Effect of Exercise Training and Sucrose Feeding on Insulin-stimulated Glucose Uptake in Rats with Streptozotocin-induced Insulin-deficient Diabetes

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SUMMARY

The effect of exercise training and a sucrose-rich diet on insulin-stimulated glucose disposal was studied in rats with streptozotocin-induced insulin deficiency. Rats were injected with streptozotocin (40 mg/kg), and 3 days later divided into three groups with equal degrees of hyperglycemia. One group of rats was allowed to run spontaneously on exercise wheels, another group remained sedentary but ate a sucrose-rich diet (66% sucrose), and the third also remained sedentary but consumed conventional rat chow. Three weeks later, we determined the effect of these various programs on postabsorptive plasma glucose and insulin levels, as well as on the ability of exogenous insulin to stimulate disposal of a glucose load during a period in which endogenous insulin was suppressed by epinephrine and propanolol. Basal plasma insulin levels were the same in all three groups, but plasma glucose levels were significantly lower ($P < 0.001$) in exercise-trained rats, and significantly higher ($P < 0.05$) in sucrose-fed rats, than in chow-fed diabetic rats. The inference that exercise training markedly enhanced insulin action in rats with insulin deficiency was borne out by direct estimation of insulin-stimulated glucose disposal. In contrast, sucrose-fed diabetic rats seemed to be more insulin-resistant than chow-fed diabetic rats. These results provide direct evidence that spontaneous exercise can dramatically attenuate the severity of diabetes in insulin-deficient rats by enhancing insulin action. DIABETES *32*:165–168, February 1983.

Experimentally-induced insulin deficiency leads to the development of in vivo insulin resistance in normal dogs,[1] and a decrease in insulin-stimulated glucose utilization has been observed in adipocytes isolated from insulin-deficient rats.[2,3] Consequently, it might be anticipated that environmental manipulations that enhance insulin sensitivity would ameliorate the diabetic state in subjects with insulin deficiency. In this regard, reports have recently appeared that demonstrated that insulin-stimulated glucose uptake was increased in exercise-trained normal rats, and this effect seemed to be due to a potentiation of insulin action on muscle.[4,5] Given these observations, it seemed possible that exercise training would modulate severity of insulin-deficient diabetes, and we have recently published evidence in support of this view. Specifically, moderately insulin-deficient rats, allowed to run spontaneously, had lower postabsorptive plasma glucose, free fatty acid, and triglyceride levels than did sedentary diabetic rats.[6] We argued that the most likely explanation for our findings was that in vivo insulin action had been enhanced in the exercised rats. Similar results and conclusions as to the beneficial effects of exercise training on streptozotocin-induced diabetes in the rat have also recently been reported by Tancrede et al.[7] However, neither study contained any direct experimental evidence of the putative ability of exercise training to alleviate the insulin resistance associated with experimentally-induced insulin deficiency. In light of the potential therapeutic utility of exercise training in the management of diabetes, we thought it essential to provide experimental verification of the beneficial effects of exercise training on insulin-stimulated glucose utilization in insulin-deficient rats. Therefore, we have used a recently developed technique for estimating in vivo insulin action in the rat,[8] and attempted to quantify the effects of exercise and diet on insulin-stimulated glucose utilization by rats with experimentally-induced insulin deficiency of a relatively severe degree.

MATERIALS AND METHODS

These experiments were conducted on male Sprague-Dawley rats, 6 wk old at the beginning of the studies. Insulin-

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deficient diabetes was induced by i.v. injection of streptozotocin, 40 mg/kg, diluted in 0.01 M citrate buffer (pH 4.5). Blood was obtained from the tail vein 3 days later and analyzed for glucose. Rats with glucose values ≤ 250 and ≥ 400 mg/dl were excluded from further study. The remaining animals were randomly divided into three groups with comparable mean plasma glucose values, e.g., 342 mg/dl versus 336 mg/dl versus 341 mg/dl. One group was maintained in standard laboratory cages, and fed conventional chow which contained (as percent calories) 60% vegetable starch, 29% protein, and 11% fat. The second group was also housed in standard cages, but fed a diet containing 66% sucrose, 22% protein, and 12% fat (Teklad Labs). The third group ate conventional chow, but were housed individually in exercise wheel cages (Wahman Co., Timonium, Maryland, USA), and allowed to run at their own pace. The cages, as supplied by the manufacturer, consist of a rotating wheel cage with the number of revolutions/day recorded by a cyclometer attached to the wheel axis plus an adjoining feed cage. To maximize exposure to the wheel, we replaced the feed cage attachment with a small feeding trough large enough to hold 4–5-days food supply. Thus, the exercising animals remained entirely in the wheel portion of the cage to run at will and feed on laboratory chow ad lib from the attached feeding trough. Preliminary studies indicated that approximately two out of three of the rats placed in the modified exercise wheel cages showed a progressive increase in running activity and average between 2.0–3.0 miles/day after 10 days exposure. Animals unable to attain a level of 2.0 miles/day were excluded from the study.

All experiments were performed 21 days later, starting at 1400 h, 6 h after withdrawal of food and removal from the exercise wheels. Blood samples were collected from the tail vein for determination of postabsorptive glucose[9] and insulin[10] concentrations, and the rats then anesthetized with sodium thiamyal. Following this, a constant infusion of epinephrine (0.8 μg/kg/min), propanolol (0.0017 mg/kg/min), glucose (8 mg/kg/min), and porcine insulin (5 mU/kg/min) were given for 180 min through a cannula inserted into the right jugular vein for 150 min.

Endogenous insulin is suppressed under these conditions,[11] and steady-state plasma glucose (SSPG) and insulin (SSPI) levels are reached by 90 min. Tail blood samples were collected before the infusion was started, and at 10-min intervals during the last 60 min of the infusion. The mean value of these seven measurements was used to estimate the SSPG and SSPI values of each experiment, and studies in which the coefficient of variation exceeded 10% were excluded. Insulin radioimmunoassays used rat insulin as standard when determining insulin concentration in the basal state, and porcine insulin for measurement of insulin levels during the infusion.

Mean (±SEM) SSPI values were 100 ± 8 μU/ml, 104 ± 6 μU/ml, and 98 ± 7 μU/ml for diabetic sedentary rats, diabetic exercise-trained rats, and diabetic sucrose-fed rats, respectively. Thus, similar levels were achieved in all groups, and any differences in glucose disposal rates reflect differences in the ability of insulin to stimulate glucose utilization.

Insulin-stimulated glucose disposal was compared in two ways. In the first place, we have determined the SSPG levels achieved during the infusion. Secondly, we have calculated the difference between the plasma glucose concentration before the infusion and the SSPG concentration.

RESULTS

The initial and final weights of the three groups of rats are seen in Table 1. Exercise-trained diabetic rats ran (mean ± SEM) 2.88 ± 0.4 miles/day, and gained slightly less weight (47 g) than did sedentary diabetic rats (63 g). Sucrose-fed diabetic rats gained the least (25 g), and their final weight was significantly lower than that of sedentary diabetic rats.

Postabsorptive plasma glucose and insulin concentrations for the three experimental groups are seen in Figure 1, which also displays values for healthy rats of comparable age and weight studied in our laboratory. The data in the left panel indicate that the magnitude of hyperglycemia was significantly lower ($P < 0.001$) in the exercise-trained diabetic rats than in the other two groups of diabetic rats. On the other hand, the degree of hyperglycemia was accentuated in the sucrose-fed rats ($P < 0.05$).

Plasma insulin levels are depicted in the right panel, and document the fact that the streptozotocin led to a fall in insulin concentration to about 1/3 the normal value. Although mean plasma insulin levels were lower in sucrose-fed diabetic rats, the change as compared to chow-fed diabetic rats was of marginal significance ($0.05 < P < 0.1$).

Estimates of insulin-stimulated glucose disposal are seen in Figure 2, which also includes results for normal rats of comparable size. SSPG levels are seen in the left panel, and it is obvious that exercise-trained diabetic rats had much lower ($P < 0.001$) values than either chow-fed or sucrose-fed sedentary diabetic rats. Indeed, SSPG levels of exercise-trained diabetic and normal rats were quite comparable. The highest SSPG levels were seen in sucrose-fed rats, but the differences between sucrose-fed and chow-fed sedentary diabetic rats was of questionable significance ($0.05 < P < 0.1$).

The SSPG levels were lower than the basal postabsorptive glucose levels in all groups, and the degree of this decrease is seen in the right panel of Figure 2. The fall in glucose level resulting from the infusion was approximately twice as great in the exercise-trained diabetic rats ($P < 0.01$) than in the other two diabetic groups.

DISCUSSION

The results presented confirm our previous demonstration[6] that hyperglycemia is ameliorated in exercise-trained diabetic rats with moderate degrees of experimentally-induced

TABLE 1
Mean (± SEM) weights of the three groups of rats at the time of injection (day 0) and at the conclusion (day 21)

Group	No. of rats	Day 0 (g)	Day 21 (g)
Sedentary	11	267 ± 8	330 ± 10
Exercise-trained	10	259 ± 4	306 ± 10
Sucrose-fed	10	259 ± 6	284 ± 12

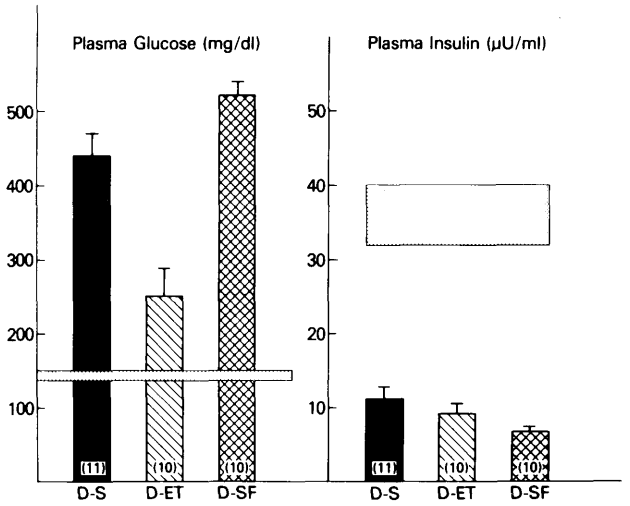

FIGURE 1. Mean (±SEM) postabsorptive plasma glucose and insulin concentrations. The horizontal stippled bars represent mean ± SEM values for normal rats of similar age and weight. The experimental groups are designated as D-S (diabetic, sedentary), D-ET (diabetic, exercise-trained), and D-SF (diabetic, sucrose-fed). The digits in parentheses refer to the number of rats in each group.

insulin deficiency. As before, circulating insulin levels were similar in all groups of diabetic rats, suggesting that exercise training exerts its beneficial effect by augmenting in vivo insulin action. Essentially identical results have recently been reported by Tancrede and associates.[7] The data illustrated in Figure 2 offer direct experimental support for the inference that insulin sensitivity is enhanced in exercise-trained diabetic rats, and dramatically document the magnitude to which

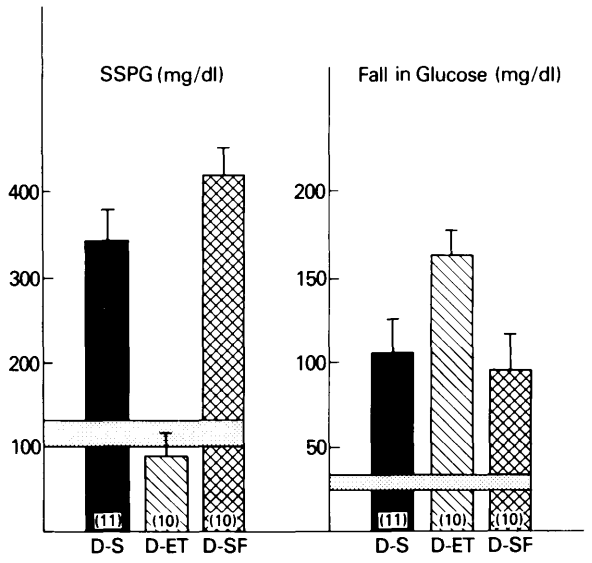

FIGURE 2. Estimates of insulin-stimulated glucose disposal for the three experimental groups: D-S (diabetic, sedentary), D-ET (diabetic, exercise-trained), and D-SF (diabetic, sucrose-fed). The left panel illustrates the SSPG concentrations attained during the infusion, and the right panel the net fall in glucose concentration (basal glucose concentration—SSPG concentration). The horizontal stippled bar defines the values obtained in normal rats of similar age and weight. Data are expressed as mean ± SEM, and the digits in parentheses refer to the number of rats in each group.

insulin-stimulated glucose utilization can be increased by exercise training in rats with insulin deficiency.

Although the ability of exercise training to enhance insulin action seems unequivocal, the effect of sucrose feeding is less clear. Feeding insulin-deficient rats high sucrose diets seemed to accentuate the severity of the diabetic state, i.e., these rats were more hyperglycemic and gained less weight. This was associated with a reduction in both circulating insulin levels and in in vivo insulin action. Both of these latter changes were of borderline statistical significance, and probably were equally responsible for aggravating the severity of the hyperglycemia. In either event, these data emphasize the fact that high carbohydrate diets do not necessarily lead to an improvement in the diabetic state.[12] Although we did not attempt to see if exercise training would have improved in vivo insulin action in sucrose-fed diabetic rats, results of a previous study would suggest that this would be the case. Thus, normal rats fed sucrose-rich diets became hyperinsulinemic in the absence of any significant change in plasma glucose levels.[13,14] However, when such rats are allowed to run spontaneously, as in the present experiment, the sucrose-induced hyperinsulinemia is markedly reduced.[14] In addition, triglyceride secretion rates and plasma triglyceride concentrations also fall.[14] These results are most consistent with the view that exercise training enhanced insulin action in sucrose-fed rats. As such, we think it most likely that exercise training would also ameliorate the severity of diabetes in sucrose-fed rats with moderate insulin deficiency.

In this study, we have attempted to estimate in vivo insulin-stimulated glucose disposal by calculating our results in two different ways. The simplest approach was to determine SSPG concentration, under conditions in which circulating levels of insulin were identical, both qualitatively and quantitatively. When this was done, it became clear that SSPG levels were significantly lower in exercise-trained diabetic rats than in the other two groups of diabetic rats. Indeed, it was clear that SSPG levels of diabetic rats fell within the value observed in nondiabetic rats.

On the other hand, it can be argued that using the SSPG level to compare insulin action of rats with different basal glucose concentrations ignores the potential impact that variations in initial degree of hyperglycemia might have on the SSPG value achieved. Since previous studies[15,16] from our laboratory in normal and diabetic men have shown that differences in degree of insulin resistance as estimated by SSPG are independent of glucose level before the infusion, we did not think this was a likely possibility. However, for completeness sake, we have also compared the three groups of diabetic rats by calculating the difference between basal (pre-infusion) glucose level and SSPG (Figure 2, right panel). These data indicate that the insulin infusion lowered the plasma glucose level in all three groups of diabetic rats, but the magnitude of the effect was approximately twice as great in the exercise-trained rats. Thus, in vivo insulin action in diabetic rats was also shown to be increased by exercise training with this approach. On the other hand, we do not wish to leave the impression that we believe this way of calculating in vivo action to be a useful one. As stated earlier, our previous experience has demonstrated the contrary,[15,16] and the results of Figure 2 indicate that the use of this measurement

leads to the conclusion that normal rats are more insulin resistant than diabetic rats. This seems most unlikely, and there is ample evidence[1–3] that supports the view that experimentally-induced insulin deficiency results in the development of insulin resistance. Thus, we would suggest that comparisons of insulin resistance based on the net fall in plasma glucose are likely to be misleading if the initial levels are widely disparate.

The rat is a nocturnal animal and, in an attempt to simulate the overnight fasting state in man, all measurements were made in the early afternoon, 6–8 h after the rats had last been allowed to eat or run. As a consequence, we cannot quantify the relative impact of the chronic effects of training versus the acute effect of running. Unfortunately, willingness to run in the exercise wheel takes time to develop, and we cannot simply define the effect of only one night's running on insulin action. We do have unpublished evidence which indicates that the ability of exercise training to enhance insulin sensitivity declines with time over 7 days, but is still clearly present for at least 3 days after animals have been removed from the exercise wheels. The situation is further complicated by the fact that the increased food intake of exercised-trained rats persists even after they have become sedentary, and the rapid weight gain that immediately ensues almost certainly contributes to the rate at which the beneficial effect of exercise training on insulin action disappears when rats are sedentary. Given the above information, we would suggest that the improvement in severity of diabetes and insulin action that occurs in exercised-trained rats with diabetes is primarily a function of the chronic effects of training, augmented by the acute effects of the last bout of running.

Finally, it is of interest that there is relatively little information as to the impact of exercise training on glucose homeostasis in diabetic humans, and what data are available[17–19] suggest that the beneficial effects are, at best, marginal. However, the patients in two of the studies had fasting euglycemia,[17,18] and mean fasting plasma glucose was only 135 mg/dl in the third study.[19] The diabetes was obviously much less severe in these patients than in our rats with streptozotocin-induced insulin deficiency, and this may account for the lack of a substantial beneficial effect of exercise training in these patients. Alternatively, the intensity of the exercise training needed to lead to significant changes in insulin sensitivity cannot be easily accomplished in diabetic humans. Obviously, there are many other possible explanations for the apparent discrepancy between the effect of exercise training in diabetic humans as opposed to diabetic rats, and it will be necessary to carry out relevant clinical studies on diabetic humans to resolve this issue.

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