Rat Liver Glucose 6-Phosphate Dehydrogenase

REGULATION BY CARBOHYDRATE DIET AND INSULIN*

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SUMMARY

Rates of rat liver glucose 6-phosphate dehydrogenase synthesis and degradation in vivo have been measured by following the time course for the induction of the enzyme by carbohydrate diets and insulin. If rats previously adapted to a commercial pellet diet are fed a 60% carbohydrate diet, the rate of glucose-6-P dehydrogenase synthesis increases up to 80-fold, and the first order rate constant for the degradation in vivo of the enzyme is increased 4.6-fold. The rate of synthesis of glucose-6-P dehydrogenase in these rats increases in proportion to the caloric consumption of carbohydrate, whether or not that carbohydrate is able to stimulate the release of insulin from the pancreas. Although large doses of insulin are able to induce the synthesis of glucose-6-P dehydrogenase, this induction can be attributed to the ability of insulin to stimulate the appetite. It is concluded that insulin has no direct effect on the regulation of glucose-6-P dehydrogenase levels under our experimental conditions.

Rat liver glucose 6-phosphate dehydrogenase (p-glucose 6-phosphate:NADP oxidoreductase, EC 1.1.1.49) levels appear to be regulated by a complex interaction of diet and hormones. Levels of dietary protein, fat, and carbohydrate have been implicated in regulating levels of this enzyme (1-3). Feeding fasted rats a diet high in carbohydrate causes dramatic increases in the level of liver glucose-6-P dehydrogenase (1) which are proportional to the caloric consumption of carbohydrate (4). However, under similar conditions, the final enzyme level is decreased by including fat in these diets (3). Hormonal involvement in this regulation is indicated by the fact that thyroxine, 17-β-estradiol, dihydroepiandrosterone, cortisone, and insulin (5-7) have all been shown to alter the level of this enzyme in liver. Finally, interactions between these dietary and hormonal factors were suggested by the ability of insulin to augment the effect of high carbohydrate diets on the level of the enzyme in liver (8, 9). The diversity of agents which apparently can regulate the level of rat liver glucose-6-P dehydrogenase suggested this enzyme as a model system to study interrelationships between diet and hormones in the regulation of enzyme levels in rat liver.

The experiments reported here were designed to elucidate the nature of the interaction between high carbohydrate diets and insulin on the rates of glucose-6-P dehydrogenase synthesis and degradation in vivo. Our approach has been to follow the time course of the change in enzyme specific activity between several different steady state levels of enzyme. Berlin and Schimke (10) have demonstrated that by following the time course of change in the activity of an enzyme from one steady state to a second steady state, it is possible to determine the half-life of the enzyme. The steady state level of an enzyme is determined by the ratio of the rate constants for enzyme synthesis and degradation. We have studied the half-life of rat liver glucose-6-P dehydrogenase during transitions from one steady state to another, both increasing and decreasing, as perturbed by dietary or hormonal manipulations. With the knowledge of such half-lives and the eventual steady state levels produced by these dietary or hormonal manipulations, calculations of rates of synthesis can be made. These results show that increases in enzyme specific activity are due to increases in the rate of synthesis of this enzyme. In addition, our data indicate that insulin has no direct effect in regulating the level of this enzyme under our experimental conditions.

METHODS AND MATERIALS

Analytical Methods—The supernatant fraction from a 20% liver homogenate in 0.15 M KCl was prepared by centrifuging at 66,000 × g for 60 min. Glucose-6-P dehydrogenase activity was assayed according to a modification of Procedure 2 by Bottomley et al. (11), which corrects for the 6-P-gluconate dehydrogenase present in liver homogenate. One unit of glucose-6-P dehydrogenase activity is the amount required to form 1 μmole of NADPH in 1 min in the assay employed. The assay contained 120 μmoles of Tris-chloride buffer, pH 8.0, 2 μmoles of glucose-6-P, 0.9 μmole of NADP+, 10.4 μmoles of MgCl₂, 0.6 μmole of 6-P-gluconate, and enzyme in a final volume of 1.0 ml. The activity of 6-P-gluconate dehydrogenase was determined by eliminating glucose-6-P from the above assay. All assays were run at 30°. Protein was assayed by the method of Lowry et al. (12), and enzyme specific activity is expressed as units of enzyme per mg of protein.

Treatment of Animals—Young male rats of the Sprague-
The experimental results confirm the large increase in liver glucose-6-P dehydrogenase specific activity in the livers of rats fasted for 48 hours and then fed a 60% glucose diet for 7 days. Our results also revealed that after feeding a new diet or injecting insulin, there is an initial lag phase followed by an immediate change in the rate of enzyme synthesis, showing a selective stimulation. The term induction is used here to mean a selective stimulation of the rate of enzyme synthesis; no genetic or other mechanism is implied.

Materials—Salt mixture P-H, vitamin diet fortification mixture, dextrose, vitamin-free casein, cellulose (Alphacell), and dextrose were obtained from Nutritional Biochemicals; fructose, sucrose, and magnesium chloride were products of Mallinckrodt Chemical Works; glucose-6-P, A-P-gluconate, and Tris base were obtained from Sigma; NADP' and protamine zinc insulin were purchased from Boehringer Mannheim and Lilly, respectively. The metabolism feeders were purchased from the Wahman Manufacturing Company.

RESULTS

The experiment presented in Fig. 1 was designed to measure the rate of glucose-6-P dehydrogenase degradation and synthesis in vivo at an induced steady state by following the time course of change in enzyme specific activity in the livers of rats fasted for 48 hours and then fed a 60% glucose diet for 7 days. Our results confirm the large increase in liver glucose-6-P dehydrogenase previously reported for these conditions (2, 3, 14).

In each figure illustrating a time course for glucose-6-P dehydrogenase induction, a theoretical curve is superimposed over the experimental data. The fit of this theoretical curve is a graphical representation of the agreement of the calculated half-life with the experimental data. In Fig. 1, the excellent fit of the theoretical curve to the experimental data provides convincing evidence for the agreement of the calculated half-life with the experimental data.
Evidence that liver glucose-6-P dehydrogenase is degraded in vivo with a half-life of 15 hours. This fit between the experimental data and the theoretical curve also provides additional verification of the validity of Berlin and Schimke's equations (10) and illustrates the usefulness of this experimental approach for the determination of rates of enzyme synthesis and degradation in vivo.

Insulin, as well as diet, has been implicated in the regulation of the level of this enzyme. Because glucose causes the release of insulin from the pancreas (15), glucose diets could possibly be acting in a secondary fashion by stimulating the release of insulin. To test this hypothesis, one can feed a diet in which the glucose is replaced by fructose. Fructose diets result in very low levels of liver glucokinase (16), an enzyme which requires insulin for its synthesis (17). Presumably, this is because fructose is unable to cause the release of enough insulin to maintain normal levels of this enzyme (18). In contrast to the results with glucokinase, fructose diets are able to increase levels of glucose-6-P dehydrogenase at least as well as glucose diets (19, 20). Because the two diets produced similar levels of this enzyme, it seemed likely that both carbohydrates were acting by a similar mechanism which did not involve insulin. If this were true, both carbohydrate diets should have similar effects on the rates of glucose-6-P dehydrogenase synthesis and degradation. We have calculated these parameters in rats fasted for 2 days before being fed a 60% fructose diet for 7 days (Fig. 2). The rates of enzyme synthesis were 0.025 and 0.028 unit per mg of soluble protein per hour for the glucose and fructose diets, respectively; the half-life for glucose-6-P dehydrogenase degradation was 15 hours for both diets.

Interactions between insulin and carbohydrate diets were reported by Weber and Convery (8) and Freedland, Cunliff, and Zinkl (9), who found that insulin increased the levels of this enzyme in rats fed high glucose diets. In order to determine the nature of the interaction between diet and insulin in the regulation of glucose-6-P dehydrogenase levels, we determined how much insulin was required to produce a change in the level of this enzyme (Fig. 3). In rats at a low steady state level of enzyme (pellet-fed rats), 4 units of insulin per day per 100 g of rat or less failed to cause a significant increase in the specific activity of this enzyme, whereas 8 units caused a maximal response. In Table I, we report the effect of a combination of insulin and diet on the level of glucose-6-P dehydrogenase. In these experiments, insulin was given to rats previously adapted to an intermediate (specific activity = 0.409) or a high (specific activity = 0.605) steady state level of enzyme. The combination of insulin and diet resulted in approximately the same final steady state specific activities of 0.604 and 0.278, respectively.

![Graph: Effect of insulin on specific activity of liver glucose-6-P dehydrogenase in rats previously adapted to two different steady state levels of enzyme](http://www.jbc.org/)

**Table I**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Previous treatment</th>
<th>Number of animals</th>
<th>Glucose-6-P dehydrogenase activity (units/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pellet control</td>
<td>Fed</td>
<td>3</td>
<td>0.048 ± 0.005</td>
</tr>
<tr>
<td>Sucrose</td>
<td>Fed</td>
<td>4</td>
<td>0.409 ± 0.017</td>
</tr>
<tr>
<td>Sucrose + insulin</td>
<td>Fed</td>
<td>2</td>
<td>0.750 ± 0.002</td>
</tr>
<tr>
<td>Sucrose</td>
<td>Fasted</td>
<td>4</td>
<td>0.605 ± 0.032</td>
</tr>
<tr>
<td>Sucrose + insulin</td>
<td>Fasted</td>
<td>3</td>
<td>0.778 ± 0.040</td>
</tr>
</tbody>
</table>

* Standard error.

Fig. 3. Liver glucose-6-P dehydrogenase specific activity as a function of insulin dose. Rats were fed the pellet diet for 9 days prior to injection of the indicated dose of protamine zinc insulin and were then maintained on the pellet diet for the remainder of the experiment. Rats weighed from 225 to 240 g and were killed 48 hours after the initial injection of insulin. Each point represents the mean (±standard error) of two rats for the 0-, 0.5-, or 8-unit groups or three rats for each of the other groups.

Fig. 4. Time course of induction of liver glucose-6-P dehydrogenase in nonfasted rats. Rats were fed the pellet diet for 8 days and weighed from 185 to 230 g prior to being fed a 60% glucose diet. Each point represents the mean (±standard error) of three (8 hours, 5, and 7 days), four (0, 3, and 4 days), or five (24, 36, and 49 hours) animals. The solid line shows the theoretical curve calculated by means of a half-life of 15 hours for glucose-6-P dehydrogenase degradation in vivo and initial and final steady state specific activities of 0.054 and 0.279, respectively.
lishes that the half-life of glucose-6-P dehydrogenase degradation was 15 hours in this experiment. We next injected similar rats with insulin and followed the time course of enzyme increase in order to calculate the effect of the hormone on rates of glucose-6-P dehydrogenase synthesis and degradation. These data, presented in Fig. 5, establish that the half-life of enzyme degradation in vivo was unchanged by insulin. A new steady state enzyme level can result from changing either the rate of its synthesis or degradation. Because the increase in glucose-6-P dehydrogenase specific activity in this experiment was not caused by a change in the rate of enzyme degradation (the half-life was 15 hours before and after insulin), insulin must have increased the rate of synthesis of this enzyme.

Although the data we have presented in Figs. 4 and 5 indicate that insulin increases the rate of glucose-6-P dehydrogenase synthesis, the changes caused by the carbohydrate diets could result from changes in either the rates of synthesis or degradation of the enzyme. In order to establish which of these parameters was being affected by carbohydrate diets, it was necessary to determine the rates of enzyme synthesis and degradation at the low steady state level (pellet-fed rats). These values were calculated from the data in Fig. 6, which shows the time course for the decrease in enzyme specific activity which results from feeding the pellet diet to rats at a high steady state level of enzyme. The rates of enzyme synthesis and degradation for the pellet-fed rats and for each of the other steady state enzyme levels are recorded in Table II. It is apparent that the rates of synthesis and degradation are both very slow in the pellet-fed rats.

![Fig. 5. Time course of insulin induction of liver glucose-6-P dehydrogenase in rats equilibrated to a 60% glucose diet. Rats initially weighing from 162 to 197 g were fed a pellet diet for 4 days and were then fed a 60% glucose diet for 7 days. Control rats not injected with insulin (0) were killed after 4 or 7 days on the glucose diet. After 4 days on the glucose diet, the remaining rats were injected with 6 units of protamine zinc insulin per day per 100 g of body weight (O) and killed at the times indicated. Each point represents the mean (±standard error) of at least four animals. The solid line represents the theoretical curve calculated by means of a half-life of 15 hours for glucose-6-P dehydrogenase degradation in vivo and initial and final steady state specific activities of 0.219 and 0.75, respectively. The final enzyme specific activity of 0.75 was calculated with the use of the equation presented under "Methods and Materials," a half-life of 15 hours, and the specific activity observed 3 days after starting the insulin injections (0.70). This small correction was necessary because 3 days is not quite long enough to reach the new steady state.]

![Fig. 6. The time course for the decrease in glucose-6-P dehydrogenase specific activity from an induced steady state to the low steady state level (pellet-fed rats). Rats were induced to a high steady state level of liver glucose-6-P dehydrogenase as in Fig. 1. After 6 days on the glucose diet, rats were given the pellet diet, and the decrease in enzyme specific activity was followed for 7 days. Each point represents the mean (±standard error) of three or four rats. The theoretical curve was calculated by means of a half-life of 69 hours for glucose-6-P dehydrogenase degradation in vivo and initial and final steady state specific activities of 0.615 and 0.034, respectively. Although the 7-day duration of this experiment was insufficient to reach the steady state level of the pellet-fed rats, this value (0.034 specific activity) had been previously determined in Fig. 4.]

**Table II**

Rates of synthesis and degradation in vivo for glucose-6-P dehydrogenase at various steady state levels of specific activity ($P'$)

<table>
<thead>
<tr>
<th>Treatment of rats</th>
<th>Origin of data</th>
<th>$P'$</th>
<th>$k'$</th>
<th>Rate of enzyme synthesis</th>
<th>Carbohydrate consumed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pellet fed.........</td>
<td>Fig. 6</td>
<td>0.034</td>
<td>$1.0 \times 10^{-4}$</td>
<td>3.4 x 10^{-2}</td>
<td>21.6</td>
</tr>
<tr>
<td>Glucose diet nonfasted</td>
<td>Fig. 5</td>
<td>0.219</td>
<td>$4.6 \times 10^{-3}$</td>
<td>1.0 x 10^{-2}</td>
<td>20.8</td>
</tr>
<tr>
<td>Glucose diet nonfasted</td>
<td>Fig. 4</td>
<td>0.279</td>
<td>$4.6 \times 10^{-3}$</td>
<td>1.3 x 10^{-2}</td>
<td>20.2</td>
</tr>
<tr>
<td>Fructose diet nonfasted</td>
<td>Not presented</td>
<td>0.207</td>
<td>$4.6 \times 10^{-3}$</td>
<td>1.4 x 10^{-2}</td>
<td>25.7</td>
</tr>
<tr>
<td>Glucose diet fasted.............</td>
<td>Fig. 1</td>
<td>0.537</td>
<td>$4.6 \times 10^{-3}$</td>
<td>2.5 x 10^{-2}</td>
<td>23.5</td>
</tr>
<tr>
<td>Fructose diet fasted.................</td>
<td>Fig. 2</td>
<td>0.615</td>
<td>$4.6 \times 10^{-3}$</td>
<td>2.8 x 10^{-2}</td>
<td>23.5</td>
</tr>
<tr>
<td>Glucose diet nonfasted, given insulin</td>
<td>Fig. 5</td>
<td>0.750</td>
<td>$4.6 \times 10^{-3}$</td>
<td>3.5 x 10^{-2}</td>
<td>27.4</td>
</tr>
</tbody>
</table>

The first order rate constant ($k'$) for the degradation in vivo of the enzyme was calculated from data presented in previous figures. The rate of enzyme synthesis in vivo equals $P'k'$. The caloric intake of carbohydrate was calculated from the grams of diet consumed by each rat at each steady state enzyme level. A least squares plot of the rate of enzyme synthesis versus the caloric intake of carbohydrate gives a straight line with a correlation coefficient $r = 0.9$. 

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Regulation of Rat Liver Glucose-6-P Dehydrogenase
The carbohydrate diets increased both the rate of synthesis and degradation of glucose-6-P dehydrogenase.

During many of the experiments, daily account was kept of the food consumption by each rat. It became apparent that rats given insulin were eating more than the corresponding controls. Sassoon, Watson, and Johnson (4) have shown a correlation between the level of rat liver glucose-6-P dehydrogenase and the caloric intake of carbohydrate. In the last column of Table II, we have recorded the caloric consumption of carbohydrate for rats at each of the steady state enzyme levels. When the rate of glucose-6-P dehydrogenase synthesis is plotted versus the caloric consumption of carbohydrate for each of the steady state enzyme levels shown in Table II, a straight line is obtained. The correlation coefficient \( r \) calculated from these data was 0.9, and the level of significance \( p \) for the correlation between these two parameters was less than 0.02. These data confirm and extend those of Sassoon et al. (4) and indicate that the steady state level of this enzyme is related to the quantity of carbohydrate consumed by these animals.

**DISCUSSION**

These investigations were undertaken to answer the following questions. Do high carbohydrate diets and insulin increase the level of rat liver glucose-6-P dehydrogenase by regulating the rate of enzyme synthesis or the rate of enzyme degradation? How do carbohydrate diets and insulin interact in this regulation? The answer to the first question would appear to be that carbohydrate diets and insulin both induce glucose-6-P dehydrogenase by increasing the rate of synthesis of this enzyme. In addition, the carbohydrate diets also increase the rate of degradation. Since the effect of an increased rate of enzyme degradation is to lower the steady state level of an enzyme, the large increases in glucose-6-P dehydrogenase levels reported in this paper could only result from even larger increases in the rate of synthesis of this enzyme. The rate of glucose-6-P dehydrogenase synthesis in the pellet-fed rat is increased 30-fold by feeding the rat a high carbohydrate diet. Much greater increases in the rate of enzyme synthesis are observed (73 to 103-fold) if the rat is made to eat more of this diet, either by fasting him for 2 days prior to feeding or by giving him insulin. These very large changes in rates of glucose-6-P dehydrogenase synthesis certainly recommend the use of this enzyme as a model system for studying mechanisms regulating enzyme synthesis in vivo in mammalian liver. In making these calculations, the assumption is made that after a lag phase, an immediate increase in the rate of glucose-6-P dehydrogenase synthesis occurs and that this new rate of synthesis is maintained throughout the duration of the experiment. The fact that the theoretical curve, calculated by using this assumption, fits the experimental data fairly well during the early phases of the induction suggests that the increase in the rate of synthesis is achieved fairly rapidly. However, a definitive answer on the validity of this kinetic method and its assumptions must await the use of a second, independent method to determine rates of glucose-6-P dehydrogenase synthesis at various times during the induction of this enzyme.

The experiments reported here also provide some insight into interactions between carbohydrate in the diet and insulin in the regulation of this enzyme. Several pieces of evidence suggest that insulin may not directly regulate the level of this enzyme in vivo. The amount of insulin required to produce a significant increase in the enzyme is greater than that which one could reasonably expect to call a physiological dose. The rat secretes from 2 to 3 units of insulin per day (21), and it takes more than this to cause a significant increase in liver glucose-6-P dehydrogenase. Also, much smaller doses of insulin (0.1 unit) are capable of increasing the rate of synthesis of rat liver tyrosine transaminase (22). Sols, Silleró, and Salas (17) have shown that rat liver glucokinase, which requires insulin for its synthesis, is very low in rats fed a fructose diet and elevated in rats fed a glucose diet (16). This difference has been attributed to the inability of fructose diets to release insulin from the pancreas (18). If rat liver glucose-6-P dehydrogenase required insulin for its induction, one would expect levels of the enzyme to be much higher in rats fed a glucose diet. The fact that rat glucose-6-P dehydrogenase levels are higher when rats are fed a fructose diet suggests that insulin is not directly involved in the induction of this enzyme.

Table II indicates a highly significant \( p < .02 \) correlation between the steady state level of liver glucose-6-P dehydrogenase and the caloric intake of carbohydrate. This strongly suggests that the effect of insulin shown in Fig. 5 can be accounted for in terms of the stimulation of the appetite by insulin and the resulting greater consumption of carbohydrate. The effect of insulin, therefore, appears to be an indirect effect upon appetite rather than a direct stimulation of glucose-6-P dehydrogenase synthesis.

There is considerable variation in the lag phase between the initiation of feeding a new diet and the initiation of glucose-6-P dehydrogenase synthesis. For example, the initiation of fructose induction (Fig. 2) takes longer (32 hours) than the initiation of glucose induction (Fig. 1) (21 hours). Our data show that rats fed fructose ate less during the first 12 hours than did rats fed glucose. Also, fructose has been reported to lower ATP levels and inhibit protein synthesis (23). Either of these observations could account for the slower effect of fructose. Prior fasting decreased the lag phase and caused a greater increase in the rate of glucose-6-P dehydrogenase synthesis (compare Figs. 1 and 4). In all of our experiments, the feeding of a new diet was initiated between 8 and 9 a.m. The fasted rats started to eat sooner and also ate more food than rats which had not been fasted. The lag phase for the insulin-treated rats was also short, and the stress of a low blood glucose probably caused these rats to start eating sooner. Thus, the duration of the lag phase prior to the initiation of an increased rate of enzyme synthesis depends, in part at least, upon how soon the animals start to eat the new diet.

As the data in Fig. 6 indicate, the half-life for glucose-6-P dehydrogenase degradation in vivo is 69 hours. When these rats are fed the high carbohydrate diet, the half-life decreases to 15 hours. Szepesi and Freedland (24) have reported that the half-life for glucose-6-P dehydrogenase degradation is 36 hours in rats shifted from a 90% glucose diet to a 90% protein diet and that the increase in glucose-6-P dehydrogenase activity when these animals are treated with cortisone can only be due to an increased rate of enzyme synthesis. It is apparent that different rates of degradation do exist for this enzyme, and the probability exists that this enzyme is regulated by changes in both the rates of synthesis and degradation.

Our results indicate that carbohydrate in the diet increases the rate of glucose-6-P dehydrogenase synthesis, and the magnitude...
of this increase is determined by the quantity of carbohydrate consumed. Obviously, the carbohydrate could be acting indirectly by stimulating the release or production of a primary inducer. For the reasons outlined previously, insulin, the most likely candidate as a primary inducer released in response to ingested carbohydrate, appears not to be involved.

REFERENCES

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