Increased Intake of Calcium Reverses Vitamin B_{12} Malabsorption Induced by Metformin

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OBJECTIVE — Of patients who are prescribed metformin, 10–30% have evidence of reduced vitamin B_{12} absorption. B_{12}-intrinsic factor complex uptake by ileal cell surface receptors is known to be a process dependent on calcium availability. Metformin affects calcium-dependent membrane action. The objective of this study was to determine the magnitude and mechanism of the reduction in serum vitamin B_{12} after metformin administration.

RESEARCH DESIGN AND METHODS — A comparative study design was employed using 2 groups (metformin and control). A total of 21 patients with type 2 diabetes received sulfonylurea therapy; 14 of these 21 patients were switched to metformin. Monthly serum total vitamin B_{12} measurements and holotranscobalamin (holoTCII) (B_{12}-TCII) were performed. After 3 months of metformin therapy, oral calcium supplementation was administered.

RESULTS — Serial serum vitamin B_{12} determinations revealed a similar decline in vitamin B_{12} and holoTCII. Oral calcium supplementation reversed the metformin-induced serum holoTCII depression.

CONCLUSIONS — Patients receiving metformin have diminished B_{12} absorption and low serum total vitamin B_{12} and TCII-B_{12} levels because of a calcium-dependent ileal membrane antagonism, an effect reversed with supplemental calcium.

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Metformin, a disubstituted biguanide, is an increasingly important agent in standard therapy of type 2 diabetes (1). Approximately 10%, but in some studies up to 30%, of patients taking metformin on a continuous basis have evidence of reduced vitamin B_{12} absorption (2–4). The mechanism of this assumed idiosyncratic effect has been unclear and, therefore, a subject of controversy. Some evidence supported the hypothesis that metformin-induced B_{12} malabsorption is due to enhanced bacterial overgrowth, especially because diabetic patients are known to exhibit alterations in small bowel motility as well as bacterial overgrowth (5–6). It may also be speculated that metformin could alter bacterial flora through an effect on motility and/or facilitation of bacterial growth by delaying absorption of glucose (4). Alternatively, metformin is known to have an effect on calcium-dependent membrane action (7). Of note, B_{12}-intrinsic factor (IF) complex uptake by the ileal cell surface receptor is known to be calcium dependent (8). Some investigators have demonstrated a direct effect on B_{12} absorption in the absence of any apparent bacterial overgrowth (2,3). These considerations prompted us to hypothesize a metformin-induced decrease in vitamin B_{12} absorption because of altered intestinal calcium metabolism.

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Abbreviations: CBC, complete blood count; holoTCII, holotranscobalamin; IF, intrinsic factor; MCTT, mouth-to-cecum transit time; TC, transcobalamin..

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

RESEARCH DESIGN AND METHODS

Subjects

A total of 21 subjects with known type 2 diabetes who were receiving therapy with an oral sulfonylurea and followed as outpatients in the diabetes clinic of the Veterans Affairs Medical Center, Bronx, New York, were recruited for study. The subjects' ages were restricted to 30–60 years. Patients were excluded if they had a history of alcoholism or other drug abuse, psychiatric disease, chronic renal failure, liver disease, cardiopulmonary disease, pernicious anemia, bowel surgery, stomach or bowel disease, acid-based disturbance, or cancer, or if they were receiving antibiotics or any medications known to influence gastrointestinal motility. An initial blood sample was obtained to exclude the possibility of occult preexisting vitamin B_{12} deficiency (serum holotranscobalamin II [holoTCII] [B_{12}-TCII]$<$50 pg/ml or serum total $B_{12}<$200 pg/ml). Any subject with low serum B_{12} or holoTCII would have been excluded; however, none was identified.

After informed consent was obtained, 14 of the 21 subjects were switched from their sulfonylurea preparation to metformin; this was accomplished in order of their entrance into the study by placing 2 subjects on metformin and 1 remaining on his or her sulfonylurea, and so forth. Thus, 7 patients continued to receive their usual sulfonylurea preparation and served as control subjects. Subjects in the experimental and control groups were men of similar age (48.6 ± 10.1 vs. 54.0 ± 4.9 years, NS) (Table 1) and proportionally similar ethnic distribution (metformin group: 3 white, 4 Hispanic, 7 African-American; control group: 1 white, 4 Hispanic, 2 African-American), duration of diabetes (6.9 ± 6.1 vs. 6.0 ± 3.6 years), and initial serum total vitamin B_{12} levels.

A physician performed a history and physical examination. Baseline laboratory

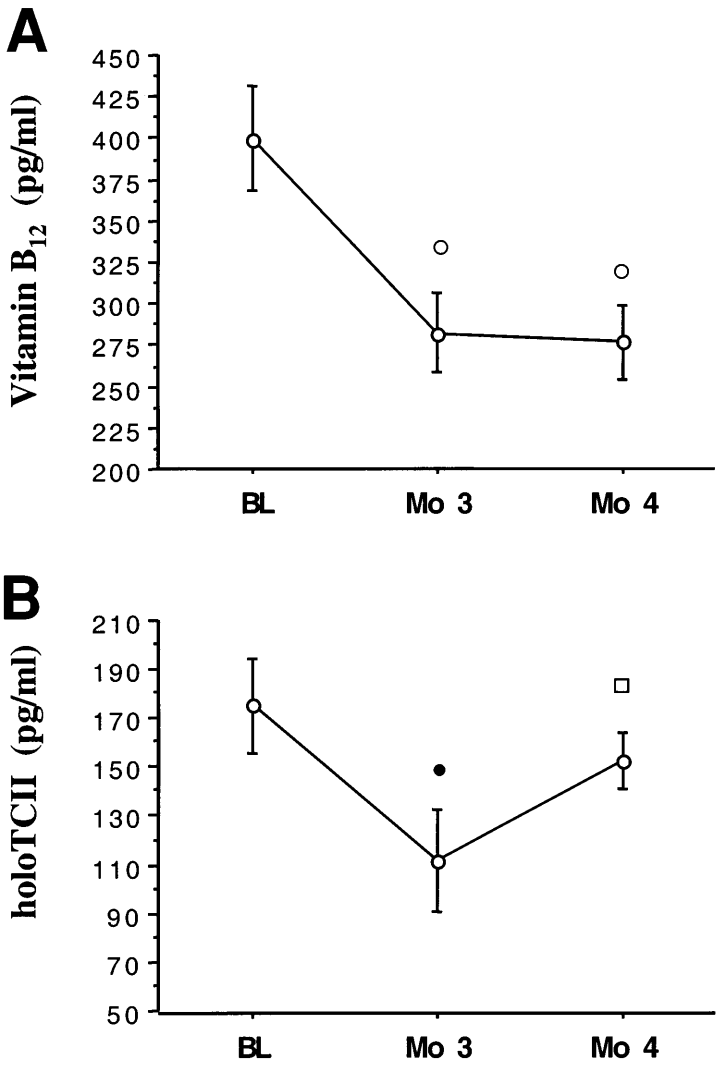

Figure 1—*Metformin-treated subjects for serum total vitamin B_{12} (A) and serum holoTCII (B). ○, P < 0.0005 between baseline and month 3 or month 4; ●, P < 0.01 between baseline and month 3; □, P < 0.005 between month 3 and month 4. Vertical bars indicate SEM.*

studies included the following: general chemistries (electrolytes, renal function, and liver function tests), complete blood count (CBC), serum folate, and lactate. A baseline hydrogen breath test (mouth-to-cecum transit time [MCTT]) was performed on all patients.

A standard protocol was used for metformin therapy. Patients began metformin at 850 mg/day for 2 weeks. Almost all subjects experienced loose bowel movements during the initial treatment period. However, the dose of medication was titrated up to 850 mg twice a day for 2 weeks and then to the maximum maintenance dosage of 850 mg 3 times a day. Because of gastrointestinal symptoms, a few subjects could not tolerate the maximum dosage and were reduced to 850 mg metformin twice a day.

Blood collections were performed for measurement of total serum vitamin B_{12} (9,10) and holoTCII in all subjects at weekly intervals for the first month and at monthly intervals for 4 months. After 3 months of metformin therapy, oral calcium carbonate (1.2 g/day) was administered to the metformin group alone for 1 month. Serum B_{12} analogs and folate were performed at baseline and 4 months and, if increased, served as serum markers for states of small-bowel bacterial overgrowth. Serum lactate and glucose were drawn with the other determinations. HbA_{1c} levels and body weights were performed at baseline and 6 months in the metformin-treated and sulfonylurea-treated groups.

Sample analysis

Blood samples obtained as part of the clinical evaluation of metformin-treated and control (sulfonylurea-treated) subjects were analyzed for total and holoTCII-B_{12}. After separation, serum samples were frozen immediately at −20°C until assayed (SimulTRAC; Becton Dickinson, Orangeberg, NY). Vitamin B_{12} and vitamin B_{12} analogs were assayed by differential radioassay according to the modification by Kolhouse et al. (9) of the method of Lau et al. (10) using purified IF to measure cobalamin and R-binder to measure total corrinoids (9,10). The analogs are measured by the difference between the 2 assays; elevated levels of the analogs indicate bacterial overgrowth of the small intestine, except in rare cases of ingestion of the analogs themselves from alternative medicine sources, e.g., herbs, algae, or seaweed.

HoloTCII was measured by determining the difference between serum total vitamin B_{12} and B_{12} remaining after absorption onto microfine silica, as previously described (11). HoloTCII levels fall when absorption of B_{12} is reduced, regardless of the etiology of the decreased B_{12} absorption (11–15).

Hydrogen breath tests

To ascertain bacterial overgrowth, a lactulose-stimulated hydrogen breath analysis was performed in patients before enrollment and after 4 months of treatment in the experimental or control groups. A Quintron 12i breath analyzer was used. After an overnight fast, a baseline hydrogen breath test was obtained before and after administration of 10 g lactulose in 100 ml water. Readings were taken every 10 min for 2 h. An increased basal level, as well as an increased early peak, was considered consistent with small intestinal bacterial overgrowth. A delayed peak on the MCTT was taken to indicate slowed intestinal transit.

Other determinations

Serum folate levels were determined by competitive inhibition radioassay using a commercial kit (SimulTRAC). Serum glucose and lactate determinations were performed on an automated Glucose-Lactate Analyzer II (Yellow Springs Instruments, Yellow Springs, OH). Serum HbA_{1c} was performed by the clinical laboratory using high-performance liquid chromotography methodology (Variant; Bio-Rad, Hercules, CA).

Statistical analysis

The dependent variables (serum total vitamin B_{12} and holoTCII) were analyzed for the between-group comparisons (control vs. metformin) by using an unpaired *t* test and a multiple regression analysis. A nonparametric test (Mann-Whitney *U* test) was used to determine the difference in the distribution between the control and metformin groups for the percent change from baseline to month 3 in the holoTCII levels. The results are reported as means ± SEM. Within the control and metformin groups, comparisons were performed using a repeated-measures analysis of variance to determine significance of differences for the baseline, month 3, and month 4 time points.

Consent

Before being performed, the study was approved by the research and development committee as well as the subcommittees on animal and human studies at the Veterans Affairs Medical Center.

RESULTS

General laboratory values

Baseline laboratory studies for general chemistries (electrolytes, renal function, and liver function tests), CBC, and serum lactate were within normal limits for all subjects enrolled.

Baseline and follow-up values

The initial serum total vitamin B_{12} levels were not significantly different between the metformin and control groups (400 ± 119 vs. 335 ± 120 pg/ml, NS) (Table2). Throughout the course of the study, no significant changes in serum B_{12} levels were noted in the control group at baseline, 3 months, or 4 months (335 ± 45 vs. 364 ± 51 vs. 375 ± 90 pg/ml, NS). After 3 months of metformin therapy, subjects in this group demonstrated a significant decrease in serum total vitamin B_{12} (400 ± 32 vs. 282 ± 24 pg/ml, $P < 0.0005$) (Fig. 1*A*) and holoTCII (175 ± 19 vs. 111 ± 21 pg/ml, $P < 0.01$) (Fig. 1*B*). Of the 14 subjects receiving metformin, with the exception of 2 subjects who had a slight increase, 12 subjects had a fall in serum total vitamin B_{12} levels; 11 of 14 subjects had a decrease in holoTCII concentration (Table 2). During the initial 3 months of treatment with metformin, none of the patients had a serum holoTCII value <40 pg/ml. Controlling for baseline variation by use of a multiple regression model, at 3 months, the metformin-treated group had lower serum total B_{12} (−61 ± 19 pg/ml, $P < 0.005$) than the control group. Significantly more subjects in the metformin-treated group had greater percent reductions in holoTCII than subjects in the control group ($P < 0.05$).

Table 1—*Subject characteristics*

	Metformin	Control subjects	*P*
n	14	7	—
Age (years)	49 ± 10	54 ± 5	NS
Weight (lb.)	232 ± 31	190 ± 45	0.053
Duration of diabetes (years)	6.9 ± 6.1	6.0 ± 3.6	NS
HbA_{1c} (%)	9.9 ± 2.0	8.7 ± 2.1	NS

Data are *n* or means ± SEM. Control subjects continued on sulfonylurea therapy.

Intervention with oral calcium carbonate

Dietary supplements of oral calcium carbonate (Tums, 1.2 g/day) in the metformin-treated group partially reversed the decreased serum holoTCII levels. The serum holoTCII increased from 111 ± 21 to 153 ± 11 pg/ml—a 53 ± 15% increase after calcium supplementation from month 3 to month 4 ($P < 0.005$; Fig. 1*B*)—but the serum total vitamin B_{12} level did not change significantly (Fig. 1*A*).

Absence of evidence of bacterial overgrowth or malabsorption

No patient had evidence of bacterial overgrowth before or at the conclusion of the study period, as assessed by serial MCTT hydrogen breath tests. The concentrations of the serum B_{12} analogs were variable. After 4 months of therapy, they were not significantly different from baseline in either the metformin or control groups (metformin group: 142 ± 60 vs. 126 ± 101 pg/ml; control group: 188 ± 44 vs. 101 ± 78 pg/ml; baseline vs. 4 month values, respectively), also suggesting that bacterial overgrowth was not present. Similarly, at 3 and 4 months, serum folate levels were not significantly different from baseline values (metformin group: 8.8 ± 3.3 vs. 7.8 ± 2.8 vs. 7.0 ± 1.8 pg/ml; control group: 8.7 ± 1.9 vs. 9.6 ± 4.9 vs. 8.6 ± 1.3 pg/ml; baseline vs. 3-month value vs. 4-month value, respectively). None of the serum folate values were below the lower limit of normal in any of the subjects.

Metabolic considerations and body weight

The average HbA_{1c} level in the metformin group was significantly lower at 6 months compared with the baseline value (9.9 ± 1.9 vs. 9.1 ± 2.0%, $P < 0.05$). In the control (sulfonylurea-treated) group, no significant difference in HbA_{1c} was found (8.7 ± 2.1 vs. 8.4 ± 1.6%, NS). No patient developed lactic acidosis that mandated withdrawal from the study. From baseline to 6 months, body weight remained stable in the control group (190 ± 45 vs. 187 ± 43 lb., NS) but significantly fell in the metformin group (232 ± 31 vs. 222 ± 28 lb., $P < 0.05$).

CONCLUSIONS

— Because ileal vitamin B_{12} absorption is a calcium-dependent process, in our study, patients with type 2 diabetes developed a marked reduction in serum holoTCII while being treated with metformin. A fall in serum total vitamin B_{12} also occurred. Serum holoTCII represents the bioavailable form of vitamin B_{12} and has a half-life of 6 min and, as such, is responsive to dietary pertubation (11,15,16). In the normal adult, ~80% of total serum vitamin B_{12} is on haptocorrin (formerly called transcobalamin [TC] I and TC III), a circulating vitamin B_{12} storage protein with a half-life of 2 weeks and with cell surface receptors only on reticuloendothelial storage cells (15,16). We further demonstrated that this malabsorption of vitamin B_{12} is reversible by oral calcium supplementation. A potential limitation of this study was that the enrollment of subjects was not truly random and thus may have permitted assignment bias to group membership.

The hydrophobic tail of biguanides, such as metformin, extends into the hydrocarbon core of membranes. The protonated biguanide group gives a positive charge to the surface of the membrane, which acts to displace divalent cations (7). Thus, biguanides alter membrane potentials and affect divalent cation membrane functions, such as those that are calcium dependent, and may act in general as a calcium channel blocker. Adhesion of many substances to cell surface membranes is

Table 2—*Vitamin B_{12} and holoTCII values*

	B_{12} (pg/ml)			HoloTCII (pg/ml)		
Group	Baseline	Month 3	Month 4	Baseline	Month 3	Month 4
Metformin	328	258	203	142	61	143
Metformin	358	351	—	138	259	—
Metformin	521	376	347	239	271	233
Metformin	332	359	378	239	50	118
Metformin	344	183	264	166	73	123
Metformin	525	335	316	276	125	172
Metformin	598	441	315	311	211	155
Metformin	463	323	392	198	64	194
Metformin	488	304	231	188	118	164
Metformin	270	291	238	136	78	167
Metformin	543	210	351	158	64	185
Metformin	256	130	138	92	48	74
Metformin	332	204	262	110	66	121
Metformin	235	184	166	56	72	136
Mean ± SEM	400 ± 32	282 ± 24	277 ± 22	175 ± 19	111 ± 21	153 ± 11
Control subjects	580	605	868	249	88	403
Control subjects	302	343	259	128	85	181
Control subjects	330	493	509	112	341	300
Control subjects	386	331	264	128	107	140
Control subjects	242	238	248	58	81	141
Control subjects	261	275	270	98	165	96
Control subjects	242	262	207	117	202	207
Mean ± SEM	335 ± 45	364 ± 51	375 ± 90	127 ± 22	153 ± 36	210 ± 40

Control subjects continued on sulfonylurea therapy.

affected by calcium. Specifically, the cell surface TCII receptors on all DNA synthesizing cells are calcium dependent, and metformin may interfere with the delivery of vitamin B_{12} to these cells (16). Calcium-dependent processes in general may potentially be altered by metformin, and whether such effects are significant physiologically has yet to be determined. However, in the study herein, there is no evidence that supplemental calcium has an adverse effect on metformin's hypoglycemic effects. There is a known calcium dependence of B_{12}-IF binding to the ileal B_{12}-IF receptor (8,17,18). This finding is further supported by our in vivo observation of a reversal of metformin-induced serum holoTCII depression by oral calcium.

As evaluated by differential radioassay, noncobalamin vitamin B_{12} analogs constitute a significant percentage of total corrinoids in human serum (9,16). The absence of change in serum analogs or that of serum folate in our study suggests that bacterial overgrowth was not a significant factor in the depression of serum vitamin holoTCII or total vitamin B_{12} levels (19).

The serum total vitamin B_{12} level was once considered to be a sensitive index for the detection of negative vitamin B_{12} balance and the clinical disorders caused by vitamin B_{12} deficiency. However, in the past decade, it has been increasingly appreciated that normal total serum vitamin B_{12} levels may be found in a significant proportion of patients with varying clinical features of this vitamin deficiency (9,11,12,16,20,21). Herzlich and Herbert (11) were the first to report the selective depletion of that portion of total serum vitamin B_{12} that is on TCII (i.e., reduction of holoTCII or TCII-B_{12}) in early negative vitamin B_{12} balance. This indicates that the earliest serum marker of subnormal vitamin B_{12} absorption, and therefore of negative vitamin B_{12} balance, is low serum holoTCII (11–16). In our study, serum holoTCII and vitamin B_{12} concentrations were affected similarly by metformin; however, levels of serum holoTCII increased with oral calcium supplementation, just as they do in elderly individuals given oral vitamin B_{12} supplementation (12). No patient exhibited overt clinical hematological or neurological evidence of vitamin B_{12} deficiency because it takes over a year of negative vitamin B_{12} balance caused by subnormal vitamin B_{12} absorption to result in the elimination of nerve and blood vitamin B_{12} stores (16,22). A state of severe B_{12} deficiency, as a consequence of prolonged metformin administration, may result in peripheral nerve damage. This may be confused with that of the peripheral neuropathy of diabetes, a clinical scenario that could lead to permanent nerve loss of a potentially reversible etiology.

In conclusion, individuals with type 2 diabetes receiving metformin develop low bioavailable B_{12} (holoTCII), which, if allowed to progress, would be expected to be followed by low serum total vitamin B_{12} levels and, presumably, eventual clinical deficiency; this sequence of events was not addressed in our study. Because the peripheral neuropathy of diabetes may present with symptoms that may be indistinguishable from that of vitamin B_{12} deficiency, the condition of metformin-induced low serum vitamin B_{12} is of great concern if not recognized and treated appropriately. Ionic calcium is obligatory for the B_{12}-IF complex to attach to ileal cell surface receptors, and metformin competes with calcium for the mucosal cell membrane. This form of vitamin B_{12} malabsorption was reversible with an oral calcium supplement. Patients with type 2 diabetes treated with metformin, especially those who do not consume milk or milk products on a daily basis or do not take supplemental calcium should be encouraged to increase their intake of calcium as well as be closely monitored for vitamin B_{12} deficiency.

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