

Mechanism of Exercise-Induced Hypoglycemia in Depancreatized Dogs Maintained on Long-Acting Insulin

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ABSTRACT Human diabetics on intermediate and long-acting insulin occasionally become hypoglycemic during exercise. We have shown previously that during exercise, hypoglycemia did not occur in depancreatized insulin-infused dogs because the increments in glucose production and utilization were proportional and of the same magnitude as in normal dogs. Therefore, to elucidate the mechanism of the glucose-lowering effect of strenuous exercise, we measured glucose production and utilization, metabolic clearance of glucose, and serum immunoreactive insulin in postabsorptive depancreatized dogs 8 h after a subcutaneous injection of protamine zinc and crystalline insulin. During rest, plasma glucose was stable, but ranged between hypoglycemia and hyperglycemia. Hyperglycemia was associated with overproduction of glucose, indicating insulin deficiency despite normal or elevated serum immunoreactive insulin. Glucose clearance, as in normal dogs, increased threefold but glucose production increased only marginally (50%) and, consequently, glucose decreased in plasma. The decrease of plasma glucose was directly proportional to the pre-exercise concentration and production of glucose. The magnitude of inhibition glucose production was not correlated with the serum immunoreactive insulin indicating either that some released insulin was not active or that a moderate immunoreactive insulin increment induced a near-maximal inhibition. It is concluded that in depancreatized dogs injected with protamine zinc insulin, exercise accelerates mobilization of insulin from its injection site presumably because of increased blood and lymph flow. Glucose utilization did not exceed that in normal dogs, but hepatic glucose production failed to increase sufficiently to meet the needs of muscle in exercise.

INTRODUCTION

Diabetics on intermediate and long-acting insulin occasionally become hypoglycemic during exercise. However, the mechanism of exercise-induced hypoglycemia has not as yet been elucidated. We have shown previously that strenuous exercise did not decrease plasma glucose levels in depancreatized dogs infused with crystalline insulin into the portal vein, because the increments in the rates of glucose production (R_a)¹ and utilization (R_d) were proportional and of the same magnitude as in normal dogs. During exercise, both in normal and depancreatized dogs, serum immunoreactive insulin (IRI) levels were below normal (1); a low serum IRI is one of the factors which can lead to stimulation of glucose production by the liver. A critical amount of insulin appeared to be essential for the control of glucose uptake in the working muscle because R_d increased only marginally when insulin infusion was discontinued in depancreatized dogs (1); even a subnormal level of IRI could be important because relatively larger quantities of the hormone could become available to the muscle when the increased blood flow and capillary surface area are associated with exercise. The necessity of insulin for glucose metabolism was also demonstrated by Berger et al. (2), in perfused working muscle. Sanders et al. (3) and Wahren et al. (4) have observed normal increments of glucose utilization during exercise in diabetics 24 h after insulin injection. Such findings could indicate that the remaining insulin was still adequate.

Our aim was to determine whether strenuous exercise would decrease plasma glucose when depancreatized dogs were injected with protamine zinc (pz) and crystalline insulin. A decrease in plasma glucose could occur if exercise increased glucose utilization in muscle more in diabetic than in normal dogs, or if in-

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¹Abbreviations used in this paper: IRI, immunoreactive insulin; PZ, protamine zinc; R_a , glucose production; R_d , glucose utilization.

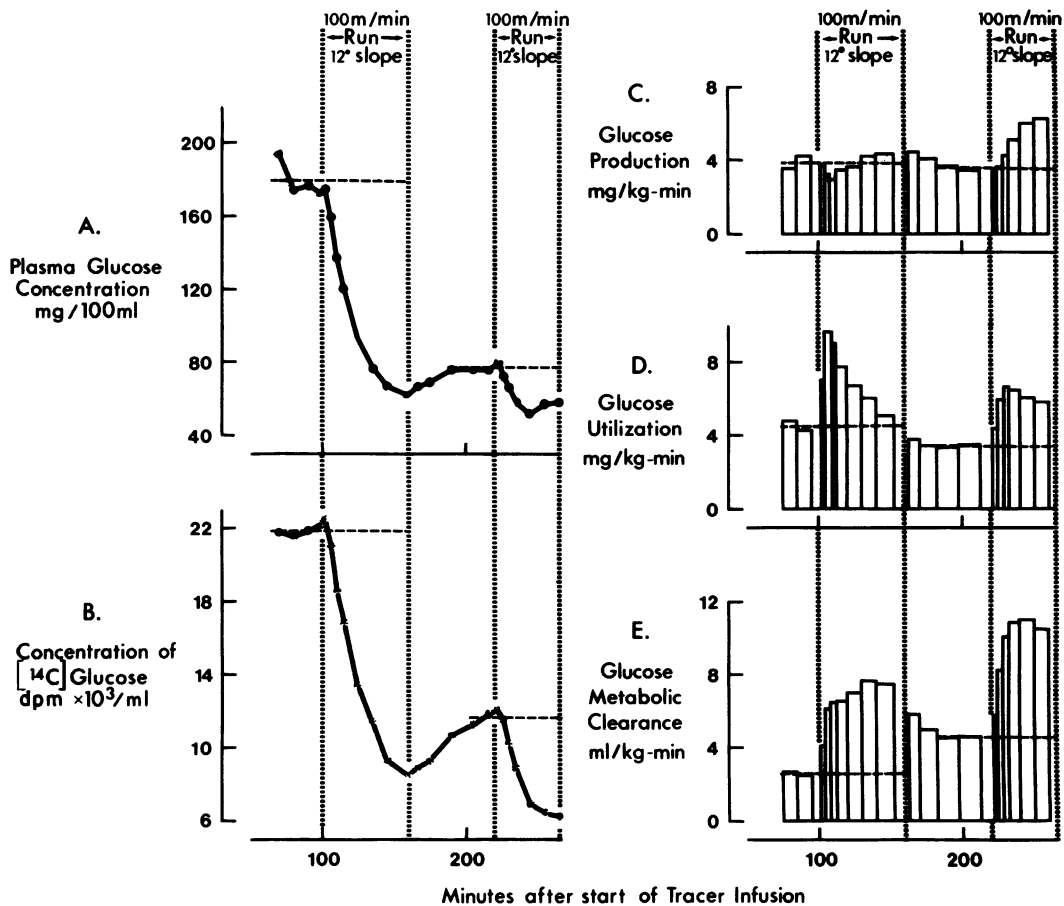


FIGURE 1 The effects of exercise (Run: 100 m/min, 12° slope) on (A) plasma glucose concentration; (B) concentration of [¹⁴C]glucose; rates of (C) production, (D) utilization, and (E) metabolic clearance of glucose in one depancreatized dog (no. 1) 8 h after injections of PZ (11 U) and crystalline insulin (11 U), and feeding. Exercise induced a decrease in plasma glucose levels because during both exercise periods the increments in glucose production did not match the increments in glucose utilization.

crements in R_a were insufficient to meet the fuel requirements of the working muscle. Either or both of these effects could result if serum IRI levels were substantially higher in depancreatized than in normal exercising dogs. Therefore, glucose concentration, glucose turnover, and serum IRI were determined during rest and exercise in depancreatized dogs 8 h after an injection of PZ insulin.

METHODS

Animals and surgical procedures. 3–4 wk before pancreatectomy, five male mongrel dogs (12–19 kg) were trained to lie quietly or to run on the treadmill for 60 min (100 m/min at a slope of 12°). Such a strenuous exercise increases the overall oxygen uptake five to sevenfold (1, 5). The dogs were given a high protein diet supplemented by raw pancreas and tablets containing pancreatic enzymes before and after pancreatectomy (1). 9–17 days after total pancreatectomy, the dogs were treated with porcine insulin (Connaught Laboratories, Toronto, Canada), which has an amino acid sequence

identical to that of canine insulin, and does not induce formation of antibodies (6). 11–14 U, PZ insulin was administered subcutaneously each morning and 8–14 U crystalline insulin was given subcutaneously twice daily after each feeding; glycosuria was usually less than 0.1 g/kg per day; ketone bodies were not detected in the urine and the mean body weight decreased by less than 4%. The first post-pancreatectomy experiment was performed 10–17 days after pancreatectomy. On the day of the experiment a vinyl cannula was inserted through the jugular vein into the right atrium for sampling of blood. The cannula for the infusion of [¹⁴C]glucose was inserted through a saphenous vein into the inferior vena cava. After completion of the first experiment, the dogs were fed and given subcutaneous injections of PZ and crystalline insulin. The second experiment was then performed. At autopsy, the patency and site of all catheters were verified; no pancreatic tissue was found in any dog, and there was no evidence of infection.

Experimental design. Two experiments (exercise and control, respectively) were performed in each of the five dogs, 8–9 h after food intake and the last subcutaneous crystalline insulin injection into the thigh. Each exercise experiment consisted of (a) a 100-min control resting period, (b) a 60-

min interval of treadmill running (100 m/min on a slope of 12°), (c) a postrun rest period of 60 min, and (d) a second treadmill run for 45–60 min. For the controls, measurements were made in dogs which had been lying quietly for 5 h, thus providing information about glucose turnover and plasma IRI concentration 8–14 h after the last insulin injection but in the absence of exercise.

Processing of blood samples and tracer methods. The collection and processing of blood samples and the tracer methods have been described in detail previously (1). Glucose was isolated with the aid of an ion-exchange resin (Ag-2 × 8, Bio-Rad Laboratories, Richmond, Calif.), its plasma concentration was determined by a glucose oxidase method, and the concentration of labeled glucose by liquid scintillation counting. Serum IRI was determined in triplicate using the AmershamSearle Kit (AmershamSearle Corp., Arlington Heights, Ill.). In all experiments, a priming dose (37 μ Ci) of [14 C]glucose (New England Nuclear, Boston, Mass.) was injected intravenously at the beginning of the initial rest period ($t = 0$) and a tracer dose (2 μ Ci/ml isotonic saline) of labeled glucose was infused throughout the experimental period at 0.167 ml/min. The rate of glucose production ("rate of appearance", R_a), utilization ("rate of disappearance", R_d), and metabolic clearance (R_d divided by the prevailing concentration of glucose in plasma) were calculated by the method of primed tracer infusion (7), as modified and substantiated previously (8–10). In our previous paper the glucose turnover rates were corrected for recycling of radioactive glucose. In resting, depancreatized insulin-treated dogs, such a correction yielded 17% higher glucose turnover rates, but exercise had only a marginal further effect (7%) on the contribution of recycling to the turnover rates (1). It was felt that such a small correction was not necessary for the scope of the present paper.

RESULTS

Exercising dogs. Fig. 1 illustrates the metabolic effects of exercise in one representative experiment. During rest, 8 h after insulin injection and feeding, stable hyperglycemia prevailed, and during both exercise periods, plasma glucose decreased (Fig. 1A). A constant infusion of the tracer maintained the concentration of labeled glucose in the steady state during rest but [14 C]glucose declined markedly during exercise. It increased in the postrun resting period, and declined again during the second exercise (Fig. 1B); the decrease of [14 C]glucose corresponded to the increase in the metabolic clearance of glucose (Fig. 1E). The increments in glucose clearance were of the same magnitude in both exercise periods and were comparable to those in normal running dogs (1). Glucose production did not change during the first exercise and increased slightly during the second exercise (Fig. 1C). The increments in glucose utilization were smaller than the increments in the metabolic clearance; in both periods plasma glucose concentration decreased progressively. Glucose concentration influences the rate of glucose utilization through its mass effect, but does not affect its metabolic clearance (11). In normal dogs, exercise induced the same increase in both glucose production and utilization (1), whereas in depancreatized dogs maintained on PZ and regular insulin,

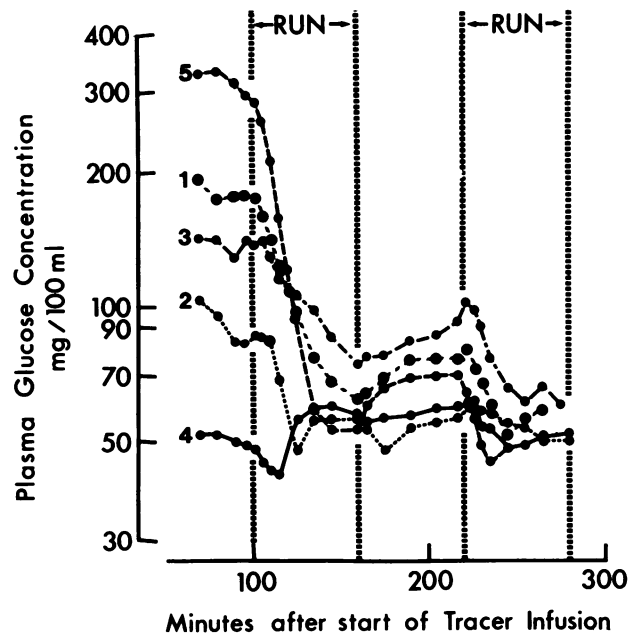


FIGURE 2 The effect of exercise on plasma glucose concentration in five depancreatized dogs 8–9 h after injections of PZ (11–18 U) and crystalline insulin (8–14 U), and feeding. The ordinate (glucose concentration) is on a logarithmic scale. Numbers identify each dog.

exercise induced hypoglycemia because R_d did not match R_a .

Fig. 2 illustrated plasma glucose concentrations in the five exercising dogs. The pre-exercise glucose concentrations were relatively stable but ranged between hypoglycemia and hyperglycemia. As expected, statistical analysis showed that a higher glucose concentration was directly correlated with glucose production ($r = 0.83$, $P < 0.02$). During both exercise periods

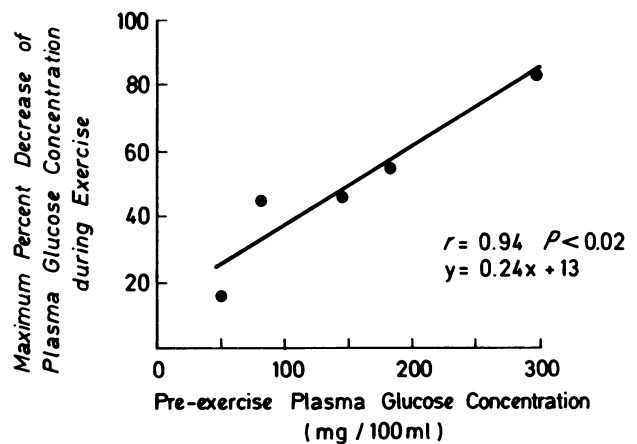


FIGURE 3 Positive correlation between pre-exercise glucose concentration and the maximum exercise-induced decrease of glucose in plasma (percent) during the first exercise period in five dogs.

plasma glucose fell in four of five animals. (Only a small transient decrease occurred in dog 4, which was hypoglycemic during the rest period.) Glucose concentrations leveled off at 50–60 mg/100 ml. The magnitude of the fall in the glucose concentration was greater when the resting value had been high; a significant positive correlation between the initial glucose levels and its maximum percent decrease in plasma is shown in Fig. 3. As shown in Fig. 4 the mean pattern of R_a , R_d , and metabolic clearance during rest and exercise in

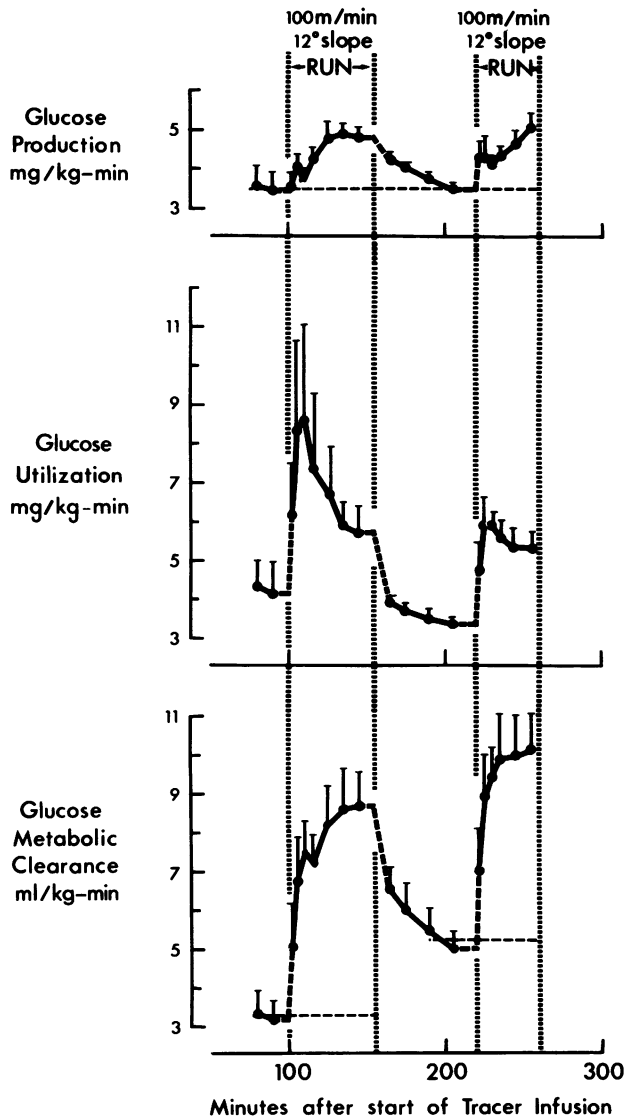


FIGURE 4 The effect of exercise on mean (\pm SEM) production, utilization, and metabolic clearance of glucose in five depancreatized dogs 8–9 h after injection of insulin and feeding. During the first exercise period glucose production, utilization, and clearance were significantly increased at 35 ($P < 0.05$), 15 ($P < 0.05$), and 6 min ($P < 0.025$), and during the second period at 10 ($P < 0.05$), 6 ($P < 0.025$), and 6 min ($P < 0.025$), respectively.

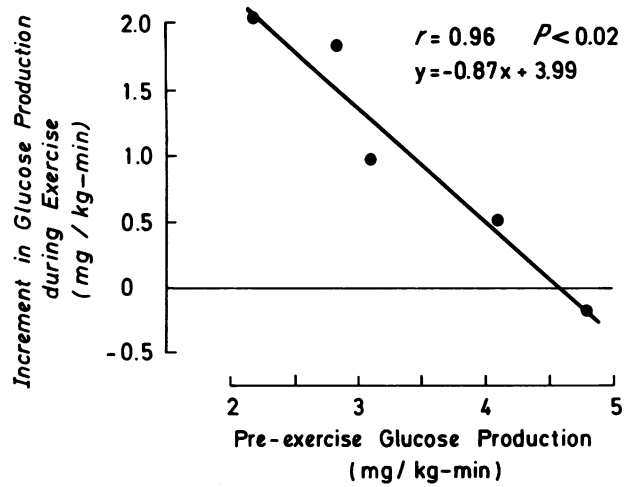


FIGURE 5 Negative correlation between pre-exercise glucose production and the average glucose production increment during the first exercise period in five depancreatized dogs 8–9 h after administration of insulin and food.

five dogs was the same as in the dog described in Fig. 1. During both exercise periods, R_a increased to a small extent only, and it did not match the larger increase in R_d . The increments of the metabolic clearance indicate that running had essentially the same effect on glucose uptake by the muscle during both exercise periods. The exercise-induced increments in R_a were larger when the pre-exercise R_a was smaller (Fig. 5), but there was no relationship between the resting and exercise-induced increments of the clearance rates of glucose. Thus, exercise induced a moderate decrease in plasma glucose when glucose levels and R_a were normal, and a precipitous fall when they were high.

Fig. 6 illustrates individual serum IRI values in the five exercising dogs. 8 or 9 h after the last injections of insulin, serum IRI was stable (20–40 μ U/ml), but increased during the 10 exercise periods. This increase was marked during the first exercise period in three dogs (nos. 1, 2, and 4) and moderate in the remaining two dogs. We have observed previously that mean serum IRI decreased 40% during strenuous exercise in normal dogs (from $20 \pm 3 \mu$ U/ml to $11 \pm 2 \mu$ U/ml) (1) and these values are also shown in Fig. 6. The shaded area (difference between IRI values in the PZ insulin-treated and normal dogs) thus indicates the excess concentration of insulin. Depancreatized dogs had more serum IRI than normal dogs, not only because they could not decrease IRI release during exercise, but also because the mobilization from the depot was accelerated. The exercise-induced inhibition of R_a and the stimulation of metabolic clearance were not larger when IRI was higher (first exercise period nos. 1, 2, and 4), indicating that even a moderate increase of insulin was sufficient to induce hypoglycemia.

Nonexercising dogs. To determine whether signifi-

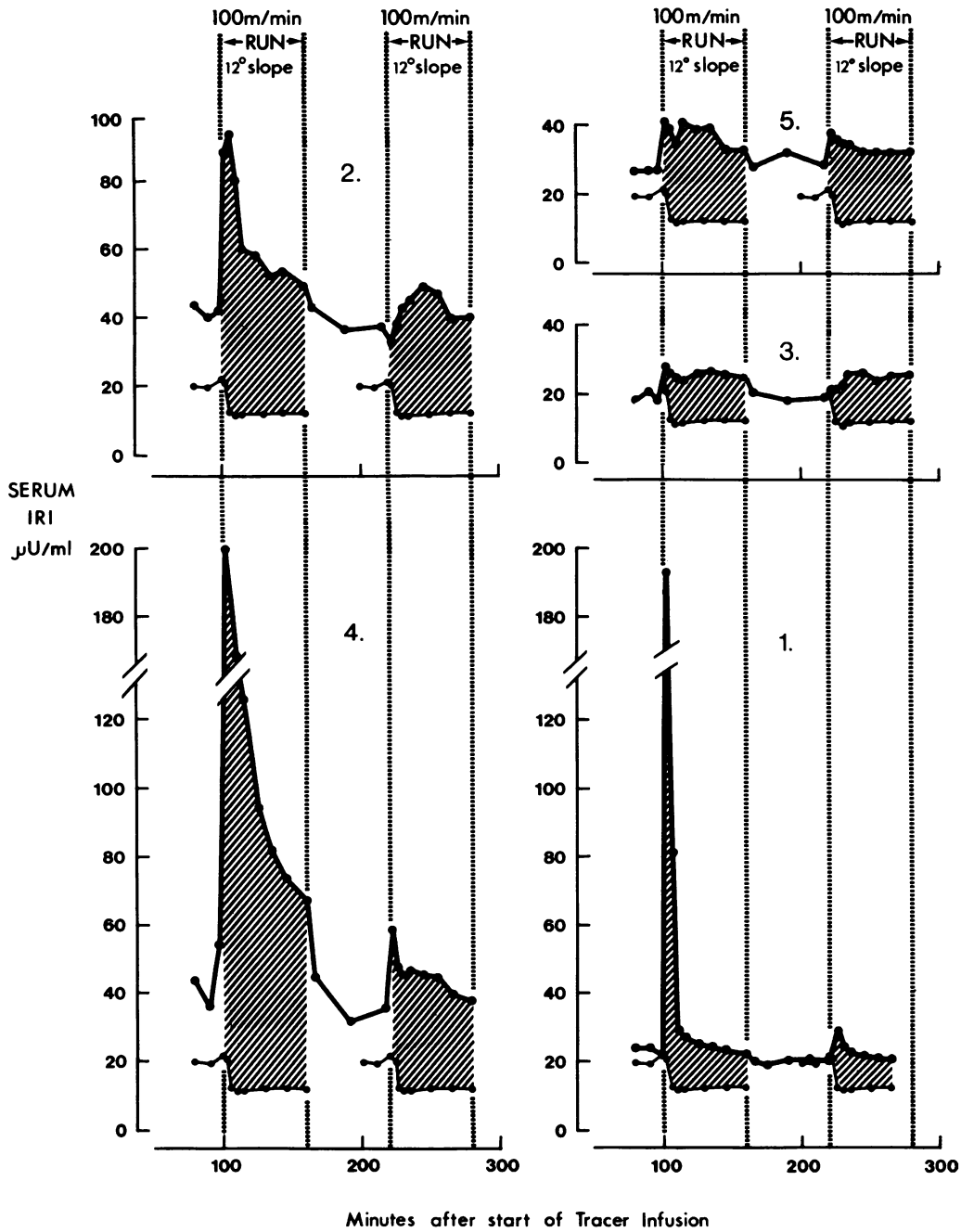


FIGURE 6 Serum concentrations of IRI during rest and exercise in five depancreatized dogs 8–9 h after administration of food and insulin. Shaded areas designate the differences between individual IRI levels in each depancreatized dog (●), and mean levels of IRI in five normal dogs (○). Mean IRI during rest and exercise in normal dogs are replotted from our previous paper (1). Numbers identify each dog. In each depancreatized dog, IRI increased during exercise and all exercise IRI values were higher than in normal dogs.

cant variations in concentration and glucose turnover occur in the absence of exercise, five depancreatized dogs were allowed to rest for 13 h after the insulin injection and blood was sampled from $t = 8$ –13 h. The mean data of three dogs in which glucose turnover was

studied are shown in Fig. 7. IRI ranged between 35 and 50 $\mu\text{U/ml}$ and plasma glucose decreased slightly. The concentration of radioactive [^{14}C]glucose was stable, indicating that glucose turnover was in a steady state. In another two resting dogs in which turnover was not

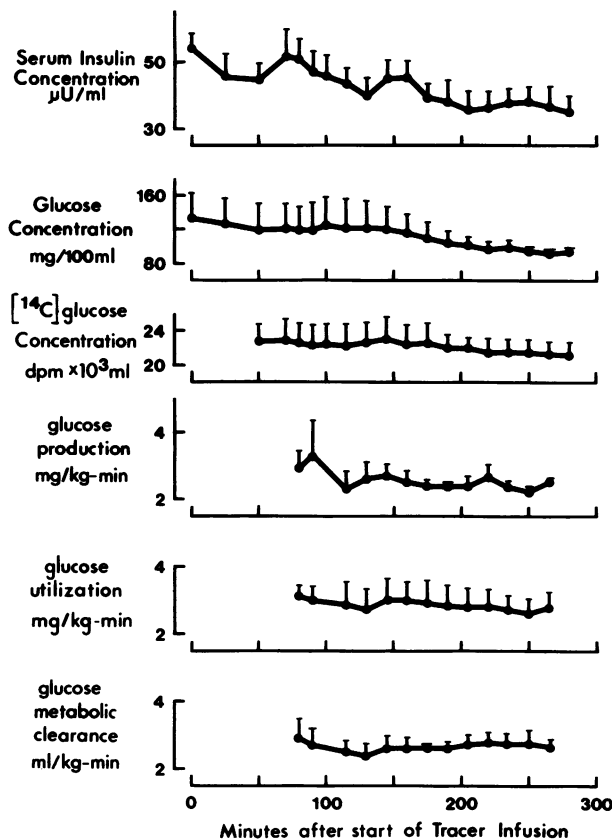


FIGURE 7 Mean (\pm SEM) serum IRI, unlabeled and labeled glucose concentrations, production, utilization, and metabolic clearance rates in three depancreatized resting dogs 8–13 h after injections of insulin (8–9 U) and feeding. All the parameters measured were near a steady state.

studied, the mean plasma glucose was stable at 290 and 70 mg/100 ml, and the mean IRI at 23 and 37 μ U/ml, respectively.

DISCUSSION

Exercise is important in the management of diabetics; increased physical activity can lead to a diminution in insulin requirements and to improvement in glucose tolerance (12). Some of these effects may be attributed to the redistribution of blood flow during exercise, so that a greater fraction of circulating insulin is channeled into the muscle. Exercise-induced hypoglycemia can, however, cause management problems. We have shown that exercise induces a decrease in plasma glucose in depancreatized PZ insulin-injected dogs. IRI rose in plasma indicating that exercise might accelerate mobilization of the hormone from its depot. To illustrate the metabolic effects of excess insulin we compared these data to those previously published in normal and in depancreatized dogs (Fig. 8). In depancreatized dogs on PZ and crystalline insulin, the

exercise-induced R_a increment was only marginal; it was much lower than in normal dogs or in depancreatized dogs either untreated or given crystalline insulin by constant infusion into the portal vein. The increment in metabolic clearance was not significantly higher than in normal or depancreatized insulin-infused dogs, but it was much higher than in diabetic insulin-deprived dogs. Thus, the increase in serum IRI did not induce an increase in glucose utilization larger than in normal exercising dogs, but it inhibited the regulatory mechanisms which promote R_a .

We feel that increased plasma IRI reflects an increased IRI mobilization rather than a decrease in its degradation because serum IRI did not change in depancreatized running dogs infused with insulin at a constant rate (1). The effect of exercise on the release of insulin from its depot might be caused by increased blood and lymph flow of the exercising limb and this could deliver, rather abruptly, some of the free insulin (which had accumulated in the extracellular fluid) into the bloodstream. Surprisingly, highest IRI levels did not induce the largest decreases in plasma glucose. For example, in dog 5, glucose decreased by 250 mg/100 ml at a time when only a moderate increment in IRI occurred. Thus, either some mobilized insulin was not biologically active or even moderate increments in IRI exerted a near-maximal effect. The

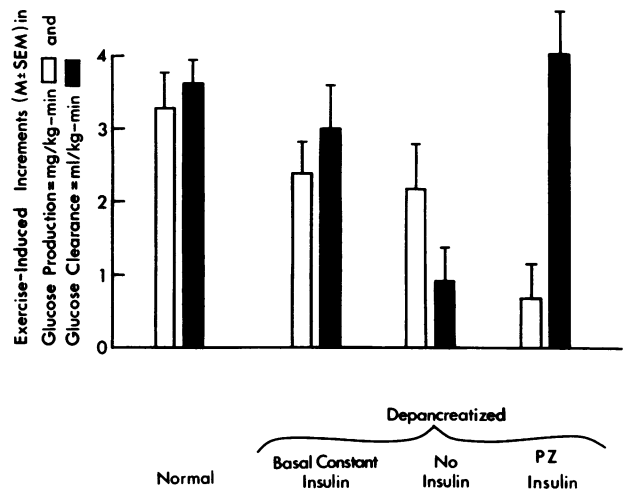


FIGURE 8 Exercise-induced average increments ($M \pm SEM$) in glucose production (R_a) and metabolic clearance. The mean turnover data in five depancreatized dogs treated with PZ insulin are compared to normal and depancreatized dogs recalculated from (1). In the previous work (1) R_a and metabolic clearance were measured in depancreatized dogs, either maintained on an infusion of insulin (230 μ U/kg-min) or deprived of insulin. In dogs on PZ insulin, R_a increments were lower than in normal dogs ($P < 0.01$), or in other depancreatized dogs ($P < 0.05$); metabolic clearance increments were higher than in insulin-deprived dogs ($P < 0.005$), but were not different from those observed in normal or depancreatized insulin-infused dogs.

magnitude of the hypoglycemic effect of exercise was, however, related to the resting "diabetic state" of the dogs. The rate of decrease of plasma glucose was directly correlated to the pre-exercise concentrations (Fig. 3) and production rates of glucose (Fig. 5), but not to resting metabolic clearance.

It is known that the regulation of glucose production during exercise is multifactorial (1), and therefore a reduced activity of one or more factors can be compensated by others. Similar R_a increments occurred when plasma immunoreactive glucagon increased or remained unchanged in dogs (1) and humans (13) as well as in humans under circumstances where a decrease in IRI was prevented during exercise (14). It is surprising, therefore, that R_a was inhibited even when only a moderate amount of insulin was mobilized in our PZ insulin-injected dogs, and when endogenous insulin secretion was only slightly increased in diabetic patients (15). The sensitivity of the liver to small IRI increments in the depancreatized dogs was perhaps due to unchanged immunoreactive glucagon levels during exercise (1), because when glucagon was infused into depancreatized resting (11) or exercising dogs (16), much higher IRI increments could not prevent an increase in R_a .

We conclude that exercise induces a decrease in plasma glucose in depancreatized dogs on PZ insulin, because exercise does not suppress but enhances the mobilization of insulin from its injection site. The blood glucose decrease did not result from a larger than normal increase in glucose uptake by muscle, but from a failure of hepatic glucose production to meet the increased energy needs of the exercising muscle. Our preliminary data indicate that a similar mechanism of hypoglycemia occurs also in diabetic patients. When exercising diabetic patients received a constant insulin infusion, glucose turnover increased, but glycemia remained unchanged; however, after a subcutaneous injection of isophane or lente insulin (an insulin preparation more commonly used than PZ insulin) plasma glucose fell because glucose utilization increased and glucose production was suppressed (17).

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