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Alteration of Bile Acid Metabolism and Vitamin-B_{12}-Absorption in Diabetics on Biguanides*

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Summary. Since vitamin B_{12}malabsorption has been described in diabetics on biguanides and inhibition of bile acid absorption found in rat ileum the effect of treatment with different biguanides (phenformin, buformin, metformin) on bile acid metabolism and vitamin B_{12} absorption was assessed in maturity onset diabetics. Biguanides did not alter faecal weight or faecal fat excretion, but they decreased faecal bile acid excretion. All biguanides tested increased deconjugation of glycocholic acid, as determined by a simple breath test technique. Vitamin B_{12} malabsorption was most prominent in patients on metformin. Discontinuation of biguanide treatment, or administration of antibiotics, normalized or improved the increased deconjugation of bile acids and the Schilling test. Decreased faecal bile acid excretion, positive ^{14}C-glycocholate breath tests, pathological Schilling tests and the reversal of pathological tests by antibiotic treatment suggest that small intestinal bacterial overgrowth, leading to binding of the intrinsic-factor-vitamin B_{12}-complex to bacteria, is responsible for the previously observed pathological Schilling tests in diabetics on biguanides. Bile acid malabsorption, possibly responsible for the cholesterol-lowering effect of biguanides, does not occur in diabetics on biguanides. Whether qualitative changes in small intestinal bile acid composition might affect cholesterol metabolism remains to be determined.

Key words: Biguanides, bile acids, vitamin-B_{12}-absorption, phenformin, buformin, metformin, intestinal bacterial overgrowth, cholesterol.

Biguanides have been shown to exert an inhibitory effect on intestinal absorption of hexoses [1, 6, 7, 15, 16, 17, 24, 36], amino acids [8, 9], myo-inositol [9] and calcium [9] in the proximal small intestine. It has recently been reported that biguanides also inhibit absorption of bile acids in rat ileum in vitro [10] and in vivo [11]. Since bile acids are synthesized in the liver from cholesterol and biguanides have been reported to have a cholesterol-lowering effect [2, 10, 23, 26, 28, 29], it was assumed that bile acid malabsorption might be responsible for the cholesterol-lowering effect of these drugs [10]. Malabsorption of vitamin-B_{12} occurs in diabetics on treatment with biguanides [2, 3, 4, 15, 32, 33, 35]. This study was therefore undertaken to assess the effect of biguanides on bile acid metabolism and absorption of vitamin-B_{12} which, like conjugated bile acids, is absorbed preferentially by the distal small intestine.

The intraluminal fate of the conjugated bile acid, glycocholic acid, was examined in maturity onset diabetics under treatment with different biguanides (phenformin, buformin, metformin) using the ^{14}C-glycocholate breath-analysis technique [12, 13, 19, 30]. This non-invasive test will detect an increased deconjugation of conjugated bile acids induced either by bacterial overgrowth of the proximal small bowel, or by colonic bacteria in ileal dysfunction, when bile acids are spilled over into the colon [12, 13, 19, 30]. A differentiation between bile acid malabsorption and bacterial overgrowth of the proximal small intestine responsible for the increased deconjugation of bile acids can be performed by bile acid analysis in the faeces [12, 13, 19]. Bile acid malabsorption due to ileal dysfunction is associated with increased faecal bile acid excretion [12, 25], whereas normal or decreased amounts of bile acids are found in the faeces of patients with small intesti-

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nal bacterial overgrowth [12, 31. This is because deconjugated bile acids will be reabsorbed in the proximal small bowel already by a diffusional transport mechanism (ionic- and non-ionic diffusion) [21, 22].

This study will demonstrate that biguanides do not induce bile acid malabsorption, but increase deconjugation of bile acids, as in the bacterial overgrowth-syndrome. The results to be presented suggest that vitamin-B_{12} malabsorption in patients on treatment with biguanides is due to bacterial binding rather than to an inhibitory effect on the specific ileal vitamin-B_{12}-intrinsic-factor transport mechanism.

Methods

Studies were performed in maturity onset diabetics on long-term treatment with oral antidiabetics, including biguanides. Patients with clinical or laboratory evidence of gastrointestinal or liver disease were excluded. Previous antidiabetic treatment was discontinued and 10 patients each were pretreated for 4 days with: 1.) 4 × 100 mg buformin (Silubin®), 2.) 4 × 50 mg phenformin (Dipar®), 3.) 4 × 850 mg metformin (Glucophage®), 4.) 3 × 100 mg buformin (Silubin®), 5.) 3 × 850 mg metformin (Glucophage®). More detailed data on patients, pretreatment and biguanide administration before performing the appropriate tests are given in Table 1. During this period of treatment faecal fat analysis, measurement of stool weight, and faecal bile acid analysis were performed in 4 × 24-h stool specimens. On the 5th day patients received the whole daily dose of biguanides (group 1–3) one hour before the ^{14}C-glycocholate breath test and the Schilling test were performed. In patients of groups 4 and 5 the above tests were performed one hour after administration of 100 mg buformin or 850 mg metformin. Pathological breath tests or Schilling tests were repeated one week to 10 days after discontinuation of treatment with biguanides. In two groups of patients (group 5 and 6) on pretreatment with 3 × 850 mg metformin or 4 × 100 mg buformin pathological breath tests or Schilling tests were repeated 8 days after additional treatment with 100 mg doxycycline (Vibramycin®) daily, maintaining the treatment with biguanides.

^{14}C-glycocholate breath tests

The test was performed according to the original method of Fromm and Hofmann [19], as reported earlier [12]. One hour after administration of biguanides 5 μCi of ^{14}C-glycocholate (spec. act.: 10–20 mCi/mol, Buchler-Amersham, Braunschweig) was given in 50 ml of tap water. After two hours patients are given an ordinary breakfast. Specific activity of $^{14}CO_2$ was analyzed by a discontinuous breath analysis technique, described by several authors [12, 13, 19, 30]. Breath samples were collected 1, 2, 3, 4, 5 and 6 h after administration of ^{14}C-glycocholate by direct exhalation into a liquid scintillation vial through a Pasteur pipette. The trapping solution in the sampling vial contained 1 ml of 1 M hyamine hydroxide (Fa. Zinsser, Frankfurt), 2 ml of methanol, and 3 drops of phenolphthalein (1%) in ethanol. Conversion from purple to colourless indicates that 1 mmol of CO_2 has been trapped by 1 mmol of hyamine hydroxide. After addition of 10 ml of Instagel (Fa. Packard, Frankfurt) radioactivity was assayed in a liquid scintillation system (Packard TRI-CARB), with automatic standardization for quench-correction. Since specific activity of $^{14}CO_2$ might be altered by increased endogenous CO_2 production patients were not allowed ambulatory activities with exception of "bathroom privileges". In order to correct specific activities of $^{14}CO_2$ for endogenous CO_2 production increasing with body weight results were expressed as % $^{14}CO_2$ exhaled/mmol CO_2 × kg body weight, according to Fromm and Hofmann [19]. Cumulative $^{14}CO_2$ exhalation was calculated assuming a constant CO_2 production of 9 mmol/kg per hour [13, 19] and calculated over 6 h.

Schilling-test

Schilling tests were performed one hour after administration of biguanides by oral administration of a capsule of 0.5 μCi ^{57}Co-vitamin B_{12} (specific activity: 0.5 μCi/mg) and an intramuscular flushing dose of 1000 μg vitamin B_{12} i. m. two hours later.

24 h urinary excretion of ^{57}Co was measured and the results expressed as % excretion of the orally administered dose in 24 h. Normal values in our laboratory are:

> 10% of the administered dose excreted in the urine in 24 h. Values < 8% excretion are considered to represent vitamin B_{12} malabsorption; values between 8 and 10% excretion are considered equivocal [35].

Faecal analysis

Faecal fat analysis was performed by the method of van de Kamer et al.]34]; faecal bile acid excretion was measured by the method of Reimold and Kattermann [27]. Statistical analysis was performed by Student's 't'-test.

Results

Analysis of faecal weight, faecal fat and bile acid excretion in patients under treatment with biguanides revealed that faecal fat and weight were normal (Table 2). Faecal bile acid excretion was decreased in patients on biguanides compared to a group of normal controls reported previously [12]. Cumulative $^{14}CO_2$ exhalation in patients on biguanides revealed an increased appearance of $^{14}CO_2$ in breath following administration of ^{14}C-glycocholic acid, which is consistent with an increased deconjugation of this conjugated bile acid in patients on biguanides (Fig. 1). Measurement of the cumulative $^{14}CO_2$ exhalation over 6 h showed that an increased deconjugation of glycocholate occurred in patients on biguanides (buformin, phenformin, metformin) who received the total daily dose one hour before the test, but could also be observed in the group of patients receiving only 100 mg buformin one hour before the test (Fig. 4). This group of patients had been pre-treated with 3 × 100 mg of buformin for 4 days.

5 of 10 patients on metformin had a pathological Schilling test; in 2 patients the Schilling test was in the equivocal range. One patient of 10 on phenformin had a pathological and one an equivocal Schilling test, whereas only one patient on buformin was found to have an equivocal test (Fig. 2). Discontinuing treatment with biguanides normalized the previously pathological Schilling tests in all but one patient after 7–10 days (Fig. 2). In order to exclude other factors than biguanides possibly responsible for the increased deconjugation of glycocholate, breath-test analysis was repeated one week after discontinuation of treatment with biguanides in those patients with a positive breath test. Complete normalization of previously increased bile acid deconjugation was observed after discontinuation of treatment with buformin and metformin, whereas deconjugation of glycocholate was still increased in patients previously treated with phenformin (Fig. 3).

After discontinuation of biguanides $^{14}CO_2$ exhalation rates were within the normal range in patients previously treated with metformin and buformin, whereas, as expected from the data in Figure 3, discontinuation of treatment with phenformin did not result in complete normalization of the increased deconjugation rate.

In order to demonstrate indirectly that increased deconjugation of glycocholate was due to bacterial overgrowth in the small intestine breath tests were repeated in those patients on buformin and metformin with a previously positive (pathological) test after additional treatment with 100 mg doxycycline for one week. The treatment with buformin (4 × 100 mg) and metformin (3 × 850 mg) was continued and patients received 400 mg buformin or 850 mg metformin one hour before the breath test was performed. The previously increased deconjugation of glycocholate was either normalized or reduced in all patients after antibiotic treatment despite continuing the treatment with the biguanide (Fig. 4).

Antibiotic treatment with doxycycline also exerted marked improvement on the pathological Schilling test in patients on treatment with metformin (Fig. 5). The group of patients on metformin subjected to the Schilling test was the same as that in which the breath test (Fig. 4) was performed.

Table 1. Data on maturity onset diabetics subjected to treatment with different biguanides

Pretreatment (4 days)	Dose administered 1 h before the tests	No. of patients	Sex: male	Sex: female	Weight (kg)	Age
1. buformin, 4 × 100 mg	400 mg	10	4	6	78.45	57.1
2. phenformin, 4 × 50 mg	200 mg	10	7	3	81.43	61.1
3. metformin, 4 × 850 mg	3400 mg	10	6	4	78.42	56.6
4. buformin, 3 × 100 mg	100 mg	10	4	6	74.5	58.1
[a]5. metformin, 3 × 850 mg	850 mg	10	7	3	81.76	57.3
[a]6. buformin, 4 × 100 mg	400 mg	9	2	7	74.45	62.6

[a] in these groups of patients pathological breath tests and Schilling tests were repeated after additional antibiotic treatment with doxycycline

Table 2. Effect of treatment with biguanides on faecal weight, fat and bile acid excretion. Faecal weight, faecal fat and bile acid excretion were measured under treatment with 4 × 100 mg buformin, 4 × 50 mg phenformin, or 4 × 850 mg metformin

	Patients on treatment with		
	Buformin (4×100 mg)	Phenformin (4×50 mg)	Metformin (4×850 mg)
Faecal weight (normal: < 200g/24h)	131,9±61,0	56,7±42,9	89,9±56,7
Faecal fat (normal: < 7g/24h)	2,93±0,62	3,21±1,3	2,84±1,03
Faecal bile acids (normal: 0,3±0,2/24h)	0,088±0,078*	0,040±0,037**	0,036±0,039**

* $p < 0{,}005$
** $p < 0{,}001$

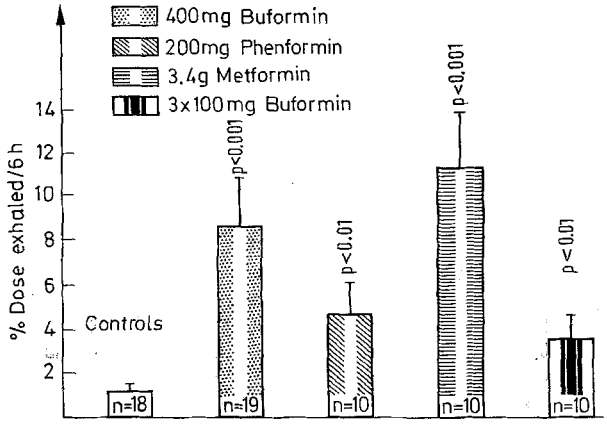

Fig. 1. Cumulative $^{14}CO_2$ exhalation in maturity onset diabetics on biguanides after oral administration of 5 μCi ^{14}C-glycocholic acid. Patients were pretreated with biguanides for 4 days and received the total daily dose of biguanides (400 mg buformin, 200 mg phenformin, 3400 mg metformin) one hour before administration of the labelled bile salt. One group of patients was pretreated with 3 × 100 mg buformin and received 100 mg buformin one hour before performing the ^{14}C-glycocholate breath test (column No. 5). Results are means ± SEM

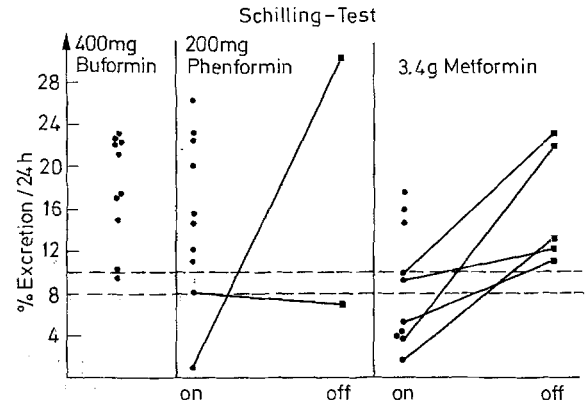

Fig. 2. Vitamin B_{12} absorption (Schilling-test) in maturity onset diabetics on treatment with biguanides. Patients were pretreated for 4 days with 4 × 100 mg buformin, 4 × 50 mg phenformin or 4 × 850 mg metformin and received the total daily dose one hour before performing the Schilling-test. In patients with a pathological or equivocal test a second Schilling-test was done 7–10 days after cessation of treatment with biguanides (off). – – – indicates the lower limit of normal (10%) or – ˙ – borderline range (8–10%) of vitamin B_{12} absorption

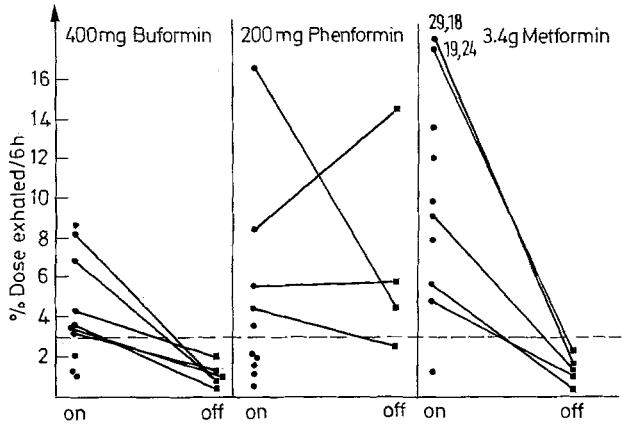

Fig. 3. Deconjugation of glycocholate measured by cumulative (6 h) $^{14}CO_2$ exhalation after oral administration of 5 μCi ^{14}C-glycocholate in maturity onset diabetics on biguanides (on) and one week after discontinuation of treatment (off). Patients were pretreated with 4 × 100 mg buformin, 4 × 50 mg phenformin or 4 × 850 mg metformin. In most of the patients with an increased deconjugation of glycocholate the ^{14}C-glycocholate breath-test was repeated one week after discontinuation of treatment with biguanides. The first breath test (on) was performed one hour after administration of the total daily dose of biguanides

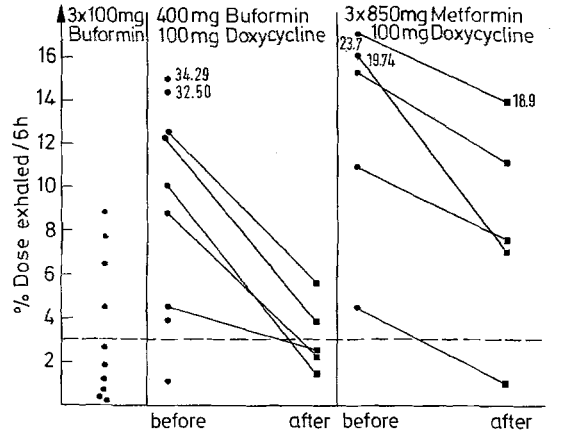

Fig. 4. Deconjugation rate of glycocholate (^{14}C-glycocholate breath test) in maturity onset diabetics on treatment with buformin and metformin before and after additional antibiotic treatment with 100 mg doxycycline/day. Ordinate: cumulative $^{14}CO_2$ exhalation over 6 h. Patients were pretreated with 3 × 100 mg buformin, 4 × 100 mg buformin, or 3 × 850 mg metformin. Patients on the left and the right part of the graph received 100 mg buformin or 850 mg metformin one hour before the test, patients in the centre part of the graph received the total daily dose (400 mg) of buformin one hour before the test

Discussion

Malabsorption of bile acids in patients on biguanides, previously assumed from animal experiments to be responsible for the cholesterol-lowering effect of biguanides, could be excluded by the finding of a decreased excretion of bile acids in the faeces. A slight underestimation of the amount of faecal bile acid excretion could have occurred since the method used [27] will miss 3-keto bile acids. The low faecal weights, however, also suggest that bile acid malabsorption is unlikely to occur in patients on treatment with biguanides. The positive breath tests and low faecal bile acid excretion do suggest, however, an increased deconjugation of bile acids [13, 14, 19]. The data presented do not direct-

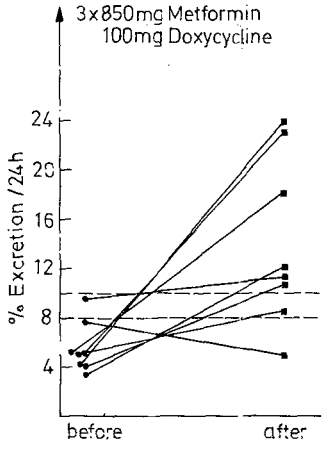

Fig. 5. Vitamin B_{12} absorption in maturity onset diabetics on treatment with metformin before and after additional treatment with 100 mg of doxycycline

ly prove the presence of bacterial overgrowth in the small intestine of diabetics on biguanides, but clearly demonstrate one of the consequences of the bacterial overgrowth syndrome; namely increased deconjugation of bile acids [13, 14, 19, 31]. Since bile acid deconjugation occurs by bacterial action alone, an increased deconjugation of a conjugated bile acid may reasonably be attributed to an increased number of deconjugating bacteria within the intestinal lumen.

Normalization of breath tests after discontinuation of treatment with biguanides suggested that the antidiabetic drug itself was responsible for the increased deconjugation of glycocholate rather than other intrinsic or extrinsic factors. Further evidence concerning the bacterial origin of increased bile acid deconjugation was obtained by the normalization or improvement of previously pathological breath tests after antibiotic treatment and continuation of treatment with buformin and, to a lesser degree, with metformin (Fig. 4).

The extent of bile acid deconjugation was greatest in patients on treatment with metformin, previously shown to exhibit considerable vitamin B_{12} malabsorption [2, 3, 4, 15, 32, 33, 35]. An increased deconjugation of glycocholate was observed after 4 days of pretreatment and subsequent administration of the total daily dose of biguanides, but could be detected, too, after pretreatment with lower doses of buformin or metformin and administration of one tablet (100 mg) of buformin one hour before the breath test was performed. This suggests that increased deconjugation of bile acids will be present in patients on treatment with normal doses of biguanides.

The decreased faecal output of bile acids during the pretreatment period with 4 divided doses of biguanides can also be considered a consequence of increased deconjugation of bile acids. Increased bacterial deconjugation of bile acids results in premature disappearence of free bile acids from the intestinal lumen via a passive diffusion mechanism [12, 21, 22, 31]. Decreased D-xylose absorption and a pathological Schilling test, which can be rendered normal by treatment with antibiotics, are considered to be indicative of bacterial overgrowth of the small intestine [12, 31]. Since D-xylose may be metabolized by bacteria [31] and vitamin B_{12} can be firmly bound by bacteria in the gut lumen [31] the intraluminal bacterial overgrowth can result in a pathological 'absorption' test. Inhibition of D-xylose absorption in diabetics on high doses of metformin (3 × 1000 mg) has been reported by one group [3], but could not be demonstrated by others [35]. Whether the former finding was due to increased catabolism of D-xylose by intestinal bacteria or to inhibition of D-xylose absorption cannot be decided, since the tests were not repeated after antibiotic treatment.

In accord with other observations [2, 3, 4, 15, 32, 33, 35] vitamin B_{12} absorption, as measured by the Schilling test, was decreased mainly in patients on treatment with metformin. Increased bile acid deconjugation and decreased vitamin B_{12} absorption improved or were completely normalized after discontinuation of treatment with biguanides or after antibiotic treatment, while continuing biguanides. This rules out a possible depletion of intrinsic factor induced by biguanides. The latter results are in disagreement with the findings of Tomkin et al. [32], who did not observe an improvement of vitamin B_{12} malabsorption in patients on metformin after a 7 day course of treatment with tetracycline, but who showed that substitution of metformin by chlorpropamide resulted in improvement of vitamin B_{12} absorption. Unfortunately their paper does not say whether the patients improving vitamin B_{12} absorption on chlorpropamide were the same who failed to respond to treatment with tetracycline. The results presented, however, clearly demonstrate that patients on biguanides do exhibit two of the consequences of small intestinal bacterial overgrowth: increased or premature deconjugation of bile acids and pathological Schilling tests, both of which improve or completely normalize after antibiotic treatment.

Increased folate levels in patients on metformin with vitamin B_{12} malabsorption [32] could also be attributed to bacterial overgrowth, since high serum folate levels have been reported in patients with the blind-loop syndrome [31].

Berger et al. [5], in contrast to others [2, 3, 4,

15, 32, 33, 35], did not observe vitamin B_{12} malabsorption in patients on lower doses of metformin, using a whole body counter technique. Their findings on the basis of the results presented may suggest that the ordinary Schilling test may not be the appropriate method to assess vitamin B_{12} absorption in the bacterial overgrowth syndrome. If the intrinsic-factor-vitamin B_{12}-complex is bound to bacteria, there will be decreased urinary excretion of labelled vitamin B_{12}. Information is scarce, however, on whether bacteria-bound vitamin B_{12} is completely unavailable for absorption at a later stage. The results of Berger et al. [5] could be due to the lower dose of biguanides administered, or they could suggest a prolonged retention of the ^{58}Co-labelled vitamin B_{12} in the lumen of the gut by bacteria, thus indicating apparently normal vitamin B_{12} absorption. Performing a Schilling test with urinary collections over several days may solve this question.

The underlying cause for the increased deconjugation of bile acids and pathological Schilling tests, indicating bacterial overgrowth of the proximal small intestine of patients on biguanides, is most likely due to the inhibitory effect of biguanides on intestinal motility, which has been demonstrated especially for gastric emptying [14, 17, 18]. Bile acid malabsorption as a possible cause of the cholesterol-lowering effect of biguanides could be excluded by the data presented. Since cholesterol absorption requires micellar formation and free bile acids resulting from increased deconjugation have been shown to be less effective in promoting cholesterol absorption than conjugated bile acids [30 a], a decrease of cholesterol absorption due to increased bile acid deconjugation induced by biguanides could be responsible for the cholesterol-lowering effect of biguanides.

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