# STIMULATION BY INSULIN OF RAT LIVER β-HYDROXY-β-METHYLGLUTARYL COENZYME A REDUCTASE AND CHOLESTEROL-SYNTHESIZING ACTIVITIES

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## SUMMARY

β-Hydroxy-β-methylglutaryl coenzyme A reductase activity in rat liver increased 2 to 7-fold after subcutaneous administration of insulin into normal or diabetic animals. Reductase activity began increasing after one hour, rose to a maximum in two to three hours, and then declined to the control level after six hours. This response was elicited during the time of day when the normal diurnal variation in reductase activity approached a minimum. It was also elicited when animals did not have access to food. This stimulation of reductase activity was completely blocked when glucagon was administered in conjunction with insulin. The increase in reductase activity after insulin administration was accompanied by a proportionate increase in activity for the conversion of acetate to cholesterol.

It is well established that insulin exerts a marked effect on carbohydrate utilization, protein synthesis, and lipogenesis (1). The stimulatory effect of insulin on lipogenesis has been attributed to its antilipolytic action (2). However, another mode of action of this hormone, namely the induction of lipid-synthesizing enzymes, may be extremely important in increasing lipogenesis. Thus, a recent study from this laboratory (3) demonstrated that insulin induces a lipogenic enzyme, rat liver fatty acid synthetase, to a supranormal concentration and that glucagon acts antagonistically to insulin with respect to this induction (3). In view of these results it is of interest to learn whether other key lipogenic enzymes are also induced by insulin to higher concentrations. Of particular interest is the effect of insulin on the rate-limiting

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enzyme in cholesterol synthesis, β-hydroxy-β-methylglutaryl coenzyme-A (HMG-CoA) reductase, because of the correlation between serum cholesterol levels and atherosclerosis and the increased incidence of atherosclerosis in the human diabetic (4).

In this communication we demonstrate that insulin markedly stimulates hepatic HMG-CoA reductase activity with a concomitant rise in overall synthesis of cholesterol from labeled acetate. This effect of insulin is completely blocked by glucagon.

## METHODS

Treatment of Animals - Male albino rats from Holtzman, fed ad libitum for at least two days prior to experimentation, were housed in wire cages in a room that was artificially illuminated from 0600 to 1800 during each 24-hour period. The rats were maintained on a normal diet of Wayne Lab-Blox and weighed 140-160 g at the time of experimentation. Animals to be made diabetic were injected with 65 mg per kg of body weight of streptozotocin (courtesy of Dr. W. E. Dulin, Upjohn Co.) in the tail vein two days prior to experimentation. Insulin (regular Iletin) and glucagon (both obtained from Eli Lilly) were administered subcutaneously.

Preparation of Rat Liver Sub-Cellular Fractions - Animals were killed by decapitation and the excised livers were homogenized in three volumes of the indicated buffer by three strokes of a motor-driven teflon pestle in a Potter-Elvehjem homogenizer. When HMG-CoA reductase activity only was assayed, microsomes were prepared as described by Dugan et al. (5). When cholesterol synthesis from acetate was assayed, a 500 x g supernatant fraction of liver homogenate was prepared as reported by Lakshmanan et al. (6). After aliquots were removed for assay for the synthesis of cholesterol, microsomes were prepared as reported previously (5).

Enzymatic Assays - [$^{14}$C]HMG-CoA (815 dpm/nmole) was prepared from [3-$^{14}$C]dl-β-hydroxy-β-methylglutaric acid (obtained from New England Nuclear) by the method of Goldfarb and Pitot (7). Enzymatic reduction of HMG-CoA to mevalonic acid was carried out as described by Dugan et al. (5). The reaction was terminated and mevalonic acid was lactonized by adding hydrochloric acid to pH 1. The reaction mixture was lyophilized and the residue was extracted with acetone. Radioactive mevalonolactone was purified by thin-layer chromatography (8) and then assayed for radioactivity in

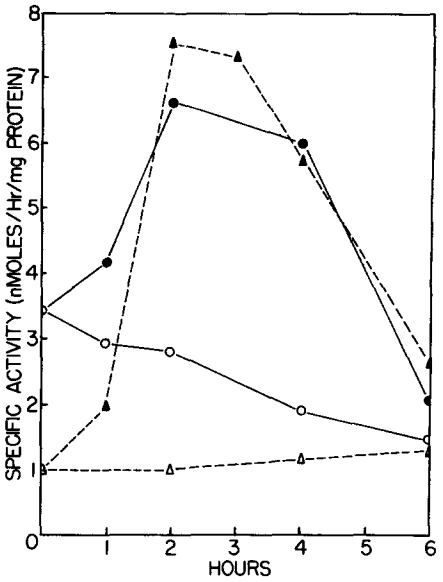


Fig. 1. Effect of insulin on HMG-CoA reductase activity as a function of time. At 0800 hrs insulin was administered to normal and 2-day diabetic rats in doses of 3 units and 6 units per 100 g body weight, respectively. The animals were killed at the specified time intervals and their hepatic HMG-CoA reductase activities were determined as described in Methods. Each point represents the average of values obtained from assaying 2 to 4 rats individually. o——o, normal control rats; •—•, normal rats treated with insulin; Δ---Δ, diabetic rats; ▲---▲, diabetic rats treated with insulin. The groups of normal and diabetic rats treated with insulin for 2 hrs were significantly different ($p < 0.05$) from their corresponding control groups by Student's t test.

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dioxane phosphor in a Packard liquid scintillation spectrometer. The enzymatic conversion of [2-$^{14}$C]acetate (550 dpm/nmole) to non-saponifiable compounds and the preparation of the digitonin precipitable fraction were carried out as described by Lakshmanan et al. (6). Digitonin precipitates were assayed for radioactivity in dioxane-phosphor solution. Gas-liquid chromatography of the radioactive, non-saponifiable fraction, with cholesterol added as a mass marker, was carried out at 212° on a 6 ft x 6 mm column of 1% SE 30 on Gas Chrom Q in a Barber Colman Model 10 instrument. Eluate fractions were collected on glass wool in Z-shaped glass tubes that were cooled in a dry ice-acetone bath.

## RESULTS

The time course of hepatic microsomal HMG-CoA reductase activity

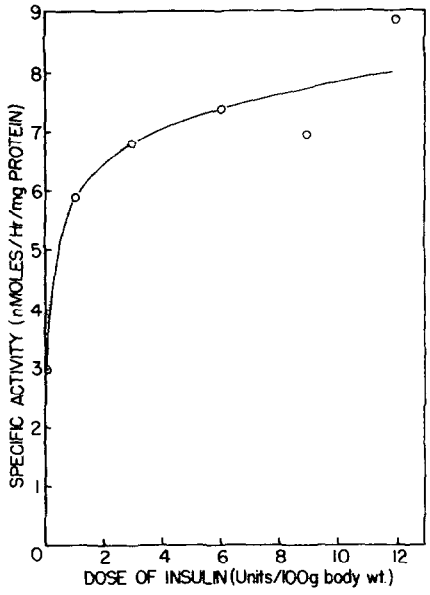


Fig. 2. Effect of insulin dose on HMG-CoA reductase activity. The insulin was administered to normal rats at 0800 hrs at the indicated dosages and the animals were killed 2 hrs later. Liver HMG-CoA reductase activity was determined as described in Methods. Each point represents the mean obtained from assaying 3 rats individually. The group treated with 3 units or more of insulin were significantly different ($p < 0.05$) from the untreated group.

after subcutaneous administration of insulin is shown in Fig. 1. It is apparent that insulin increased hepatic HMG-CoA reductase activity by 2 to 3-fold in ad libitum-fed normal rats within two hours. The rapid rise in enzyme activity was followed by a rapid decline to control levels approximately six hours after insulin administration. The stimulatory effect of insulin was more pronounced in ad libitum-fed diabetic rats with a 7 to 8-fold increase in reductase activity. Both diabetic and normal rats showed a maximal response two hours after insulin treatment. In the experiments in which the time response of HMG-CoA reductase activity was measured, insulin was administered subcutaneously at 0800. Thus, the observed rise in hepatic HMG-CoA reductase activity was elicited at a time when the activity of this enzyme was declining towards the minimum in its normal daily cycle (5).

The activity of hepatic HMG-CoA reductase was measured two hours after administration of varying quantities of insulin, Fig. 2. Three units of

insulin per 100 g of body weight gave nearly maximum stimulation. Hence, this dosage was employed in subsequent experiments.

In order to show that the observed rise in HMG-CoA reductase activity following insulin injection is not produced as a result of food intake, six normal rats were deprived of food from 0800 to 1400. Three of them received insulin (3 units/100 g) at 1200 hours and all were killed at 1400 hours. Strikingly, the insulin-treated rats showed a 5-fold increase in HMG-CoA reductase specific activity (nmoles of mevalonic acid formed per hour per mg of microsomal protein) over that of the corresponding untreated rats (insulin treated 3.88 ± 0.96; control 0.78 ± 0.12). In this experiment and all others reported in this communication the change in the activity of HMG-CoA reductase per liver and per gram liver was proportional to the change per mg of microsomal protein.

An experiment was carried out in which the effects of insulin on the cholesterol-synthesizing activity from acetate and HMG-CoA reductase were measured. Livers of four normal rats, two of which were treated with three units of insulin per 100 g of body weight two hours prior to killing, were assayed for HMG-CoA reductase and cholesterol synthesizing activities. The specific activity for HMG-CoA reductase was 3.1 ± 0.5 for insulin-treated as compared to 1.5 ± 0.3 for untreated animals. In these same animals the specific activity for cholesterol synthesis (nmoles acetate incorporated into digitonin precipitable non-saponifiable compounds per mg of 500 x g supernatant fraction of liver homogenate per hour) was 0.88 ± 0.21 for insulin-treated and 0.41 ± 0.10 for untreated animals. In each case the ratio (insulin-treated/untreated) is slightly more than 2. Analogous results were obtained with diabetic rats. Gas-liquid chromatography of selected samples established that the major radioactive component of the non-saponifiable fraction chromatographed coincident with cholesterol.

Since glucagon is a physiological antagonist of insulin, an experiment was performed to ascertain whether glucagon can block the insulin-mediated increase in HMG-CoA reductase activity. The results in Table I show that glucagon completely blocked the effect of insulin on HMG-CoA reductase activity, when injected two hours prior to insulin administration. Glucagon, by itself, had little effect on HMG-CoA reductase activity. However, it should be noted that it was administered at the time of day when the activity of this

TABLE I

ELIMINATION BY GLUCAGON OF THE STIMULATORY EFFECT OF INSULIN ON HMG-CoA REDUCTASE ACTIVITY

| Treatment | Enzyme Activity (nmoles mevalonate /hr/mg protein) |
|---|---|
| None | 2.18 ± 0.24 |
| + Insulin | 6.10 ± 0.20 |
| + Glucagon | 2.37 ± 0.45 |
| + Insulin and glucagon | 1.76 ± 0.49 |

Insulin was administered subcutaneously to normal rats at a dosage of 3 units per 100 g of body weight two hours prior to killing. Glucagon was also administered subcutaneously at a dosage of 200 μg per 100 g of body weight four hours prior to killing. All rats were killed at 1400 hours. Each value represents the mean (± S.E.) from individual assays on each of three rats.

diurnally varying enzyme is minimal and when there is little or no synthesis of the reductase (9).

## DISCUSSION

The present data clearly show that insulin administration promotes a rapid and marked increase in hepatic HMG-CoA reductase and cholesterol-synthesizing activity in both normal and diabetic rats. The rapid (2-hour) response in HMG-CoA reductase activity to insulin administration is in marked contrast to the previously reported responses of this enzyme to noradrenalin (10) and to thyroxine (11) which required 12 and 30 hours, respectively.

Since it was previously established (9) that the rapid rise in the activity of HMG-CoA reductase during its diurnal cycle is due to an increased rate of synthesis of the enzyme and not to a decreased rate of degradation or to activation of pre-existing enzyme, it seems probable that the insulin mediated response is also due to a change in the rate of synthesis of this enzyme. The results of preliminary studies with cycloheximide carried out in our laboratory are consistent with this conclusion. The rapid decline observed in HMG-CoA reductase after insulin administration is also consistent with the short half-life (2 to 3 hours) of this enzyme (5, 9).

The marked rise in the level of liver HMG-CoA reductase and cholesterol-synthesizing activity as a result of insulin administration may have clinical implications. There is a high incidence of cardiovascular disease in diabetics (4) and indeed, hyperinsulinism in non-diabetic animals on a normal diet has been shown to lead to vascular lesions (12). It has also been observed that human subjects who secrete abnormally large amounts of insulin in response to dietary carbohydrate have a particularly high incidence of vascular disease (13).

Investigations are currently in progress to determine if the stimulation of HMG-CoA reductase activity by insulin results from new protein synthesis of the enzyme, and if so, whether insulin controls the synthesis of the reductase at the level of transcription or translation. We are also investigating the role of insulin in relation to the normal diurnal rise in HMG-CoA reductase activity, especially since the level of blood insulin (14), like HMG-CoA reductase (5), varies diurnally and rises following a meal.

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