Ventilatory response to exercise in diabetic subjects
with autonomic neuropathy

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PREVIOUS EXPERIMENTAL STUDIES in humans on the role
of the pulmonary autonomic nervous system on the
modulation of the breathing pattern during stimulated
ventilation were invasive (vagal blockade) (16, 17) or
limited to the trachea and large bronchi (airway anes-
thesia) (36, 40). Because of these technical limitations,
data were sparse and sometimes conflicting. In investigat-
ing heart-lung transplanted patients, some investiga-
tors have recently claimed that pulmonary neuro-
genic mechanisms essentially influence the level, but
not the pattern, of ventilation during exercise (23),
although both tidal volume (VT) (2, 13) and respiratory
rate (RR) (2, 13, 23) exhibited a steeper increase in
these patients compared with normal subjects. Con-
versely, another study (33) in similar patients, who
were compared only with heart transplant recipients
without a matched group of normal subjects, seems to
indicate that during exercise pulmonary nerves are
more important in regulating the breathing pattern
(deeper and slower in heart-lung transplanted pa-
tients) than is the absolute level of ventilation.

Apart from the disruption of bronchial circulation
and pulmonary lymphatics, heart-lung transplanted
patients frequently have chronic muscle deconditioning
due to long-term pretransplant debilitation (39) and
may also have a restrictive ventilatory defect (23).
Moreover, despite the indirect evidence provided for the
persistence of pulmonary denervation after transplan-
tation (1, 23), reinnervation several months from sur-
ery cannot be completely ruled out (12, 26). All of these
findings may be confusing factors when heart-lung
transplant recipients are regarded as a unique model of
pulmonary autonomic denervation to be compared with
normal subjects for analysis of the ventilatory response
during exercise.

These patients, however, do not constitute the sole
model of pulmonary autonomic denervation in humans,
since a defect of the autonomic nervous system is not
uncommon in diabetes mellitus (15); indeed, in the
study of the ventilatory response to exercise, diabetic
patients with autonomic neuropathy may provide a
simple model of chronic pulmonary autonomic denerva-
tion (8, 35).

In the attempt to further investigate the role of the
autonomic system on ventilatory response to exercise, a
group of diabetic subjects suffering from moderate to
severe autonomic neuropathy was studied during stress-
ful exercise.

Our results suggest that diabetic autonomic dysfunc-
tion is associated with a greater ventilatory response to
exercise. Although this finding can be partially ex-
plained by the increment of dead space (Vd) ventilation
and by a faster RR, a progressively higher inspiratory
activity is likely to be involved, as indicated by a
steeper relationship for mouth occlusion pressure and
alveolar ventilation against exertional CO2 production
(VCO2). Increased CO2 chemosensitivity or decreased
autonomic (probably sympathetic) inhibitory influence
on the central drive could represent the main mecha-
nism underlying the abnormally elevated inspiratory
output observed in these patients.

Possible problems with diabetic neuropathy as a
model for pulmonary denervation are fully considered
in DISCUSSION.

METHODS

Subjects. Twenty male diabetic patients, 10 without and 10
with diabetic autonomic neuropathy, henceforth referred to
as Dan− and Dan+, respectively, were recruited from the
Istituto di Medicina Interna e Scienze Endocrine e Metaboli-

che, Department of Internal Medicine, University of Perugia and were enrolled in the study after giving fully informed consent. The protocol was approved by the local ethics committee and was in accordance with the Declaration of Helsinki. All patients were on insulin treatment with four daily insulin injections (regular insulin before each meal and intermediate-acting insulin at bedtime). No drugs likely to interfere with the pulmonary or cardiovascular function were taken by the patients. Autonomic neuropathy was assessed by means of the standard battery of cardiovascular tests (14); each test was scored according to the literature (5), and patients were considered positive for autonomic neuropathy if the total score was ≥ 4. Five out of 10 Dan+ patients had severe postural hypotension with upright fall in systolic blood pressure of ≥ 30 mmHg. Postural hypotension is thought to be the consequence of impaired splanchnic vasoconstriction due to overt sympathetic damage of the autonomic nervous system.

In the Dan+ group, two patients had preproliferative retinopathy and one patient had proliferative retinopathy; four of the ten Dan− patients had background retinopathy. Four Dan− patients had microalbuminuria, whereas in the Dan+ group microalbuminuria was detected in five patients and proteinuria in three. In regard to the symptoms related to the autonomic dysfunction, only one Dan+ patient complained of nocturnal watery diarrhea.

No patient reported respiratory symptoms at the time of the study, and all had normal physical examination, electrocardiogram, and chest radiography. None of the patients studied was suffering from anemia or had signs or symptoms of endocrine or metabolic diseases other than diabetes. All were normotensive, and none had evidence of ischemic heart disease or S-T segment depression during previous submaximal or symptom-limited incremental exercise. To exclude dilated or restrictive cardiomyopathy and valvular heart disease, all patients underwent a bidimensional echocardiographic study. Left ventricular systolic or diastolic dysfunction was not observed at rest, and morphological and/or functional valvular abnormalities were absent. Despite the presence of diabetic nephropathy in some patients, none suffered from renal failure at the time of the study.

Ten (five male normal subjects recruited from the University staff, matched for age and weight, were studied as the control group.)

Study design. Patients and control subjects had pulmonary function tests, including determination of maximal voluntary ventilation (MVV) and measurements of maximal inspiratory (MIP) and expiratory (MEP) mouth pressures. Spirometry, flow-volume curves, lung volumes (by the multiple-breathe nitrogen washout technique), single-breathe diffusing capacity for carbon monoxide (D_{CO}) adjusted for hemoglobin, and MVV were performed with the subjects in a sitting position while wearing a noseclip and breathing through a mouthpiece on a Medical Graphics 1070 computerized system. MVV was performed with the subjects in a sitting position while wearing a noseclip and breathing through a mouthpiece on a Medical Graphics 1070 computerized system.

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premeal and bedtime insulin doses so as to avoid premeal blood glucose levels of <5 mmol/l, based on capillary blood glucose measurements by chemists. Each exercise test was performed in the afternoon between 5:00 and 5:30 P.M., 5 h after the patients had their prandial dose of regular insulin.

Analysis. To provide comparisons among groups at similar relative levels of physiological function, Ve was normalized as a percentage of MVV (Ve/MVV) and Vt as a percentage of inspiratory capacity (IC) (Vt/IC). These variables are referred to as adjusted Ve and adjusted Vt.

The means of lung function, exercise, and ventilatory timing variables of the three groups were compared by analysis of variance and by orthogonal comparisons adopting a two-tailed Student's t-test for unpaired data when allowed by analysis of variance. The linear correlations were calculated by the least squares method. Comparisons among groups of the mean slopes were performed according to the above-mentioned procedure. Data are expressed as mean ± SE. P < 0.05 was considered to be statistically significant.

RESULTS

Characteristics and lung function. Anthropometric and clinical data of diabetic and control subjects are shown in Table 1. Patients and control subjects were not significantly different for age, weight, and smoking habit. The long-term control of hyperglycemia was similar and satisfactory in both groups of patients.

Lung function tests, including MVV and DLCO, expressed as a function of the alveolar volume (VA) were in the normal range for both Dan− and Dan+ subjects and were not significantly different from those of control subjects. The indexes of respiratory muscle strength (MIP and MEP), reported both as absolute values and as percentages of predicted values, were also normal in the Dan− and Dan+ groups and were not dissimilar from those of control subjects.

Neuromuscular and ventilatory responses to progressive hypercapnic stimulation, as assessed by a relationship of P0.1 and Ve against PETCO2, respectively, were not different in Dan− and control subjects and were 0.50 ±0.10 vs. 0.46 ± 0.06 cmH2O/Torr for the P0.1/PETCO2 slope (not significant) and 3.71 ± 0.73 vs. 3.27 ± 0.35 l·min−1·Torr−1 for the Ve/PETCO2 slope (not significant).

Exercise performance. The maximum workload and VO2 at peak of exercise (here referred to as DLCO/VA) (VO2max) achieved by the three groups are reported in Table 2. At peak of exercise, the workload, as percentage of predicted value, was not significantly different in the three groups. Instead, VO2max was much lower in diabetic than in control subjects, both as absolute value and as percentage of predicted value. In this respect, although Dan− subjects had a lower VO2max as percentage of predicted value, compared with Dan+, the difference was not statistically significant. As absolute value of VO2, control subjects had the highest gas-exchange AT (VO2 at AT), which, in turn, was not different between the Dan− and Dan+ groups. VO2 at AT, expressed as a percentage of VO2max, was higher in the Dan+ group.

The LAT (VO2 at LAT) was almost identical in the diabetic groups (Table 2). In addition, the exertional increment of lactate plasma levels was superimposable in the Dan− and the Dan+ subjects, as shown in Fig. 1. At peak of exercise, VCO2 was significantly greater in control than in diabetic subjects but was similar in Dan− and Dan+ subjects.

Two of the Dan+ subjects stopped the exercise test because of leg fatigue. None of the diabetic subjects suffered from hypoglycemia during the exercise.

Exercise ventilation. Ve at peak of exercise was similar in the three groups both in absolute value and as a percentage of MVV (Table 2). The ventilatory

<table>
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<tr>
<th>Table 1. Anthropometric and clinical data of subjects and patients</th>
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<tr>
<td><strong>Control</strong></td>
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<td>Age, yr</td>
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<tr>
<td>Weight, kg</td>
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<tr>
<td>Smoking habit, pack · yr</td>
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<td>Duration of disease, yr</td>
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<td>HbA1c, %</td>
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<td>IDDM/NIDDM, no.</td>
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<td>DM score</td>
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<td>VC, % predicted</td>
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<td>IC, % predicted</td>
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<td>FRC, % predicted</td>
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<td>TLC, % predicted</td>
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<td>FEV1/FVC, %</td>
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<td>FEV25–75, % predicted</td>
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<td>DLCO/VA, % predicted</td>
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<td>MVV, % predicted</td>
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<td>MIP at FRC, % predicted</td>
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<td>MEP at FRC, cmH2O</td>
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<td>MEP at FRC, % predicted</td>
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<td>MIP at FRC, % predicted</td>
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Values are means ± SE. Dan−, diabetic subjects without autonomic neuropathy; Dan+, diabetic subjects with autonomic neuropathy; HbA1c, hemoglobin A1 glycosylate (values in nondiabetic subjects: 3.8–5.5%); IDDM/NIDDM, insulin-/non-insulin-dependent diabetes mellitus; DAN, diabetic autonomic neuropathy; VC, vital capacity; IC, inspiratory capacity; FRC, functional residual capacity; RV, residual volume; TLC, total lung capacity; FEV1, forced expiratory volume; FEFR, forced expiratory flow; VEFR in 1 s; FEF25–75, 25–75% of FEFR, diffusing capacity for carbon monoxide; Vt, alveolar volume; MVV, maximal voluntary ventilation; MIP, maximal inspiratory pressure; MEP, maximal expiratory pressure.

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<th>Table 2. Workload, ventilation, and gas-exchange parameters of diabetic and control subjects at peak of exercise</th>
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<tr>
<td><strong>Control</strong></td>
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<tr>
<td>Workload maximum, W</td>
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<td>Workload maximum, % predicted</td>
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<td>VO2max, ml/min</td>
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<td>VO2max, % predicted</td>
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<td>V˙Emax/ml/min</td>
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<td>V˙E at AT, ml/min</td>
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<td>V˙E at AT, %VO2max</td>
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<td>VO2 at LAT, ml/min</td>
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<td>V˙E at LAT, %predicted</td>
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*Values are means ± SE. VO2, O2 consumption; VO2max, maximal VO2; V˙CO2max, maximal CO2 production; VE, minute ventilation; AT, gas-exchange anaerobic threshold; LAT, lactate anaerobic threshold. Significantly different compared with *Dan− and Dan+ (P < 0.001); †Dan− and Dan+ (P < 0.01); ‡Dan−; §control and Dan+ (P < 0.05).
response to increasing \( V_{\text{CO}_2} \) was linear in all subjects (\( r \) values ranging from 0.98 to 0.99) (Fig. 2A). The slope of \( V_{\text{E}}/V_{\text{CO}_2} \) was 0.032 ± 0.002 ml/min in Dan+ and was steeper than that of both control (0.025 ± 0.001 ml/min; \( P < 0.001 \)) and Dan− (0.027 ± 0.001 ml/min; \( P < 0.05 \)) subjects.

The \( P_{0.1}/V_{\text{CO}_2} \) relationship was essentially linear in patients and control subjects during exercise (\( r \) values ranging from 0.90 to 0.99) and had a mean slope of 0.0031 ± 0.0002, 0.0049 ± 0.0005, and 0.0061 ± 0.0007 cmH\(_2\)O·ml\(^{-1}\)·min\(^{-1}\) in control, Dan− (\( P < 0.05 \)), and Dan+ (\( P < 0.002 \)) subjects, respectively. Thus at corresponding exertional values of \( V_{\text{CO}_2} \), \( P_{0.1} \) was progressively greater in the two groups of patients than in the control group (Fig. 2B).

The \( P_{0.1}/V_{\text{E}} \) relationship, which reflects the impedance of the respiratory system, is illustrated throughout the exercise in Fig. 3 for patients and control subjects.

Even though the computed physiological \( V_0/V_{\text{T}} \) value was not significantly different at rest in the three groups, during exercise the rate of decrease of the physiological \( V_0/V_{\text{T}} \) in relation to adjusted ventilation was substantially lower both in Dan+ (0.0030 ± 0.0017%\%/\%\%) and Dan− (0.0037 ± 0.0017%\%/\%\%) compared with control (0.0050 ± 0.0023%\%/\%\%) subjects (\( P < 0.05 \)). The physiological \( V_0/V_{\text{T}} \) was significantly greater in both groups of patients than in the control group at progressively higher values of \( V_{\text{E}}/MVV \) (Fig. 4A). This was mainly due to an abnormal exertional increase in physiological \( V_0 \) in the diabetic patients (Fig. 4B).

The relationship between computed \( VA \) and metabolic \( V_{\text{CO}_2} \) during exercise increased progressively more in Dan+ subjects, whereas it remained almost identical at a lower level for the Dan− and control groups (Fig. 5). The slope of this relationship was 0.027 ± 0.001 \( 1\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1} \) in the Dan+ group and 0.023 ± 0.001 \( 1\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1} \) in both the control and Dan− groups (\( P < 0.01 \)).

At rest, \( PET_{\text{CO}_2} \) was similar in all groups, amounting to 37.4 ± 0.7, 38.9 ± 0.7, and 38.0 ± 1.2 Torr in the control, Dan−, and Dan+ groups, respectively. The corresponding values of estimated \( P_{\text{ACO}_2} \) were 37.5 ± 0.7, 38.8 ± 0.8, 38.1 ± 1.1 Torr in the control, Dan−, and Dan+ groups, respectively. Conversely, at peak of exercise \( PET_{\text{CO}_2} \) was significantly lower in Dan+ than in control (\( P < 0.005 \)) and Dan− (\( P < 0.02 \)) subjects, amounting to 35.9 ± 1.6, 42.1 ± 0.9, and 42.1 ± 1.7 Torr in Dan+.
control, and Dan− groups, respectively. The corresponding values of estimated \(P_{aCO_2}\) were 33.5 ± 1.4, 37.6 ± 1.0, and 38.6 ± 1.7 Torr in Dan+, control (\(P < 0.005\)), and Dan− (\(P < 0.02\)) groups, respectively.

The resting hemoglobin \(O_2\) saturation was normal in the Dan−, Dan+, and control subjects (97.9 ± 1.5, 97.7 ± 1.6, and 98.5 ± 1.2%, respectively); \(O_2\) desaturation did not occur in any subject during the exercise.

Pattern of breathing. The rate of exponential increase of adjusted \(V_t\) (\(V_t/IC\)) during exercise was similar in the three groups (Fig. 4C).

On the contrary, the rate of RR linear increase during exercise was significantly higher in the Dan+ compared with the control and Dan− groups (\(P < 0.05\)), amounting to 0.52 ± 0.16 vs. 0.39 ± 0.14 and 0.36 ± 0.14 breaths/min, respectively (Fig. 4D). RR was similar at rest among the groups but faster at peak of exercise in the Dan+ compared with Dan− and control subjects (Table 3). Although \(T_i\) and \(T_e\) were significantly shorter at peak of exercise in Dan+ compared with Dan− and control subjects (Table 3), the rate of \(T_i\) and \(T_e\) decrease during exercise was not different among the groups.

There were no significant differences among control, Dan−, and Dan+ subjects in the rate of increase of mean inspiratory flow (\(V_t/T_i\)), which displayed similar values at rest and peak of exercise for the three groups (Table 3).

**DISCUSSION**

Before the results of the study are discussed, some problems with diabetic autonomic neuropathy as a model of pulmonary autonomic denervation should be pointed out.
superimposable levels of $V_{\dot{A}}$ throughout effort. Symbols closest to presence of remarkable pathological alterations in the dysautonomy, however, have consistently shown the unmyelinated fibers.

Expected to have an incomplete degree of autonomic functioning, therefore, our patients are variable and supports the pathological findings of a (25). Their expression in diabetics is inherently symmetric and distal neuropathy and produces diverse symptoms that cannot be exactly determined in patients in vivo.

It is implicit that the extent of the autonomic neuropathy and related pulmonary autonomic denervation cannot be exactly determined in patients in vivo. Diabetic autonomic neuropathy is by definition a symmetric and distal neuropathy and produces diverse and somewhat selective neural alterations, both in the parasympathetic and sympathetic nervous systems (25). Their expression in diabetic subjects is inherently variable and supports the pathological findings of a frequently partial process. Therefore, our patients are expected to have an incomplete degree of autonomic denervation with differential effects on myelinated and unmyelinated fibers.

Autotopic findings in diabetic subjects with severe dysautonomia, however, have consistently shown the presence of remarkable pathological alterations in the sympathetic ganglia and either in the vagus or sympathetic nerves, with predominant loss of myelinated fibers (11). In this respect, our patients were suffering from marked autonomic neuropathy, as demonstrated by the quantitative analysis of the reflex cardiovascular responses, the only widely accepted and standardized criteria to clinically assess the extent of the dysautonomy (5, 15).

Concerning the pulmonary involvement, it has been repeatedly observed that diabetic patients exhibit almost invariably some degree of functional impairment reflecting a specific damage to the autonomic innervation (8, 10, 19, 30, 35). In these patients, a significant correlation has also been found between cardiac and pulmonary (airways) indexes of autonomic neuropathy (8).

Thus we are aware that this model cannot assume complete pulmonary autonomic denervation but a wide clinical spectrum of autonomic dysfunction, with an inevitable heterogeneity within Dan+ subjects regarding the prevalence of cholinergic or sympathoadrenergic damage of the autonomic nervous system in the whole body, including the lungs. On the other hand, this is a quite common clinical condition where a chronic, although incomplete, autonomic neuropathy is well recognized. In this context, we attempted to categorize our diabetic subjects and to score their autonomic neuropathy by state-of-the-art methods to select patients with a high degree of dysautonomy in whom previous reports predict an involvement of the pulmonary indexes of autonomic innervation (8, 10, 19, 30, 35).

The results of this study show that during stressful exercise $V_{\dot{E}}$ increased progressively much more in diabetic subjects with autonomic neuropathy than in normal subjects, whereas diabetic subjects without autonomic neuropathy exhibited an intermediate behavior. The greater increase in $V_{\dot{E}}$ in the Dan+ subjects was associated with an abnormally high increment of RR, without differences in the rise of the adjusted $V_{\dot{T}}$. Hence, Dan+ subjects had more elevated $V_{\dot{E}}$ at corresponding $V_{\dot{CO}_2}$ and faster RR values and comparable adjusted $V_{\dot{T}}$ values at similar relative levels of ventilatory response. In addition, both groups of patients displayed a significantly lesser decrease in $V_{\dot{O}_2}/V_{\dot{T}}$ throughout the effort than control subjects and consequently higher $V_{\dot{O}_2}/V_{\dot{T}}$ values at similar relative levels of ventilatory response.

In line with studies performed in heart-lung transplanted patients (23, 31, 34), there were no differences in $V_{\dot{E}}$ levels or indexes of breathing pattern at rest in Dan+ compared with Dan− and control subjects, which suggests that pulmonary neurogenic mechanisms are not important in determining the resting ventilatory pattern in humans.

$V_{\dot{O}_2 max}$ in both diabetic groups was significantly lower than in the control group. This is believed to reflect the interaction between the impaired cardiac performance and the skeletal muscle deconditioning observed in diabetes (20). Despite lower $V_{\dot{O}_2 max}$ and maximal $V_{\dot{CO}_2}$ than in control subjects, at peak of exercise Dan− and Dan+ subjects attained similar $V_{\dot{O}_2}$

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Table 3. Breathing pattern at rest and peak of exercise

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<th>Control</th>
<th>Dan−</th>
<th>Dan+</th>
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<tr>
<td>$V_{\dot{E}}$, ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>796 ± 203</td>
<td>800 ± 319</td>
<td>735 ± 155</td>
</tr>
<tr>
<td>Exercise</td>
<td>2,745 ± 641*</td>
<td>2,274 ± 505</td>
<td>2,070 ± 472</td>
</tr>
<tr>
<td>RR, breaths/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>15.3 ± 4.6</td>
<td>16.3 ± 3.9</td>
<td>16.9 ± 4.6</td>
</tr>
<tr>
<td>Exercise</td>
<td>30.4 ± 4.5</td>
<td>31.4 ± 8.5</td>
<td>40.3 ± 9.9†</td>
</tr>
<tr>
<td>$V_{\dot{E}}/V_{\dot{T}}$, ml/s</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>436 ± 59</td>
<td>442 ± 143</td>
<td>446 ± 92</td>
</tr>
<tr>
<td>Exercise</td>
<td>2,717 ± 431</td>
<td>2,518 ± 763</td>
<td>2,801 ± 514</td>
</tr>
<tr>
<td>$T_{\dot{E}}$, s</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>2.07 ± 0.56</td>
<td>2.00 ± 0.94</td>
<td>1.74 ± 0.35</td>
</tr>
<tr>
<td>Exercise</td>
<td>1.01 ± 0.17</td>
<td>0.94 ± 0.18</td>
<td>0.76 ± 0.17†</td>
</tr>
<tr>
<td>$T_{\dot{E}}$, s</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>2.23 ± 0.74</td>
<td>2.07 ± 0.60</td>
<td>2.24 ± 0.64</td>
</tr>
<tr>
<td>Exercise</td>
<td>0.95 ± 0.14</td>
<td>1.01 ± 0.27</td>
<td>0.79 ± 0.20†</td>
</tr>
</tbody>
</table>

Values are means ± SE. $V_{\dot{E}}$, tidal volume; RR, respiratory rate; $T_{\dot{E}}$, inspiratory time; $T_{\dot{T}}$, expiratory time. Significantly different compared with: *Dan+ (P < 0.05); † control and Dan− (P < 0.05).
and \( V\dot{O}_2 \) values and had comparable levels of both \( V\dot{E} \) and \( V\dot{E}/MVV \) with respect to the control group.

As mentioned in RESULTS, the two diabetic groups, which had almost identical LAT values, achieved the gas-exchange AT at a lower absolute \( V\dot{O}_2 \) value than did the control group but at a similar or even higher percentage of \( V_{O2\text{max}} \). Moreover, the changes in lactate plasma levels overlapped in the Dan− and the Dan+ groups throughout the exercise, as shown in Fig. 1. Therefore, exertional metabolic acidosis did not seem to occur earlier and/or to progress differently in our diabetic groups, and an increasingly larger accumulation of acids cannot account for the steeper relationship between \( V\dot{E} \) and \( V\dot{CO}_2 \) observed in Dan+ compared with Dan− subjects.

Lactate concentrations during exercise were not measured in our control subjects. It is conceivable, however, that exertional metabolic acidosis began later in this group, as suggested by the higher gas-exchange AT, and perhaps increased less rapidly. Thus we cannot exclude that the higher \( V\dot{E} \) (and \( P_{0.1} \)) values recorded at corresponding \( V\dot{CO}_2 \) levels in diabetic subjects compared with control subjects could in part be induced by metabolic acidosis. Despite a similar degree of metabolic acidosis in both groups of patients, however, \( V\dot{E} \) was significantly greater in the Dan+ group, whereas it was only slightly higher in the Dan− than in the control group. Indeed, considering the higher \( V\dot{O}_2/V\dot{T} \) value in the Dan− than in the control group, the contribution, if any, of acidosis on \( V\dot{E} \) in diabetic subjects should be modest.

In fact, three mechanisms could better account for the higher ventilatory output for a given \( V\dot{CO}_2 \) observed during incremental exercise in diabetic patients affected by autonomic neuropathy: 1) the smaller reduction in \( V\dot{O}_2/V\dot{T} \), 2) the greater increment of the RR, and possibly 3) an increased neural drive.

The relative increase in \( V\dot{O}_2/V\dot{T} \) in Dan+, at rest or during exercise, could be ascribed to the alterations in the bronchomotor tone resulting from bronchial cholinergic denervation (8), with an augmentation of the anatomic and, through ventilation-perfusion mismatch, alveolar \( V\dot{O}_2 \). In our population, however, a similar lesser reduction in \( V\dot{O}_2/V\dot{T} \) during exercise occurred in both groups of patients, and therefore appears to be linked more to diabetes per se than to the autonomic dysfunction. An impaired cardiovascular response to exercise, irrespective of the presence of autonomic dysfunction, has been recently shown in diabetic patients and can be sustained by factors other than overt autonomic neuropathy, such as diabetic cardiomyopathy (6). Thus a reduced exertional increase in cardiac output, as suggested by the depressed \( V\dot{O}_2/\text{work} \) relationship found in both Dan− and Dan+ subjects during incremental effort (5), could impair the total lung perfusion and the distribution of pulmonary blood flow, inducing a lesser uniformity of the regional ventilation-perfusion relationships and a smaller reduction in the physiological \( V\dot{O}_2/V\dot{O}_2\).

The greater increment of RR observed in Dan+ compared with Dan− and control subjects could reflect the influence of the pulmonary autonomic (essentially cholinergic) nerve dysfunction on the ventilatory pattern, at least during stressful exercise. In this respect, our results contrast with those obtained in experimental animals, where vagotony leads to an increase in \( V\dot{T} \) and a decrease in RR, while maintaining \( T\text{i}/T\text{t} \) and \( V\dot{T}/T\text{i} \) unchanged, both at rest and during exercise (7, 27). The suppression of the Hering-Breuer inflation reflex due to the lack of inputs from lung stretch receptors has been invoked as the main underlying mechanism. It is well known, however, that in humans the Hering-Breuer inflation reflex is rather weak and demonstrable only at high inflation volumes (17); hence, a smaller influence, if any, on the breathing pattern during stimulated ventilation would be expected in humans after a cholinergic nervous system damage in the lungs.

Several investigators have recently studied the effects of pulmonary denervation in heart-lung transplanted patients compared with matched control subjects (2, 23) or heart-transplanted patients (33). In general, these studies have shown that during exercise the RR, and sometimes also the \( V\dot{T} \), tend to increase more rapidly after the assumed severance of cholinergic lung innervation, in contrast with the slower and deeper breathing pattern observed in classic experiments in animals (2, 13, 23). Despite the completely different model of pulmonary denervation used in the present study, our results are in line with previous observations of abnormal increments of RR associated with relatively normal increases in \( V\dot{T} \) in Dan+ patients during submaximal incremental exercise. Very similar findings have been reported by Kimoff et al. (23) in four heart-lung transplanted patients, although these investigators stressed that in normal humans autonomic pulmonary innervation has a more important role on the level of ventilation rather than on the ventilatory pattern and timing during exercise.

Pulmonary congestion can affect the breathing pattern both by reducing \( V\dot{T} \) and by increasing RR. We cannot rule out pulmonary congestion as a cause of the observed tachypnea in Dan+ during exercise. In this situation, however, the effect on the RR should occur mainly in the last part of the exercise, together with a brisk reduction in \( V\dot{T} \). This was not the case in our Dan+ patients and, anyway, it would hardly have contributed to explain the different breathing frequency of Dan+ and Dan− patients, who showed similar impairment of the cardiovascular response to exercise (6).

Also, a higher respiratory drive could induce by itself an increased ventilatory output during exercise in Dan+ patients.

The progressively greater \( P_{0.1} \) recorded in these patients during exercise could be entirely explained by the need for a progressively larger \( V\dot{E} \), due to higher \( V\dot{O}_2/V\dot{T} \) and RR, and for a greater neuromuscular support for any given \( V\dot{E} \), as shown by the \( P_{0.1}/\text{VE} \) relationship (Fig. 3). In fact, this relationship is slightly steeper and mostly shifted upward in Dan+ and even in Dan− compared with control subjects, probably because of minor mechanical constraints in diabetic subjects that
do not alter lung function at rest while becoming crucial in the presence of larger ventilatory requirements (32).

On the other hand, when $V_A$ was computed in relation to increasing $VCO_2$ during incremental effort to eliminate the confusing effects of the above-mentioned variables, a progressively larger amount of $V_A$ was found in Dan+ compared with both Dan− and control subjects, who, in turn, had identical $V_A/VCO_2$ relationships (Fig. 5).

The difference between the peak values of $PETCO_2$ (and estimated $PaCO_2$), which were significantly lower in Dan+ and very similar in Dan− and control subjects, is in line with a disproportionate increase in alveolar ventilation in Dan+ subjects. An excellent correlation has been found between $PaCO_2$ and $PETCO_2$ during exercise in normal subjects (37). Because all subjects had a normal resting ventilatory function, as shown by the pulmonary function tests, $PETCO_2$ probably reflects well the $PaCO_2$ values throughout the effort also in diabetic subjects, suggesting that Dan+ subjects were indeed hyperventilating in relation to their exertional $VCO_2$.

An earlier and perhaps greater metabolic acidosis cannot be responsible of the higher $V_A$ and lower $PETCO_2$ observed in Dan+ than in control subjects. If this had been the case, the peak $PETCO_2$ value and the $V_A/VCO_2$ relationship would have been similar in Dan+ and Dan− subjects, who showed almost identical AT values and overlapping lactate concentrations during exercise (Table 2, Fig. 1).

In our opinion, this hyperventilation could reflect, although indirectly, an excessive respiratory drive, which could in part be responsible for the higher $P_{0.1}$ and $VE$ observed in Dan+ subjects during exercise.

This phenomenon can be regarded as the expression of an altered control of breathing, possibly due to chronic lung autonomic denervation and/or to increased chemosensitivity.

The presence of pulmonary sympathetic afferents has been demonstrated in dogs and monkeys (24), and the stimulation of nerves carrying pulmonary sympathetic afferents has been proven to inhibit the phrenic nerve discharge in these anesthetized animals (24). Although there are no data on the influence of these nervous pathways on the control of breathing in humans, it can be hypothesized that in Dan+ subjects a loss or reduction of sympathetic inhibition following autonomic (sympathetic) neuropathy could lead to an excessive increase in the respiratory drive and contribute to the augmentation of the ventilatory output, at least during exercise.

In this respect, five Dan+ subjects had overt signs of sympathetic dysautonomia, i.e., significant postural hypotension. Splitting the exercise response in terms of the $V_A/VCO_2$ relationship into two subsets of Dan+ subjects, with and without postural hypotension, the highest $V_A$ is substantially exhibited by Dan+ subjects with postural hypotension, which supports the hypothesis that in Dan+ subjects, respiratory control is altered mainly in the presence of sympathetic damage of the autonomic nervous system (Fig. 6).

Alterations in central or peripheral chemosensitivity could also explain an increasing respiratory drive. We did not measure the carotid body response in our patients, but the neuromuscular and ventilatory responses to CO2 rebreathing were not different between Dan+ subjects as a group and in control subjects. Nevertheless, when the $P_{0.1}/PETCO_2$ and $VE/PETCO_2$ slopes of the five Dan+ subjects with postural hypotension were computed, their values, amounting to $0.77 \pm 0.05 \, mH_2O/Torr$ and $4.95 \pm 1.271 \, \text{min}^{-1} \cdot \text{Torr}^{-1}$, respectively, were much higher than those recorded in the five Dan+ subjects without postural hypotension and the control subjects ($P < 0.01$). Hence, a significant increase in central chemosensitivity can be suspected, at least in diabetic subjects with more severe autonomic neuropathy involving the sympathetic nerves.

In conclusion, our results suggest that chronic lung denervation, as occurring in severe diabetic dysautonomia, can induce alterations of the ventilatory response to exercise by influencing the breathing pattern and possibly by determining an abnormally high inspiratory drive through mechanisms still unclear, which appear to involve a damage to the sympathetic autonomic nervous system. In this context, an increased chemosensitivity may actually contribute to sustain the greater central inspiratory activity, leading to an excessively high ventilatory output during heavy exercise. It follows that pulmonary autonomic innervation seems to play a nontrivial role in modulating both the pattern and the level of stimulated ventilation in normal humans.

The technical assistance of Rita Fraboni and Gianpiero Cipiciani has been invaluable.

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