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The Effect of Short-Term Administration of Antidiabetic Biguanide Derivatives on the Blood Lactate Levels in Healthy Subjects*

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Summary. In three groups of healthy subjects (n = 56) changes in blood lactate, pyruvate and bicarbonate concentrations and pH were determined during three different loading tests. These were an oral ethanol load (0.5 g/kg body wt), an IV fructose load (1 g/kg body wt over 60 min), and a 15 min submaximal exercise load. The same tests were repeated after administration of biguanides for 3 days in the following doses: phenformin 150 mg, buformin 300 mg and metformin 2.55 g daily. All three derivatives induced a significant rise in blood lactate level as well as a significant increase in blood [lactate]/[pyruvate] ratio in relation to control tests. The differences in the effect of individual biguanides were minimal. It was observed, on the other hand, that increments in blood lactate concentrations depended markedly on the type of load given. The highest rise in blood lactate level was found after fructose loading; in the 60th minute of the test after phenformin it was 1.60 ± 1.29 (SD), after buformin 1.32 ± 0.79, and after metformin 1.31 ± 0.64 mmol/l. The smallest rise of lactate was observed after oral ethanol loading: in the 1st hour of the test the respective values were 0.41 ± 0.24, 0.52 ± 0.18, and 0.91 ± 0.86 mmol/l. In the exercise test the highest increment of the blood lactate level was observed 15 min after the end of the exercise, being 1.06 ± 0.37, 1.21 ± 0.25 and 1.26 ± 0.33 mmol/l, respectively. The results of these investigations show that all three biguanide derivatives used in treatment of diabetes – phenformin, buformin and metformin – are risk factors which may induce lactic acidosis under suitable conditions.

Key words: Biguanides, blood lactate, ethanol, fructose, exercise.

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Although rises in blood lactate concentration in diabetics treated with biguanide derivatives were reported within a few years of their introduction in the treatment of diabetes [1, 2, 3] and the first reports of dangerous complications resulting from lactic acidosis in these patients were published at the beginning of the 1960s [4, 5], only in the last few years has this problem aroused general concern. Lactic acidosis is an uncommon complication of biguanide therapy of diabetes, being very dangerous and often fatal. Most observations on this complication have concerned diabetics treated with phenformin. It has been claimed also that treatment of diabetics with other biguanide derivatives – buformin and metformin – causes no such complication [8, 9]. Although development of lactic acidosis has been reported recently during treatment with buformin [10, 11] and metformin [12, 13], the number of such cases so far published is much lower.

In the present investigations we tried to examine this problem by comparing the effects of all three biguanide derivatives (e.g. phenformin, buformin and metformin) on blood lactate levels in healthy subjects submitted to loading tests known to cause hyperlactacidaemia [1, 2, 14].

Material and Methods

The studies were carried out in three groups and the following loading tests were done:

1. Oral Ethanol Load

Ethanol was administered in a dose of 0.5 g/kg of body weight as a 40% solution. Blood samples were taken in the fasting state and 1, 2, 4 and 6 hours after alcohol ingestion. The test was performed in 20 subjects, including 15 men and 5 women aged

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from 24 to 62 years (mean age 36 years) and was repeated in 8 subjects after phenformin administration, in 6 after buformin, and in 6 after metformin.

2. Intravenous Fructose Load

Fructose (20 g/100 ml) was given over 60 min as a continuous intravenous infusion in the total dose of 1 g/kg body wt. Blood samples were taken fasting pre-infusion and at intervals of 15 min for 90 min. The test was carried out in 18 subjects: 8 men and 10 women, 15 of them aged 16–60 years and three over 60 years (mean age 42 years) and was repeated in groups of 6 subjects after administration of phenformin, buformin and metformin.

3. Exercise Test

It was performed on a cycle ergometer (Zimmermann, GDR) in 18 subjects: 7 men and 11 women aged 19 to 45 years (mean age 29 years). The mean exercise load was 109.3 watts for men and 78.7 watts for women. The exercise accounted for 50% of maximum oxygen utilization which was determined earlier from the pulse rate in the steady state during three submaximal exercises of known severity, using Astrand-Ryhming nomogram [15]. The duration of exercise was 15 min. The mean pulse rate during the first exercise was 148/min in men and 152/min in women, and during the second exercise the respective rates were 142/min and 148/min; it was thus somewhat lower (not significantly) during the second test. Blood samples for determinations were obtained from fasting subjects 10 and 15 min after the beginning of exercise (steady state) and then twice at an interval of 15 min and twice at an interval of 30 min. The second exercise load was repeated in groups of 6 subjects after administration of phenformin, buformin and metformin.

In all, the determinations were performed in 56 healthy non-obese subjects, 30 men and 26 women, and in each subject only one double test was carried out, that is before biguanide administration (control test) and after its administration. All persons were volunteers, and were previously informed about the nature and the aim of these investigations. Each biguanide derivative was administered for 3 days in the sustained action form (dragées) in the following daily doses: phenformin 150 mg, buformin 300 mg, metformin 2.55 g, and on the fourth day, that is on the day of the second test, the daily dose was given one hour before the test in the form of short-action tablets.

The blood samples were obtained after complete rest lasting at least 30 min. For determination of lactate and pyruvate free-flowing venous blood was collected into test tubes containing 0.6 M glacial perchloric acid. Blood glucose was determined by the method of King [18], fructose by the method of Roe [19], alcohol, lactate and pyruvate (the latter in exercise tests only) enzymatically (Boehringer), pH and bicarbonates with the Micro-Astrup apparatus. Since each subject served as his own control the effect of biguanide derivatives on the various blood components was calculated as net increment ($x_{after} - x_{before} = d$) and results were assessed using the paired "t" test at the significance level $p < 0.05$. For determining the significance of differences in the lactogenic effects of various biguanide derivatives one way analysis of variance was used.

Results

Oral Ethanol Load

After oral administration of the test dose of ethanol the highest alcohol concentration in the blood, about 0.6 g/l, was observed one hour after ingestion. Over the following 5 hours the blood alcohol level decreased gradually to below 0.1 g/l. The administration of biguanides had no effect on the maximal rise of ethanol concentration in the blood or on the rate of its disappearance. On the other hand, a slight but repeatedly occurring effect of these drugs on blood sugar level was demonstrated: one hour after ethanol ingestion blood glucose concentration rose slightly and previous administration of biguanides prevented this.

In the reported tests a rise in blood lactate level was always observed, reaching a peak value one hour after alcohol ingestion. Hyperlactacidaemia persisted for some period of time, returning to normal gradually within the following 5 hours. After previous administration of biguanide derivatives the rise in blood lactate after ethanol ingestion was significantly higher than in control tests (Table 1). This was particularly evident when the results were calculated as difference of increments in blood lactate after the test dose of ethanol.

The fall in plasma bicarbonate after ethanol ingestion was small and transient and administration of biguanides failed to influence this. The blood pH was not significantly changed either in control tests or in tests after administration of biguanide derivatives.

Intravenous Fructose Load

Following intravenous infusion of fructose the blood fructose level increased on average up to 50 mg/dl

Table 1. The effect of antidiabetic biguanide derivatives on the blood lactate level in healthy subjects after oral ethanol intake

Subjects treated with:	Phenformin				Buformin				Metformin			
	n = 8		$\bar{d}$		n = 6		$\bar{d}$		n = 6		$\bar{d}$	
Time hours	Before	After Blood lactate mmol/l	(After-Before)	p	Before	After Blood lactate mmol/l	(After-Before)	p	Before	After Blood lactate mmol/l	(After-Before)	p
0	1.00 ± 0.16[a]	1.11 ± 0.19[a]	0.12 ± 0.08[a]	ns	1.07 ± 0.27[a]	1.06 ± 0.28[a]	0.01 ± 0.06[a]	ns	1.17 ± 0.25[a]	1.21 ± 0.26[a]	0.04 ± 0.03[a]	<0.05
1	1.59 ± 0.23	2.01 ± 0.23	0.41 ± 0.24	<0.01	1.73 ± 0.40	2.26 ± 0.40	0.52 ± 0.18	<0.01	1.95 ± 0.56	2.87 ± 1.28	0.91 ± 0.86	=0.05
2	1.33 ± 0.18	1.65 ± 0.15	0.32 ± 0.19	0.01	1.41 ± 0.30	1.81 ± 0.21	0.40 ± 0.18	0.01	1.62 ± 0.50	2.18 ± 0.66	0.56 ± 0.38	<0.01
4	1.17 ± 0.13	1.34 ± 0.14	0.16 ± 0.07	0.01	1.20 ± 0.28	1.27 ± 0.27	0.10 ± 0.14	ns	1.40 ± 0.38	1.79 ± 0.58	0.38 ± 0.26	0.02
6	1.09 ± 0.14	1.13 ± 0.11	0.07 ± 0.06	0.02	1.13 ± 0.27	1.11 ± 0.24	0.01 ± 0.06	ns	1.22 ± 0.34	1.32 ± 0.34	0.10 ± 0.07	0.05

[a] SD

Table 2. The effects of antidiabetic biguanide derivatives on the blood lactate level in healthy subjects during and after IV fructose infusion

Subjects treated with:	Phenformin				Buformin				Metformin			
	n = 6		$\bar{d}$		n = 6		$\bar{d}$		n = 6		$\bar{d}$	
Time min	Before	After Blood lactate mmol/l	(After-Before)	p	Before	After Blood lactate mmol/l	(After-Before)	p	Before	After Blood lactate mmol/l	(After-Before)	p
0 ↓ i. v. fructose infusion	1.22 ± 0.38[a]	1.18 ± 0.27[a]	−0.04 ± 0.15[a]	ns	1.23 ± 0.37[a]	1.31 ± 0.39[a]	0.07 ± 0.05[a]	<0.02	1.22 ± 0.09[a]	1.19 ± 0.11[a]	−0.03 ± 0.07[a]	ns
15	1.80 ± 0.29	2.68 ± 0.68	0.87 ± 0.52	<0.01	1.75 ± 0.53	2.41 ± 0.64	0.66 ± 0.53	ns	1.99 ± 0.36	2.47 ± 0.30	0.47 ± 0.19	<0.01
30	2.48 ± 0.54	3.67 ± 1.10	1.19 ± 0.77	0.05	2.36 ± 0.75	3.02 ± 0.72	0.66 ± 0.49	<0.01	2.73 ± 0.92	3.46 ± 0.76	0.64 ± 0.41	0.01
45	2.88 ± 0.90	4.29 ± 1.41	1.40 ± 0.75	0.01	2.42 ± 0.67	3.28 ± 1.00	0.70 ± 0.40	0.01	3.17 ± 1.31	4.53 ± 1.40	1.40 ± 0.81	0.01
60	3.10 ± 1.01	4.71 ± 2.04	1.60 ± 1.29	0.05	3.17 ± 1.15	4.21 ± 1.85	1.32 ± 0.79	0.05	3.53 ± 1.45	4.84 ± 0.82	1.31 ± 0.64	0.01
75	2.51 ± 0.91	3.88 ± 1.67	1.37 ± 0.89	0.01	2.39 ± 0.87	3.94 ± 1.90	1.05 ± 0.88	0.05	3.14 ± 1.04	4.02 ± 0.84	0.95 ± 0.57	0.01
90	1.93 ± 0.40	2.55 ± 0.99	0.68 ± 0.67	0.01	1.55 ± 0.39	3.34 ± 0.48	1.02 ± 1.02	ns	2.53 ± 0.72	3.09 ± 0.77	0.56 ± 0.76	0.01

[a] SD

Table 3. The effect of antidiabetic biguanide derivatives on the blood lactate level in healthy subjects during and after exercise

Subjects treated with:	Phenformin				Buformin				Metformin			
	n = 6		$\bar{d}$		n = 6		$\bar{d}$		n = 6		$\bar{d}$	
Time min	Before	After Blood lactate mmol/l	(After-Before)	p	Before	After Blood lactate mmol/l	(After-Before)	p	Before	After Blood lactate mmol/l	(After-Before)	p
0 ↓ exercise	1.21 ± 0.16[a]	1.29 ± 0.25[a]	0.08 ± 0.07[a]	<0.05	1.13 ± 0.10[a]	1.17 ± 0.10[a]	0.04 ± 0.02[a]	<0.01	1.19 ± 0.22[a]	1.22 ± 0.24[a]	0.03 ± 0.03[a]	ns
10	4.27 ± 0.67	5.36 ± 0.62	1.08 ± 0.74	0.02	3.85 ± 0.56	4.62 ± 0.85	0.77 ± 0.31	0.01	4.49 ± 1.07	5.05 ± 0.76	0.56 ± 0.50	ns
15	4.63 ± 0.64	5.65 ± 0.78	1.02 ± 0.52	0.01	4.60 ± 0.83	5.66 ± 0.78	1.20 ± 0.70	0.01	4.59 ± 0.47	5.72 ± 0.82	1.12 ± 0.51	<0.01
30	2.63 ± 0.41	3.69 ± 0.47	1.06 ± 0.37	0.01	2.60 ± 0.33	3.81 ± 0.39	1.21 ± 0.25	0.01	2.41 ± 0.18	3.68 ± 0.37	1.26 ± 0.33	0.01
45	1.82 ± 0.16	2.82 ± 0.23	0.99 ± 0.32	0.01	1.87 ± 0.23	2.90 ± 0.32	1.03 ± 0.32	0.01	1.80 ± 0.21	2.94 ± 0.80	1.14 ± 0.77	0.02
75	1.52 ± 0.68	2.15 ± 0.26	0.63 ± 0.26	0.01	1.51 ± 0.22	2.20 ± 0.36	0.68 ± 0.22	0.01	1.43 ± 0.21	2.11 ± 0.25	0.67 ± 0.25	0.01
105	1.32 ± 0.45	1.73 ± 0.21	0.41 ± 0.14	0.01	1.25 ± 0.13	1.87 ± 0.33	0.62 ± 0.21	0.01	1.27 ± 0.21	1.75 ± 0.21	0.47 ± 0.17	0.01

[a] SD

Table 4. Net increments ($\bar{d} \rightarrow$) in blood [lactate]/[pyruvate] ratio induced by phenformin, buformin and metformin on fasting, during and after exercise in healthy subjects

Biguanide derivative	Time	on fasting	10 min	15 min[a]	30 min	45 min	75 min	105 min
Phenformin[b]	$\bar{d} \rightarrow$	0.89	6.15	6.77	9.91	11.14	8.90	7.94
n = 6	SD	±1.54	±3.37	±4.11	±2.18	±2.15	±4.64	±4.08
	p	ns	ns	<0.05	<0.01	<0.01	ns	ns
Buformin[b]	$\bar{d} \rightarrow$	−0.11	5.72	9.47	16.36	15.55	10.23	8.75
n = 6	SD	±1.10	±2.43	±6.36	±11.41	±12.18	±6.65	±4.82
	p	ns	<0.02	<0.05	<0.05	<0.05	<0.05	<0.02
Metformin[b]	$\bar{d} \rightarrow$	0.11	6.42	9.50	11.53	12.59	9.36	7.26
n = 6	SD	±1.80	±5.06	±2.96	±2.30	±5.85	±4.19	±3.72
	p	ns	ns	<0.02	<0.01	<0.01	ns	ns

[a] Termination of exercise

[b] The differences between the particular biguanide derivatives were not significant at any compared point of observation

Table 5. The effect of short-term administration of antidiabetic biguanide derivatives on the fasting blood lactate level in healthy subjects

Subjects treated with:	n	Fasting blood lactate mmol/l		$\bar{d} \rightarrow$ (After-Before)	
		Before	After		
Phenformin	20	1.13±0.77[a]	1.19±0.38[a]	0.08±0.09[a]	p<0.05
Buformin	18	1.14±0.30	1.18±0.54	0.03±0.06	
Metformin	18	1.19±0.17	1.20±0.20	0.01±0.05	

[a] SD

at 15 min and then it rose more slowly to 80 mg/dl at the end of the infusion, falling rapidly thereafter. At the same time the blood glucose level increased on the average to 140 mg/dl. Administration of biguanide derivatives had no effect on the blood fructose changes. Similarly, phenformin and metformin failed to modify the blood glucose curve in these tests. Unexpectedly, after buformin the mean blood glucose curve was significantly below the control from 15 till 75 min of the test.

The maximum rise in blood lactate occurred usually in the 60th minute of the test and raised values of blood lactate persisted for 30 min after the end of fructose infusion. After previous administration of biguanides the rise in blood lactate level was significantly higher than in control tests (Table 2).

The greatest decrease of plasma bicarbonate level, on the average to 20 mEq/l, was observed at the end of fructose infusion and it was slightly (not significantly) greater in tests after administration of biguanides. The blood pH fell significantly to about 7.30 in the 60th minute of the test and biguanides did not increase this.

Exercise Test

During exercise a slight (but significant) decrease of blood glucose concentration occurred, and it persisted till the end of the test. Biguanides caused no significant modification of the blood glucose curve.

The mean maximum rise in blood lactate concentration occurred at the end of exercise. Levels then decreased but were still elevated above fasting concentration 90 min later. All biguanide derivatives tested caused a significantly higher rise in the lactate level (Table 3) and also a significant rise of blood [lactate]/[pyruvate] ratio (Table 4). Between 10 and 15 min of the test a significant fall in plasma bicarbonate occurred to a value of 18 mEq/l and then the base reserve increased. Apart from a slight fall in plasma bicarbonate in the fasting state in one set of determinations, the administration of biguanides had no effect on the base reserve in the exercise test. The blood pH was not significantly changed in control tests and in tests after previous administrations of biguanide derivatives.

Comparison of Various Biguanide Derivatives on Blood Lactate Concentration

Table 5 shows that administration of various biguanide derivatives over 3 days caused a very slight rise in fasting lactacidaemia which was, however, significantly higher after phenformin than after buformin or metformin. In the tests, the effect of individual biguanides on blood lactate rise depended to some extent on the type of load used. Thus, the blood lactate levels after fructose load and exercise were significantly higher than after ethanol ingestion. Consequently the increments in blood lactate level after administration of antidiabetic bigua-

nide derivatives were also higher in the fructose and exercise tests than in the ethanol test. The mean values of maximum increment of blood lactate in the tests after pooling results for all three biguanides were significantly different (the mean maximum blood lactate increment in the first hour of the ethanol test was 0.59 ± 0.52 (SD) mmol/l, with fructose 1.41 ± 0.91 mmol/l, while after exercise it was 1.11 ± 0.55 mmol/l).

The lactogenic effect of various antidiabetic biguanide derivatives should be considered separately for each kind of test. A comparison of net increments of lactacidaemia showed that the effects of the biguanides were very similar in the exercise test. In the ethanol test the greatest net increment of blood lactate was observed after metformin (in the 4th and 6th hour of the test the values were significantly higher than after phenformin and buformin).

Discussion

The mechanism of increased accumulation of lactate in the blood after administration of antidiabetic biguanides has not been elucidated completely. The mechanisms considered are overproduction due to increased rate of anaerobic glycolysis [18, 19] as well as underutilization due to inhibition of gluconeogenesis [20] and/or inhibition of lactate oxidation in peripheral tissues [21]. These effects are a common feature of the pharmacological action of all antidiabetic biguanide derivatives, and they were to be expected after administration of various biguanide derivatives [22]. The results of the present investigations confirm these effects in humans. The differences in blood lactate rise and blood [lactate]/[pyruvate] ratio rise after therapeutic doses of phenformin, buformin and metformin are very small and due probably to differences in the pharmacokinetics of these drugs. It may be concluded, therefore, that these differences are without any practical significance and that in cases with conditions predisposing to hyperlactacidaemia every one of the above mentioned biguanide derivatives could cause fatal lactic acidosis.

The presently reported results stress also the importance of the factor predisposing to development of lactic acidosis in diabetics treated with biguanides. The fructose load and exercise load tests which caused a greater rise in lactacidaemia than oral ethanol loading, induced also a greater increase in lactacidaemia after administration of biguanides. A considerable rise in blood lactate level and blood [lactate]/[pyruvate] ratio after biguanide derivatives in the exercise test deserves attention. Our results differ in this respect from certain earlier reports [23, 24] which may be due to differences in methods.

The investigations on the development of lactic acidosis after phenformin treatment of patients with concomitant renal failure suggested a correlation between the level of phenformin in the blood and the degree of blood lactate rise [25]. However, it has been stressed recently that in some cases phenformin caused development of lactic acidosis despite absence of a predisposing factor and with no increased level of the drug in the blood [26–30]. In this connection it is worth while to emphasize the presence of considerable individual differences observed in our healthy volunteers in their responses to administration of antidiabetic biguanides. In some cases quite unexpectedly high blood lactate rises were found after administration of any of these agents, exceeding blood lactate increments observed on the average in a given group. It may be that this individual predisposition to development of hyperlactacidaemia after administration of antidiabetic biguanides may explain cases in which lactic acidosis developed during diabetes treatment with these agents despite absence of known predisposing factors. Finally it must be emphasized that our studies were performed in normal subjects and one should extrapolate from such data with care. None the less the results are highly suggestive and useful therapeutic guidelines may be derived.

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