per day	Multicentred DBRPCT	B_{12} levels (< 180 pmol/L)	B_{12} levels (< 180–700 pmol/L) Folate (nmol/L) HCy (μmol/L)	Baseline to 16 weeks: 47.4 pmol/L drop (−16% change) (95% CI −12% to −19%)	Baseline to 16 weeks: 4.4 pmol/L drop [–2% (95% CI −6% to 2%)]	5 1A	Reduction in levels (14%) (95% CI −4.2% to −24%) ($P < 0.001$)

All data are mean ± SD unless otherwise stated. NS: not specified.

[a] Exclusions – history of alcoholism/drug abuse, psychiatric/liver/stomach/bowel/cardiopulmonary disease, chronic renal failure, pernicious anaemia, pre-existing vitamin B_{12} deficiency, bowel surgery, acid-based disturbance, or cancer, relevant medication (antibiotics/gastric motility drugs).

[b] Exclusions – smoking, cardiac arrhythmia, CCF, stroke, chronic renal disease, microalbuminuria, dyslipidemia, relevant medication, severe illness.

[c] 16 week interim result from the same study as de Jager et al., 2010 [40].

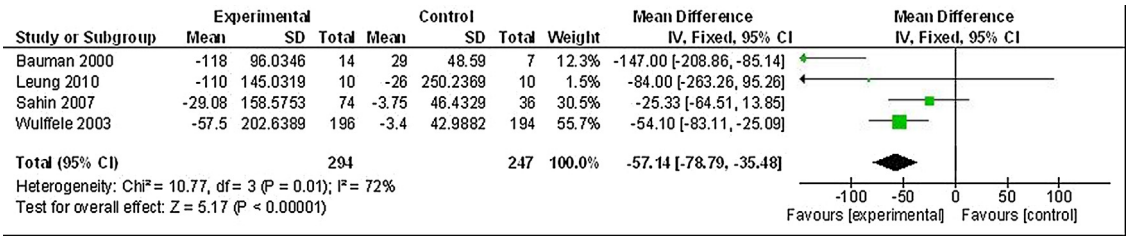

Fig. 2. The effect of metformin usage on serum B_{12} concentration (pmol/L), with fixed-effects weighted mean difference used to compare vitamin B_{12} levels between studies. Legend: experimental: Metformin; control: placebo.

3.2. Meta-analysis

Four studies [37–39,41] were suitable for inclusion in the meta-analysis. Three trials were of short duration [38,39,41] ($\leq$ 4 months) and as the HOME trial data were present in two papers (interim analysis at 4 months [37] and final analysis at 4 years [40]), the HOME trial 4 months measurement [37] was chosen for the meta-analysis rather than the 4-year measurement [40]. This was chosen in order for the HOME trial to be homogenous with the other trials in the meta-analysis. Due to the potential for significant study heterogeneity, a meta-analysis was conducted using both fixed and random effects models. The I^2 statistic was used to assess heterogeneity between study outcomes.

Fig. 2 summarises the meta-analysis with 95% confidence intervals indicating statistical significance at both individual study levels for Bauman et al. [39] and Wulffele et al. [37] and for the overall effect estimate [$WMD_{(fixed)} = -57.1$ (95% CI –35.5 to –78.8) $P < 0.001$ $I^2 = 72\%$]. The wide confidence intervals in some studies provide a cautionary note on interpretation. The mean difference in serum B_{12} levels suggests that taking metformin for 6 weeks to 3 months leads to a statistically significant reduction in B_{12} concentration by 57 pmol/L. There was significant heterogeneity, with $I^2 = 72\%$.

A sensitivity analysis, with removal of each study in turn, showed a $WMD_{(fixed)}$ estimate of –44.6 (95% CI –67.7 to –21.46, $P = 0.0002$, $I^2 = 0\%$) (Bauman et al. [39]), –56.7 (95% CI –78.6 to –35.0, $P < 0.00001$, $I^2 = 81\%$) (Leung et al. [41]), –71.1 (95% CI –97.1 to –45.1, $P < 0.00001$, $I^2 = 72\%$) (Sahin et al. [38]) and –60.1 (95% CI –93.5 to –28.4, $P = 0.0002$, $I^2 = 81\%$) (Wulffele et al. [37]). Therefore, the removal of the largest study [37] only had a small influence on the effect size estimate, albeit with wider 95% confidence intervals and slightly increased heterogeneity. Conducting the meta-analysis of the four studies with a random effects model also led to a statistically significant effect, but with a slightly larger effect size and wider 95% confidence intervals [$WMD_{(random)} = -70.1$ (95% CI –120.5, –19.7), $P = 0.006$, $I^2 = 72\%$].

4. Discussion

Observational data in this review has shown lower B_{12} levels, and an increased risk of borderline or frank B_{12} deficiency with metformin use. Most intervention trials in this review were only of short duration (up to 4 months) but indicated a statistically significant reduction in B_{12} levels beyond the 6-week period and in some cases, led to frank deficiency. Our fixed-effects meta-analysis of four intervention trials showed a statistically significant lowering of vitamin B_{12} levels by 57 pmol/L (95% CI: –35 to –79).

This reduction in B_{12} levels could be clinically significant in causing frank deficiency (< 150 pmol/L) or a borderline status (150–220 pmol/L) in some patients who are in the range of 207–277 pmol/L before metformin treatment. Indeed, 20% of men and 27% of women (19–64 years old) in the UK National Diet and Nutrition Survey (2004) [42] had a serum B_{12} concentration of < 200 pmol/L [42]. Accordingly, the EPIC (European Prospective Investigation into Nutrition and Cancer) cohort in Europe has shown that 5% of adults (< 60 years old) have a B_{12} concentration of < 175 pmol/L [43,44]. According to the effect size found in our meta-analysis, it would be predicted that a significant proportion of the patients with type 2 diabetes in European population are in danger of developing frank B_{12} deficiency or at least borderline levels of deficiency during the first four months of metformin treatment.

In terms of previous research, our results supports the recent meta-analysis by Niafar et al. which found a reduction of 66 pmol/L [–65.8 (95% CI –78.1 to –53.6 pmol/L), $P < 0.00001$] for metformin use in an analysis of a more heterogeneous group of patients (including studies of specific groups of patients with hyperlipidaemia and polycystic ovarian syndrome) [8]. Our result is also similar to that of Liu et al. [9], who found a reduction of 54 pmol/L in a meta-analysis, but included rosiglitazone as a control in the pooled estimate. The fact that our estimate is similar to these other meta-analyses suggests that the effect of metformin on B_{12} may not vary by patient population studied and thus separate guidance might not be required for different clinical populations. The concordance with the other meta-analysis also supports the reliability of our effect size. Since our electronic searches were completed, there have been 8 observational studies published that are relevant to our review [45–51]. All of these studies have supported a reduction in B_{12} status with metformin use, or higher than expected levels of deficiency in metformin using patients. Of note, Beulens et al. found both reduced vitamin B_{12} and holotranscobalamin in metformin users [51]. Therefore, inclusion of these articles would have been unlikely to change the overall results of our systematic review, although it is important to note that the study by de Groot-Kamphuis et al. found that metformin use was not associated with risk of neuropathy or anaemia [46]. Importantly, there have been no new intervention studies published to the authors' knowledge since the end date of our searches, so our

meta-analysis estimate for intervention studies can be considered up to date.

4.1. Limitations

Strengths of this work include the fact that to the author's knowledge, this is the first systematic review and meta-analysis to summarise all the research evidence for the association between metformin and vitamin B_{12} deficiency in patients with type 2 diabetes specifically. However, most of the studies in this review were conducted in outpatient clinics and not in primary care, which limits generalizability. The wide variation in study design, wide confidence intervals in some studies, lack of standardisation of assessment of vitamin B_{12} status and a small number of interventional trials make clear conclusions difficult to elucidate. Usage of a random effects model due to the presence of heterogeneity made the estimate for the B_{12} reducing ability of metformin slightly larger, with wider confidence intervals. Our meta-analysis only included trial measurements at ≤ 4 months so may not be applicable for longer time scales. Effect sizes may differ by age group of trial participants (e.g. the lowering effect could theoretically be more profound in the elderly), but this could not be assessed in this analysis due to a lack of studies in different age groups.

There were also other limitations in terms of study quality and design for the intervention studies, with Jadad score ≤ 3 in all but one study. Only one of the intervention studies (HOME study, data in two papers [37,40]) met the criteria for a double-blind randomised placebo-controlled trial. This is likely to affect the accuracy of the meta-analysis estimate calculated. Most studies contained a small number of participants, leading to the HOME trial [37] receiving 55% of the weight in the meta-analysis. However, the sensitivity analysis showed that removal of this trial from the analysis did not greatly change the effect size. We could not conduct funnel plots to assess bias (e.g. publication bias) as we only had four studies in the review, and funnel plots are known to be less reliable for analyses based on a small number of trials, studying a small sample of individuals [52].

Finally, due to lack of data, secondary outcomes were not assessed as a part of this review but highlight a research gap warranting further study. Also, there was some suggestion in three studies that dose and duration of metformin could be significant factors in determining B_{12} levels [34] [26,53]. This also could not be assessed in more detail due to lack of studies reporting data for dose and duration.

4.2. Implications for research and practice

Our meta-analysis provides an assessment as to the effect size of the association between metformin and B_{12} status specifically in patients with type 2 diabetes, based on a meta-analysis of short-term therapy (up to 4 months).

Clinically, our results suggest that there is a clear need for evidence-based guidelines and for examination of the implications for practice. The traditional treatment for B_{12} deficiency has been with parenteral therapy due to poor oral absorption. However, it is currently recognised that high dose oral B_{12} (1000 μcg) appears to rectify the levels in most patients [35] being as effective as parenteral therapy [54,55]. Regular oral B_{12} or annual B_{12} injections have been recommended as safe ways to maintain levels in B_{12} deficient patients [55]. Therefore effective treatments are available, but it is unclear what the vitamin B_{12} monitoring strategy should be for patients being prescribed metformin. The results of this systematic review and meta-analysis suggest that further large cohort studies and high quality longer term intervention studies assessing clinical endpoints of vitamin B_{12} deficiency are now required, in order that evidence-based guidelines can be produced for screening and treatment of vitamin B_{12} deficiency in metformin treated patients with type 2 diabetes.

In addition, this review suggests the need for a cost–benefit analysis, comparing a 'watch and wait' strategy of clinical case finding versus regular vitamin B_{12} status checks. This should consider the management of B_{12} status in screened vs. unscreened patients, and the effects of this upon usage of healthcare services e.g. falls in the elderly secondary to neurological damage [56].

5. Conclusion

This review of patients with type 2 diabetes shows a reduction in B_{12} status by 57 pmol/L after up to 4 months of metformin use, which based on European data for B_{12} status would be predicted to lead to frank deficiency in a significant proportion of patients. Our findings support that of previously published meta-analyses of more heterogeneous populations of metformin users [8,9]. A cost–benefit analysis is now required to fully investigate the most effective protocols for treating this widespread problem (e.g. watch and wait vs. clinical screening) and appropriate clinical guidelines produced.

Disclosure of interest

The authors declare that they have no competing interest.

Acknowledgements

None, no funding was received for this work.

Appendix A. Supplementary data

Supplementary data (Appendices A and B) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.diabet.2016.03.008.

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