The effects of diabetes mellitus, exercise, and single doses of biguanides upon lactate metabolism in man

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1 The polymorphism of phenformin oxidation has been investigated in 103 non-insulin-dependent (Type II) diabetics. The frequency distribution was clearly bimodal and 14 poor metabolisers were identified. The frequency of the recessive allele (0.369) was not significantly different from that found previously in non-diabetics.

2 Six of the extensive metabolisers of phenformin were matched for age, sex and oxidiser phenotype with non-diabetic controls. All subjects underwent a standard 3-min exercise test, using a bicycle ergometer, after which plasma lactate concentration was monitored for 90 min. There was no significant difference between groups in lactate accumulation or elimination.

3 Ten extensive metabolisers, ten poor metabolisers and seven non-diabetics (matched for age, sex and phenotype with seven of the diabetic extensive metabolisers) were challenged with a fasting oral dose of phenformin (50 mg), after which plasma lactate, and blood pyruvate and glucose concentrations were monitored for 4 h. A further ten diabetics (five extensive and five poor metabolisers of phenformin) received a single dose of metformin (1 g) following an identical protocol. No significant changes were observed in any group.

Keywords phenformin metformin diabetes mellitus oxidation polymorphism

Introduction

Although a relatively uncommon adverse reaction, lactic acidosis occurring during treatment with phenformin proved fatal in about half of all patients affected (Anon, 1977). Diabetics manifesting cardiac or renal failure were generally considered to be at high risk, presumably because of impaired phenformin elimination (two-thirds of an oral dose appears in the urine unchanged, the remainder as 4-hydroxy-phenformin), and increasing age was closely associated. But the discovery that the oxidative metabolism of phenformin, like that of debrisoquine, displayed genetic polymorphism and was severely impaired in 9% of the population

(Oates *et al.*, 1982) introduced the possibility that certain diabetics might be predisposed by inheritance to experience the ultimate complication of phenformin treatment. Healthy subjects exhibiting impaired phenformin metabolism ('poor metabolisers') were shown, by Oates *et al.* (1983), to have higher concentrations of plasma lactate after a single dose of phenformin than were evident in those with normal phenformin metabolism ('extensive metabolisers').

We set out, first, to examine the frequency distribution of phenformin hydroxylation in a population of patients with non-insulin-

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dependent (Type II) diabetes mellitus; second, to evaluate the handling of an endogenous lactate load, provoked by exercise, in diabetics and matched controls; third, to assess the effect of a single dose of phenformin upon lactate concentration in diabetic subjects, selected from the population sample with respect to oxidiser phenotype; and fourth, to compare the effect of phenformin with that of metformin, administered under similar conditions.

Methods

Subjects

Diabetics were recruited from the out patient Diabetic Clinic at the North Staffordshire Hospital Centre; all were Type II diabetics, either newly diagnosed, or acknowledged and controlled adequately by diet alone. Those with biochemical abnormalities of hepatic and/or renal function which, in the clinical judgement of the investigators, would contra-indicate biguanide therapy were excluded. After oxidiser phenotyping (see below), extensive metabolisers were recruited for the exercise test and were matched for age and sex with non-diabetic control subjects (see below). All underwent electrocardiography. Diabetics of both phenotypes were recruited for the phenformin challenge test, as well as a group of non-diabetic control subjects, matched for age, sex and phenotype. Clinical screening of the controls (physical examination, serum biochemistry and electrocardiography) was the same as for the diabetics. Signed, informed consent was obtained from all subjects and the project was approved by the Ethics Committee of the North Staffordshire Health Authority.

Procedures

Oxidation phenotyping Subjects were provided with a urine receptacle and two 25 mg phenformin tablets (Dibotin, Winthrop), to be taken at a time convenient to precede a continuous 8 h urine collection. They were asked to avoid alcohol and, where possible, proprietary medicines for 24 h before and during the collection. Urine volume was measured and a 20 ml aliquot stored at -20°C pending assay.

Exercise test Subjects arrived at the laboratory after an overnight fast and an indwelling intravenous cannula (Venflon, Viggars) was inserted into a forearm vein. After 30 min rest, venous blood (5 ml) was drawn, without stasis, for

estimation of plasma lactate, and a capillary blood sample was obtained for acid-base estimation. The subjects then pedalled a bicycle ergometer for 3 min against a load (determined previously) sufficient to produce a heart rate of 120 to 140 beats min^{-1} in the third minute. Further venous and capillary samples were drawn immediately before, in the third minute of the exercise, and at 5, 10, 15, 20, 30, 40, 50, 60 and 90 min after the end of exercise. The cannula was kept patent between sampling by flushing with 2 ml heparinised saline (100 units ml^{-1}).

Biguanide challenge tests Subjects arrived at the laboratory after an overnight fast and an indwelling intravenous cannula was inserted into a forearm vein. The half-life of lactate being taken as 9 min (Phillips *et al.*, 1977), a 30 min rest period was judged sufficient to allow plasma lactate to return to basal level. Blood samples (20 ml) were then drawn, without stasis, before and at half hourly intervals for 4 h after a single oral dose of phenformin (two 25 mg tablets; Dibotin, Winthrop) or metformin (1 g; Glucophage, Lipha), swallowed with a glassful of water. Samples were divided for measurement of plasma lactate and blood pyruvate and glucose, and an aliquot was retained for measurement of plasma biguanide concentration. The cannula was kept patent as in the exercise test. Subjects were allowed gentle exercise during the study period.

Assays The aliquots of urine were thawed, shaken and centrifuged at 5000 rev min^{-1} for 3 min; the concentrations of phenformin and 4-hydroxy-phenformin were determined in the clear supernatant, using the h.p.l.c. method of Oates *et al.* (1980). From these data we derived a metabolic ratio (MR), defined as the urinary concentration of unchanged drug divided by that of metabolite.

Plasma lactate and blood pyruvate were assayed using standard kits (Boehringer Mannheim Ltd, BCL). Blood for lactate assay was collected into fluoride-EDTA, and for pyruvate into ice-cold molar perchloric acid. For both assays the samples were centrifuged at 3000 rev min^{-1} for 5 min, and the clear supernatant assayed within 3 h. Blood for the glucose assay was collected into citrate-oxalate tubes and glucose concentrations determined by the method of Trinder (1969). Acid-base determinations were carried out using a pH/blood gas analyser (Instrumentation Laboratory 413).

Statistical methods Change of concentration with time in the challenge studies was assessed

within groups by relating the mean values at each time point to the confidence interval of the overall mean concentration. Differences between groups of subjects were assessed using either Student's *t*-test, or the Mann-Whitney *U* test, depending upon the distribution of the data.

Lactate generated as a result of the exercise test was determined by calculating the area enclosed between the concentration-time curve and a line joining the first and last points. Lactate undergoes continuous re-cycling and the decay of an endogenous lactate load cannot, unlike the decay in concentration of a drug, be taken solely to reflect elimination — to do so would require assumptions of the nature of the processes involved. However, the concentration (C_t) does show an exponential decay towards the final value (C_f) and a semi-logarithmic plot of ($C_t - C_f + 10^{-2}$) against time (the 10^{-2} correction avoids a final value of minus infinity) is linear; the slope of the regression line reflects endogenous lactate clearance, and the intercept the peak concentration achieved.

Results

Oxidation phenotyping

The population studies comprised 103 Type II diabetics (62 men) aged 29–87 (mean 61) years.

The mean (s.d.) blood glucose concentration, measured about 2 h after food on the day of recruitment, was 9.8 (4.1) mmol l⁻¹ and the proportion of glycosylated haemoglobin (HbA₁) was 10.1 (2.5)%. Thirty-nine patients were receiving regular drug treatment for conditions other than diabetes (most commonly hypertension).

Figure 1 shows the frequency distribution of MR. Fourteen subjects (nine men) were identified as poor metabolisers (mean age 59 years; range 44–80); six of these had no detectable metabolite in their urine, while in the others, MR ranged from 22.6 to 336. The percentage (mean $\pm$ s.d.) of the original dose recovered in the urine showed marked variation in both extensive ($30.3 \pm 13.6\%$) and poor ($31.4 \pm 13.5\%$) metabolisers. There was no relationship between oxidiser phenotype and diabetes control. The mean (s.d.) glucose concentrations were 10 (5.1) and 9.7 (3.9) mmol l⁻¹ and the proportions of HbA₁ were 11.2 (2.6) and 10 (2.5)% for the poor and extensive metabolisers respectively. The frequency of the poor metaboliser allele was calculated (see Emery, 1976) as 0.369 ± 0.09 (s.e. mean).

Exercise test

Twelve subjects (six pairs) were studied; all were men aged 28 to 63 (mean 53) years. Three of the non-diabetic controls were the same age as their

Figure 1 Frequency distribution of metabolic ratio in 103 Type II diabetics.

diabetic pair-partners and three were 1 year younger. The mean (s.d.) body weights of the diabetic and control groups were 76.2 (10.5) and 76.2 (5.7) kg respectively. Mean (s.d.) blood glucose concentration of the diabetics at their immediate past clinic appointment was 6.2 (3.4) mmol l⁻¹. The exercise was well tolerated by all the men. Mean heart rates during the last minute of exercise varied from 125–142 beats min⁻¹ and did not differ significantly between diabetics and controls. There were no significant changes in pH, P_{CO}₂, or in standard bicarbonate during the sampling period and no differences between the two groups of subjects. Calculation of lactate generated during the test, the peak concentration achieved, and the endogenous lactate clearance (Table 1) revealed no significant differences between diabetics and non-diabetics.

Biguanide challenge

The response to a single oral dose of phenformin was assessed in twenty diabetics (ten extensive and ten poor metabolisers) and in seven non-diabetic controls, matched for age, sex and phenotype with seven of the extensive metabolisers. The response to a single oral dose of metformin was assessed in ten diabetics (five extensive and five poor metabolisers of phenformin.).

Table 2 shows the mean age and sex ratio of the groups. There was no significant difference (paired *t*-test) in fasting blood glucose between controls and their diabetic pair-partners. Both drugs were well tolerated and none of the subjects experienced any gastro-intestinal irritation, despite the empty stomach. However, one diabetic extensive metaboliser was forced to withdraw from the study two hours after taking phenformin, as a result of hypoglycaemia.

Figures 2 and 3 show the variation with time of plasma lactate, blood pyruvate, blood glucose and the lactate/pyruvate ratio for the two diabetic phenotypes, and for the non-diabetic controls,

after phenformin challenge. No significant change occurred in any group, although blood glucose tended to fall with time in all three diabetic groups. Within- and between-group variations in plasma lactate were assessed both before and after subtraction of the resting concentrations. Comparison of the raw data showed that the non-diabetics had significantly lower plasma lactate concentrations before and at 30 min after the dose ($P < 0.05$); subtraction of the resting concentration eliminated these differences. The areas under the plasma lactate curves did not differ between any of the groups, but the lactate-pyruvate ratios were significantly higher ($P < 0.05$) in the diabetics than in the non-diabetics before the dose, and at all time points from 150 min onwards. Three diabetics who took phenformin (two extensive, and one poor metaboliser) showed a marked rise in lactate concentration during the experiment (after 90, 210 and 240 min, increasing by a maximum of 130, 58 and 43% respectively). The rises did not correlate with age, degree of obesity, or diagnosis other than diabetes mellitus. In spite of our best endeavours we were unable to measure phenformin in plasma.

Figure 4 shows the results of the metformin challenge, in which no significant changes occurred in lactate, pyruvate or glucose concentrations. In both extensive and poor metabolisers of phenformin, lactate/pyruvate ratio differed significantly ($P < 0.05$) from the pre-dose estimation only after 240 min. The mean ratio was significantly lower ($P < 0.05$) in poor metabolisers than in extensive metabolisers at 60, 90 and 210 min after the dose.

Discussion

Population study

In their investigation of non-diabetics, Oates *et al.* (1982) observed an antimode (MR=20) in

Table 1 Indices (mean $\pm$ s.d.) derived from rise and fall of lactate concentration with time after ergometer exercise. The area-under-the-curve (AUC) above the baseline is an index of lactate generated; the slope of the decline phase is an index of endogenous lactate clearance; the intercept in the vertical axis (obtained by back extrapolation of the slope) is an index of the peak lactate concentration

Subjects	n	AUC (mmol min)	Slope	Intercept (mmol)
Diabetics	6	91.4 ± 23.2	0.025 ± 0.002	0.74 ± 0.10
Non-diabetics	6	67.1 ± 15.0	0.027 ± 0.003	0.57 ± 0.11

Figure 2 Plasma lactate, blood pyruvate, blood glucose and lactate:pyruvate ratio after single dose challenge with phenformin (50 mg) in 20 diabetic subjects, 10 poor metabolisers (○) and 10 extensive metabolisers (●). Values shown are mean $\pm$ s.e. mean.

Table 2 Age (mean and range) and sex ratio of groups undergoing biguanide challenges

Challenge	n	Age mean (range)	Sex ratio M:F
Phenoformin			
Diabetic E.M.	10	59 (39-74)	8:2
Diabetic P.M.	10	58 (44-80)	6:4
Non-diabetic E.M.	7	62 (55-74)	6:1
Metformin			
Diabetic E.M.	5	60 (56-63)	5:0
Diabetic P.M.	5	55 (48-69)	4:1

the distribution of metabolic ratio. The population data obtained in this study for $MR < 20$ were found to fit a probability distribution of form $P = e^{-\lambda x}$ where λ is the reciprocal of the mean of all values below 20. The fitted curve did not differ significantly from that observed and 99% of its area lay below $MR=20$. From this, one may conclude that the antimodes of the two distributions do not differ significantly. Moreover, the frequency of the poor metaboliser allele in the diabetic population (0.369) does not differ significantly from that frequency (0.303) observed previously in non-diabetics ($P < 0.05$).

Figure 3 Plasma lactate, blood pyruvate, blood glucose and lactate:pyruvate ratio after single dose challenge with phenformin (50 mg) in seven non-diabetics (Δ) and seven diabetics (▲) (all extensive metabolisers). Values shown are mean $\pm$ s.e. mean.

Two independent conclusions may be drawn from this comparison of frequency distributions: first, there is no generalised impairment of hepatic oxidation in Type II diabetics; and second, the alleles for Type II diabetes and impaired phenformin metabolism are not linked.

Exercise study

There appeared to be no difference between Type II diabetics and non-diabetics in the lactate response to ergometer exercise, although variances in the two groups were large. Our aim was

to assess whether diabetes mellitus impaired the response to an increase in endogenous lactate, independent of the effect of phenformin. Exercise was used merely as a physiological way of generating lactate. Previous investigators have examined the interacting effects of phenformin and a lactate load. We did not attempt to repeat these studies, as our main interest lay in the response to phenformin in different oxidiser phenotypes, but the earlier work is of interest, nevertheless.

For instance, Shepardson *et al.* (1962) found that phenformin had no effect upon the lactate

Figure 4 Plasma lactate, blood pyruvate, blood glucose and lactate:pyruvate ratio after single dose challenge with metformin (1g) in 10 diabetic subjects, five poor metabolisers ($\circ$) and five extensive metabolisers ($\bullet$) of phenformin. Values shown are mean $\pm$ s.e. mean.

response to exercise in six individuals (including two diabetics of unspecified type); and Güttler *et al.* (1963) showed that steady-state lactate concentration during exercise, in non-diabetics and in Type I (insulin-dependent) diabetics deprived of their morning insulin, differed neither before nor after 7 days treatment with phenformin. However, if Type II diabetics are exposed to bicycle ergometer exercise while breathing 13% oxygen, before and after treatment with phenformin (400 mg over 36 h), a higher basal lactate concentration and a slower decline of lactate

after the end of exercise are observed following treatment (Hermann *et al.*, 1973).

Other investigators have studied the response to an exogenous lactate load. It is known that lactate elimination following an intravenous infusion of lactate is slower in Type II diabetics than in non-diabetics (Doar & Cramp, 1970). In non-diabetics, daily treatment for 2 weeks with either phenformin (1.5 mg kg^{-1}) or metformin (10 mg kg^{-1}) retards lactate elimination following a lactate infusion, the effect being greater after phenformin (Phillips *et al.*, 1976). Craig

et al. (1960) gave a lactate infusion to eight diabetics, half of whom were Type I, before and after regular treatment with phenformin (100–175 mg daily) for several weeks; after treatment, lactate concentration rose to a higher peak and declined more slowly. Cesar *et al.* (1980) compared the effects of phenformin in non-diabetics and in patients with chemical diabetes mellitus, infusing lactate before and after treatment (phenformin 100 mg daily for 60 days); the drug appeared to retard lactate elimination in the non-diabetics, and to increase endogenous lactate production in the diabetics, whose fasting lactate concentrations were higher.

These studies suggest that, whereas diabetes itself does not influence the ability to handle an endogenous lactate load, exogenous lactate is removed more slowly in diabetics. Lactate elimination is retarded to an even greater extent after treatment with biguanides, which also increase the production of endogenous lactate.

Biguanide challenge

We were surprised to find no difference in the response of our diabetic poor and extensive metabolisers to a single dose of phenformin, in view of the difference observed previously by Oates *et al.* (1983), and sought explanations for this.

When we examined the profile of plasma lactate concentration in our own diabetic and Oates's non-diabetic poor metabolisers, we found a striking consistency in the way in which lactate concentration declined, irrespective of whether or not there was an initial rise. A rise in lactate concentration due to phenformin might not be apparent in diabetics, if the resting concentration (before phenformin was given) were already raised in these subjects because of diabetes mellitus *per se*. However, resting plasma lactate did not differ significantly between our diabetic poor metabolisers and the non-diabetic poor metabolisers of Oates *et al.* (1983). The difference between the basal lactate/pyruvate ratios of the diabetics and their controls is suggestive of a diabetes-linked disturbance of redox status, but the small sample size prevents this argument being advanced with any confidence. Doar *et al.* (1970) suggested that obesity was more important than diabetes mellitus in determining the resting concentrations of both lactate and pyruvate, but we found no correlation between these measurements and either body weight or ponderal index.

Our subjects all received their dose of phenformin (or metformin) on an empty stomach whereas the non-diabetics (Oates *et al.*, 1983) had a light breakfast before the challenge. Evidence

on the relationship between food and the effect of biguanides upon resting lactate concentration is conflicting. Varma *et al.* (1972) found elevated fasting plasma lactate concentrations in diabetics after treatment with phenformin, whereas Jaillard (1974) observed no effect after either phenformin or metformin. Natrass *et al.* (1977) compared metabolic rhythms in diabetics during treatment with glibenclamide, phenformin or metformin in a cross-over study and found that, although mean fasting lactate did not change, mean concentrations over 24 h (where food was allowed and gentle exercise encouraged) were elevated by both biguanides, especially phenformin, but not by glibenclamide. We cannot rule out the possibility that, had our diabetics been fed prior to receiving phenformin, our findings might have been different.

It is possible that the plasma concentration of phenformin produced by a single oral dose of 50 mg was insufficient to induce detectable metabolic abnormalities in diabetics of either phenotype. Although we were unable to assay phenformin in plasma, there seems no obvious reason why the concentration produced should be lower than that achieved by the same dose in non-diabetics; indeed, since our subjects were fasted, a higher peak concentration would have been anticipated.

In the absence of a plasma lactate response to phenformin, one would not have expected one following metformin; the pharmacological activity of the biguanides is a function solely of their lipophilicity (Schafer, 1974, 1976) and the doses of metformin and phenformin given here were judged to be equipotent.

In conclusion, there is ample published evidence demonstrating that phenformin increases the production of endogenous lactate and retards its removal in patients with diabetes mellitus, and that accumulation of lactate is a risk of treatment. Our data provide no reason to suppose that a relative inability to metabolise phenformin adds substantially to this risk, in patients with normal cardiac, hepatic and renal function.

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