Vol. 89, No. 1, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 12, 1979 Pages 108-115

REGULATION OF GLUCOSE-6-P DEHYDROGENASE SYNTHESIS IN RAT EPIDIDYMAL FAT PADS[1]

Ronald G. Wolfe*, Roderick Nakayama and Darold Holten†

Department of Biochemistry
University of California
Riverside, California 92521

Received May 22,1979

SUMMARY:

The relative rate of synthesis of glucose-6-P dehydrogenase increases up to 8-fold when fasted rats are fed a 60% carbohydrate, fat-free diet for 3 days but the specific activity of the enzyme only increases 2 to 3 fold. This suggests that the high carbohydrate diet also causes a 2 to 3 fold increase in the rate of glucose-6-P dehydrogenase degradation. The nutritional induction of this enzyme in adipose tissue is primarily due to a large increase in the rate of its synthesis.

Mechanisms regulating the induction of lipogenic enzymes have been studied extensively in rat liver, yet there is a lack of consensus as to the nature of the hormonal and/or metabolic signals which regulate the levels of this class of enzymes. Insulin has been implicated in this regulation because diabetic rats do not show the normal induction of lipogenic enzymes (1,2). However, in normal animals there does not appear to be a correlation between insulin levels and the levels of lipogenic enzymes in liver (3). Cyclic AMP can repress the synthesis of some lipogenic enzymes (4,5) but has no effect on 6-phosphogluconate dehydrogenase synthesis and only a two fold effect on glucose-6-phosphate dehydrogenase synthesis (6). Hypophysectomy decreases lipogenic enzyme levels (7) and thyroxine is synergestic with insulin in inducing malic enzyme in cultures of chick liver (5).

Abbreviations: SDS, sodium dodecyl sulfate.

1. This work was supported in part by Research Grant AM-13324 from the United States Public Health Service.

*Present address: Sigma Chemical Co., P.O. Box 14508, St. Louis, Missouri 63178

† To whom all correspondence and requests for reprints should be made.

0006-291X/79/130108-08$01.00/0

However, thyroxine may only play a permissive role in lipogenic enzyme induction in this system (5).

Adipose tissue, like liver, shows an induction of lipogenic enzymes during fasting-refeeding experiments but there are differences between the two tissues which might give some important insight into mechanisms regulating the induction of these enzymes. Insulin levels in blood correlate well with the induction of lipogenic enzymes in adipose tissue but not in liver (3). This may be due to a requirement for insulin in glucose transport in adipose tissue but not in liver (8). Dietary fructose is more effective than glucose in lipogenic enzyme induction in liver but less effective than dietary glucose in adipose tissue (3,9). This could be due to a specific fructokinase in liver which allows a very rapid utilization of fructose (10) and the absence of this enzyme in adipose tissue (9). Thyroxine effects lipogenic enzyme levels in opposite directions in the two tissues (11). Thus there are interesting differences between liver and adipose tissue in how dietary and hormonal factors regulate levels of lipogenic enzymes.

A comparison of the mechanisms regulating the induction of a specific lipogenic enzyme in these two tissues might provide some insight into the nature of the signals regulating lipogenic enzyme synthesis. This paper provides evidence that glucose-6-phosphate dehydrogenase synthesis (G6PD; E.C. 1.1.1.49) changes as much as 8 fold in adipose tissue when rats are fasted and then fed a high carbohydrate-fat free diet. This enzyme would therefore be an excellent model system for further study in both liver and adipose tissue.

Methods: Assays for G6PD and protein and composition of the diets were as previously described (12). Young male Sprague-Dawley rats (100-150 g) were fed Purina rat chow (pellet fed rats), fasted for 3 days (fasted rats) or fasted for 3 days and fed a 60% carbohydrate (fructose or glucose) fat free diet for 3 days (induced rats). One unit of G6PD is that amount of enzyme which will produce 1μ mole of NADPH/min at 30° and pH 8. Specific activity is expressed as units of enzyme/mg protein.

Preparation and incubation of adipose cells. Epididymal adipose tissues were dissected, placed in saline at room temperature and connective tissue and blood vessels were removed. Tissues were minced, incubated in digestion

buffer (9 ml/g tissue) for 1 hr at 37° in a shaking water bath (120 cycles/min) while being continually gassed with 5% CO_2/95% O_2. The digestion buffer contained 3.2% bovine albumin (fatty acid free), 0.24% collagenase in Krebs original Ringer phosphate media supplemented with glucose (0.005 M), pyruvate (0.005 M), and $NaHCO_3$ (0.025 M). The cells were harvested by centrifugation at 50 x g for 2 min and washed twice with Krebs media. The cells were taken up in Krebs media (15 ml/g tissue) containing 3.6% Bovine albumin (fatty acid free) and insulin (1 mu/ml). Radioactivity was incorporated by adding 20 µCi of L-[4,5-^{3}H]leucine (60 Ci/mmol). Each of the other 19 common amino acids were added at a final concentration of 80 µM. The cells were incubated in a shaking water bath at 37° for 1 hr while being continually gassed with 5% CO_2/95% O_2. They were then harvested by centrifugation at 50 x g for 2 min and washed twice with Krebs media. Cells were sonicated in an equal volume of cold 0.15 M KCl and centrifuged at 100,000 x g for 45 min. The supernatant fraction was assayed for G6PD and the enzyme was isolated by specific immunoprecipitation. Immunoprecipitation of G6PD and SDS polyacrylamide disc gel electrophoresis of the immunoprecipitate were as described previously (13). The radioactivity in G6PD was taken as the sum of the radioactivity in the peak which migrated with the G6PD subunit (see Figure 2). Total soluble protein radioactivity was determined as before (13) except that radioactive protein was extracted from cellulose discs by heating with protosol for 4 hr at 50°C.

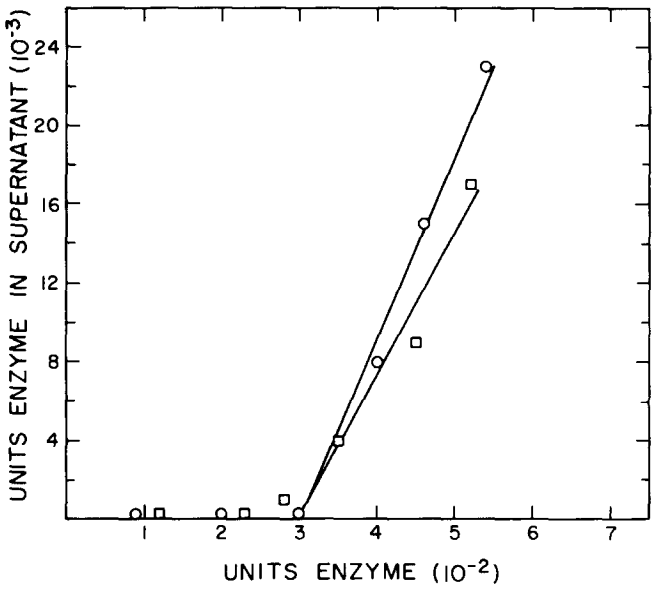

Figure 1. Quantitative immunotitration of adipose tissue G6PD from fasted and glucose induced rats. Twenty µl of antiserum was added to increasing amounts of supernatant fractions from adipose tissue of fasted (**O**) and induced (**□**) rats. The specific activity of G6PD was 0.16 and 0.40 units of enzyme/mg protein in fasted and induced animals respectively. The final reaction volume was constant at 59 µl. The supernatnat fraction from the fasted rat was concentrated by ultrafiltration to an enzyme concentration equivalent to that from the induced rat. Reactions were incubated at 30° for 30 min and then placed on ice for 1 hr prior to centrifugation to remove the immunoprecipitate and assay of the supernatant fraction for unreacted G6PD.

Results and Discussion

As a preliminary step in determining the effect of dietary carbohydrate on the synthesis of G6PD in adipose tissue, we determined if the antiserum to liver G6PD would react equally well with the enzyme from adipose tissue. Figure 1 shows a quantitative titration of adipose tissue G6PD from fasted rats and rats fasted and refed a 60% glucose, fat-free diet for 3 days. The antiserum had been raised against pure liver G6PD and had a titer of 150 units/ml against liver G6PD. Figure 1 shows that this antiserum had an identical titer of 150 units/ml when titered against G6PD from the adipose tissue of fasted or induced rats. This antiserum therefore reacts in a quantitatively identical way with either liver or adipose G6PD and gives an identical titer for G6PD from the adipose tissue of induced or fasted rats. This demonstrates that the 2.5 fold induction of G6PD in adipose tissue was due to a corresponding 2.5 fold increase in the amount of G6PD protein.

Figure 2 illustrates the specificity of the antiserum in immunoprecipitating G6PD from adipose tissue. In this experiment rats in three different

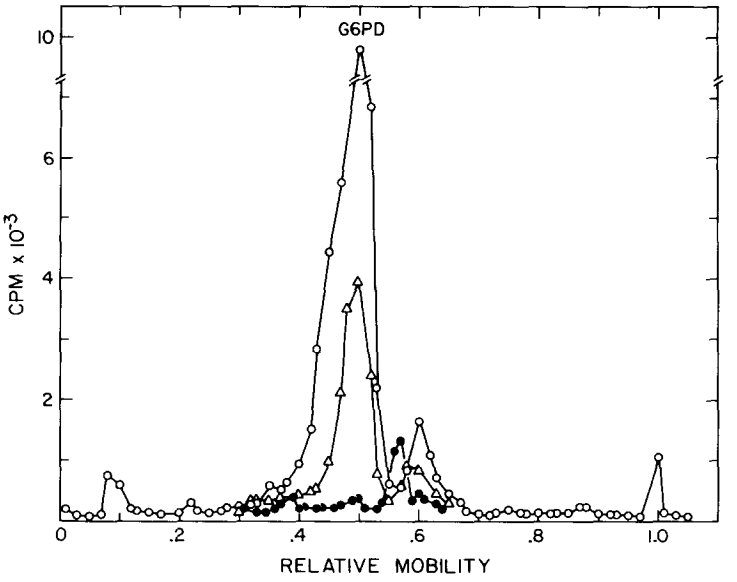

Figure 2. Polyacrylamide SDS disc gel electrophoresis of G6PD immunoprecipitated from adipocytes of rats in three different nutritional states. Adipocytes were labeled with [^{3}H]leucine for 60 min prior to sonification and centrifugation. Four units of carrier liver G6PD were added prior to immunoprecipitation. The counts in the G6PD have not been normalized to a uniform amount of radioactivity in total protein. The three nutritional states are glucose induced (○), pellet fed (△) and fasted (●).

nutritional states were used to prepare adipocytes from epididymal fat pads. These were then incubated with [^{3}H]leucine for 1 hr, sonicated and centrifuged to produce a supernatant fraction. The specific activity of the G6PD was determined and the enzyme was immunoprecipitated as described in the Methods section. The washed immunoprecipitate was electrophoresed on an SDS polyacrylamide gel which was then sliced and counted for [^{3}H]proteins. The data in Figure 2 show that there is one major band which is immunoprecipitated and that this band migrates with the subunit of pure G6PD. The small peak of relative mobility 0.58-0.6 may represent a small amount of G6PD nascent chain (14) and is not included in calculating the radioactivity in G6PD. The G6PD immunoprecipitated by the antiserum was shown previously to produce cyanogen bromide fragments of the same molecular weight as pure G6PD (15). Together these data demonstrate that this antiserum is specific for G6PD.

The data in Figure 2 also suggests that the amount of radioactive G6PD synthesized in adipose tissue increases in animals which are fasted and then fed a 60% glucose diet. We have therefore used this technique to determine the relative rate of G6PD synthesis in rats in four different nutritional states and the data are presented in Table I. When a fasted rat is fed a high carbohydrate, fat free diet for 3 days, G6PD increases 2 to 3 fold in specific activity. In these same animals there is a 5 to 8 fold increase in the relative rate of G6PD synthesis. Animals fed a Purina chow diet fall somewhere between fasted and induced animals both in the amount of G6PD present and in its relative rate of synthesis. These data demonstrate that the dietary induction of G6PD in adipose tissue, like the induction of this enzyme in liver, is due to a large change in the rate of enzyme synthesis.

The fact that the increase in G6PD synthesis is larger than the change in enzyme specific activity suggests that the rate of degradation increases in induced animals. We can calculate a relative rate of degradation of G6PD from the relationship $E = Ks/Kd$ (16) where E is the enzyme concentration at any steady state level of enzyme and Ks and Kd are the rate constants for

TABLE I

Relative Rate of Glucose-6-P Dehydrogenase Synthesis in Adipose Tissue

Nutritional State	Number of animals	Specific Activity (units/mg protein)	dpm in G6PD (10^3 dpm/mg protein)	dpm in Total Soluble Protein (10^7 dpm/mg protein)	Relative Rate of Synthesis (10^{-2})
Glucose induced	8	0.34 ± 0.09	14 ± 1.7	0.84 ± 0.12	0.17 ± 0.01
Fructose induced	6	0.28 + 0.09	11 ± 2.6	0.98 ± 0.26	0.11 ± 0.01
Pellet fed	4	0.19 ± 0.02	5.6 ± 1.0	1.0 ± 0.1	0.056 ± 0.01
Fasted	6	0.12 ± 0.04	1.2 ± 0.7	0.6 ± 0.4	0.02 ± 0.01

Rats were fed Purina rat chow (pellet fed), fasted for 3 days (fasted) or fasted for 3 days and fed a 60% carbohydrate (glucose or fructose), fat free diet for 3 days. Adipose cells were isolated and labeled with (^{3}H)leucine for 1 hr prior to being sonicated and centrifuged. G6PD was isolated by immunoprecipitation and electrophoresis on SDS-polyacrylamide gels. Other details are provided in the Methods section.

synthesis and degradation at the steady state. If we substitute the specific activity for E and the relative rate of synthesis (RRS) for Ks we can calculate a relative rate of degradation (17). When this is done the relative rate of degradation is 0.5, 0.39, 0.29 and 0.17 for glucose induced, fructose induced, pellet fed and fasted rats respectively. Our data therefore suggest that the induction of G6PD in adipose tissue is primarily due to changes in the rate of enzyme synthesis but that there may also be a 2 to 3 fold increase in the rate of G6PD degradation in the induced rat.

We have recently shown that dietary fat may decrease the rate of degradation of G6PD in liver (17) and this is consistent with an increase in G6PD degradation in adipose tissue when rats are fed a fat free diet. Geisler, Roggeveen and Hansen (18) have recently shown that adipose cells incubated with insulin degrade G6PD 2 to 3 times more slowly than cells incubated without insulin so adipose tissue appears to regulate G6PD synthesis within an 8 fold range and G6PD degradation within a 2 to 3 fold range. These differences are large enough to make adipose G6PD an attractive model for providing additional insight into the types of nutritional and hormonal signals which regulate the indcution of this enzyme in lipogenic tissues.

References

1. Lakshmanan, M. R., Nepokroeff, C. M., and Porter, J. W. (1972) Proc. Natl. Acad. Sci. USA 69, 3516-3519.
2. Nepokroeff, C. M., Lakshmanan, M. R., Ness, G. C., Muesing, R. A., Kleinsek, D. A., and Porter, J. W. (1975) Arch. Biochem. Biophys. 162, 340-344.
3. Bruckdorfer, K. R., Khan, I. H., and Yudkin, J. (1972) Biochem. J. 129, 439-446.
4. Volpe, J. J., and Marasa, J. C. (1975) Biochim. Biophys. Acta 380, 454-472.
5. Goodridge, A. G., and Adelman (1976) J. Biol. Chem. 251, 3027-3032.
6. Garcia, D. and Holten, D. (1975) J. Biol. Chem. 250, 3960-3965.
7. Kumar, S., Das, D. K., Dorfman, A. E., and Asato, N. (1977) Arch. Biochem. Biophys. 178, 507-516.
8. Froesch, E. R., and Ginsberg, J. L. (1962) J. Biol. Chem. 237, 3317-3324.
9. Volpe, J. J., and Vagelos, P. R. (1974) Proc. Natl. Acad. Sci. USA 71, 889-893.
10. Sillero, M. A. G., Sillero, A., and Sols, A. (1969) Eur. J. Biochem. 10, 345-350.
11. Roncari, D. A. K., and Murthy, V. K. (1975) J. Biol. Chem. 250, 4134-4138.

12. Rudack, D., Chisholm, E. M., and Holten, D. (1971) J. Biol. Chem. 246, 1249-1254.
13. Winberry, L., and Holten, D. (1977) J. Biol. Chem. 252, 7796-7801.
14. Adams, D. J., and Barker, K. L. (1979) Fed. Proc. 38, Abst. No. 906.
15. Sun, J. D., and Holten, D. (1978) J. Biol. Chem. 253, 6832-6836.
16. Berlin, C. M., and Schimke, R. T. (1965) Mol. Pharmacol. 1, 149-156.
17. Wolfe, R. G., and Holten, D. (1978) J. Nutr. 108, 1708-1717.
18. Geisler, R. W., Roggeveen, A. E., and Hansen, R. J. (1978) Biochim. Biophys. Acta 544, 284-293.