

EFFECT OF INSULIN AND DIET ON THE STEADY STATE CONCENTRATIONS OF INTERMEDIATES OF THE PENTOSE PHOSPHATE PATHWAY OF GLUCOSE METABOLISM IN LIVER

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1. Introduction

Measurement of the steady state concentrations of intermediates of the pentose phosphate pathway have been made in conditions where marked changes are known to occur in the pattern of glucose metabolism and where it has been established that alterations occur in both the oxidative and non-oxidative reactions of this pathway. The conditions chosen were alloxan-diabetes with and without insulin treatment, starvation and starvation followed by refeeding a high carbohydrate diet or a high fat diet. In alloxan-diabetes, the most striking change was a marked increase in sedoheptulose-7-phosphate. Changes in 6-phosphogluconate and pentose phosphate suggested an inhibition of 6-phosphogluconate dehydrogenase. Insulin treatment for 3 days partially reversed this latter effect but the sedoheptulose-7-phosphate concentration remained high. Starvation and starvation followed by refeeding a high fat diet which decreases the flux of glucose through the pentose phosphate pathway, were found to cause a lowering of metabolite levels. Refeeding high carbohydrate diet restored the pentose phosphate level to control value, the sedoheptulose-7-phosphate remained low in this condition. These results suggest control points at both the oxidative reactions of the pentose phosphate cycle and at transketolase.

2. Materials and methods

The preparation of the enzymes and estimation of

metabolites were as described previously [1]. We are indebted to Dr. B. L. Horecker for the transaldolase used in these experiments. The metabolites were determined using the standard rapid freezing technique with liquid nitrogen [2]. The metabolites were determined on perchloric acid extracts, neutralised and concentrated by freeze-drying; specific enzymic methods were used.

Starvation and refeeding high carbohydrate and high fat diets: Adult male albino rats of 170-190 g group were used. The control group received *ad libitum* the standard laboratory rat diet 41B containing 65% carbohydrate, 16% protein and 3% fat, the remaining animals were starved for 72 hours and refed a high carbohydrate diet of white bread (83% carbohydrate, 14% protein, 3% fat) for 3 days or a high fat diet (60% fat, 1% carbohydrate, 30% protein and 9% cellulose filler) for 3 days before killing the rats for estimation of metabolites.

Alloxan-diabetes and treatment with insulin: Adult male albino rats of 200 g body weight were used. Diabetes was induced by subcutaneous injection of alloxan (20 mg/100 g body weight) into rats previously starved for 48 hours. These rats were given 2 units of protamine zinc insulin/day for 5 days, this was then withdrawn and the alloxan-diabetic rats were kept for 3 weeks on the stock diet. The blood sugar was in excess of 400 mg/100 ml blood. At the end of this period one group was used without further treatment and the other given subcutaneously 2 units of protamine zinc insulin daily for 3 days before killing the rats for estimation of metabolites.

3. Results and discussion

The results of these experiments are summarized in table 1. In alloxan diabetic rats there was a tendency for 6-phosphogluconate to be increased coupled with a fall in the pentose phosphate concentration suggesting a relative decrease in 6-phosphogluconate dehydrogenase activity. This is reflected in the quotient 6-phosphogluconate/pentose phosphate which more than doubles in the diabetic rats. At the same time there is also a fall in the quotient glucose-6-phosphate/6-phosphogluconate in the alloxan-diabetic group to approximately one third of the control value. This is probably related to the more marked decrease in 6-phosphogluconate dehydrogenase than glucose-6-phosphate dehydrogenase in diabetic rat liver [3].

The most striking change in the alloxan-diabetic rats is the increase in sedoheptulose-7-phosphate to almost twice the control value. This suggests control at transketolase but at present it is difficult to interpret this change since measurements have shown a decrease in transketolase to approximately 70% of control and an unchanged transaldolase in this condition [4,5], alterations which might be expected to lead to a fall in sedoheptulose-7-phosphate. Another factor known to be concerned in the control of sedoheptulose-7-phosphate in liver and in ascites tumour cells is the concentration of glyceraldehyde-3-phosphate [1,6] but no significant differences were found in the concentration of this metabolite in liver from alloxan-diabetic rats (table 1).

Treatment of alloxan-diabetic rats with insulin for 3 days did not greatly alter the metabolite pattern compared with the untreated group, however, the pentose phosphate concentration was somewhat increased and there was a small decrease in the 6-phosphogluconate/pentose phosphate quotient. Both sedoheptulose-7-phosphate and 6-phosphogluconate were increased above the normal control value. In this context, the three-fold increase in glucose-6-phosphate dehydrogenase and two-fold increase in transketolase in alloxan-diabetic rats treated with insulin may be of importance [4].

Previous work using (1-¹⁴C)glucose and (6-¹⁴C)glucose and measuring the quotient of the incorporation of these sugars into ¹⁴CO₂, while difficult to interpret quantitatively, did suggest that there was a relative in-

Table 1
Effect of insulin and diet on the steady state concentrations of intermediates of the pentose phosphate pathway of glucose metabolism in liver

Condition	Control	Starved	Starved + high carbohydrate	Starved + high fat	Alloxan diabetes	Alloxan diabetes + insulin
Observations	18	6	12	6	5	5
Glucose-6-phosphate	178.0 ± 13.0	50.7 ± 2.6	109.0 ± 10.0	59.0 ± 3.4	123.0 ± 17.0	141.0 ± 8.6
6-phosphogluconate	18.8 ± 2.3	19.8 ± 0.7	24.9 ± 5.0	6.9 ± 0.6	32.8 ± 7.6	34.5 ± 6.8
Pentose phosphate	429.0 ± 7.2	236.0 ± 12.0	456.0 ± 38.0	165.0 ± 9.0	345.0 ± 60.0	435.0 ± 33.0
Sedoheptulose-7-phosphate	25.7 ± 1.4	16.7 ± 2.3	16.1 ± 1.8	18.5 ± 1.9	46.1 ± 7.7	40.4 ± 6.6
Glyceraldehyde-3-phosphate	10.6 ± 1.1	16.7 ± 0.8	8.3 ± 1.3	15.5 ± 0.4	10.0 ± 2.8	10.8 ± 2.5
Glucose-6-P/6-phosphogluconate	11.8 ± 1.41	2.56 ± 0.07	5.06 ± 0.74	8.72 ± 0.79	4.10 ± 0.63	4.75 ± 0.97
6-phosphogluconate/pentose phosphate	0.043 ± 0.005	0.084 ± 0.003	0.052 ± 0.007	0.042 ± 0.001	0.105 ± 0.025	0.082 ± 0.018

Results are expressed as nanomoles/g liver and are given as mean values ± S.E.M. Treatment of animals is as described in the Methods section. Erythrose-4-phosphate was too low to be measured.

crease in the metabolism of glucose by way of the pentose phosphate pathway in both alloxan-diabetic rats and alloxan-diabetic rats treated with insulin [3]; the present results are in keeping with this view.

Starvation and starvation followed by refeeding a high fat diet both resulted in decreased concentrations of key metabolites of the pentose phosphate pathway. Pentose phosphate and sedoheptulose-7-phosphate were both decreased after 72 hours starvation, again control at 6-phosphogluconate dehydrogenase is suggested by the increase in 6-phosphogluconate/pentose phosphate quotient which is increased by a factor of two.

There is a very marked fall in 6-phosphogluconate in starved rats refed a high fat diet compared with the starved group. Examination of the activity of glucose-6-phosphate dehydrogenase and of 6-phosphogluconate dehydrogenase in the two groups shows that while the latter enzyme was not altered, the glucose-6-phosphate dehydrogenase was increased from 0.89 ± 0.09 units in the starved animals to 1.46 ± 0.08 in rats refed a high fat diet. Furthermore, the glucose-6-phosphate concentration of the two groups of rats was the same and although low, $50\text{m } \mu\text{moles/g liver}$, was nevertheless still some four-fold higher than the $K_m\text{-G-6-P}$ for this enzyme in liver which is $1.3 \times 10^{-5}\text{M}$ [7]. This suggests the possibility that glucose-6-phosphate dehydrogenase is inhibited by a metabolite such as long chain acyl CoA to which this enzyme is known to be particularly sensitive [8]. A further possibility is control by the concentration of NADP^+ which may become more rate-limiting in a condition of fat oxidation as opposed to fat synthesis.

Starvation followed by refeeding a high carbohydrate diet raised the concentration of glucose-6-phosphate and pentose phosphate but did not alter neither 6-phosphogluconate nor sedoheptulose-7-phosphate. The control at 6-phosphogluconate dehydrogenase is again shown by the fall in the quotient 6-phosphogluconate/pentosephosphate which returned towards the control value.

The present results indicate that in addition to control of the pentose phosphate pathway at the oxidative reactions there is also a complex control at the transketolase reaction as indicated by the marked changes in sedoheptulose-7-phosphate in these different conditions. Kaufman, Brown, Passonneau and Lowry [9] from a study of metabolites of the pentose

phosphate pathway in brain in conditions of increased and decreased glucose utilisation have concluded that control is exerted at 6-phosphogluconate dehydrogenase. The present results are in accord with this view. The higher K_m of 6-phosphogluconate dehydrogenase for NADP^+ compared with glucose-6-phosphate dehydrogenase may be of importance in this context [7]. Another factor which may play a part in the control of 6-phosphogluconate dehydrogenase is the ratio 6-phosphogluconate: fructose diphosphate. Carter and Parr [10] have shown that fructose diphosphate is a competitive inhibitor of 6-phosphogluconate dehydrogenase and that when present in equimolar amounts, a 25% inhibition was found. In most of the present conditions studied the quotient 6-phosphogluconate/fructose diphosphate was approximately two, but in the case of starved rats refed a high fat diet this quotient is less than one (fructose diphosphate concentration of $8\text{m } \mu\text{moles/g liver}$) and this may also be a contributory factor to the low pentose phosphate concentration in liver in this condition.

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