Beneficial Effects of Physical Training in Rats with a Mild Streptozotocin-induced Diabetes Mellitus

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SUMMARY

The present studies have been designed to evaluate the effects of physical training in rats with a diminished insulin reserve. Mild diabetes mellitus was induced in rats with 45 mg/kg streptozotocin. Physical training was done on a treadmill, with a progressive program, twice daily, 5 days per week, for 10 wk in control and diabetic rats. At the end of the training program, a significant diminution in body weight gain and in epididymal fat pad weight was observed in both trained groups, as compared with sedentary controls. Sixty-four hours after the last exercise, control (N = 16), control-trained (N = 14), diabetic (N = 17), and diabetic-trained (N = 15) rats were submitted to an intravenous glucose tolerance test (0.5 g/kg). Arterial blood samples were collected at −15, 0, 2, 4, 6, 10, 15, 30, 45, and 60 min during the test in unanesthetized and precannulated rats for plasma glucose and insulin determinations. In normal rats, physical training induced a sharp decrease in the basal insulin levels (36 ± 3 vs. 101 ± 6 μU/ml; $P < 0.001$) without any significant changes in glucose levels (122 ± 4 vs. 129 ± 2 mg/dl; $P > 0.05$). After the glucose loading there was no significant change in the glucose tolerance curve, although the insulin values remained lower throughout the test in the trained group. In the diabetic rats, the elevated basal glucose levels were significantly diminished in the trained group as compared with the untrained diabetic group (177 ± 22 vs. 306 ± 37 mg/dl; $P < 0.001$), although the basal insulin values were similar in both groups (51 ± 7 vs. 54 ± 9 μU/ml; $P > 0.05$). The improvement in the glucose tolerance of the diabetic-trained rats was further confirmed by the glucose disappearance rate constant that was significantly increased (3.6 ± 0.4 vs. 2.0 ± 0.3; $P < 0.01$), although not fully restored to normal (6.3 ± 0.2; $P < 0.001$). These data clearly show that in rats with a diminished insulin reserve, a 10-wk running program greatly improved the glucose homeostasis. Measurements of circulating insulin suggest that, although an effect on insulin secretion cannot be totally excluded, the beneficial effect of physical training on diabetes mellitus is probably best explained by an increase in insulin sensitivity. **DIABETES *31*:406–409, May 1982.**

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Received for publication 18 March 1981 and in revised form 17 December 1981.

It has been suggested for many years that physical exercise was a beneficial adjunct in the treatment of diabetes mellitus.[1,2] However, as recently reviewed by Vranic and Berger,[3] the evidence to support this proposal has been limited. In normal human subjects, a physical conditioning program does not have any significant effect on glucose tolerance;[4–7] furthermore, in a population study, no significant relationship between glucose tolerance and habitual physical activity has been found.[8] The more recent demonstration that normal glucose tolerance in highly trained human subjects was associated with lower insulin levels[9–11] raised the possibility that physical training induced an increase in insulin sensitivity; this was also found in non-diabetic-trained rats.[12,13] Physical training may thus be postulated to improve glucose tolerance in subjects with a diminished insulin reserve; indeed Saltin et al.[14] have recently reported preliminary data in 25 nonobese subjects with chemical diabetes showing that physical training was associated with a better glucose tolerance. The present work was designed to evaluate the effect of a physical training program on glucose tolerance and insulin secretion in an experimental model with a diminished insulin reserve. Data will now be presented indicating that a 10-wk treadmill running program greatly improved the glucose homeostasis of rats with a mild streptozotocin-induced diabetes mellitus.

MATERIALS AND METHODS

Male Wistar rats with an average initial weight of 189 g were divided into four groups: control, control-trained, diabetic,

and diabetic-trained. Nonfasting rats were rendered diabetic by the intravenous injection of streptozotocin (Sigma, lot 79C-0027, St. Louis, Missouri) dissolved in 200 μl of an acidified citrate buffer (pH 4.5). The dose of 45 mg/kg was used; preliminary studies with this dosage have shown a moderate diabetogenic action with basal plasma glucose of about 300 mg/dl without frank ketosis nor insulin dependence. Control rats received an equivalent amount of citrate buffer. The rats were individually housed at 23°C under standard lighting (05.00–19.00 h) and fed with Purina rat chow and tap water ad libitum. The rats were weighed once a week.

Training began 20 h later on a motor-driven treadmill (Quinton instruments) set at an 8° incline according to a modification of the program of Pattengale and Holloszy.[15] The animals were exercised twice daily, 4 h apart, 5 days per week, and the program was made progressively more vigorous. They initially ran for 10 min at 22 m/min for 3 wk, then for 40 min at 28 m/min for 3 wk, and finally for 60 min at 31 m/min for 4 wk.

Sixteen hours after the last period of exercise, the rats were cannulated[16] in order to study unanesthetized and unrestrained rats. Forty-eight hours later, after a 1-h resting period, two arterial blood samples (0.4 ml) were taken at 15-min intervals for initial values in nonfasted animals. A 50% glucose solution was then injected intravenously at a dose of 0.5 g/kg over a period of 15 s; arterial blood samples were collected at 2, 4, 6, 10, 15, 30, 45, and 60 min. Each blood sample was immediately transferred to a chilled tube containing 0.5 mg EDTA and centrifuged at 4°C. The plasma was kept frozen for later glucose determination by a glucose oxidase method (A-Gent, Glucose U.V., Abbott Laboratories, Chicago, Illinois). Plasma immunoreactive insulin was measured by a modified double-antibody radioimmunoassay technique:[17] duplicate analysis was done on 40-μl plasma samples, using rat insulin as standard (Lot no. R170, Novo Industries, Copenhagen, Denmark, kindly provided by Dr. R. J. Schlichtkrull). The glucose disappearance rate constant (K value) was calculated graphically according to Conard et al.[18] At the end of the test, the rats were killed by decapitation and their epididymal adipose tissue was immediately removed and weighed.

It should be noted at this point that of the 86 rats entering the protocol, only 62 completed the study, and that this was due to either difficulties with the training (N = 10) or at cannulation (N = 14). However, the loss was similar in all four groups with no evidence that diabetes impaired either the training nor the cannulation.

The Student's *t* test was used to study the differences between groups.

RESULTS

As shown in Figure 1, the body weight of the control rats increased progressively from 190 to about 500 g during the 10-wk experimental period. Both diabetes mellitus and physical training caused a decrease in weight gain. However, a statistically significant difference ($P < 0.05$) from the control group was noted earlier with physical training (after 1 wk) than with diabetes mellitus (after 2 wk) and the overall gain of weight was less in control-trained rats than in diabetic rats after 9 wk. The physical training of diabetic rats gave a growth curve that was not statistically different from

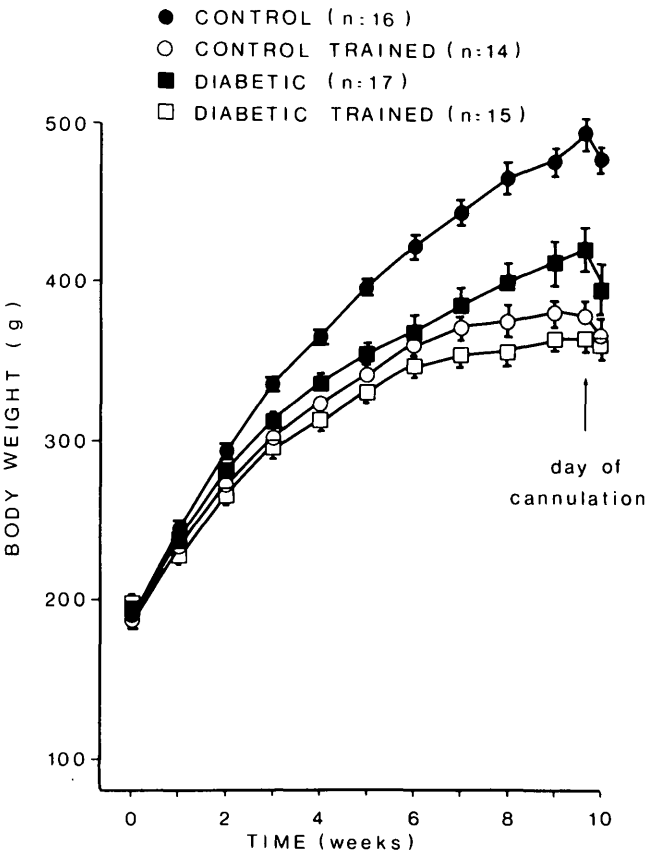

FIGURE 1. Effect of treadmill running program for 10 wk on the body weight curves in control and streptozotocin-diabetic rats. Each point and bar represents the mean ± SEM.

that of trained controls. Similar results were obtained with the epididymal fat pad weight; indeed, physical training caused an important reduction in the adipose tissue of the control animals (3.0 ± 0.2 vs. 7.3 ± 0.3 g; $P < 0.001$), while in the diabetic rats this was less marked (2.9 ± 0.2 vs. 4.4 ± 0.6 g; $P < 0.05$). Although there were no differences between the two groups of trained rats, the epididymal fat pad weight was significantly less ($P < 0.001$) in the untrained diabetic rats as compared with control animals.

The basal plasma glucose values (Figure 2) were not significantly lowered by physical training in nondiabetic rats (122 ± 4 vs. 129 ± 2 mg/dl; $P > 0.05$). However, the elevated glucose levels of diabetic rats were diminished by training (177 ± 22 vs. 306 ± 37; $P < 0.01$) even though they were not restored to normal ($P < 0.05$). After glucose loading, the glucose concentrations were slightly lower in trained than untrained control rats for the first 10 min; however, the glucose disappearance rate constants were not significantly different (6.7 ± 0.4 vs. 6.3 ± 0.2; $P > 0.05$). In diabetic animals, the glucose values were significantly lower ($P < 0.01$) throughout the test in the trained group. Furthermore, their glucose level had returned to basal values after 30 min as in normal rats, while this was not found in untrained diabetic rats even after 60 min. Calculation of the glucose disappearance rate constant also showed that the K value was significantly improved by physical training (3.6 ± 0.4 vs. 2.0 ± 0.3 mg 10^{-2} min^{-1}; $P < 0.01$) although not fully restored to normal.

As shown in Figure 2, physical training of control rats

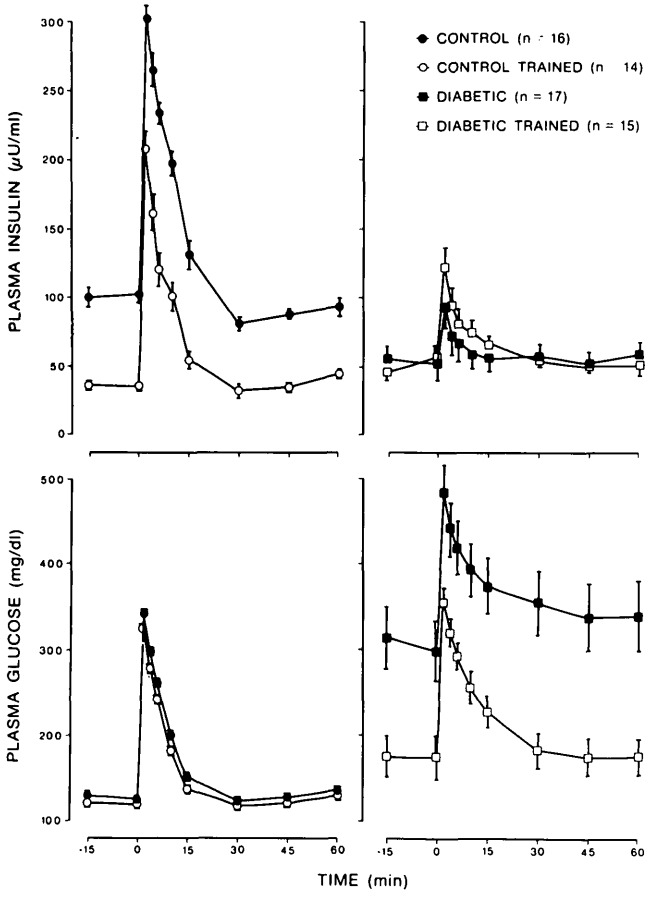

FIGURE 2. Effect of treadmill running for 10 wk on plasma glucose and insulin levels in control and streptozotocin-diabetic rats: 1.0 ml/kg of a 50% glucose solution was injected i.v. in 15 s at zero time. Each point and bar represents the mean ± SEM.

caused a diminution in the basal insulin levels (36 ± 3 vs. 101 ± 7 μU/ml; $P < 0.001$). Although a similar decrease was already found in diabetic rats (54 ± 9 μU/ml; $P < 0.001$), this was not further modified by training (51 ± 7 μU/ml; $P > 0.05$). After glucose loading in normal rats, the insulin curve was lower in trained animals throughout the test ($P < 0.001$). In diabetic rats, however, the insulin curves were not statistically different ($P > 0.05$) between the trained and untrained groups.

DISCUSSION

The results of the present study clearly show that the elevated basal glucose levels of diabetic rats were significantly diminished in a group of animals submitted to a 10-wk physical training program; the improvement in their glucose tolerance was further substantiated after an i.v. glucose load. To our knowledge, our group has been the first to report such a beneficial effect of physical training in rats with a diminished insulin reserve.[19] It has been reported in abstract form[20] that physical training could improve the glucose tolerance of fatty Zucker rats, but this is a syndrome of extreme insulin resistance. In men, Björntorp et al.[21] found that a 6-mo training period in obese nondiabetic subjects caused some improvement in their glucose tolerance. Saltin et al.[14] have also reported an improvement in the glucose tolerance of nonobese subjects with chemical diabetes mellitus after 6 mo of light training. In six men with maturity-onset diabetes mellitus of a little more severity, Ruderman et al.[22] have also found some improvement in the glucose disappearance rate after 3–6 mo of training, but the basal glucose remained at the same elevated level. The highly spectacular results obtained in our experimental model could possibly be attributed to a more intensive program and/or to the fact that the diabetes was secondary to a lack of insulin[23] rather than to a peripheral resistance to insulin as found in maturity-onset diabetes.[24]

Measurements of circulating insulin suggest that the training-induced improvement in the glucose tolerance of diabetic rats depends on an enhanced sensitivity to insulin rather than on an increased insulin secretion, since both groups of diabetic rats had similar basal levels of insulin and insulin response curves after glucose load, but lower glucose levels. The enhancing effect of physical training on insulin sensitivity is even more evident in the trained control group since a clear-cut diminution in insulin levels was observed throughout the test without any differences in plasma glucose or glucose levels. Such results in nondiabetic animals agree well with other studies done in trained rats[12,13,25] and human athletes[9,10,11] and interpreted as evidence that physical training improves the insulin sensitivity.

Although the present study does not permit us to identify the exact mechanism by which training induced an increase in insulin sensitivity, the reduction in weight gain could partly account for this phenomenon, since reduction of obesity can improve glucose tolerance and insulin sensitivity.[26] However, since diabetes mellitus in itself has already caused a diminution in weight gain and in epididymal fat pad weight in our experimental model, a mechanism linked to such changes appears to be less likely. Desensitization of adrenoceptors might be another explanation, since we have previously shown that rats chronically treated with adrenaline have an increased insulin sensitivity,[27] and it is well known that the repeated forced exercises in our training program stimulate the secretion of catecholamines.[12] However, the recent report of Mondon et al.[25] that spontaneously exercised rats also have an enhanced insulin sensitivity made this explanation less probable, since their training regimen appears to be much less stressful.

Although the improved glucose tolerance of trained diabetic rats is more likely to be explained by an enhanced insulin sensitivity, studies of insulin dynamics may also suggest that there is an increased sensitivity of the beta-cells in response to glucose. Indeed, the lower basal levels of glucose in the trained diabetic rats were associated with insulin levels similar to those observed in the untrained diabetic rats. If the glucose level was then elevated by an i.v. injection of glucose to a level comparable to the basal level of control diabetic rats, as observed 6 min after the glucose load (see Figure 2), then the insulin levels were almost twice higher. At this point, one may speculate that the increased sensitivity to insulin, which has been induced by whatever means during physical training, may preserve the insulin secretory capacity of a pancreas with a diminished reserve. Further studies will be necessary to fully evaluate this hypothesis.

In conclusion, the present studies have clearly shown that in rats with a mild streptozotocin-induced diabetes mellitus, a 10-wk running program greatly improved the glucose homeostasis, probably by an insulin sparing effect as recently

shown in nondiabetic human subjects,[28] although some effect on insulin secretion cannot be excluded.

ACKNOWLEDGMENTS

Support was provided by grants from the Juvenile Diabetes Foundation, the Canadian Diabetic Association, and the Banting Research Foundation.

Gilles Tancrède was the holder of a studentship from the Conseil de la Recherche en Santé du Québec.

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