Insulin Stimulation of Hepatic Malic Enzyme Activity in Normal and Diabetic Rats Controlled by Different Regulatory Processes*

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Richard L. Drake, William C. Parks$, and
Ed W. Thompson
From the Department of Anatomy and Cell Biology,
University of Cincinnati College of Medicine,
Cincinnati, Ohio 45267

A comparison of the processes controlling the increase in hepatic malic enzyme activity in insulin-treated normal and diabetic rats indicated the existence of two distinct regulatory mechanisms. Livers were removed at 12, 36, and 60 h after insulin treatment of normal and alloxan-diabetic rats, and the activity, quantity, and specific activity (units/nmol) of malic enzyme was determined. In normal rats, a significant increase in activity occurred 12 h after insulin, whereas 36 h of insulin treatment was required for diabetic rats to show an increase in enzyme activity. This suggested that the return of malic enzyme activity from the depleted levels measured in diabetic rats probably involved a different sequence of events. A malic enzyme specific radioimmunoassay confirmed this. The increase in activity in insulin-treated normal rats was due to an increase in the quantity of malic enzyme. In insulin-treated diabetic rats, the increase in activity resulted from increases in both enzyme quantity and the specific activity of the enzyme, which returned to levels observed in normal rats.

The availability of insulin has been shown to dramatically influence hepatic lipogenesis (1). Diabetes is associated with a decrease in the ability of the liver to convert carbohydrates into fatty acids and triglycerides (2), while providing insulin to the diabetic animal reverses this defect (3, 4). These alterations have been attributed to changes in activities of the lipogenic enzymes. The activities of fatty acid synthetase, acetyl-CoA carboxylase, citrate cleavage enzyme, 6-phosphogluconate dehydrogenase, and glucose-6-phosphate dehydrogenase are significantly depressed in diabetes, whereas 36 h of insulin treatment resulted in activity increases which returned to levels observed in normal rats.

EXPERIMENTAL PROCEDURES

Male Sprague-Dawley rats with initial body weights of 175-195 g (King Animal Labs, Oregon, WI) were used in these studies. Food and water were available ad libitum. Diabetes was induced by intravenous injection of 4% (w/v) alloxan monohydrate at a dosage of 55 mg/kg, body weight. Rats with plasma glucose levels greater than 300 mg/dl, as determined using hexokinase and glucose-6-phosphate dehydrogenase (14), and little or no weight gain were considered diabetic (15). Rats meeting these criteria were maintained for at least 10 days after alloxan administration to stabilize the diabetic condition (16). After 10 days, insulin treatment was initiated in both normal and diabetic rats. This consisted of subcutaneous injections of 4 units of regular insulin (Regular Iletin) and 4 units of NPH insulin (NPH Iletin) every 12 h for 12, 36, or 60 h. Simultaneously, rats receiving insulin were placed on a high carbohydrate, fat-free diet (AIN-76; Zeigler Brothers, Gardners, PA). The special diet was chosen to provide maximal availability of carbohydrate substrate for de novo lipogenesis.

At the specified times after the initiation of diet and insulin treatment, all rats were sacrificed at 9:00 a.m. by decapitation. The livers were removed, weighed, and homogenized in 4 volumes of 30 mM Tris-HCl, pH 7.7, 625 mM sucrose, 1.0 mM dithiothreitol, and 0.1 mM EDTA. The 100,000 X g supernatant was prepared from each liver and used for the determination of enzyme activity and quantity.

Malic enzyme activity was determined spectrophotometrically with monitoring the reduction of NADP as previously described (17). One unit of activity was defined as 1 μmol of NADPH generated per min. Protein levels were determined (18) using bovine serum albumin as a standard.

The quantity and specific activity of hepatic malic enzyme in each 100,000 X g supernatant were determined by radioimmunoassay as previously described (12, 13). Antiserum specificity was determined on Ouchterlony double-diffusion plates and by its ability to inhibit the activity of cytosolic and purified hepatic malic enzyme. The radioimmunoassay was sensitive to less than 200 ng of unlabeled enzyme, with 50% displacement of 3H-labeled malic enzyme obtained with 2.9 μg of unlabeled antigen.

RESULTS AND DISCUSSION

The diabetic rats in this study lost 0.25 ± 0.58 g/day and had plasma glucose levels of 498 ± 74 mg/dl (mean ± S.D., n = 20). In contrast, normal rats gained 6.42 ± 0.0 g/day with plasma glucose levels of 141 ± 16 mg/dl (mean ± S.D., n = 4). Therefore, the diabetic rats clearly satisfied the criteria for experimental diabetes of little or no increase in body

malic enzyme (L-malate:NADP+ oxidoreductase (oxaloacetate-decarboxylating) EC 1.1.1.40), which provides NADPH for de novo fatty acid synthesis (6, 7), is directly related to the availability of insulin. During diabetes, malic enzyme activity is depressed, with insulin reversing this defect (5, 8-11). Recently, we have shown that the increase in enzyme activity following insulin treatment of diabetic rats is due to increases in both enzyme quantity and the specific activity of the enzyme (12, 13). The sequence of these changes in relation to the increase in enzyme activity is unknown. Furthermore, the involvement of quantity and specific activity changes in the control of malic enzyme activity in insulin-treated normal animals has yet to be determined.

In the current study, the kinetics of the increase in the quantity and specific activity of malic enzyme following insulin treatment of diabetic rats was correlated with the increase in enzyme activity. Additionally, the regulation of malic enzyme activity in insulin-treated normal rats was examined to determine if the regulatory mechanisms were similar to those identified in insulin-treated diabetic rats.
Control of Malic Enzyme by Insulin

![Graph 1: Effect of Insulin on the activity of hepatic malic enzyme](graph1.png)

**FIG. 1. Effect of Insulin on the activity of hepatic malic enzyme.** Livers were removed from normal (●) and diabetic (○) rats at 0, 12, 36, and 60 h after insulin and special diet treatment. The 100,000 × g supernatant was obtained and the total activity of malic enzyme in each liver determined. Each point represents the mean ± S.D. The number of rats was the same as indicated in Table I.

Several important differences were evident in the kinetics and magnitude of the increase in hepatic malic enzyme activity in insulin-treated normal and diabetic rats (Fig. 1). A statistically significant increase in total activity was observed in normal rats 12 h after the initiation of diet and insulin treatment. This stimulation continued through the experimental period of 60 h when a 15-fold increase in total activity had occurred. These results contrasted sharply with those obtained in insulin-treated diabetic rats. The enzyme activity in untreated diabetic rats was significantly lower than in untreated normal rats (1.00 ± 0.25 units/liver versus 2.94 ± 1.15 units/liver) and stimulation was absent at 12 h post insulin. By 36 h after the initiation of insulin treatment, there was a significant increase in enzyme activity which continued for the 60-h treatment period, at which time a 32-fold increase in total activity had occurred. Expression of these results as either milliunits/mg 100,000 × g supernatant protein or units/100 g, body weight, showed the same delayed increase in activity and a greater stimulation of activity in insulin-treated diabetic rats (data not shown). While the use of alloxan to induce diabetes could have altered the ability of the liver to respond normally to insulin, the rapid increase in hepatic glycogen levels and rapid decrease in plasma glucose levels reported in previous studies (19) argues against this interpretation. Therefore, the delayed rise in enzyme activity in insulin-treated diabetic rats is probably due to the shunting of metabolites to other metabolic pathways, e.g. glycogen synthesis, before their use in hepatic lipogenesis. Also, it is noteworthy that the overall stimulation of enzyme activity following insulin treatment was greater in diabetic rats than normal rats, 32-fold versus 15-fold. Since the increases in malic enzyme activity must be due to changes in either the quantity or specific activity of the enzyme, our results suggest that the contribution of these processes in normal and diabetic rats may be unequal.

The increase in activity of a specific enzyme may be due to alterations in enzyme quantity caused by changes in rate of synthesis or degradation, to a modification of the specific activity of the enzyme, or to a combination of these processes. A radioimmunoassay specific for malic enzyme was used in this study to determine the contribution of these processes to the insulin-mediated increase in malic enzyme activity in normal and diabetic rats.

The results of the radioimmunoassay demonstrated that an increase in enzyme quantity paralleled the increase in enzyme activity (Fig. 2). There was a rapid rise in the total nanomoles of enzyme in insulin-treated normal rats, compared to a delayed stimulation in insulin-treated diabetic rats. Both increases continued for the 60-h treatment period when the insulin-treated normal rats and the insulin-treated diabetic rats showed 12- and 10-fold increases, respectively. These results clearly indicate that an increase in enzyme quantity is a factor in the insulin-mediated increase in malic enzyme activity in both experimental groups. This change in enzyme quantity is probably due to increased enzyme synthesis, which has accounted for the increase in hepatic malic enzyme quantity in refed rats (20–22) and in rats treated with a protein-deficient diet (23). However, a decrease in the degradation rate of malic enzyme cannot be ruled out as a contributing factor.

If these results were expressed as nmol/100 g, body weight, the kinetics of the increase in enzyme quantity and the overall fold stimulation were similar for both experimental groups (data not shown). However, when the concentration of enzyme (nmol/g of liver) was determined, a significant difference was evident between the two experimental groups (Table I). The insulin-treated normal rats again showed a rapid

![Graph 2: Insulin effect on the quantity of hepatic malic enzyme](graph2.png)

**FIG. 2. Insulin effect on the quantity of hepatic malic enzyme.** Livers were removed from normal (●) and diabetic (○) rats after 0, 12, 36, and 60 h of treatment with insulin and the special diet. The 100,000 × g supernatant was prepared and the quantity of malic enzyme in each liver determined using a specific radioimmunoassay. Each point represents the mean ± S.D. The number of rats was the same as indicated in Table I.

### Table I

**Insulin effect on the concentration of hepatic malic enzyme in normal and diabetic rats**

|          | Normal | Diabetic |
|----------|--------|---------|
| Untreated| 0.17 ± 0.05 (6) | 0.16 ± 0.09 (4) |
| Insulin-treated |         |         |
| 12 h     | 0.33 ± 0.01* (4) | 0.09 ± 0.04 (5) |
| 36 h     | 1.13 ± 0.23* (4) | 0.20 ± 0.04 (4) |
| 60 h     | 1.54 ± 0.24* (3) | 0.61 ± 0.19* (7) |

*p < 0.001.

Results are mean ± S.D., with number of rats/group in parentheses. All rats were fed a high carbohydrate, fat-free diet for the duration of insulin treatment. Insulin treatment was 4 units each of regular and NPH insulin every 12 h. Statistical analyses were based on a comparison with untreated values using the Student's t test.

The overall stimulation of enzyme activity following insulin treatment was greater in diabetic rats than normal rats, 32-fold versus 15-fold.
response, leading to a 9-fold increase in enzyme concentration. This contrasted sharply with the delayed response observed in insulin-treated diabetic rats and with the significantly lower overall increase in enzyme concentration, approximately 4-fold. However, there has also been a dramatic increase in liver size in insulin-treated diabetic rats (4.8 g of liver/100 g, body weight to 12.1 g of liver/100 g, body weight) which did not occur in insulin-treated normal rats (4.3 g of liver/100 g, body weight, to 5.9 g of liver/100 g, body weight). This increase in liver size in insulin-treated diabetic rats is due to a change in cell volume which decreases the number of cells/g of liver and would account for the differences in enzyme concentration between the two experimental groups. These changes in enzyme quantity also indicate the involvement of a second mechanism in the diabetic animal. The 12-fold increase in enzyme quantity in the normal animal is probably sufficient to account for the 15-fold stimulation in the experimental groups. This would indicate whether a change in enzyme concentration between the two experimental groups has also been demonstrated for pyruvate kinase (27) and acetyl-CoA carboxylase (28). No evidence suggests that malic enzyme from diabetic rats reacts differently from the enzyme from normal or insulin-treated rats to the antibody. A direct correlation exists between enzyme activity and quantity in all experimental animals. Current studies are under way to analyze further the kinetic characteristics, molecular properties, and immunoreactivity of purifed hepatic malic enzyme isolated from alloxan-diabetic rats.

In conclusion, the insulin-mediated induction of hepatic malic enzyme in normal and diabetic rats involves two distinct regulatory processes. In insulin-treated normal rats, the increase in enzyme activity was due to a single mechanism, an increase in enzyme quantity. There was no change in the specific activity of the enzyme. This contrasts with the events occurring in insulin-treated diabetic rats where the increase in enzyme activity was due to a dual mechanism consisting of an increase in the quantity and the specific activity of the enzyme.

**Table II**

| Insulin effect on the specific activity of hepatic malic enzyme in normal and diabetic rats | Normal | Diabetic |
|---|---|---|
| Untreated | 2.12 ± 0.50 (6) | 0.81 ± 0.24 (4) |
| Insulin treated | 12 h | 2.11 ± 0.37 (4) | 1.68 ± 0.80 (5) |
| | 36 h | 2.15 ± 0.26 (4) | 2.75 ± 0.32* (4) |
| | 60 h | 2.31 ± 0.4i (3) | 2.54 ± 0.37* (7) |

* p < 0.001.

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