# Altered Control of Ventilation in Streptozotocin-Induced Diabetic Rats (43809)

MICHAEL S. HEIN,* EVELYN H. SCHLENKER,*,[1] KAUSHIK P. PATEL†

*Department of Physiology and Pharmacology,* The University of South Dakota, School of Medicine, Vermillion, South Dakota 57069 and Department of Physiology and Biophysics,† University of Nebraska Medical Center, Omaha, Nebraska 68198–4575*

*Abstract.* **The purposes of this study were (i) to determine if ventilatory control is altered in streptozotocin (STZ)-induced diabetic rats and (ii) to determine whether insulin treatment of diabetic rats could prevent ventilatory abnormalities. Male Sprague-Dawley rats were randomly assigned to three groups: control ($n$ = 10), diabetic ($n$ = 9), and diabetic treated with insulin ($n$ = 9). The diabetic group exhibited a progressive reduction of tidal volume, minute ventilation, and $CO_2$ production compared with the control and diabetic treated with insulin groups over the 4 week period. Furthermore, the ventilatory responses to the hypercapnic (3%, 6%, 9% $CO_2$) and hypoxic (10% $O_2$) gas challenges were significantly less in the diabetic rats than those of the control and diabetic and insulin treated groups by the third and fourth week. Ventilation and ventilatory responses to hypercapnia and hypoxia were similar in the control group and the diabetic treated with insulin group at the end of the study. In conclusion, uncontrolled diabetes induced in rats by STZ treatment resulted in altered control of ventilation that could be prevented by insulin therapy.** [P.S.E.B.M. 1994, Vol 207]

Respiratory abnormalities characterized by alterations in lung morphometry (1–3), pulmonary function (4–8), and control of ventilation (9–12) have been reported in insulin-dependent diabetic patients. Furthermore, defective ventilatory control has been suggested as the cause of sudden cardiopulmonary arrest in diabetic patients with autonomic neuropathy (13, 14). However, Calverley and coworkers (15), and Solar and Eagleton (16), did not observe ventilatory control abnormalities in their diabetic patients with autonomic neuropathy. To date, the control of ventilation in some patients with diabetes mellitus challenged with hypoxia, hypercapnia, and exercise, remains inconsistent (9–12, 15, 16).

To examine the development of ventilatory control abnormalities in insulin-dependent diabetes mellitus, we used the streptozotocin (STZ)-induced diabetic rat as an animal model (17). The purposes of this study were (i) to determine if control of ventilation is altered during 4 weeks of STZ-induced diabetes in rats and (ii) to determine whether insulin treatment in the diabetic rats could prevent ventilatory abnormalities.

## Materials and methods

Male Sprague-Dawley rats (200–240 g, SASCO, Omaha, NE) were randomly assigned to one of three groups: control ($n$ = 10), diabetic ($n$ = 9), and diabetic rats treated with insulin ($n$ = 9). Four to five animals per cage were housed in a light (12-hr light:dark cycle) and temperature (20°–22°C) controlled animal facility. Food and water was available *ad libitum*. Diabetes was induced by a single intraperitoneal injection (65 mg/kg) of STZ in 2% solution of 0.1 *M* citrate buffer (pH 4.5). Onset of diabetes occurred rapidly and was identified 2 days after STZ injection by polyuria, polydipsia, and a blood glucose concentration of ≥14.0 mmol/liter (Accu-chek, Boehringer, Mannheim, Indianapolis, IN). Control rats received an injection of vehicle (citrate buffer) of equivalent volume. The diabetes + insulin group was treated with 2 U/rat/day of Protamine zinc insulin (Lilly) which reduced blood glucose levels by two days after treatment commenced to 9.1 ± 2.0 mmol/l (Control = 6.3 ± 0.4 mmol/l). The

[1] To whom requests for reprints should be addressed at Department of Physiology and Pharmacology, University of South Dakota, School of Medicine, Vermillion, SD 57069.

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experiments were approved by the University of South Dakota Animal Care and Use Committee.

**Ventilatory and Metabolic Measurements.** Ventilation and $CO_2$ production were measured in awake rats placed in a cylindrical 20 cm long × 14 cm diameter Plexiglas plethysmograph. One end of the plethysmograph contained two ports to measure flow rate (Gillmont rotameter) of air or test gases, and to measure $CO_2$ and $O_2$ concentrations (Beckman LB-2 $CO_2$, OM-14 $O_2$ gas analyzers). The other end was sealed with a rubber stopper (No. 15) which contained three ports: one measured chamber temperature (Digitec digital thermometer); another connected by a short (6-cm) polyethylene tube to a pressure transducer (Gould Statham) coupled to a Grass polygraph (Model 7D) was used to measure chamber pressure associated with ventilation; and the third port allowed air or test gases to be infused into the chamber. Chamber pressure was calibrated for volume displacements with a 1.0-cc glass syringe. A more in-depth description of this method has been reported (18).

Frequency of breathing ($f$, breaths/min), tidal volume ($\dot{V}_T$, ml/breath), and minute ventilation ($\dot{V}_E$, ml/min) were calculated from the average of ten consecutive breaths. Carbon dioxide production ($\dot{V}_{CO_2}$, ml/min) was calculated by measuring the fractional concentration difference between $CO_2$ entering and exiting the chamber multiplied by the flow rate through the chamber. $\dot{V}_{CO_2}$ was corrected to STPD and normalized, along with $\dot{V}_E$ and $\dot{V}_T$, by 100 g of body weight. At the conclusion of the 4 week study, the animals were sacrificed and entire diaphragms were dissected and weighed.

**Protocol.** Each rat was weighed, its body temperature determined, and its nose-anus length measured. Then it was placed into the chamber for an acclimation period while room air flowed through the chamber. The collection of the ventilatory ($\dot{V}_T$, $f$, $\dot{V}_E$) and metabolic ($\dot{V}_{CO_2}$) data began after the animals appeared to be calm and resting and chamber $CO_2$ levels were stable (approx. 6 min). Then 10% $CO_2$ in $O_2$ was introduced into the chamber. Ventilation was evaluated when $CO_2$ concentrations exiting the chamber reached 3%, 6%, and 9%. The chamber was flushed with room air for 15 min and then 100% $N_2$ was infused into the chamber. Ventilation was evaluated when the $O_2$ concentration in the chamber reached 10%. Animals were tested in the same sequence on Week 1, 2, 3 and 4.

**Statistical Analysis.** Ventilatory parameters, $\dot{V}_{CO_2}$, body weight, and length data were subjected to a two-way analysis of variance followed by Fischer's least means square method (SAS Version 5, Cary, NC). To compare the effects of vehicle, STZ-treatment and insulin replacement on ventilatory responses of the animals to hypercapnia and hypoxia, the slope and intercept of each rat were determined using linear regression analysis, and then the two parameters were subjected to the two-way analysis of variance and a post hoc Fischer's test were used. Differences among the diaphragmatic weights (uncorrected and corrected for body weight) in the three groups were evaluated using a one-way analysis of variance. The level of significance chosen was $P < 0.05$. Data are presented as mean ± SEM.

## Results

Blood glucose levels 2 days after STZ-injection were 18.4 ± 0.6 mmol/l. glucose remained elevated after 4 weeks in the diabetic animals (19.9 ± 1.0 mmol/l), was reduced to near normal levels as a result of insulin treatment in the diabetic rats (9.1 ± 2.0 mmol/l), and was 6.3 ± 0.4 mmol/l in the control group.

Growth (body weight and nose-anus length) was retarded in the diabetic rats. These differences in growth were noted during the second week and continued throughout the study, resulting in body weights and nose-anus lengths 70% and 80% of control group values at Week 4, respectively (Table I). After Week 1, the nose-anus length was less both in the diabetic and in the insulin-treated diabetic rats compared with the control animals. However, the insulin-treated rats exhibited normal growth until the fourth week at which time their body weight and nose-anus length were significantly less than those of the control animals while still 31% heavier and 9% longer than the diabetic animals. Although diaphragm net-weight in the diabetic group was only 70% of control, when adjusted by 100 g of body weight, the diaphragm weights among groups were not different.

Weight-adjusted tidal volume ($W\dot{V}_T$), minute ventilation ($W\dot{V}_E$), and frequency of breathing were comparable among the three groups after Week 1 (Table II). A decrease in weight-adjusted tidal volume and minute ventilation occurred in the STZ-treated group by Week 2 and continued throughout the 4-week period compared with increases of these parameters in the two other groups. Frequency of breathing, in contrast, showed neither an age nor treatment effect. Insulin-treated STZ and control rats exhibited comparable ventilatory parameters throughout the 4-week period.

Not only ventilation, but also $CO_2$ production was affected by STZ treatment (Table III). After the first week, the diabetic rats' weight-adjusted $CO_2$ production ($W\dot{V}_{CO_2}$) was 38% less than that of the control animals. By the conclusion of the study, the diabetic group's $W\dot{V}_{CO_2}$ was 56% of their initial value and was 28% of the control group's value. There were no significant differences in $W\dot{V}_{CO_2}$ between the control and insulin-treated diabetic animals throughout the 4 weeks of the study.

After the first week of treatment, the $W\dot{V}_E$ re-

**Table I.** Body Weight, Nose-Anus Length, and Post-Study (4 Week) Diaphragm Weight of Control, Diabetic + Insulin and Diabetic Rats

| Week | Control | Diabetic + insulin | Diabetic |
|---|---|---|---|
| Body weight (g) | | | |
| 0 | 212.80 ± 3.93 | 205.89 ± 2.82 | 215.70 ± 3.96 |
| 1 | 227.85 ± 4.51 | 221.54 ± 3.99 | 205.79 ± 4.81 |
| 2 | 264.34 ± 6.63 | 253.29 ± 7.13 | 213.12 ± 6.51[a,b] |
| 3 | 290.80 ± 8.94 | 279.62 ± 8.95 | 211.18 ± 8.30[a,b] |
| 4 | 312.15 ± 9.71 | 284.16 ± 10.79[a] | 217.03 ± 12.84[a,b] |
| Nose-anus length (cm) | | | |
| 1 | 19.60 ± 0.27 | 18.72 ± 0.37[a] | 18.65 ± 0.35[a] |
| 2 | 19.85 ± 0.26 | 20.05 ± 0.13 | 18.90 ± 0.31[a,b] |
| 3 | 21.15 ± 0.39 | 21.22 ± 0.24 | 19.10 ± 0.35[a,b] |
| 4 | 22.60 ± 0.18 | 21.75 ± 0.35[a] | 19.90 ± 0.34[a,b] |
| Diaphragm weight (g) | | | |
| 4 | 0.84 ± 0.04 | 0.80 ± 0.04 | 0.59 ± 0.04[a,b] |
| Body weight adjusted diaphragm weight (g/100 g body wt) | | | |
| 4 | 0.27 ± 0.01 | 0.28 ± 0.01 | 0.28 ± 0.01 |

*Note.* Values are mean ± SEM, obtained each week for four consecutive weeks, with the exception of diaphragm weight.
[a] Significant difference from control ($P < 0.05$).
[b] Significant difference from diabetic + insulin ($P < 0.05$).

**Table II.** Weight Adjusted Minute Ventilation, Weight Adjusted Tidal Volume, and Frequency at Basal Room Air of Control, Diabetic + Insulin and Diabetic Rats

| Week | Control | Diabetic + insulin | Diabetic |
|---|---|---|---|
| Minute ventilation (ml/min/100 g body wt) | | | |
| 1 | 159.10 ± 13.51 | 176.30 ± 12.08 | 139.09 ± 10.83 |
| 2 | 281.00 ± 13.58 | 304.00 ± 40.30 | 174.58 ± 10.89[a,b] |
| 3 | 306.94 ± 27.24 | 342.13 ± 36.35 | 182.65 ± 17.81[a,b] |
| 4 | 274.36 ± 15.87 | 307.04 ± 25.69 | 148.09 ± 14.26[a,b] |
| Tidal volume (ml/breath/100 g body wt) | | | |
| 1 | 1.25 ± 0.10 | 1.36 ± 0.09 | 1.18 ± 0.10 |
| 2 | 2.14 ± 0.11 | 2.18 ± 0.33 | 1.36 ± 0.11[a,b] |
| 3 | 2.21 ± 0.16 | 2.51 ± 0.19 | 1.31 ± 0.16[a,b] |
| 4 | 2.04 ± 0.09 | 2.49 ± 0.22 | 1.13 ± 0.09[a,b] |
| Frequency (breaths/min) | | | |
| 1 | 128.06 ± 3.91 | 130.62 ± 5.06 | 118.60 ± 4.77 |
| 2 | 133.00 ± 6.65 | 141.55 ± 5.08 | 129.95 ± 4.90 |
| 3 | 138.27 ± 5.51 | 135.25 ± 4.81 | 144.59 ± 9.90 |
| 4 | 134.82 ± 6.21 | 124.95 ± 5.44 | 131.19 ± 5.83 |

*Note.* Values are mean ± SEM, obtained each week for four consecutive weeks. Minute ventilation, tidal volume, and frequency of breathing were obtained during acclimation period of protocol and adjusted by body weight of each individual animal.
[a] Significant difference from control ($P < 0.05$).
[b] Significant difference from diabetic + insulin ($P < 0.05$).

sponses (both slope and intercept) of the three groups to hypercapnia were similar (Fig. 1). Throughout the next 3 weeks the intercept values of the diabetic group were lower ($P < 0.001$) than those of the other two nondiabetic groups, and there were no significant differences between the control and the insulin-treated diabetic group (Fig. 1 and 2). By Week 2, the slope values of the STZ-treated ($P < 0.001$) and insulin-treated STZ rats ($P < 0.02$) were significantly lower than that of the control group values. Over the next 2 weeks, the slope values of the diabetic animals were markedly lower than those of the other two groups.

The first week after treatment the $W\dot{V}_E$ (slope and intercept) of the three groups to hypoxia were similar

**Table III.** Weight Adjusted Carbon Dioxide Production

| Week | Carbon dioxide production (ml/min/100 g body wt) | | |
|---|---|---|---|
| | Control | Diabetic + insulin | Diabetic |
| 1 | 8.61 ± 0.82 | 7.87 ± 0.93 | 5.36 ± 0.43[a] |
| 2 | 9.51 ± 1.37 | 9.49 ± 0.64 | 5.09 ± 0.55[a,b] |
| 3 | 11.33 ± 1.03 | 11.22 ± 1.45 | 3.61 ± 0.32[a,b] |
| 4 | 10.79 ± 0.77 | 8.45 ± 1.52 | 3.02 ± 0.47[a,b] |

*Note.* Values are mean ± SEM, obtained each week for four consecutive weeks during the acclimation period of the protocol and adjusted by body weight of each individual animal.
[a] Significant difference from control ($P < 0.05$).
[b] Significant difference from diabetic + insulin ($P < 0.05$).

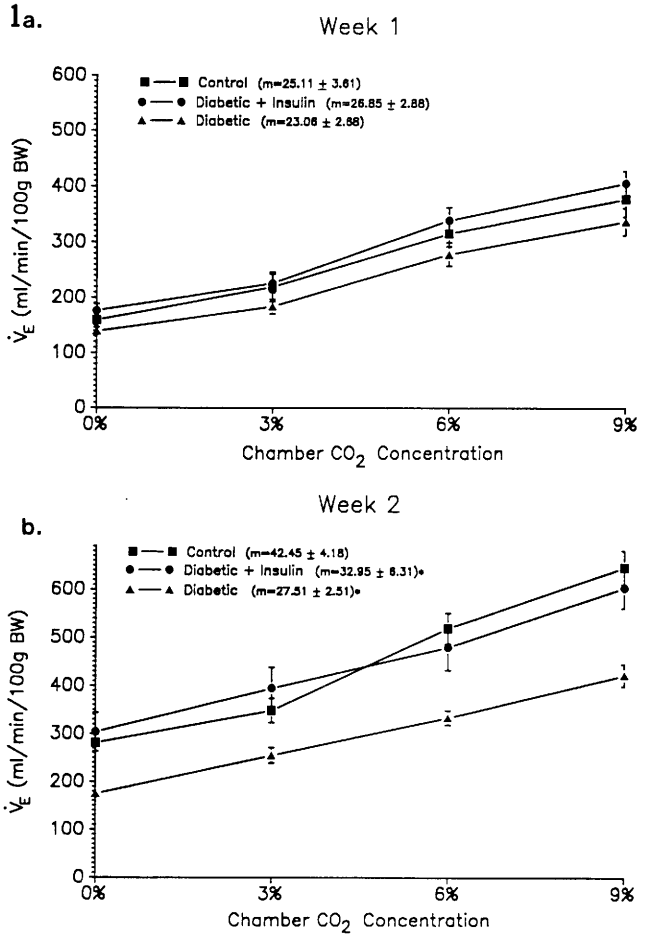


**Figure 1.** Weight-adjusted minute ventilation response to a hypercapnic challenge in control (black squares), diabetic + insulin (black circles), and diabetic rats (black triangles) during Week 1 (a) and Week 2 (b). Figures represent the mean values and bars are ±SEM. The values in parentheses are the value of the mean slope and standard error of the slope for animals in each group. An asterisk denotes a significant value compared with the control group value. An exclamation mark (!) denotes a significant difference between the diabetic and the insulin-treated diabetic group.

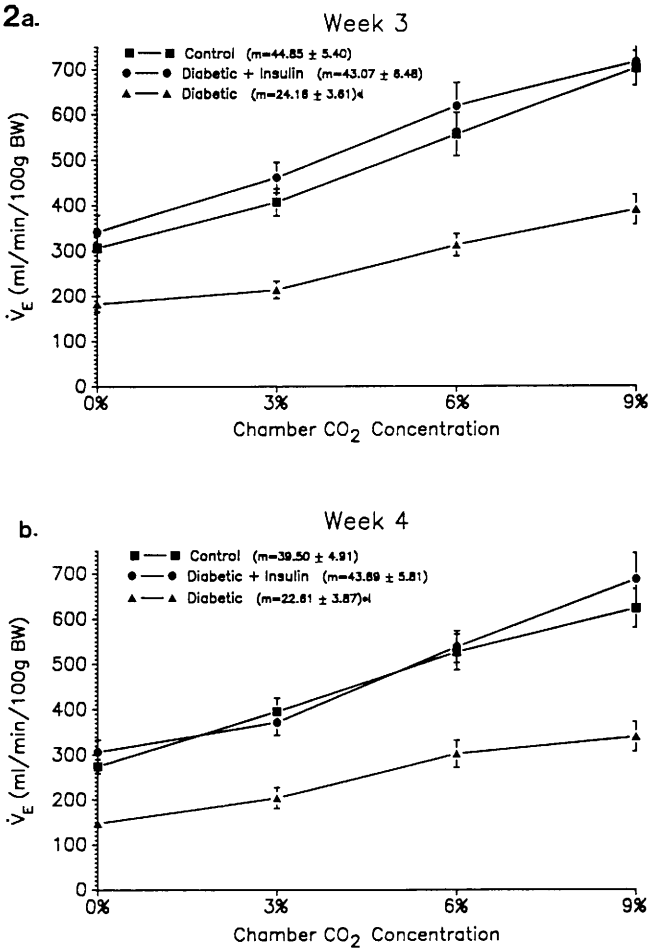


**Figure 2.** Weight-adjusted minute ventilation response to a hypercapnic challenge in control, diabetic + insulin, and diabetic rats during Week 3 (a) and Week 4 (b). See Figure 1 for explanation of the symbols.

(Fig. 3). Intercept values were significantly lower in both the control and insulin-treated diabetic groups compared with the STZ-treated group ($P < 0.001$) the following three weeks (Fig. 3 and 4). Slope values were greater in the control group than either the insulin-treated STZ or the diabetic groups by Week 2. During Week 3 and 4 the slope values in the control and the insulin-treated STZ groups were not different from each other, but higher than ($P < 0.02$) that of the diabetic group.

## Discussion

The results of this study indicate that minute ventilation and ventilatory responses to hypoxia and hypercapnia were decreased in STZ-induced diabetic rats relative to those of the other groups. Although there were some differences between the insulin-treated group and the control group in ventilatory control (Week 1) and body growth (Week 1 and 4), insulin treatment of diabetic rats prevented the development of alterations in minute ventilation and ventilatory response to hypercapnia and hypoxia.

Precisely how insulin treatment or insulin deprivation affects the control of ventilation in diabetic rats remains unclear at present. However, the literature suggests that both structural and functional changes in muscle and nervous tissues due to altered delivery of oxygen at the tissue level, abnormal transcellular glucose transport, depressed cellular metabolism, and changes in endogenous opioid modulation of ventilation may contribute to altered ventilatory function in diabetic rats.

**Autonomic Neuropathy.** One major contributor to the diminished ventilatory responses in diabetic rats may be associated with development of autonomic neuropathy. Navarro and coworkers (19) documented abnormalities of cardiopulmonary reflexes in 75% of the 232 insulin-dependent diabetic patients. Many of these patients had evidence of somatic and autonomic

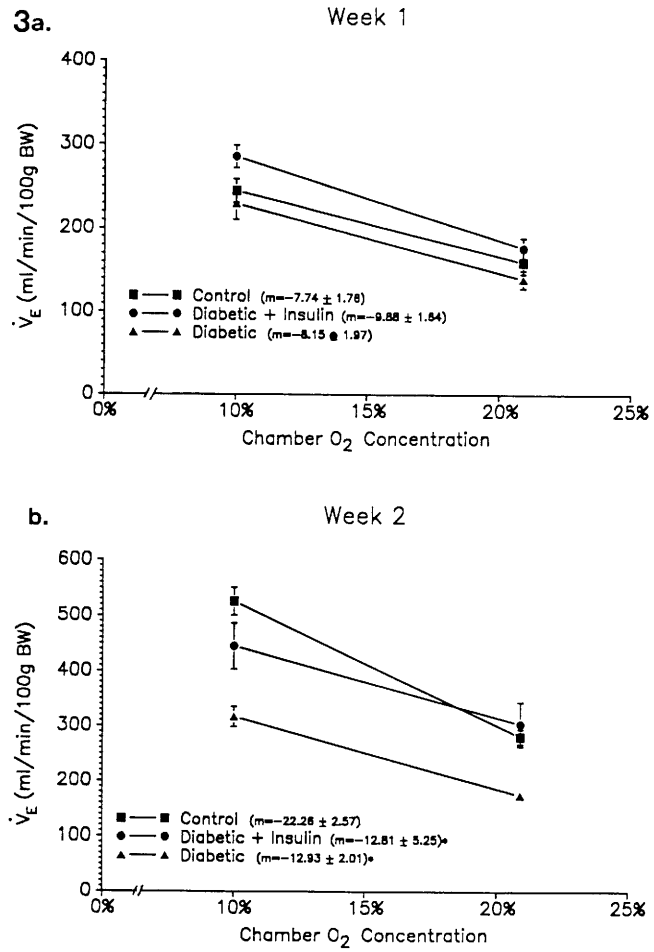


**Figure 3.** Weight-adjusted minute ventilation response to a hypoxic (10% $O_2$) challenge in control, diabetic + insulin, and diabetic rats on Week 1 (a) and Week 2 (b). See Figure 1 for further explanations of the symbols.

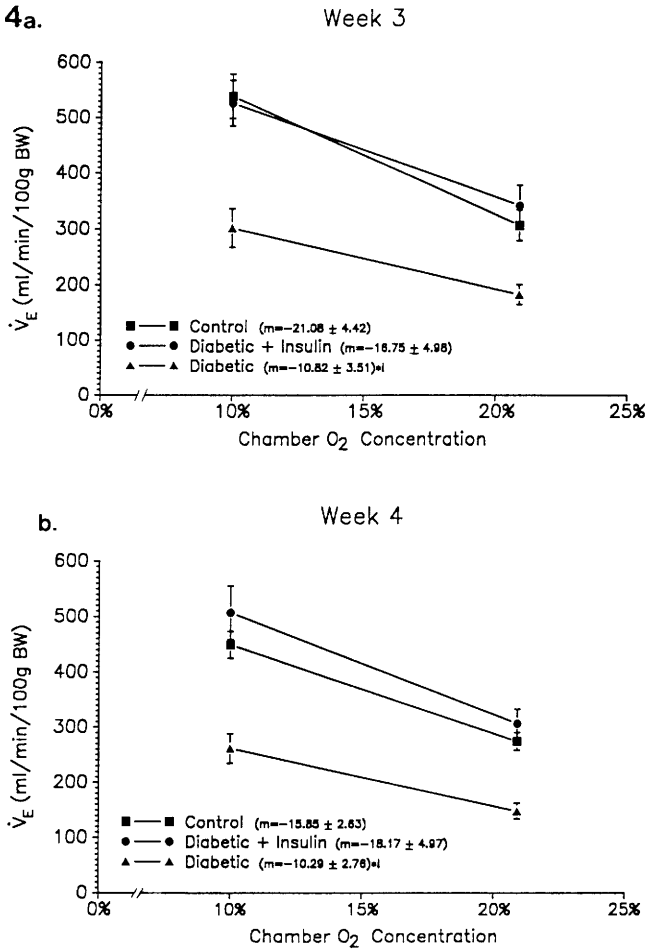


**Figure 4.** Weight-adjusted minute ventilation response to a hypoxic (10% $O_2$) challenge in control, diabetic + insulin, and diabetic rats on Week 3 (a) and Week 4 (b). See Figure 1 for further explanation of the symbols.

neuropathy and also had higher mortality rates than patients without abnormal autonomic tests. Patients with neuropathy who received a functioning pancreas transplant exhibited a decreased rate of mortality. Moreover, insulin-dependent diabetic patients were reported by O'Donnell and coworkers (20) to have diminished perception of inspiratory-resistive loads. This may lead to delayed recognition of the development of pulmonary disease in patients. Furthermore, diminished ventilatory responses to hypercapnic and hypoxic challenges, as noted in the present study, together with decreased perception of loads may lead to an inability of individuals with diabetes to respond appropriately to apneic episodes during sleep and thereby increase the likelihood of sudden death (13). A possible mechanism for altered control of ventilation in diabetic patients is suggested by a recent study by Wanke and coworkers (21). In that study, diabetic patients with autonomic neuropathy exhibited reduced ventilatory responses to hyperoxic hypercapnia, compared with normal responses in diabetic patients without autonomic neuropathy and matched control subjects. Of further importance is that the control group exhibited a stimulation of ventilation after naloxone administration, but neither diabetic group showed a stimulation of ventilation after naloxone. Moreover, respiratory muscle function and pulmonary mechanics were comparable in three groups. The authors suggest that central modulation of ventilation in diabetic patients by endogenous opioids appears to be abnormal. Whether the STZ-induced diabetic rat also exhibits blunted ventilatory responses to naloxone remains to be determined. Interestingly, Schlenker and Farkas (35) noted that the obese Zucker rat, an animal model of insulin independent diabetes mellitus, shows a marked stimulation of ventilation after naloxone administration suggesting that the two types of diabetes may have different effects on opioid modulation of ventilation.

**$CO_2$ Production and Ventilatory Responses.** Another factor that may explain the depressed ventilation and the ventilatory response of diabetic rats to hypoxic and hypercapnic gas challenges is their low metabolic rate. For example, Doekel and coworkers

(22) reported that semistarvation in otherwise healthy human subjects resulted in a 60% decrease in their $CO_2$ production, accompanied by a concomitant fall in minute ventilation, and a markedly decreased hypoxic, but not hypercapnic, ventilatory response. Moreover, Smith and Schlenker (unpublished results) observed that food restriction of Sprague Dawley rats resulted in decreased oxygen consumption, decreased ventilation in response to hypoxia, but no effect on ventilation in response to hypercapnia compared to these parameters in *ad libitum* fed animals. In the present study, $CO_2$ production dropped dramatically by the first week, however both minute ventilation and responses to hypoxia and hypercapnia in the diabetic group were similar to those of the control and insulin-treated diabetic groups. The pattern of lower $CO_2$ production relative to ventilation was also noted on subsequent weeks in the diabetic group, but then ventilatory responses to both hypoxia and hypercapnia became significantly lower in this group relative to control animals' responses. Thus, in the diabetic group ventilation, ventilatory responses to gas challenges, and metabolic rate were not always matched. Moreover, the insulin-treated diabetic rats in the present study exhibited a normal $W\dot{V}_{CO_2}$ production and $W\dot{V}_E$ on Week 2, but had similar ventilatory response (slope) as did the diabetic animals. These results suggest that in this animal model of diabetes $CO_2$ production most likely is not the only factor affecting ventilation and ventilatory responses to gas challenges. Moreover, the effects of food restriction on ventilation in response to hypoxia and to hypercapnia are not the same as in rats with STZ-induced diabetes.

**Insulin Deprivation and Skeletal Muscle Function.** In untreated diabetes, insulin deprivation mimics the effects of semistarvation on skeletal muscle (23, 24). Studies on the effects of starvation or semistarvation in skeletal muscle demonstrate that strength decreases proportional to weight loss as a result of decreased muscle contractility (25). Alterations in diaphragm muscle structure in rats due to nutritional deprivation, such as decreased fiber diameters, may impair the ability of the diaphragm to generate force.

Studies in diabetic patients indicate that diaphragm muscle strength may be reduced (26) and that decreased diaphragm weight and thickness may well correlate with weight loss in diabetic rats. We showed that the diabetic rat's diaphragm weight and body weight were both reduced by 30% when compared with control animals, but, when adjusted for 100 g of body weight, there was no difference between the diaphragm weight of the diabetic rats and the diaphragm weight of the controls. Since the maintenance of ventilation is dependent on the ability of the inspiratory muscles to generate force during hypoxic and hypercapnic conditions, atrophy of muscle fibers and also alterations in phrenic nerves may contribute to decreased ventilatory response in diabetic rats. In an extensive morphometric and histochemical study of the phrenic nerves and diaphragms from 12-month-old STZ-treated rats, Bestetti and coworkers (27) found reductions in phrenic nerve fiber cross-sectional areas and severe lesions in Schwann cells, endoneural mesenchymal cells, and in the motor endplate. The diaphragms from STZ-treated rats contained smaller white fibers and increased lipid content compared with control animals. Although this study was conducted in animals 12 months after STZ treatment, a number of studies in rats treated with STZ for shorter time periods (from a couple of days to a few weeks) also reported similar disruptions of structure and function in somatic nerves, autonomic nerves, and skeletal muscles in diabetic animals (28–33). Callis and his coworkers showed that rats treated with STZ for 3 weeks had smaller gastrocnemius muscles compared with control rats muscles (34). The diabetic muscle, compared with the control muscle, developed less twitch tension, showed greater drops in intracellular pH, and adenosine and phosphate levels, as well as impaired pyruvate oxidation during contraction. These differences were not noted between the two groups at rest.

In conclusion, control of ventilation is altered progressively over 4 weeks of STZ-induced diabetes in rats, and insulin treatment ameliorates the ventilatory abnormalities observed in the diabetic group. This animal model may be useful to elucidate mechanisms in various segments of the ventilatory reflex loop that may be responsible for the development of ventilatory control abnormalities in diabetes.

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