Exercise and Posture-Related Changes of Atrial Natriuretic Factor and Cardiac Function in Diabetes

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To study whether the release of atrial natriuretic factor (ANF) was altered in diabetic cardiac autonomic neuropathy (CAN), we determined plasma ANF concentrations during exercise and changes of posture in three groups of age- and sex-matched subjects (9 healthy subjects, 7 diabetic patients with CAN, and 7 diabetic patients without CAN). During exercise, plasma ANF concentrations rose threefold ($P < .001$), and this increase was similar in the three groups. However, heart-rate response to exercise was impaired in the two groups of diabetic patients ($P < .004$ vs. healthy subjects) but was more severely impaired in patients with CAN ($P < .03$ vs. patients without CAN). In healthy subjects and patients without CAN, the increases of ANF during exercise correlated significantly with those of heart rate, systolic blood pressure, and rate-pressure product ($P < .01$). In patients with CAN, the correlation was found exclusively with heart rate ($P < .01$). An increase of ventricular ejection fraction occurred in all groups ($P < .001$) but without showing statistical differences between groups. After 30 min of standing, a similar postural drop of plasma ANF concentrations ($P < .002$) was observed in all subjects, reflecting preserved sympathetic control of vessels. In conclusion, exercise induces an increase of plasma ANF in diabetic patients with CAN. This increase, occurring similarly to healthy subjects, indicates that autonomic activation plays a minor role in ANF release during exercise. Impaired heart-rate response to exercise in patients without CAN suggests early damage of autonomic function, undetected by conventional rest tests. *Diabetes Care* 12:475–80, 1989

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The atrial natriuretic factor (ANF) is released from cardiocytes by volume expansion and increased in plasma in several pathological conditions such as heart and kidney failure, tachyarrhythmias, or the syndrome of inappropriate secretion of antidiuretic hormone (1–7). Atrial distension is the principal determinant of ANF release, although adrenergic stimulation has been suspected to directly elicit the release of ANF (8–12). The latter mechanisms could account for the increase in plasma ANF concentrations observed during exercise and explain the previously reported correlations between changes of ANF and heart rate or blood pressure during exercise (13–16). Cardiac autonomic neuropathy (CAN), resulting from long-term diabetes mellitus, is characterized in rest conditions by abnormal cardiovascular autonomic tests and during exercise by a lower response in catecholamines, heart rate, and blood pressure (17–22).

To evaluate whether the autonomic nervous system plays a dominant role in the ANF response to exercise, we determined plasma ANF levels during graded exercise in diabetic patients with and without CAN. We also studied whether CAN affected the relationships between the changes of ANF and those of heart rate and blood pressure. Moreover, because a postural drop of plasma ANF has been described in the standing position and postural hypotension in CAN, we studied the changes of plasma ANF after assumption of the upright position (23).

RESEARCH DESIGN AND METHODS

Subjects. Three age- and sex-matched groups were studied: 9 healthy subjects (6 men, 3 women), 7 diabetic patients without CAN (5 men, 2 women), and 7

diabetic patients with CAN (5 men, 2 women). Their ages (mean ± SE with ranges in parentheses) were, respectively, 41 ± 2 (29–54), 41 ± 4 (33–68), and 44 ± 4 (29–56) yr. Characteristics of the diabetic patients are shown in Table 1. Diabetic patients with and without CAN were matched for type of diabetes, endogenous insulin secretion, insulin therapy, and glycemic control (HbA_1). As expected, diabetic patients with CAN had a longer duration of diabetes.

All subjects gave oral informed consent and the study was approved by the institutional review board. Before the test, subjects stayed in the hospital and consumed a 4-day constant sodium diet (150 mM/day) supplied by the dietetic unit and supervised by a dietitian. During this period, subjects abstained from alcohol and medicines other than insulin. Control subjects were healthy with regard to history, clinical examination, electrocardiogram (ECG), chest X ray, and maximal exercise stress test.

Diabetic patients were selected by the following criteria: *1*) no clinical evidence of heart failure or coronary artery disease; *2*) normal blood count, electrolytes, urea and creatinine concentrations; *3*) normal ECG and blood pressure at rest and during maximal exercise stress test; *4*) normal chest X ray; and *5*) absence of intercurrent illness or infection. Diabetic patients were classified as having CAN if at least 2 of the 5 following tests were abnormal, as previously described by Ewing et al. (18): *1*) resting heart rate; *2*) heart-rate response to deep breathing: E/I ratios (defined as the longest R-R interval in expiration [E] to the shortest in inspiration [I]) were calculated from ECG tracings during 6 breaths/min (normal range was found in age-related tables); *3*) Valsalva maneuver (ratios of the R-R interval after the maneuver to the shortest during the maneuver were calculated) values were considered abnormal with a ratio ≤1.10; *4*) heart-rate response to standing up (30:15 ratios were established as the longest R-R interval at the 30th beat to the shortest interval at the 15th beat after standing up; abnormal was defined as ≤1.00; and *5*) blood pressure response to standing up (blood pressure was measured with a sphygmomanometer, and a postural drop of systolic blood pressure was considered abnormal if ≥30 mmHg) (19). Peripheral neuropathy was detected by electromyography and nephropathy by a proteinuria >150 mg/24 h. These assessments revealed that complications of retinopathy, peripheral neuropathy, and nephropathy were present more frequently in patients with CAN (Table 1). In the same group, three of five autonomic tests (heart rate, E/I, Valsalva ratio) were found to be significantly different from those in patients without CAN.

Exercise protocol. A preliminary exercise test was performed on a bicycle ergometer in the sitting position to determine the maximal physical capacity and to exclude patients with coronary vessel disease or hypertension. The initial work load of 20 W was increased by 20 W every minute until the subject became exhausted. Exhaustion was defined as the last stage of effort possible to achieve due to severe dyspnea and weakness of the legs. Data obtained during the first test were used to select similar exercise levels during the second test. The latter was performed on the following day in the morning (between 0900 and 1100) after a light breakfast.

For diabetic patients on insulin, the dose of short-acting insulin was reduced by 50% to avoid hypoglycemia during exercise. Subjects remained seated for 30 min before starting the exercise in a semisupine position (70%) on a specially designed table fitted with an ergometer (Ergomed 740 L, Siemens, Uithoorn, The Netherlands). Three levels of 5-min exercises were performed, corresponding to 20, 40, and 60% of the maximal work load achieved the day before. After exercise, patients were asked to stay in a supine position for 30 min and then to stand up for another 30 min. The ECG was continuously monitored throughout the exercise test. Blood pressure measurements, ECG tracings, and blood samples for glucose and ANF determination were taken 10 min and just before the exercise test, during the last 30 s of each exercise level, and after 5 min recovery, 30 min lying, and 30 min standing. Blood pressure was measured with a standard mercury sphygmomanometer. Isotopic data were acquired at rest, during the last 150 s of exercise, after 5 min recovery, and at the end of the 30-min lying period.

TABLE 1
Characteristics of diabetes and autonomic function tests in diabetic patients with and without cardiac autonomic neuropathy

	Cardiac autonomic neuropathy	
	Without	With
n	7	7
Type I (*n*)	5	5
C-peptide (pM)	<0.03	<0.03
Type II (*n*)	2	2
C-peptide (pM)	0.97 ± 0.25	0.83 ± 0.11
Duration of diabetes (yr)	6 ± 1 (2–10)	19 ± 6* (2–52)
Insulin therapy (*n*)	6	6
HbA_1 (%)†	9.7 ± 0.5	10.5 ± 0.8
Complications (*n*)		
Retinopathy	1	6
Peripheral neuropathy	3	6
Nephropathy	1	4
Autonomic tests		
Heart rate (beats/min)	73 ± 3	82 ± 3*
E/I	1.36 ± 0.08	1.05 ± 0.01‡
Valsalva ratio	1.23 ± 0.05	0.99 ± 0.02‡
30:15 ratio	1.02 ± 0.03	1.01 ± 0.01
Postural drop of blood pressure ≥30 mmHg (*n*)	0	1

E/I, the longest R-R interval in expiration (E) to the shortest in inspiration (I).
Results are means ± SE; ranges in parentheses.
*$P < .05$, ‡$P < .01$ vs. diabetic patients without cardiac autonomic neuropathy.
†Normal range of HbA_1: 8.1 ± 0.3%.

Measurements. Blood glucose was determined during the test with reagent strips (Dextrostix, Ames, Brussels) and a Glucometer (Ames). Total glycosylated hemoglobin (HbA_1) was measured by electrophoresis (Corning, Palo Alto, CA) and C-peptide by radioimmunoassay with K6 antiserum (Novo, Bagsvaerd, Denmark). All blood samples were taken from a peripheral vein into 10-ml plastic tubes containing potassium EDTA (12 mg) and benzamidine (14 mg) in 200 μl of 0.05 M phosphate-buffered saline. The blood samples were stored on ice and centrifuged for 10 min at 4°C. The plasma was then frozen and stored at −20°C until measurements were made. ANF was measured as previously described (14). Briefly, a radioimmunoassay, performed after plasma extraction on Sep-Pak C18 cartridges (Waters, Milford, MA), used antibodies from Peninsula (ref. no. 8798; Belmont, CA). Fifty percent tracer binding inhibition was obtained with 4.2 ± 0.3 fmol ANF/tube. Mean ± SE recovery was 75 ± 4%. Results were corrected for recovery. Intra-assay coefficient of variation was 6.7% (tested at the dose level of 14.9 pM; n = 12) and interassay coefficient of variation was 8.7% (tested at the dose level of 15.6 pM; n = 9). Cardiac volumes and ejection fraction were calculated as previously described (14,24,25).

Statistics. Characteristics of patients were compared between groups by Mann-Whitney-Wilcoxon rank-sum tests for unpaired data. The exercise test data were analyzed by nonparametric analysis of variance with the Kruskal-Wallis test with BMDP statistical software (Department of Biomathematics, Univ. of California, Los Angeles, CA). Correlations were tested by the correlation coefficient of Pearson (r) and confirmed or not confirmed by the nonparametric rank correlation coefficients of Kendall (τ) and Spearman (r_s). Linear regressions were compared by F tests. All tests were two sided; $P \leq .05$ was considered statistically significant. Data are expressed as means ± SE.

RESULTS

Exercise tests. No complications resulted from exercise tests. The maximal physical capacity (W) was not different between healthy subjects and diabetic patients without CAN. However, it was reduced in diabetic patients with CAN compared with healthy subjects ($P <$ .05) but not significantly compared with patients without CAN (healthy subjects, 180 ± 13; diabetic patients without CAN, 200 ± 28; patients with CAN, 150 ± 8). Therefore, the work loads chosen at each exercise level during the second test were also lower in diabetic patients with CAN (at the 3rd level, 82 ± 9 W in diabetic patients with CAN vs. 100 ± 4 W in healthy subjects, $P <$ .05, and 117 ± 14 W in patients without CAN, NS). However, all subjects performed similar exercise related to their physical capacity (at the 1st level in percentage of maximal physical capacity, 18.5% for healthy subjects, 17.5% for patients without CAN, and 17% for patients with CAN; at the 2nd level, 37, 34.5, and 35%, respectively; and at the 3rd level, 55.6, 58.5, and 55%, respectively).

In the three groups, exercise was accompanied by a rise in ANF concentrations ($P <$ .001) that was significant at the second ($P <$ .02 for healthy subjects and patients without CAN) and third ($P <$ .03 for the 3 groups) exercise levels (Fig. 1). After 5 min recovery, values were still above those of basal conditions (healthy subjects, $P <$ .004; diabetic patients without CAN, $P <$ .04; patients with CAN, $P <$ .02). A return to baseline values was achieved after 30 min recovery except for healthy subjects, whose ANF levels were still above basal values ($P <$ .04). If there was a tendency for diabetic patients with CAN to display higher increases of ANF, no statistical differences between groups could be found. Changes of ANF were parallel between the three groups, apart from the 5-min recovery period. During this period, a significant difference (presence of an interaction exercise level × group on ANF; $P <$ .04) was found between the decrease of ANF occurring in both diabetic groups and the increase in control subjects.

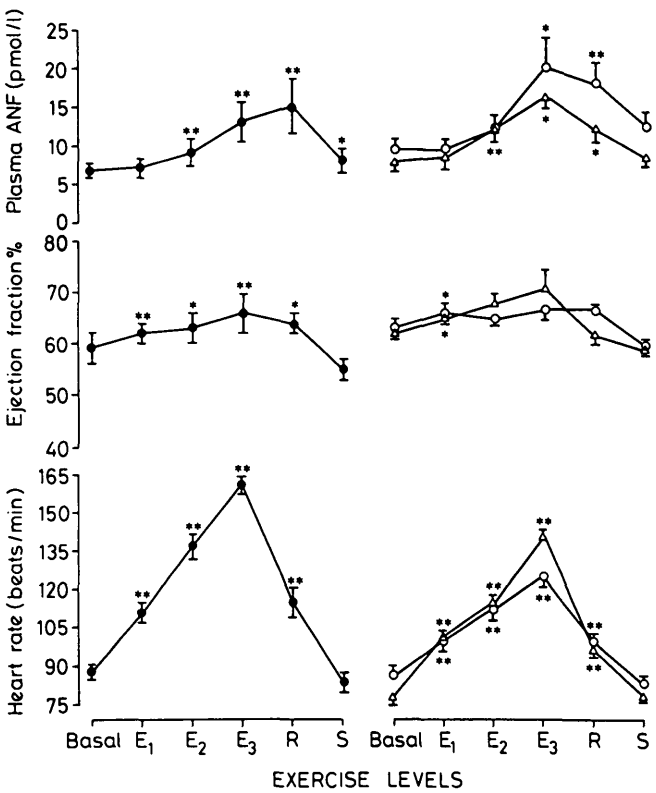

FIG. 1. Changes of plasma atrial natriuretic factor (ANF) concentrations, ejection fraction, and heart rate during 3 graded exercise levels (E_1, E_2, E_3 corresponding to 20, 40, and 60% of maximal work load) after 5 min recovery (*R*) and 30 min supine (*S*) in healthy subjects (*left*) and diabetic patients with (○) and without (△) cardiac autonomic neuropathy (*right*). Readings are compared with basal readings. Data are expressed as means ± SE. *$P <$ .05, **$P <$ .02.

Heart rate. In the three groups, there was an increase of heart rate during exercise ($P < .001$). At the second and third levels, heart rate was significantly higher in healthy subjects than in diabetic patients without CAN (2nd level, $P < .01$; 3rd level, $P < .004$) and with CAN (2nd level, $P < .01$; 3rd level, $P < .002$). At the third level, heart rate was also higher in patients without CAN than with CAN ($P < .03$). Changes of heart rate were not parallel in the three groups (presence of a significant interaction work load × group on heart rate; $P < .001$). This resulted from a higher increase of heart rate at the second level for healthy subjects and a lower increase at the third level for patients with CAN. There was no correlation between changes of heart rate and duration of diabetes in either group of diabetic patients (without CAN $r = -.02$, $\tau = -.03$, $r_s = -.03$; with CAN $r = -.05$, $\tau = .00$, $r_s = .01$).

Blood pressure and rate-pressure product. Blood pressure rose from 131 ± 4 to 191 ± 8 mmHg in healthy subjects at maximal exercise level, from 120 ± 4 to 179 ± 10 mmHg in patients without CAN, and from 125 ± 21 to 175 ± 35 mmHg in patients with CAN. If there was a trend for blood pressure increases to be less pronounced in patients with CAN, no statistical differences were found. Rate-pressure product also increased in the three groups ($P < .001$) but without showing statistical differences between groups.

ANF. As shown in Fig. 2, significant correlations were observed between increases of ANF concentrations and those of heart rate, systolic blood pressure, and rate-pressure product in healthy subjects and diabetic patients without CAN ($P < .01$). A significant correlation of ANF changes was also found with increases of heart rate in diabetic patients with CAN ($P < .01$) but disappeared with those of blood pressure and rate-pressure product. No statistical difference could be found between the linear regressions ΔANF/Δheart rate of the three groups.

A mean 10% postural drop of plasma ANF concentration was observed in all subjects ($P < .002$) and was not different between groups.

In healthy subjects, a correlation between ANF and age was suspected ($r = .44$, $P < .01$) but not confirmed by nonparametric correlation coefficients ($\tau = .25$, NS; $r_s = .35$, NS). In patients with and without CAN, ANF did not correlate with age: $r = -.17$, $\tau = -.16$, $r_s = -.24$ and $r = .17$, $\tau = -.15$, $r_s = -.17$, respectively. The correlation coefficients between ANF and duration of diabetes were also not significant ($r = .06$, $\tau = .15$, $r_s = .20$ for patients with CAN and $r = -.10$, $\tau = -.11$, $r_s = -.14$ for patients without CAN).

Ejection fraction. Left ventricular ejection fraction increased in the three groups ($P < .001$) without showing statistical differences between groups (Fig. 1). However, analysis of individual groups disclosed that the ejection fraction significantly increased in each group at the first level ($P < .03$). At the third level, a further increase occurred in healthy subjects ($P < .02$) that was not significant in the two groups of diabetic patients.

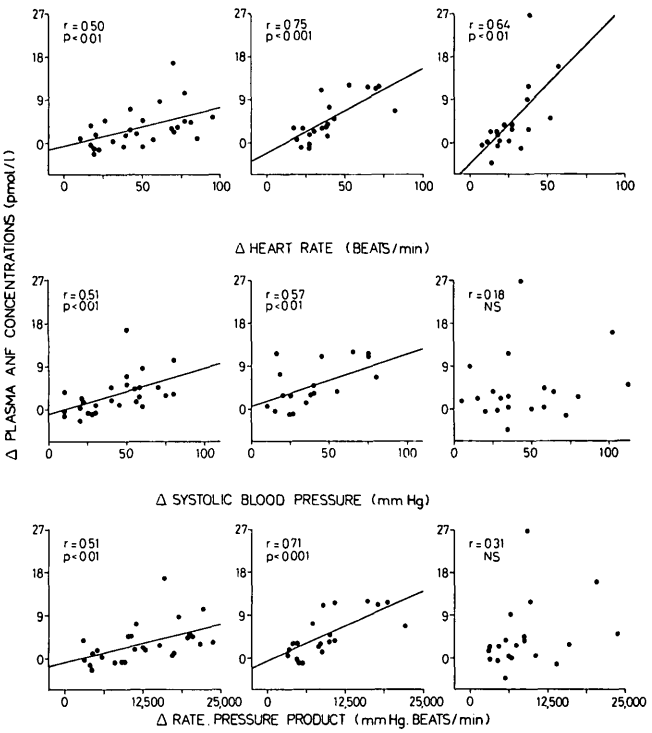

FIG. 2. Relationship between increase in plasma atrial natriuretic factor (ANF) concentrations and respective increase in heart rate, systolic blood pressure, and rate-pressure product during exercise in healthy subjects (*left*) and diabetic patients without (*middle*) and with (*right*) cardiac autonomic neuropathy.

Blood glucose concentrations remained unchanged during exercise. None of the patients had symptoms of hypoglycemia. Mean ± SE glucose concentrations in diabetic patients with and without CAN were, respectively, 14.2 ± 1.7 and 14.8 ± 1.0 mM at rest and 12.8 ± 2.0 and 14.3 ± 1.8 mM at the third exercise level.

DISCUSSION

This study has shown that exercise induced an increase of plasma ANF concentrations in both healthy subjects and diabetic patients (with and without CAN). The rise of plasma ANF, clearly related to the intensity of exercise, was similar in the three groups despite the presence of diabetes or CAN. This finding shed new light on the potential mechanisms that might be responsible for the release of ANF during exercise. Indeed, impairment of nervous autonomic activity or of catecholamine release, known to characterize diabetic CAN, did not influence the release of ANF (17–22). Thus, the data suggest that these factors probably play a minor role in ANF release, whereas the atrial stretch might be a more important stimulus. However, this remains a hypothesis made by exclusion because no reliable techniques are available to measure the de-

gree of atrial distension during exercise. Measurements of atrial pressures would have been too invasive in our healthy subjects and diabetic patients. Moreover, atrial distension, rather than atrial pressure increases, has been described as the principal determinant of ANF release (8).

Higher basal and exercise-stimulated ANF levels have been reported in patients with congestive cardiac failure (4). Ejection fractions in the two diabetic groups were similar to those of healthy subjects, indicating that diabetic patients did not have major diabetic cardiopathy. The normal basal and exercise-stimulated ANF levels in diabetic patients are also consistent with the absence of cardiac failure.

The data demonstrated a significant correlation between the increase of plasma ANF and those of heart rate, systolic blood pressure, and rate-pressure product in healthy subjects and diabetic patients without CAN. Such correlations, also reported by others on healthy subjects (13,16), might have been considered as resulting from the common adrenergic stimuli on both the cardiovascular parameters and ANF release. This view is supported by various studies showing stimulatory effects of adrenergic agonists on ANF release, both in vivo and in vitro (9–12). A recent study, however, shows that endogenous sympathetic activation might reduce ANF release (26). In our study, when autonomic function was impaired, ANF release did not correlate with the changes of blood pressure and rate-pressure product. In the same group, the increases of plasma ANF still correlated with heart-rate changes, but ANF was released similarly to healthy subjects despite a lower increase in heart rate. This shows that ANF release in patients with CAN is dissociated from the cardiovascular parameters under autonomic control.

Another important finding of this study was an impaired rise in heart rate observed in patients classified as without CAN on the basis of the conventional tests. This feature, recognized as being typical for CAN, reinforces the concept of a gradation of autonomic damage already suggested by Ewing and Clarke (17,21,22). During exercise, the increase in heart rate results from withdrawal of vagal tone at low work loads and from sympathetic stimulation at high work loads (22,27). The reduction of exercise-related tachycardia observed in our patients would then correspond to an impairment of both para- and orthosympathetic control of the sinoatrial node. The difference between both diabetic groups at high work loads suggests greater damage of sympathetic nerves in patients with overt CAN. Involvement of the sympathetic innervation controlling vascular resistance was probably mild, because no statistical differences of blood pressure response could be established, despite a tendency for patients with CAN to display an attenuated response. This abnormal response of heart rate during exercise, found in the two diabetic groups, did not result from differences in the intensity of effort. Indeed, each exercise level, calculated from the same percentage of maximal physical capacity, was equivalent. Maximal work loads of healthy subjects and patients without CAN were also identical. Because all subjects were matched for age and sex, differences did not result from one of these factors, and because diabetic patients were also matched for type of diabetes, insulin therapy, and glycemic control, the more pronounced impairment of heart-rate response to exercise observed in patients with CAN must be attributed to more severe neuropathy, a consequence of a longer duration of diabetes. Duration of diabetes itself seems unlikely to account for differences between diabetic groups because no correlation could be established with heart-rate changes.

The postural drop of plasma ANF levels was studied in relation to the possible dysfunction of vessels existing in CAN. Indeed, the postural drop of plasma ANF in healthy subjects has been explained by a decrease of atrial distension in the standing position. We did not observe any differences between groups, indicating that adaptation of vessels in the standing position was still preserved.

In conclusion, this study shows that in diabetic patients with CAN, ANF response to exercise is similar to that observed in diabetic patients without CAN and in control subjects. This finding suggests that autonomic activity does not play a determinant role in the release of ANF during exercise. This study also reveals an abnormal heart-rate response to graded exercise in patients considered free of neuropathy, indicating early autonomic damage undetected by conventional rest tests.

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