Vol. 127, No. 1, 1985 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 28, 1985 Pages 136-142

Regulation of the Multiple Molecular Forms of Rat Liver Glucose 6-Phospnate Dehydrogenase by Insulin and Dietary Restriction[1]

Ralph N. Martins*, Gilbert B. Stokes§ and Colin L. Masters*

*Laboratory of Molecular and Applied Neuropathology
Neuromuscular Research Institute, Department of Pathology, and
§Department of Biochemistry, University of Western Australia,
Nedlands, Western Australia 6009

Received January 4, 1985

Insulin treatment of virgin female rats increased the hepatic activity of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase to levels 3.4 and 1.5 fold higher than controls. The increase in glucose 6-phosphate dehydrogenase activity was attributed to increased activity of all three dimer species. Thus dimer bands, 1, 2 and 3 of insulin-treated animals were 5, 3 and 2-fold higher respectively than controls.
The activity of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase decreased with fasting to 55% and 72% respectively of controls. The decrease in glucose 6-phosphate dehydrogenase activity reflected a lower activity of dimer bands 2 and 3 only, which were 62% and 39% of control activity respectively after three days fasting.
A shift towards band 1 was observed under both conditions of starvation as well as under conditions of insulin treatment.

The rat liver hexose monophosphate dehydrogenases, glucose 6-phosphate dehydrogenase (D-glucose 6-phosphate-$NADP^+$ oxidoreductase, EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (6-phospho-D-gluconate:$NADP^+$ oxidoreductase (decarboxylating), EC 1.1.1.44) have been demonstrated to change in activity under various metabolic and hormonal conditions (1,2,3,5,6). The male rat model has been used in most of the experimental studies on the nutritional and hormonal regulation of glucose 6-phosphate dehydrogenase activity and little information is available on the responses in the female rat where basal activity is about double that observed in male rat liver.

Rat liver glucose 6-phosphate dehydrogenase has been shown to occur as at least 6 different activity forms (2). It has not been established whether these molecular

[1]Supported in part by Grants from the National Health and Medical Research Council of Australia, and the Telethon Research Foundation.

*To whom correspondence should be addressed.

0006-291X/85 $1.50

forms are different gene products, though there is some evidence to suggest that they represent different molecular forms of the same enzyme protein (2, 3 and 5). However, Hori and Matsui (8) have shown under in vitro conditions that one particular form of glucose 6-phosphate dehydrogenase is selectively inhibited by the sex steroid dehydroepiandosterone.

In this paper we describe the response of specific molecular forms of hepatic glucose 6-phosphate dehydrogenase to insulin levels in the female rat that were raised by insulin administration or lowered by fasting.

MATERIALS AND METHODS

Materials. D-Glucose 6-phosphate, 6-phosphogluconate, $NADP^+$, Trizma base, nitroblue tetrazolium and phenazine methosulphate were purchased from Sigma (St. Louis, Mo). All other materials were of reagent grade and obtained locally.

Rats of the Wistar strain were obtained from the Animal Resources Centre of Western Australia. Fourteen week old virgin rats were used in all experiments. They were housed in an airconditioned room under a controlled 12h light (7.15 am - 7.15 pm) 12 h dark (7.15 pm - 7.15 am) cycle. All animals were fed ad libitum a commercial pelleted diet, (Milne Feeds Pty. Ltd., Perth, W.A.) containing 3-6% fat (60% polyunsaturated) and about 68% carbohydrate, for seven days prior to commencement of the experimental period.

Animal treatment and tissue preparation. Rats were divided into three groups at the commencement of experimentation. Groups A and B were fed ad libitum and group C was starved for 72h. Group A and C were injected subcutaneously with physiological saline every 12h. Group B was injected with protamine zinc insulin (3 Units/100g/12h). Animals were sacrificed between 10am and 12 noon. The liver was rapidly excised, weighed and homogenized in a cold Perspex homogenizer of the Potter-Elvehjem type with three volumes of 10mM Tris-HCl, buffer pH 7.4 containing 0.25M sucrose and 1mM EDTA. The homogenates were immediately centrifuged at 130000 x g for 60 min, and the high speed supernatant fraction was collected.

Assays of enzyme activities. Glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities were measured spectrophotometrically at 25°C according to the procedure of Rudack et al. (1). 6-phosphogluconate dehydrogenase was assayed in the presence of 0.6mM 6-phosphogluconate and 0.9mM $NADP^+$, pH 7.6. The combined activity of both dehydrogenases was measured in the presence of 0.6mM 6-phosphogluconate, 0.9mM $NADP^+$ and 2mM glucose 6-phosphate at pH 7.6. Glucose 6-phosphate dehydrogenase activity was estimated as the difference between the combined activity and the 6-phosphogluconate dehydrogenase activity. The amount of the high speed supernatant fraction assayed for enzyme activity corresponded to 5mg of liver. One unit of enzyme activity is that amount of enzyme which catalyses the reduction of 1 μmole of $NADP^+$ per minute under the conditions of the assay. Enzyme activity is expressed as U/g tissue wet wt.

Electrophoresis. Electrophoresis of the high-speed liver supernatant fractions was performed within 3h of preparation using a 1.5mm thick slab gel containing 10% (w/v) acrylamide and 0.26% (w/v) bisacrylamide polymerized in the presence of 0.017% (w/v) ammonium persulphate and 0.033% (v/v) tetramethylene diamine. A 7.5% acrylamide stacking gel was used and samples were applied directly in a 5mM Tris-HCl, pH 7.4 loading buffer containing 20% (w/v) sucrose and bromophenol blue as the tracking dye. The elctrophoresis buffer was 25 mM Tris, and 200 mM glycine, pH 8.3. $NADP^+$ (10 μM) was included in the cathode buffer. Samples were electrophoresed at a constant current of 15 mA/gel and then stained for activity in pH 8.0. 50 mM Tris-HCl, containing 0.58 mM glucose 6-phosphate, 0.13 mM $NADP^+$, 10 mM $MgCl_2$, 33 μM phenazine methosulphate and 0.1 mM nitroblue tetrazolium. The gel was incubated in this staining solution at 4°C for 16h. The reaction was then stopped by immersing the gel in 7% acetic acid, after which it was photographed and scanned using a GS 300 gel scanner (Hoeffer Scientific Instruments, San Francisco) (Figure 1).

RESULTS

In this study the activity of both hexose monophosphate dehydrogenases decreased in the liver to 55% and 72% of controls respectively when rats were starved for 72h ($P<.01$). The activity of glucose 6-phosphate dehydrogenase and 6-phosphogluconate

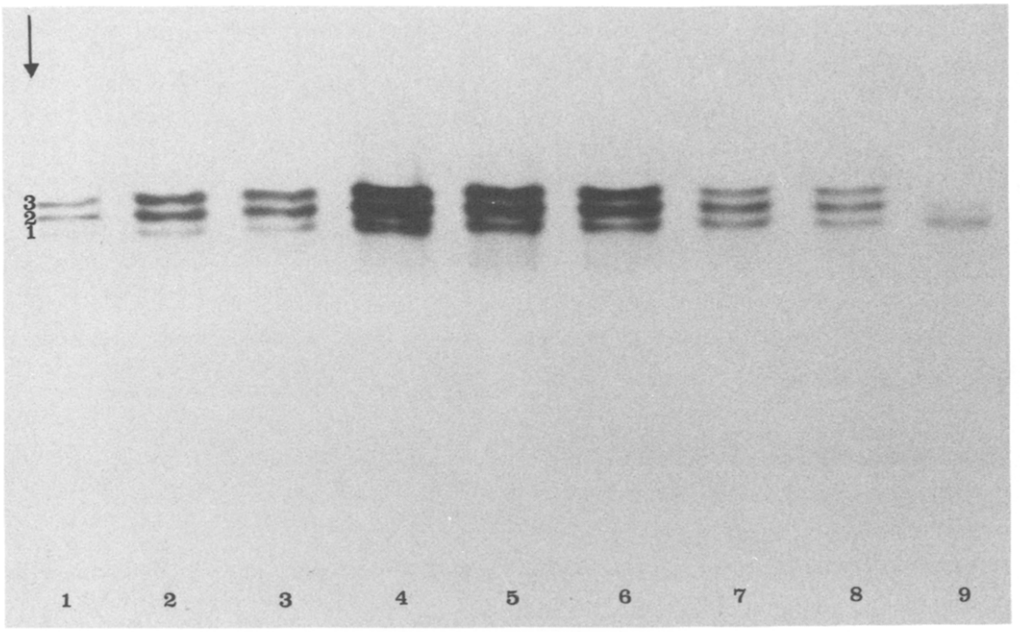

Fig. 1. Non-denaturing gel electrophoresis

Activity staining of rat liver glucose 6-phosphate dehydrogenase on a 7.5% polyacrylamide slab gel. Lanes, 1, 2, 3 contain samples from control animals. Lanes 4,5,6 contain samples from animals treated with insulin for 72h. Lanes 7,8,9 represent samples from rats fasted for 72h. An average of 750±50 µg total protein was loaded in each well. Electrophoretic conditions and enzyme activity staining are described in methods.

dehydrogenase increased in the insulin-treated group to 244% and 150% of control values respectively ($P < 0.01$) (Table 1). Non-denaturing gel electrophoresis of high speed supernatants demonstrated that total liver glucose 6-phosphate dehydrogenase activity (as determined densitometrically) increased to 258% of controls ($P<.01$). However, though starvation caused a decrease in total activity, this decrease was not significant ($P > 0.05$) (Table 2A). The insulin-promoted increase in liver glucose 6-phosphate dehydrogenase activity was due to increases in activity of all three dimer activity bands. However, densitometric scanning of the enzyme bands from starved animals revealed that none of the bands, 1, 2 or 3 was significantly altered when compared with control values. To determine whether the relative ratios of the bands 1, 2 and 3 were altered by

TABLE 1. Changes in hepatic G6PD activity under different experimental conditions

Treatment	Enzyme activity (Units/g tissue wet wt.)	
	6PGD	G6PD
Control	$6.60\pm.35^{a}$	$3.40\pm.39^{d}$
Fasted	4.76 ± 0.3^{b}	1.87 ± 0.17^{e}
Insulin	9.98 ± 1.07^{c}	8.3 ± 1.45^{f}

Each value represented the mean $\pm$ SE of 8 animals. The differences between (a) and (b), (a) and (c), (d) and (e), and (d) and (f) are all statistically significant ($P<0.01$).

either insulin treatment or starvation, we have expressed band activities as percentages of total activity. Only band 3 from insulin-treated animals exhibited a significant decrease (Table 2B).

DISCUSSION

In this paper, we chose to restrict our study of glucose 6-phosphate dehydrogenase to the dimer molecular forms. Higher aggregates were observed on the gels, but their

TABLE 2. A. Quantitation of the molecular forms of liver G6PD activity as measured densitometrically

Band activity in mg.	Control	Insulin Treated	Fasted
Band 1	14 ± 4.6^{a}	68 ± 24^{b}	13.7 ± 14^{c}
Band 2	47 ± 15.7^{d}	130 ± 22^{e}	29 ± 12.7^{f}
Band 3	56 ± 20^{g}	105 ± 12.7^{h}	22 ± 7^{i}

B. Comparison of liver G6PD molecular forms as percentage of total activity

Band activity % of total	Control	Insulin Treated	Fasted
Band 1	12.2 ± 2.1^{j}	22 ± 7.3^{k}	16.7 ± 14.7^{l}
Band 2	40 ± 1^{m}	42.6 ± 4^{n}	46.5 ± 4^{o}
Band 3	47.6 ± 1.5^{p}	35 ± 7^{q}	36.8 ± 10^{r}

Each value represents the mean $\pm$ S.D. of 3 rats. (a) is significantly different from (b) ($P<.05$) but not from (c); (d) is signficantly different from (e) ($P<.01$) but not from (f) ($P>.05$); (g) is significantly different from (h) ($P<.05$) but not from (i) ($P>.05$); (j) is not significantly different from (k) or (l) ($P>.05$). (m) is not signficantly different from (n) or (o) ($P>.05$). (p) is signficantly different from (q) ($P<.05$) but not from (r) ($P>.05$).

activity never exceeded 5% of the total activity, in accordance with earlier reports (2,3).

Our findings of decreased activity of the hexose monophosphate dehydrogenases following fasting and the increased activity following insulin treatment of the female rat is in agreement with previous reports for the male rat (1,4). In our electrophoresis experiments we always used similar amounts of sample protein to prevent any protein concentration-dependent shift in the glucose 6-phosphate dehydrogenase molecular forms that might occur. The results demonstrate that the insulin-promoted increase in glucose 6-phosphate dehydrogenase activity is due to an increase in all three dimer bands. The changes in the intensity of the glucose 6-phosphate dehydrogenase activity bands both in response to fasting and insulin treatment, are similar for all the three dimer bands. However, there does appear to be a decrease in band 3 and an increase in band 1 for both the insulin treated and the fasted animals, though only the change associated with band 3 of the insulin treated group was significant (Table 2B).

Taketa and Watanabe (1971) demonstrated that sulfhydryl reagents can alter the distribution of the dimer species (5). They have also shown that band 1 activity increased in aged preparations of rat liver glucose 6-phosphate dehydrogenase as compared to freshly prepared rat liver glucose 6-phosphate dehydrogenase. Recently, Grigor (3) confirmed these earlier observations for rat mammary gland glucose 6-phosphate dehydrogenase and proposed that band 1 and band 3 represent fully oxidized and fully reduced forms of the enzyme respectively, and that band 2 represents a partially oxidized form (3). Grigor (3) also showed that band 1 was most susceptible to proteolytic inactivation and that when mammary gland glucose 6-phosphate dehydrogenase activity decreases on weaning, a concomitant selective shift in the dimer banding pattern towards band 1 occurred. Chang et al. (6) observed a slight shift towards band 1 in the fasted refed induced male rat. Their finding and that of ours with the insulin induced female rat may be explained by earlier reports that the induction of glucose 6-phosphate dehydrogenase results in an increase in both the rate of synthesis as well as the rate of degradation of the enzyme (1,7). Taken

together, our results lend support to Grigor's proposal (3) that a shift in the dimer banding pattern towards band 1 may be an early event in the degradation of this enzyme.

REFERENCES

1. Rudack, D., Chisolm, E.M., and Holten, D. (1971) J. Biol. Chem. 246, 1249-1254.

2. Holten, D. (1972) Biochem. Biophys. Acta 268, 4-12.

3. Grigor, M. (1984) Arch. Biochem. Biophys. 229, 612-622.

4. Weber, G. (1963) in Advances in Enzyme Regulation (Weber, G., ed) Vol. 1, pp 1-20, Permagon Press, London.

5. Taketa, K., and Watanabe, A. (1971) Biochim. Biophys. Acta 235, 19-26.

6. Chang, H., Holten, D. and Karin, R. (1979) Can. J. Biochem. 57, 396-401.

7. Morikawa, N., Nakayama, R. and Holten, D. (1984) Biochem. Biophys. Res. Commun. 120, 1022-1029.

8. Hori, S.H., and Matsui, J. (1968) J. Histochem. Cytochem. 16, 62-63.