The Effect of Phenformin-HCl on Patients with Diabetes Mellitus, Studied under Strict Balance Conditions

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Received: February 19, 1975, and in revised form: July 11, 1975

Summary. Under strict balance conditions we studied the effect of phenformin in 5 patients with diabetes mellitus. In all cases phenformin lowered the blood glucose values, and all patients showed a reduction of glycosuria. Contrary to other reports body weight increased during phenformin treatment. This was accompanied by positive nitrogen, phosphorus and calcium balances. The weight gain can be explained by the positive caloric balance, mainly caused by the diminished glycosuria. No change in B.M.R. or R.Q. was seen. During phenformin treatment there was a drop in cholesterol and total lipid levels in 4 patients. No conclusions could be drawn about the effect of phenformin on triglycerides,

phospholipids and lipoprotein spectra. Phenformin treatment did not affect the disappearance of glucose, nor the insulin levels after intravenous glucose loading. During oral glucose loading phenformin caused a significant fall in blood glucose levels, accompanied by an increased insulin response in one patient. In the other 4 patients phenformin had no effect on either parameter.

Key words: Phenformin, biguanides, weight reduction, caloric balance, balance study, lipid spectra, glucose tolerance, insulin levels.

Biguanides are used mainly to treat the elderly obese diabetic. According to many investigators biguanides reduce the elevated blood glucose level [7, 8, 11, 13, 17, 21, 37], body weight [1, 4, 11, 17, 18, 34, 37, 42] and glucose excretion in urine [11]. A concomitant reduction of elevated cholesterol [1], triglyceride and insulin levels [24, 28, 34] has been reported.

Since reduction of glycosuria without diminishing the diet should cause an increase in the available energy in the body, the weight loss is difficult to un-

derstand. By investigating patients under strict balance conditions, we hoped to learn more about the mechanism by which biguanides reduce both the blood sugar level and the body weight.

Materials and Methods

We studied 5 otherwise healthy diabetics from our out-patient department (Table 1). They were hospitalized in the metabolic ward during the whole study.

Table 1.

patient	duration	treatment	height(m)	weight(kg) ^a	composition of the diets(g) ^b			caloric value of the diets (kcal.)	
date of birth	diab. (years)	before study			carbohydr.	protein	fat	bombcal.	table ^b
1. ♀ A. K.-v. R. 03-01-22	7	diet + tolbutamide	1.55	51.9	216	82	72	2246	1843
2. ♀ HC. v. V.-V. 18-01-17	3	diet	1.65	72.5	243	85	83	2413	2096
3. ♂ H. v. V. 06-09-00	10	diet	1.76	75.8	274	101	89	2493	2312
4. ♂ W. B. 22-11-22	< 1	diet	1.67	84.0	287	143	134	3410	2925
5. ♂ JJ. v. V. 04-12-05	< 1	diet	1.75	76.0	209	116	109	2846	2529

^a At the beginning of the first A period.

^b Calculated from the official table of the "Nederlandse Voedingsraad".

To exclude gastrointestinal side-effects phenformin capsules (3×50 mg) were prescribed before the final selection of the patients, but gastrointestinal disturbances forced us to omit one patient from the study. Two weeks before admission oral hypoglycemic therapy was withdrawn.

The diet was individually adjusted such that the body weight would remain constant during the

non-phenformin periods. At identical times 3 isocaloric meals and 3 isocaloric snacks were given. The meals for each balance study were all prepared and divided at one time before the start of the study and kept frozen until consumption. No other food or drinks than those included in the diet were allowed except a measured amount of distilled water. The patients were required to consume all meals com-

Fig. 1. Data on body weight, glucose levels and glycosuria in pat. 1-5

pletely. We tried to keep the activities of the patients identical to that at home.

After a period of 7 days, in which the patient became accustomed to the diet and slight modifications could be made, the study was carried out according to the A-B-A system. During the A periods (10–15 days) the patients received a placebo capsule 3 times a day and in the B period (15 days), a capsule of 50 mg phenformin 3 times a day. Body weight (at 8.30 a.m.), blood glucose values (at 8 a.m., 1 p.m., 5 p.m., using the glucose oxidase method [41] and 24 hrs sugar (polarimeter) and creatinine excretion in the urine were determined daily. Faeces were collected over 5-day periods, marked by carmine red. Chromium oxide (Cr_2O_3 , capsules of 500 mg) was given 3 times a day to quantify the faecal collection [40]. Faeces, urine and three thoroughly mixed daily diets were analyzed chemically and in the bomb calorimeter according to Miller and Payne [33]. Calcium was estimated by complexon III (Na_2EDTA) titration, using murexide as an indicator, phosphorus according to a modification of Fiske and Subbarow [20], and nitrogen according to Kjeldahl [23].

At the end of every period the cortisol levels during that day were determined (8 a.m., 5 p.m., 12 p.m.) [31], as well as total lipid, cholesterol and triglyceride levels and lipoprotein spectra in fasting blood samples [38]. At the start and the end of each period serum osmolarity, urea, total protein, hemoglobin and hematocrit were determined as a measure of the hydration of the patient.

An oral and an intravenous glucose tolerance test on subsequent days were performed at the end of each period, and blood sugar (Nelson-Somogyi [35]) and plasma insulin levels (Yalow and Berson [44]) were determined. All blood samples were collected in heparin via an indwelling needle. An hour before testing, one placebo or phenformin capsule (50 mg) was given. On these days the entire daily diet was consumed but the times of consumption were changed. For the intravenous glucose tolerance test 25 grams of glucose were injected in 4 min. Samples were drawn at zero (in the middle of the injection), 2, 4, 6, 9, 12, 20, 30, 45 and 60 min. The rate constant for the fractional disappearance of the injected glucose (K-value) was calculated from the least square regression line. For the oral glucose tolerance test 50 grams of glucose were given. Blood was sampled in the fasting state and 30, 60, 90, 120 and 150 min after glucose administration.

Results

Fig. 1 shows the effect of phenformin (B period) on body weight, blood glucose and glycosuria in patients

1–5. During the phenformin period a decrease and stabilization in blood glucose levels, with an accompanying reduction in glycosuria, occurred in all patients within a few days. When phenformin was withheld, the return to pre-phenformin levels also took several days. In Fig. 2 and Table 2 we see that during phenformin therapy there is an absolute or relative (patient 2) increase in body weight accompanied by an absolute or relative (patient 2) positive N, Ca and P balance.

The calculated caloric balance (Table 3) shows that phenformin induces a positive balance. This is most marked in the patients with a substantial decrease in glycosuria. The caloric value of the faeces is practically unchanged during phenformin therapy, indicating the absence of malabsorption. Table 4 indicates that it is not likely that metabolites of high caloric value are excreted during phenformin substitution.

In 4 patients (1, 3, 4 and 5) no effect of phenformin on blood sugar or insulin levels after oral glucose loading is seen (Fig. 3). The insulin levels in these patients were already low before phenformin treatment. The only patient (2) with an increased insulin response during phenformin and a concomitant reduction of the blood sugar levels showed higher insulin levels before phenformin than the other patients.

The intravenous glucose loading tests revealed that none of the patients showed a significant change in K values during phenformin treatment (Table 5).

In patients 1, 2, 3 and 4, but not patient 5, there was a decrease in cholesterol and total lipid levels during phenformin treatment (Table 6). The phospholipids, triglycerides and free fatty acids exhibited variable changes during phenformin therapy. The only change seen in the lipoprotein spectra is a decrease in the β -lipoproteins during phenformin treatment.

Fig. 2. Weight changes during both control (A) periods and the phenformin (B) period, related to the body weight at the beginning of the first A period (zero)

Table 2. Nitrogen, calcium and phosphor balances in the three periods (A, B and A)

Pat.	N-balance g/24 h			Ca-balance mg/24 h			P-balance mg/24 h		
	A	B	A	A	B	A	A	B	A
1	-2.0	-1.3	-2.1	-56	-41	-64	-174	-60	-163
2	+0.4	+1.3	+0.9	-106	-6	-174	-109	+73	-174
3	+0.7	+1.5	+0.5	+133	+202	+160	+35	+141	+37
4	-1.4	-0.0	-1.0	+94	+108	+158	-26	+166	+23
5	+1.4	+1.7	-1.0	+106	+130	+87	-72	+106	-118

Table 3. Caloric balance Kcal./24 h. (bombcalorimetre)

	Pat. 1			Pat. 2			Pat. 3			Pat. 4			Pat. 5		
	A	B	A	A	B	A	A	B	A	A	B	A	A	B	A
Intake (diet)	2246	2246	2246	2413	2413	2413	2493	2493	2493	3410	3410	3410	2846	2846	2846
Output															
feces	157	144	166	118	127	112	113	143	139	137	157	141	144	111	129
urine	818	550	685	203	a	244	368	137	233	753	397	596	144	131	150
total	975	694	851	321		356	481	280	372	890	554	737	288	242	279
Balance	1271	1552	1395	2092		2057	2012	2213	2121	2520	2856	2673	2558	2604	2567

^a For technical reasons the bombcalorimetric estimation of the urine (B period) in pat. nr 2 failed.

Table 4. Caloric value of the urine after subtraction of the caloric value of the glucose (Kcal./24 h)

	Pat. 1			Pat. 2			Pat. 3			Pat. 4			Pat. 5		
	A	B	A	A	B	A	A	B	A	A	B	A	A	B	A
total cal. val. urine (bombcalorimetre)	818	550	685	203		244	368	137	233	735	397	596	144	131	150
cal. val. glucose (g. glucose $\times$ 4.23 kcal)	739	434	597	135	58	158	244	32	76	524	219	346	-	-	-
Balance	79	116	88	68		86	144	105	157	211	178	250	144	131	150

Table 6. Total lipids (mg/100 ml) and cholesterol (mg/100 ml)

Pat.		A	B	A
1	total lipids	1071	1006	968
	cholesterol	323	273	300
2	total lipids	732	701	744
	cholesterol	210	181	227
3	total lipids	897	767	905
	cholesterol	290	244	288
4	total lipids	1011	828	905
	cholesterol	300	245	269
5	total lipids	841	844	898
	cholesterol	279	300	316

Table 5. Glucose assimilation coefficient (K-value) in the intravenous glucose tolerance tests

Pat.	A	B	A
1	0.32	0.23	0.23
2	0.25	0.43	0.34
3	0.71	0.76	0.72
4	0.66	0.41	0.46
5	0.71	0.75	0.67

Fig. 3. Plasma insulin and blood sugar levels during the oral glucose tolerance test in pat. 1-5

Discussion

In contrast to other published data, all 5 of our patients gained weight during phenformin treatment with respect to the control periods. Many investigators report that biguanides induce a weight loss in obese patients with diabetes mellitus [1, 4, 11, 17, 18, 34, 37, 42]. This is attributed to anorexia [37], intestinal malabsorption [42, 43], suppression of high insulin levels resulting in a reduction of lipogenesis [18, 34] or stimulation of thermogenesis [26, 27]. In none of

these studies were the patients observed under strict balance conditions. Thus the loss of weight might be the result of a reduced caloric intake due to the anorexic effect of the biguanides or a closer adherence to the prescribed diet because of the more elaborate control. Apart from the direct interactions of energy uptake and release, weight reduction may also be caused by a shift in body composition from protein to fat. Protein calories are approximately 10 times heavier than fat calories, due to the adhering water mantle and the lower energetic value of protein. We thought it necessary therefore to observe our patients under strict balance conditions to exclude the effect of changes in food intake.

In 4 patients (1, 3, 4 and probably 2) there was a positive caloric balance in the phenformin period. The caloric gain can be explained by the reduced glycosuria. The increased retention of N, Ca and P during the phenformin period, compared to the control periods, also indicates that there is an increase in body tissue. For patient 5, the lack of glycosuria and the approximate equilibrium of the caloric balance under phenformin (+ 40 kcal./24 hrs) make it more difficult to explain the weight changes. Decreased physical activity during phenformin treatment can be excluded in this patient. An increase in body tissue is not likely, as there was almost no caloric gain. The nitrogen balance does not support an important shift of fat to protein. The weight gain might be explained by fluid retention. However we could not estimate the fluid balance with sufficient accuracy, since we had no facilities to measure total body water. Attempts to calculate fluid balances from the total intake and the urinary excretion of water gave too variable results to be of any use.

Direct determinations of the caloric content of feces and urine are not reported in the literature, except by Stowers [43], who found during clinical observation of one overweight patient with diabetes mellitus treated with 225 mg phenformin per day, that the caloric value of the feces increased by 28%. We found that such high doses of phenformin can induce gastro-intestinal disturbances, e.g. diarrhea. The results of the bomb calorimetric estimations indicate that in our patients there is probably no loss of substrates with a high caloric value in faeces or urine.

The theory that the weight loss during phenformin therapy is due to an increased metabolic rate is not supported by the findings of Stowers *et al.* [42] since neither B.M.R. nor R.Q. changed significantly from the values measured during the control periods. We also found no changes in B.M.R. or R.Q. (not shown here). Moreover the positive nitrogen balance during phenformin excludes increased catabolism.

To eliminate anorexia as a possible cause of weight loss during phenformin treatment, food intake during our study was constant. Nevertheless most patients felt more discomfort during the phenformin period as a result of the consumption of their entire diet. In one patient (not included in this report) anorexia was so strong that vomiting developed and we had to discontinue the study. We feel that if our patients had not been required to consume their whole diet, they would definitely have reduced their food intake.

Theoretically phenformin may lower the blood glucose level by reducing intestinal resorption [9, 10, 13, 14, 25], enhancing peripheral glucose utilization [7, 8, 19, 22] or reducing endogenous glucose production [2, 5, 32]. A reduced intestinal glucose resorption is not supported by the bomb calorimetric data which show no increase in the caloric content of faeces and urine during phenformin treatment. This is in agreement with Olsen [36], who could not demonstrate carbohydrate malabsorption during phenformin treatment. Also the blood glucose values after oral glucose loading during phenformin administration are virtually unchanged in 4 out of 5 patients in comparison with the control periods. In the only patient who showed a significant decrease in glucose levels during phenformin treatment, the insulin values were also significantly higher. This does not support the malabsorption theory.

Another often stated assumption is that biguanides stimulate peripheral glucose utilization [7, 8, 19, 21, 22]. The mechanisms which might be involved are not clear. Biguanides uncouple the oxidative phosphorylation in mitochondria [15]. A stimulating effect of biguanides on glucose transport has never been demonstrated. Phenformin does not stimulate the transport of glucose in isolated fat cells and it inhibits the stimulation of glucose uptake induced by insulin in such cells [29].

A more reasonable explanation, which is also supported by experiments in animals [2, 12], might be a decrease in internal glucose production, although we could not prove this from our data.

After an oral glucose load phenformin is said to reduce the rise in blood glucose and insulin levels [3, 6, 14, 24]. Only patient 2 showed a drop in blood glucose concentration, but with a concomitant rise in insulin levels. In the remaining 4 patients (1, 3, 4 and 5) phenformin did not have any effect (Fig. 3). This may be due to the fact that these patients were no longer able to secrete much insulin. Moreover, our patients received their regular dose of one capsule of phenformin (50 mg) one hour before the test, in contrast to the total daily dose of 100–150 mg administered by other investigators. The lack of effect

may also be explained by the fact that in our study the patients had received phenformin for only ten days prior to the glucose tolerance tests. Boshell [6] observed no reduction of insulin levels in 4 patients ("hypersecretors") after oral glucose loading when they had taken phenformin for only one week (100 mg/day), but there was a significant decrease after 3 weeks of treatment. However in our patients the hypoglycemic effect of phenformin was already fully established after a few days. Since no effect of phenformin could be seen on the intravenous glucose tolerance test, this might indicate an action of phenformin in the gut (mucosa), possibly by stimulation of a hormonal factor(s) which enhances insulin secretion. In this connection it is interesting to note Loubatière's [30] report that phenformin stimulated insulin secretion in the dog (*in vivo*) and in the perfused rat pancreas.

There are many reports of a phenformin-induced drop in cholesterol and triglyceride levels in diabetic patients [1, 16, 28, 34, 39]. In 4 patients we found a decrease in both cholesterol and total lipid levels during phenformin treatment. No effect on phospholipids and triglycerides was seen. With regard to the lipoprotein spectra the only reproducible effect we observed was a reduction of the β -lipoprotein fraction, probably related to the decrease in cholesterol levels. Since most of our patients showed almost no insulin response to glucose loading, it does not seem likely that a decrease in insulin levels induces the reduction in cholesterol and total lipid levels. The caloric gain observed in our patients during phenformin treatment does not support the theory that a diminished supply of substrate for lipogenesis can explain the phenformin effect.

Conclusion

In patients with diabetes mellitus, observed under strict metabolic balance conditions, phenformin caused a decrease in blood glucose levels but no reduction of body weight. It also reduced the cholesterol and the total lipid content in the serum. The slight gain in weight during phenformin treatment can be explained by the diminished glycosuria in this period.

Acknowledgements. The technical assistance of Mrs. A. Cornelius – von Franquemont and the secretarial help of Miss M. G. A. T. Paal are gratefully acknowledged. We also thank Mr. W. Zuidervaart for the drawing of figures. The phenformin and placebo capsules used in this study were a gift of Winthrop B. V.

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