Characterization of Exercise-Induced Hyperketonemia in Streptozotocin-Diabetic Rats and the Effects on It of Prolonged Training

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Abstract

Exercise-induced hyperketonemia was investigated using streptozotocin (STZ)-diabetic rats subjected to running exercise on a treadmill. The degrees of hyperketonemia after 50, 55 and 60% \( V_{O_{2\max}} \) of exercises were similar in mild diabetic rats (fasting plasma glucose; FPG < 11 mM). The degree of hyperketonemia (especially an increase in acetoacetate; AcAc) after 60% \( V_{O_{2\max}} \) of exercise was correlated with FPG (\( P < 0.01 \)) and basal plasma ketone bodies (\( P < 0.01 \)). Prolonged training with 60% \( V_{O_{2\max}} \) of exercise for 30 min 3 times per week for 6 wks reduced the increase in plasma ketone bodies induced by the exercise in both mild (FPG < 11 mM) and severe (FPG > 22 mM) diabetic rats. The exercise-induced increase in plasma glucagon in mild diabetic rats and free fatty acids (FFA) in severe diabetic rats are also reduced by the training. These results demonstrate that exercise-induced hyper-AcAc-emia correlated with the FPG level is reduced by prolonged training in diabetic rats, and might suggest that exercise-induced hyperketonemia is reduced by long-term exercise training also in diabetic patients.

A moderate hyperketonemia is sometimes found in poorly controlled diabetic patients. This is largely ascribed to the exaggerated hepatic ketogenesis mainly due to a decreased insulin/glucagon molar ratio (McGarry and Foster, 1977). In these patients, exercise further elevates the plasma ketone body level, while the exercise therapy is accepted as a important tool for glycemic control in diabetic patients (Berger et al., 1977).

Also in normal subjects, prolonged exercise induces moderate hyperketonemia (Corbett et al., 1969; Féry and Balasse, 1986; Johnson et al., 1969; Wahren et al., 1984). However, this hyperketonemia is reduced by long-term exercise training (Corbett et al., 1969; Johnson and Walton, 1972; Johnson et al., 1969). Similar results also appear in normal rats (submitted for publication). These observations might suggest that long-term exercise training reduces exercise-induced hyperketonemia also in diabetic patients. But no study

Received February 28, 1989
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concerning this hypothesis has been reported heretofore.

Therefore, using STZ-induced diabetic rats, this study was designed to investigate 1) the relationship between the intensity of exercise and the degree of exercise-induced hyperketonemia, 2) the relationship between the severity of diabetes mellitus (DM) and the degree of exercise-induced hyperketonemia, and 3) the effects of training on exercise-induced hyperketonemia.

Materials and Methods

Animals

Five-day-old Wistar rats were injected with STZ (60–100 mg/kg, ip) in 25 μl of citrate buffer (0.05 M, pH 4.5) according to the method of Portha et al. (1974). All the animals were weaned 3 wks after birth and only male rats continued to be housed with free access to a commercial chow. To evaluate the severity of DM, at 16 wks of age, blood samples were taken from the tail vein after an overnight fast.

Protocol 1

This protocol was designed to study the relationship between the intensity of exercise and the degree of exercise-induced hyperketonemia. Twenty-four rats with a mild DM (FPG <11 mM, 7.6±0.3 mM) were divided into one sedentary control group and three exercise groups. There was no significant difference in the FPG level and fasting body weight among the four groups. At 19 wks of age, the rats in the exercise groups were subjected to forced running on a treadmill at different intensities (treadmill speeds of 5 m/min, 10 m/min and 15 m/min) for 30 min. Running at 5 m/min, 10 m/min and 15 m/min corresponds to exercise intensities of 50% \( \dot{V}_{O_2}\text{max} \), 55% \( \dot{V}_{O_2}\text{max} \) and 60% \( \dot{V}_{O_2}\text{max} \), respectively, in normal rats (Abe et al., 1986). The experiment was started at 1100 h after a 4-h fast. Blood samples (0.35 ml) were taken from the tail vein at the start and the end of the exercise, and 20 min, 40 min and 60 min after the end of the exercise. Samples were collected in chilled tubes containing 6 mg EDTA/ml blood and centrifuged at 3000 rpm for 15 min at 4°C. The resulting plasma was stored at ~80°C until analyzed.

Protocol 2

This protocol was designed to study the relationship between the severity of DM and the degree of exercise-induced hyperketonemia. At 19 wks of age, 13 rats with more than 11 mM FPG were subjected to forced running on a treadmill at a speed of 15 m/min for 30 min. The experimental procedure was the same as protocol 1. The data were summarized separately in three groups according to the FPG: the mild DM group from protocol 1 (FPG<11 mM, 7.9±0.7 mM, n=6), a moderate DM group (11 mM<FPG<22 mM, 15.6±1.6 mM, N=6), and severe DM group (FPG>22 mM, 24.5±0.5 mM, N=7).

Protocol 3

This protocol was designed to study the training effects on exercise-induced hyperketonemia. Fourteen rats with mild DM (FPG<11 mM, 8.5±0.3 mM) and 16 rats with severe DM (FPG>22 mM, 27.8±0.6 mM) were divided into untrained and trained groups. These 30 rats were different from those in protocols 1 and 2. At 19 wks of age, the following training was started: a treadmill speed of 15 m/min, a duration of 30 min and a frequency of 3 times every week. After 6 wks of training, the four groups of rats were subjected to running exercise on the treadmill at a speed of 15 m/min for 30 min at 1100 h after a 4-h fast. To accustom the untrained rats to treadmill running, they were run for 10–20 min at a speed of 5–10 m/min three times one week before the experiment. The blood was taken from the tail vein at the start and the end of exercise, and 60 min after the end of exercise. Samples were collected in chilled tubes containing 6 mg EDTA and 500 units of aprotinin/ml blood. After centrifugation at 3000 rpm for 15 min at 4°C, the resulting plasma was stored at ~80°C until analyzed.

Assays

Plasma AcAc, 3-hydroxybutyrate (3-OHB) and total ketone body (TKB) concentrations were determined by the method of Harano et al. (1982) (Ketone Test, Sanwa Kagaku Co., Nagoya, Japan). 3-OHB was calculated by subtracting the AcAc value from the TKB value. The plasma FFA concentration was assayed...
with a commercial kit (NEFA C-Test, Wako Junyaku Co., Osaka, Japan). The plasma glucose concentration was determined by the glucose oxidase method (Glucose Analyzer 2, Beckman Instruments, Fullerton, CA, USA). The plasma immunoreactive insulin (IRI) level was measured by radioimmunoassay (RIA) with a commercial kit (Rat Insulin Kit, Incstar Co., Stillwater, MN, USA). The plasma immunoreactive glucagon (IRG) level was determined by RIA according to Faloona and Unger (1974) using E-7 antibody (kindly donated by Dr. H. von Schenck).

**Statistical analysis**

The data were analyzed by analysis of variance followed by group comparisons (Student’s t-test) when a significant F value was obtained. Linear regression analysis was also used. P<0.05 was considered to be significant. All data in this paper are expressed as means ± SE.

**Results**

**Relationship between intensity of exercise and degree of exercise-induced hyperketonemia**

The basal values for AcAc, 3-OHB and TKB were not significantly different among the four groups (running at 5 m/min, 10 m/min and 15 m/min, and the sedentary control) (Fig. 1).

The plasma AcAc level was significantly increased at the end of 5 m/min of exercise (from the basal level of 68.2±14.7 µM to 174.2±37.0 µM, P<0.05), and maintained a plateau even 60 min after the end of exercise. The plasma 3-OHB level was more severely increased at the end of 5 m/min of exercise (from basal level of 108.5±19.9 µM to 432.3±89.8 µM, P<0.05), and tended to increase even 60 min after the end of exercise. The mode of change in the plasma TKB level induced by the 5 m/min of exercise was similar to that in the plasma 3-OHB level (Fig. 1).

Both 10 m/min and 15 m/min of exercise induced changes in the plasma AcAc, 3-OHB and TKB levels similar to those with 5 m/min of exercise (Fig. 1).

As shown by the dotted lines in Fig. 1, the plasma AcAc, 3-OHB and TKB concentrations in the sedentary control group were gradually increased. Significant rises in 3-OHB and TKB levels continued from 30 min after the start of the experiment (P<0.05), but these rises were very slight as compared with those of the exercise groups.
Relationship between severity of DM and degree of exercise-induced hyperketonemia

The basal values for AcAc in rats with mild DM (FPG<11 mM), moderate DM (11 mM<FPG<22 mM) and severe DM (FPG>22 mM) were 63.3±8.3 μM, 204.7±51.0 μM and 350.7±85.2 μM, respectively. Similarly, the basal values for 3-OHB and TKB also significantly correlated with the FPG level (P < 0.01) (Fig. 2).

Running at 15 m/min for 30 min significantly increased plasma AcAc, 3-OHB and TKB in all groups (P<0.05 or P<0.01) (Fig. 2). The net increases in AcAc at 60 min after the end of exercise in mild, moderate and severe DM rats were 117.8±29.2 μM, 391.7±109.8 μM and 783.4±142.5 μM, respectively. The value in the severe DM group was significantly greater than those in the mild and moderate DM groups (P<0.05 and P<0.01, respectively). Similarly, the net increase in TKB at 60 min after the exercise was dependent on the FPG level. On the other hand, there

Fig. 2. Effects of different FPG levels on plasma ketone body levels after exercise.

Fig. 3. Relationship between FPG level and the degree of exercise-induced hyperketonemia.

A; (between FPG and \( \Sigma \Delta \text{AcAc} \), \( r=0.66 \), P<0.01),
B; (between FPG and \( \Sigma \Delta \text{3-OHB} \), \( r=0.26 \), not significant),
C; (between FPG and \( \Sigma \Delta \text{TKB} \), \( r=0.61 \), P<0.01).
was no significant difference in the net increase in 3-OHB at 60 min after the exercise among the three groups (426.8±92.6 μM, 509.7±80.3 μM and 566.0±56.2 μM, respectively) (Fig. 2).

As shown in Fig. 3-C, the level of FPG was positively correlated with the sum of the net increase in the TKB level through the experimental period (ΣΔ TKB) (r=0.61, P<0.01). Also, the level of FPG was positively correlated with the sum of the net increase in AcAc through the experimental period (ΣΔ AcAc) (r=0.66, P<0.01), whereas ΣΔ3-OHB was independent of the FPG level (Fig. 3-A and B).

In addition, the basal TKB level was positively correlated with ΣΔTKB (r=0.60, P<0.01). Also, the basal AcAc level was positively correlated with ΣΔAcAc (r=0.71, P<0.01), whereas ΣΔ3-OHB was independent of the basal 3-OHB level (Fig. 4).

**Effects of training on exercise-induced changes in plasma concentrations of ketone bodies, FFA, IRI and IRG**

Before the exercise training, the levels of plasma glucose, plasma ketone bodies and body weight after an overnight-fast were not significantly different in the untrained group from those in the trained group in both mild and severe DM rats (Table 1).

After 6 wks of training, the body weight of the trained group was not significantly different from that of the untrained group in both mild and severe DM rats (Table 2).

Six wks of training did not affect the basal plasma levels of ketone bodies (Fig. 5-A), FFA, glucose, IRI and IRG (Fig. 6-A) in mild DM rats.

The 15 m/min of exercise for 30 min caused significant increases in plasma AcAc, 3-OHB and TKB in both untrained and trained rats with mild DM (P<0.01) Fig. 5-A). However, the values for AcAc, 3-OHB and TKB at 90 min in the trained group were significantly smaller than those in the untrained group (P<0.05 or P<0.01) (Fig. 5-A). Thus, the sum of the net increases in TKB through the experimental
Table 1. Fasting body weight, glucose and ketone bodies in each group before the start of training (at 16 wks after birth)

<table>
<thead>
<tr>
<th></th>
<th>Mild DM</th>
<th></th>
<th>Severe DM</th>
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<tbody>
<tr>
<td></td>
<td>Untrained</td>
<td>Trained</td>
<td>Untrained</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>385.9 ± 28.0</td>
<td>366.6 ± 25.1</td>
<td>260.9 ± 7.8</td>
</tr>
<tr>
<td>Plasma glucose (mM)</td>
<td>8.3 ± 0.4</td>
<td>8.8 ± 0.5</td>
<td>28.6 ± 0.9</td>
</tr>
<tr>
<td>Plasma AcAc (μM)</td>
<td>286.6 ± 42.4</td>
<td>266.6 ± 13.1</td>
<td>785.8 ± 112.4</td>
</tr>
<tr>
<td>Plasma 3-OHB (μM)</td>
<td>659.4 ± 115.6</td>
<td>679.1 ± 103.0</td>
<td>1000.3 ± 115.2</td>
</tr>
<tr>
<td>Plasma TKB (μM)</td>
<td>946.0 ± 157.3</td>
<td>945.7 ± 121.7</td>
<td>1786.0 ± 222.0</td>
</tr>
</tbody>
</table>

Values are means ± SE.
Table 2. The sums of the net increases in plasma concentrations of ketone bodies, other substrates and hormones in Figs. 5 and 6

<table>
<thead>
<tr>
<th></th>
<th>Mild DM</th>
<th>Severe DM</th>
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<tr>
<td></td>
<td>Untrained (N=7)</td>
<td>Trained (N=7)</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>463.7± 28.6</td>
<td>442.3± 29.8</td>
</tr>
<tr>
<td>ΣJDAcAc (µM)</td>
<td>216.7± 46.0</td>
<td>169.0± 25.3</td>
</tr>
<tr>
<td>ΣJD3-OHB (µM)</td>
<td>728.1± 116.3</td>
<td>436.6± 85.8</td>
</tr>
<tr>
<td>ΣJTKB (µM)</td>
<td>989.9± 99.5</td>
<td>605.6± 96.1*</td>
</tr>
<tr>
<td>ΣJFFA (g/L)</td>
<td>0.073± 0.022</td>
<td>0.072± 0.027</td>
</tr>
<tr>
<td>ΣJGlucose (mM)</td>
<td>0.4± 1.8</td>
<td>-2.5± 1.9</td>
</tr>
<tr>
<td>ΣJIRI (PM)</td>
<td>-80.8± 46.7</td>
<td>-95.1± 69.8</td>
</tr>
<tr>
<td>ΣJIRG (ng/L)</td>
<td>-0.1± 96.5</td>
<td>-57.9± 76.0</td>
</tr>
</tbody>
</table>

ΣJ means the sum of the net increases at 30 min and 90 min from basal levels. Values are means±SE. *; P<0.05, trained group vs. untrained group.

period (ΣJTKB) in the trained group was significantly smaller than it was in the untrained group (P<0.05) (Table 2). There was a similar tendency in ΣJDAcAc and ΣJD3-OHB in the mild DM rats (Table 2).

There was also a significant rise in the plasma FFA level in both the untrained and trained groups of mild DM rats (P<0.05, Fig. 6-A). However, the sum of the net increase in FFA in the two groups was not significantly different (Table 2). The plasma levels of glucose and IRI did not change significantly during the experimental period in either group (Fig. 6-A). In the untrained group, the plasma IRG level tended to rise at the end of exercise and then decreased 60 min after the exercise. But in the trained group, the IRG level was not increased at the end of exercise and it was lower than that in the untrained group throughout the experiment (Fig. 6-A).

In the severe DM rats, the basal plasma 3-OHB in the trained group was significantly lower than it was in the untrained group (Fig. 5-B, P<0.05) and the levels of AcAc, TKB, glucose and IRG also tended to be lower than they were in the untrained group (Fig. 5-B and 6-B). The plasma levels of AcAc, 3-OHB and TKB at the end of exercise in the trained rats with severe DM were significantly lower than they were in the untrained rats (P<0.05 or P<0.01). Also, the sums of the net increases in AcAc and TKB through the experiment (ΣJDAcAc and ΣJTKB) in the trained group were significantly smaller than they were in the untrained group (Table 2).

The plasma FFA concentration in the trained group was lower than it was in the untrained group throughout the experimental period. There was a significantly lower FFA value at the end of exercise (Fig. 6-B). The plasma glucose was significantly decreased just after and 60 min after the exercise (P<0.05 or P<0.01) in both the trained and untrained groups (Fig. 6-B). But the degree of decrease in the groups was not significantly different. The plasma IRI was very low in severe DM rats and did not change during the experiment in either the trained or the untrained groups (Fig. 6-B). The plasma IRG was significantly decreased just after and 60 min after the exercise (P<0.01) in the untrained group. A similar tendency also appeared in the trained group. But the IRG
levels in the two groups were not significantly different at each point (Fig. 6-B).

Discussion

There has been no previous report referring to the relationship between exercise intensity and the degree of exercise-induced hyperketonemia in diabetes mellitus. At first, we tried to evaluate the small difference in moderate-intensity exercises (treadmill speeds of 5 m/min, 10 m/min and 15 m/min corresponding to intensities of 50% \( \dot{V}O_{\text{max}} \), 55% \( \dot{V}O_{\text{max}} \) and 60% \( \dot{V}O_{\text{max}} \), respectively (Abe et al., 1986)), since such moderate intensity exercises are recommended for diabetic patients. Probably because of the small differences in the exercise intensities, the degrees of hyperketonemia after such different exercises were unexpectedly not different among the groups (Fig. 1). But in normal rats (report submitted for publication), the degree of hyperketonemia was increased more by the higher intensity exercise (the treadmill speed of 30 m/min) than at the speed of 15 m/min. It has been demonstrated that an anaerobic threshold (AT) level appears at 55–65% \( \dot{V}O_{\text{max}} \) in normal subjects (Davis et al., 1976; Davis et al., 1982; Robinson and Sucec, 1980). Thus, the 30 m/min of exercise corresponding to 75% \( \dot{V}O_{\text{max}} \) (Abe et al., 1986) may be beyond the AT level in our normal rat study. So the larger difference in intensity of exercise should produce the different degree of hyperketonemia also in diabetic rats.

Secondly, in this study, we clearly demonstrated that the degree of increase in plasma ketone body after the moderate exercise (15 m/min) was correlated with the FPG level and the basal plasma ketone body level (Fig. 3 and 4). Sato et al. (1983) similarly reported that the degree of post-exercise ketosis in poorly controlled diabetic patients (FPG = 15.1 ± 0.7 mM) was greater than it was in well controlled diabetic patients (FPG = 4.3 ± 0.1 mM).

Finally, we have clarified the training effects on exercise-induced hyperketonemia in mild and severe diabetic rats. No such study has been reported for diabetic subjects or animals, although some previous reports were performed in normal subjects (Corbett et al., 1969; Johnson and Walton, 1972; Johnson et al., 1969) and normal rats (submitted for publication).

In mild DM rats, 6 wks of training reduced exercise-induced hyperketonemia (Fig. 5-A). This is consistent with previous studies in normal men (Corbett et al., 1969; Johnson et al., 1965; Johnson and Walton, 1972) and normal rats (submitted for publication). The training tended to inhibit the exercise-induced rise in plasma IRG, whereas the levels of plasma FFA were the same in the untrained and trained groups (Fig. 6-A). Glucagon has a direct effect on ketogenesis in the liver through the reduction of malonyl-CoA and the stimulation of carnitine acyltransferase (McGarry et al., 1977). Therefore, these results suggest that the training effect on exercise-induced hyperketonemia in mild DM rats may be caused partly by the reduction of hepatic ketogenesis due to the smaller increase in plasma glucagon during the exercise.

The plasma FFA level of the trained group of mild DM rats and that of the untrained group of severe DM rats was increased by the exercise without an increase in the plasma glucagon concentration (Fig. 6-A and 6-B). These results suggest the possibility that catecholamines mainly contribute to the increase in plasma FFA during the exercise. Nilsson et al. (1975) reported an evidence supporting this speculation. Namely, in their human study, short-term submaximal exercise caused increases in plasma catecholamines and glycerol without a change in plasma glucagon.
In the present study, both untrained and trained rats were subjected to running at the same absolute intensity after 6 wks of training period. Therefore, the relative intensity of exercise for trained rats seemed to be lower than that for untrained rats. However, to evaluate the relative intensity of exercise especially in untrained rats is very difficult (Shepherd and Gollnick, 1976). Furthermore, as shown in Fig. 1, the degree of hyperketonemia was the same after 50, 55 and 60% \( \dot{V}O_{2\text{max}} \) of exercise. In the literature, Gyntelberg et al. (1977) demonstrated in their human study that the increase in plasma glucagon in response to exercise was reduced after 10 wks of training, and that this effect was evident not only in an exercise at the same absolute intensity but also at the same relative intensity. Therefore, we designed a protocol to evaluate the training effects on ketone body metabolism at the same absolute exercise intensity in both trained and untrained rats, and we believe that the results obtained from the present study does not ascribe to the decrease in relative intensity of exercise.

In untrained severe DM rats, plasma AcAc increased markedly after the exercise (Fig. 5-B). This result does not seem to be consistent with the general concept that augmentation of hepatic ketogenesis causes an increase in the 3-OHB/\( \text{AcAc} \) ratio due to an increased NADH/NAD\(^+\) ratio in the mitochondria in hepatocytes (Sato et al., 1983). However, our previous study (Okuda et al., 1986) demonstrated that both the basal AcAc output and the growth hormone-stimulated AcAc production from perfused rat liver were larger than those of 3-OHB. This observation supports the possibility that AcAc production is dominantly increased by exercise in severe DM rats. In addition, hyperketonemia in the diabetic state is caused not only by increased hepatic ketogenesis but also by some reduction of ketone body utilization in peripheral tissues (Sherwin et al., 1976). Ruderman et al. (1974) demonstrated that the AcAc uptake by perfused hindlimb muscle in diabetic ketoacidosis rats was diminished by 50% compared with 48-h fasted normal rats. Furthermore, Féry et al. (1987) recently suggested that the hyperketonemic effect of prolonged exercise in ketotic diabetic patients does not result from exaggerated ketogenesis, but from some removal defect. Thus, the fact that plasma AcAc was dominantly increased by exercise in severe DM rats may be due to increased AcAc production and reduced AcAc utilization.

As shown in Fig. 5-B, the exercise-induced increase in plasma AcAc was more noticeably reduced than 3-OHB by 6 wks of training in severe DM rats. The training also reduced the exercise-induced increase in plasma FFA, whereas there was no definite effect of training on plasma IRG (Fig. 6-B). These results suggest that the training effect on exercise-induced hyperketonemia in severe DM rats may be caused mainly by the reduction of hepatic ketogenesis (dominantly \( \text{AcAc} \)) due to the decreased supply of FFA from the adipose tissue to the liver, which is regulated by norepinephrine (Schade and Eaton, 1979; Keller et al., 1984) and epinephrine (Weiss et al., 1984). These catecholamine responses to exercise are lower in the trained subjects than in the untrained subjects (Koivisto et al., 1982; Péronnet et al., 1981).

In severe DM rats, as mentioned above, the exercise-induced increase in plasma AcAc was more clearly reduced than 3-OHB by 6 wks of training (Fig. 5-B). This result suggests that a defect in AcAc utilization by peripheral tissues may be attenuated also by prolonged exercise training in severe DM rats.

In summary, it has been elucidated that 1) the degrees of hyperketonemia after three different intensity of moderate exercises (50, 55 and 60% \( \dot{V}O_{2\text{max}} \)) are not different in
mild DM rats. 2) the degree of hyperketonemia (especially an increase in AcAc) after 60% \( V_{\text{O}_{2\text{max}}} \) of exercise is correlated with the FPG and the basal plasma ketone body levels. 3) the exercise-induced increases in the plasma ketone bodies and glucagon are reduced by prolonged training in mild DM rats. 4) the exercise-induced increase in the plasma ketone bodies (especially AcAc) and FFA are reduced by prolonged training in severe DM rats.

These results show that exercise-induced hyper-AcAc-emia correlated with the fasting plasma glucose level is reduced by prolonged training in diabetic rats and these training effects may be caused by both the reduction of hepatic ketogenesis and the amelioration of defect of ketone body utilization in peripheral tissues. The present study might suggest that exercise-induced hyperketonemia is reduced by long-term exercise training in diabetic patients. However, further investigations concerning the intensity and duration of exercise are necessary for application of the exercise therapy, especially to severe diabetic patients.

**Acknowledgements**

We thank Miss Sanae Nakata for her expert technical assistance. We also express our gratitude to Dr. Shigeru Katsuta for his advice. This study was supported partly by a grant for project research from the University of Tsukuba.

**References**


