Diabetologia 9, 47–49 (1973)


## SHORT COMMUNICATIONS

# A Differential Action of Phenformin in Normal and Diabetic Rat Livers

J.J. Connon

Department of Medicine, The Queen's University of Belfast, N. Ireland

Received: September 19, 1972, accepted: October 26, 1972

*Summary.* Phenformin inhibited gluconeogenesis by livers from both normal and diabetic rats. However, the concentration of phenformin which inhibited gluconeogenesis by the diabetic livers was not effective in normal livers. It is suggested that an action which is differentially effective in the diabetic state is likely to be clinically relevant.

*Key words*: Phenformin, gluconeogenesis, inhibition, diabetic rat livers.

## Introduction

Although phenformin has been used therapeutically for 14 years its mode of action is not yet fully understood. It is claimed to increase glucose uptake in peripheral tissues, possibly through the Pasteur effect (Unger *et al.*, 1957), to decrease intestinal absorption of glucose (Czyzyk, 1968) and to inhibit hepatic gluconeogenesis (Altschuld and Kruger, 1967; Haeckel and Haeckel, 1970). The inhibitory effect of phenformin on gluconeogenesis by guinea-pig livers (Altschuld and Kruger, 1968) has also been shown in rat livers (Toews *et al.*, 1970) and the site of inhibition localised to 3-phosphoglycerate kinase. Unlike the sulphonylureas, the biguanides are not effective hypoglycaemic agents in normal subjects. Therefore in the present study a differential effect of phenformin on gluconeogenesis by normal and diabetic rat livers was sought.

## Methods

Overnight fasted Wistar rats (180–200 g) were used in most experiments (in one experiment the rats were fasted for 48 h). The perfusion technique was modified from Miller *et al.*, (1951). The rats were anaesthetised with intraperitoneal Nembutal, the abdomen opened by a midline incision and the portal vein cannulated with a polythene catheter connected to a source of oxygenated Krebs-Ringer bicarbonate buffer, to ensure the least possible anoxia during further dissection. After the liver had been dissected free of neighbouring structures, the cannula was clamped and cut about one inch from the porta hepatis, and the liver transferred to the perfusion apparatus. This consisted of a multi-bulb oxygenator which delivered the medium (Krebs-Ringer bicarbonate buffer containing 4 g% serum albumin) into the portal vein with a hydrostatic pressure of 15 cm $H_2O$. The perfusate dripped from the hepatic veins into a collecting vessel and was then pumped by a Watson-Marlow pump back to the oxygenator. The apparatus was kept in an enclosed cabinet at 37°C and gassed with a mixture of 95% $O_2$: 5% $CO_2$.

Perfusate samples were taken at 15-min intervals and glucose measured by the hexokinase/glucose-6-phosphate dehydrogenase method.

Table 1. *The effect of phenformin on gluconeogenesis from 10 mM pyruvate by normal rat livers*

| Phenformin concentration | Glucose production (μ moles/g/45 min ± SEM) | |
|---|---|---|
| 0 | 28±3 | (8) |
| 12 mg/100 ml | 23±2 | (7) |
| 40 mg/100 ml | 1±0.1 | (6) |

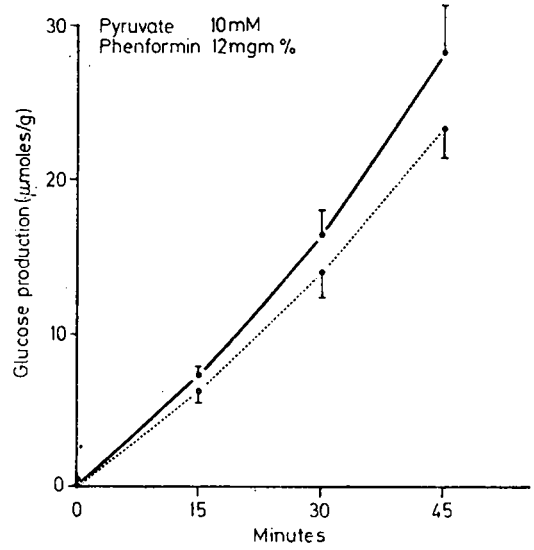


Fig. 1. Effect of phenformin on gluconeogenesis by normal livers

The rats were made diabetic by intraperitoneal injection of streptozotocin 12.5 mg/100 g body weight. Their subsequent blood sugars ranged from 200–300 mg% and they were used for perfusion experiments 3–10 days after the injection.

The gluconeogenic substrate in all experiments was 10 mM pyruvate. Phenformin was kindly donated by Bayer Laboratories and since the inhibitory effect of phenformin took about 30 min to develop (Toews *et al.*, 1970), all livers were pre-perfused for this period before the addition of substrate.

## Results

Phenformin 40 mg% completely inhibited gluconeogenesis by normal rat livers. When the concentration of phenformin was reduced step-wise, there was a corresponding increase in gluconeogenesis until at 12 mg% phenformin, glucose production was not significantly different from the control livers with phenformin (Table 1, Fig. 1).

Gluconeogenesis by livers from diabetic rats 3 days after streptozotocin was also completely inhibited by 40 mg% phenformin, but when the dose of phenformin was reduced to 12 mg%, the inhibition persisted (Table 2, Fig. 2).

Table 2. *The effect of phenformin on gluconeogenesis by diabetic and 48-h fasted rat livers*

| Phenformin concentration | Glucose production (μ moles/g/45 min + SEM) Diabetic | | Fasted | |
|---|---|---|---|---|
| 0 | 32±1.3 | (9) | 29±2.8 | (4) |
| 12 mg/100 ml | 5±1.2 | (10) | 6±1.2 | (3) |

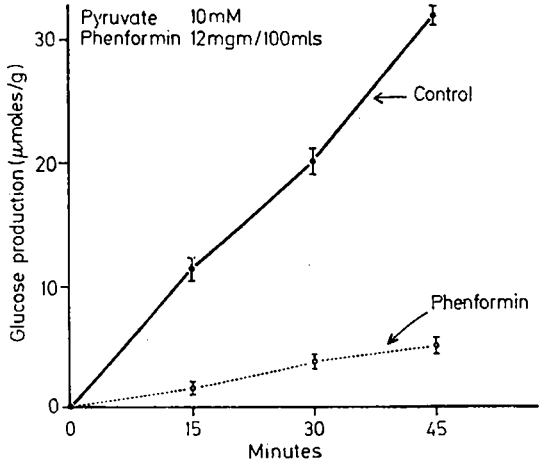


Fig. 2. Effect of phenformin on gluconeogenesis by diabetic livers

The rate of gluconeogenesis by livers from diabetic rats 3 days after streptozotocin was not significantly different from those of normal rats, but 10 days after diabetes was induced, the rate had almost doubled (Fig. 3).

When 10 mg% phenformin was added to livers from normal rats fasted for 48 h, gluconeogenesis was virtually completely inhibited (Table 2).

## Discussion

These results indicate that the selective hypoglycaemic action of phenformin in diabetic subjects is paralleled by its inhibitory effect on gluconeogenesis by livers from diabetic rats. Livers taken from starved rats, whose metabolic state is not dissimilar from diabetes, also show inhibition of gluconeogenesis in the presence of much smaller concentrations of phenformin than are effective in normal rats. A common denominator in both diabetes and starvation is a reduction in circulating insulin. Thus it may be that in some way insulin protects the liver from the effects of phenformin; against this is the fact that insulin itself normally restrains hepatic gluconeogenesis. It is intended in future experiments to add insulin to the perfusate to see whether the metabolic changes produced by phenformin can be reversed.

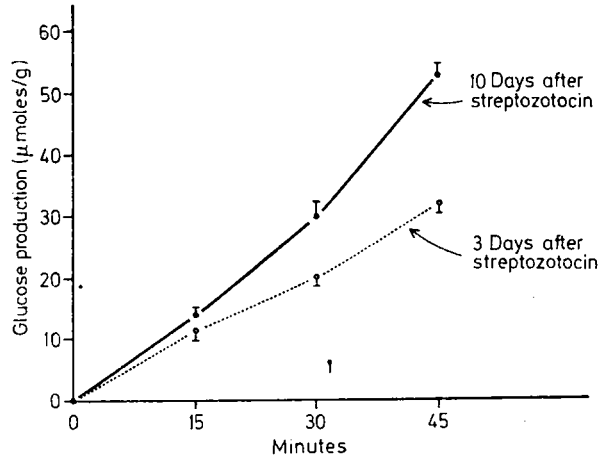


Fig. 3. Gluconeogenesis by livers from diabetic rats

The phenformin-induced inhibition of gluconeogenesis in the perfused liver (Altschuld and Kruger, 1968; Toews *et al.*, 1970) is at variance with measurements of gluconeogenesis in normal and diabetic humans treated with phenformin (Searle and Cavalieri, 1968, Kreisberg *et al.*, 1970). These workers, using labelled $C^{14}$, showed that both glucose turnover and Cori cycle activity were increased by phenformin. On the other hand, Lyngsoe (1970) has shown that there is decreased gluconeogenesis in normal starved subjects treated with phenformin.

The concentration of phenformin used in the current experiments is within the range used by other workers in *in vitro* experiments (Altschuld and Kruger, 1968; Haeckel and Haeckel, 1970). Although therapeutic drug levels of phenformin are not as high as

those used *in vitro*, it must be remembered that rats are notably resistant to the action of phenformin (Altschuld and Kruger, 1968). In addition, the degree of inhibition of gluconeogenesis in the present experiment, if transposed to the *in vivo* situation, would produce a much greater reduction of the blood sugar level than is produced by phenformin therapy.

It is unlikely that the hypoglycaemic action of phenformin is due to a single mechanism. In view of the many different effects that have been demonstrated, a multifactorial action is more plausible. However, the different effects may not be of equal importance in lowering the blood sugar and a mechanism which is more effective in the diabetic state may have greater clinical significance.

## References

Altschuld, R.A., Kruger, F.A.: Inhibition of hepatic gluconeogenesis in guinea-pig liver by phenformin. Ann. N.Y. Acad. Sci. **148**, 612–623 (1968).

Czyzyk, A., Tawecki, J., Sadowski, J., Ponikowska, I., Szczepanik, Z.: Effect of biguanides on intestinal absorption of glucose. Diabetes **17**, 492–502 (1968).

Haeckel, R., Haeckel, H.: On the inhibition of gluconeogenesis by biguanides in the perfused guinea pig liver. Konferenz der Gesellschaft für Biologische Chemie Bd. 351 (1970).

Hems, R., Ross, B.D., Berry, M.N., Krebs, H.A.: Gluconeogenesis in the perfused rat liver. Biochem. J. **101**, 284–292 (1966).

Kreisberg, R.A., Pennington, L.F., Boshell, B.R.: Lactate turnover and gluconeogenesis in obesity. Effect of phenformin. Diabetes **19**, 64–69 (1970).

Lyngsoe, J., Trap-Jensen, J., Bitsch, V.: Metabolic effects of phenformin in starving normal subjects. Diabetologia **6**, 551 (1970).

Miller, L.L., Bly, C.G., Watson, M.L., Bale, W.F.: The dominant role of the liver in plasma protein synthesis. J. exp. Med. **94**, 431–453 (1951).

Searle, G.L., Cavalieri, R.R.: Glucose kinetics before and after glucose in the human subject. Ann. N.Y. Acad. Sci. **148**, 734–742 (1968).

Toews, C.J., Kyner, J.L., Connon, J.J.: The effect of phenformin on gluconeogenesis in isolated perfused rat liver. Diabetes **19**, 368 (1970).

Ungar, G., Freedman, L., Shapiro, S.L.: Pharmacological studies of a new oral hypoglycaemic drug. Proc. Soc. exp. Biol. Med. **95**, 190–194 (1957).

J.J. Connon, M.D.
The Queens University of Belfast
Institute of Clinical Science
Grosvenor Road
Belfast
N. Ireland