Difference Between Diabetic and Nondiabetic Smokers in the Pituitary Response to Physical Exercise

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The growth hormone (GH), cortisol, and arginine vasopressin (AVP) responses to bicycle ergometry (with increasing workload until exhaustion) were measured in 20 patients affected by insulin-dependent diabetes mellitus (IDDM) (10 habitual smokers and 10 nonsmokers) and 20 nondiabetic subjects (normal controls) (10 habitual smokers and 10 nonsmokers). Cardiorespiratory parameters such as heart rate, blood pressure, ventilation, frequency of breathing, tidal volume, oxygen consumption (Vo_2), carbondioxide production (Vco_2), and respiratory exchange ratio (R) were monitored before and during exercise tests. No significant differences between groups were observed; furthermore, there were no differences in circulating somatomedin-C (SM-C) and free fatty acids (FFA) levels between groups. Blood glucose levels were similar before exercise and followed a similar pattern during tests in diabetic smokers and nonsmokers. Basal GH, cortisol, and AVP levels were similar in diabetic smokers, diabetic nonsmokers, normal smokers, and normal nonsmokers. In all groups, exercise induced a significant increase in the serum concentrations of all examined hormones. Increments were significantly higher in diabetic than in nondiabetic groups. No significant differences were observed between diabetic smokers and nonsmokers for all examined hormones. AVP responses during tests were similar in normal smokers and nonsmokers. In contrast, exercise-induced GH and cortisol increments were significantly lower in normal smokers than in normal nonsmokers.These data support the hypothesis that in normal subjects habitual nicotine consumption may attenuate both GH and cortisol responses to a releasing stimulation, such as physical exercise. This phenomenon may represent an expression of adaptation of nicotinic neurotransmission to chronic stimulation. Furthermore, the data show that the effect induced by habitual smoking is absent in diabetics, probably because of diabetes-induced neuroendocrine alterations in the central nervous system.

IN HEALTHY HUMANS, nicotine inhaled with cigarette smoking is a well-known direct stimulator of growth hormone (GH), adrenocorticotropin (ACTH)/cortisol, and arginine vasopressin (AVP) release.[1] On the other hand, lower GH and cortisol responses in smokers than in nonsmokers to stimulations unrelated to nicotine have been described.[2,3,7] These findings suggested that chronic nicotine consumption reduces the ability of neuroendocrine pathways at hypothalamic-pituitary level to respond to challenging stimulations, probably through a downregulation of nicotinic receptors.[3]

In previous studies, we have reported significantly higher GH, cortisol, and AVP responses to cigarette smoking in patients affected by insulin-dependent diabetes mellitus (IDDM) than in healthy control subjects, suggesting interactions between nicotine inhaled with cigarette smoking and diabetes-induced neuroendocrine alterations at the hypothalamic-pituitary level.[4] On the other hand, at present there are no studies available of the effects of cigarette smoking on the pituitary hormone responses to nicotine-unrelated stimulations in diabetes mellitus. The present study was performed to gain better insight into the involvement of nicotine in hypothalamic-pituitary neuroendocrine control of pituitary and adrenal function in diabetes mellitus. For this purpose, the GH, cortisol, and AVP responses to physical exercise were measured in diabetic habitual smokers and nonsmokers. Results were compared with those obtained in normal habitual smokers and nonsmokers.

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MATERIAL AND METHODS

Twenty insulin-dependent diabetic men and 20 normal control men participated in the study. Diabetics and normal controls were matched for age and weight. Each patient gave informed consent for participation in the study, which was approved by the local hospital ethics committee. Diabetic and normal controls were selected in order to constitute 4 groups of 10 subjects (diabetic smokers, diabetic nonsmokers, normal smokers, and normal nonsmokers). The number of 10 for each group was chosen because in previous studies[4] statistically significant differences were obtained between groups of this size in similar experimental conditions. Nondiabetic and diabetic smokers had been smoking cigarette for at least 10 years (range, 10 to 18 cigarettes per day, corresponding to about 180 to 280 packs per year). All parameters were similar in diabetic smokers and nonsmokers. Also, normal smokers and nonsmokers showed similar parameters. Clinical and biochemical data of the diabetic men (smokers and nonsmokers) and normal subjects (smokers and nonsmokers) are shown in Table 1.

Diabetics had been treated with insulin from the onset of their illness and at the time of this study they were outpatients under constant routine control. The mean (±SE) dose of insulin was 34.9 ± 2.1 IU/24 h in smokers and 31.1 ± 2.8 in nonsmokers. Therapy consisted of intermediate-duration monocomponent insulin and short-acting monocomponent insulin administered together once or twice daily. All patients had satisfactory metabolic control; none had clinical or laboratory evidence of ketosis or associated endocrine or other intercurrent diseases. No signs of autonomic nerve dysfunction were present in our patients according to the results of a group of tests for cardiovascular reflexes, and none showed signs of peripheral neuropathy. All patients had creatinine clearance values in the normal range (80 to 120 mL/min) without microalbuminuria. All patients underwent ophthalmologic examination to detect the presence of diabetic retinopathy.

Diagnostic Criteria

After pupillary dilatation, all patients underwent ophthalmoscopic examination performed by the same experienced ophthalmologist. The

Table 1. Clinical and Biochemical Data (mean ± SE) of the Diabetic Men (smokers and nonsmokers) and Normal Controls

	Age (yr)	Weight (kg)	BMI	Duration of IDDM (yr)	HbA_{1c} (%)
Diabetic smokers (n = 10)	32.6 ± 1.6	67.8 ± 1.5	22.0 ± 0.5	6.8 ± 0.9	7.2 ± 0.6
Diabetic nonsmokers (n = 10)	34.0 ± 1.5	66.4 ± 1.3	21.9 ± 0.4	6.3 ± 0.7	7.4 ± 0.5
Control smokers (n = 10)	31.3 ± 1.4	68.4 ± 1.4	22.5 ± 0.4		5.0 ± 0.3
Control nonsmokers (n = 10)	31.9 ± 1.4	69.1 ± 1.5	22.7 ± 0.6		4.8 ± 0.4

Abbreviation: BMI, body mass index.

findings of at least 3 microaneurysms was considered a sign of retinopathy. Patients with retinopathy were excluded from the study.

Insulin therapy remained unchanged during the period of this investigation. The last injection of insulin before the test was given on the day preceding the experiment. As indices of control of the metabolic status of these patients, blood levels of glucose were measured at 7 AM, 11 AM, and 5 PM on the days preceding the test; on the experimental day, blood samples were taken for measurement of blood glucose and glycosylated hemoglobin (HbA_{1c}).

Furthermore, 24-hour urinary glucose excretion was measured at the time of the tests.

Exercise Test

Diabetic smokers and nonsmokers and normal smokers and nonsmokers exercised for a certain period of time on a electrically braked cycle ergometer. An initial load of 50 W was increased by 50 W every 3 minutes until subject exhaustion. The subjects with a low maximal capacity (as established in a preliminary test performed at least 1 week before the study) pedalled for 3 to 4 minutes against no workload at the beginning of the test, so that the exercise lasted about the same duration (15 minutes) in all individuals.

Experiments started at 8 AM after an overnight fast. Exercise started 15 minutes after the insertion of an intravenous (IV) catheter into an antecubital vein, which was used for blood sampling. Basal blood samples were taken at time 0 (just before exercise). Further specimens were withdrawn at time 10, 15, 20, 30, 40, 50, and 60 minutes.

During exercise the subjects breathed through a low-resistance 1-way valve connected to a PK Morgan measurement system (Avinton, Seattle, WA) that had been calibrated appropriately. The following parameters were measured: ventilation, frequency of breathing, tidal volume, oxigen consumption (V_{O_2}), carbon dioxide production (V_{CO_2}), and respiratory exchange ratio (R). Heart rate and blood pressure were determined by an experienced cardiologist. Heart rate was monitored continously by an electrocardiograph (Hellige, Milan, Italy) and by auscultation over the precordium; blood pressure was evaluated with a sphygmomanometer.

Assays

The blood samples obtained from all experiments were used for evaluation of glucose, cortisol, GH, AVP, osmolality, hematocrit, somatomedin-C (SM-C), and free fatty acids (FFA). On each experimental day, blood samples were collected at time 0 for measurements of HbA_{1c}. Blood samples were placed in chilled tubes containing Na_2 EDTA (1 g/L) and aprotinin (100 mIU/L) for measurement of AVP, into plastic tubes containing potassium fluoride (KF) plus Na_2 EDTA and perchloric acid for evaluation of blood glucose and in plain tubes for cortisol and GH determinations. Blood samples were immediately centrifuged and plasma or serum frozen at −20°C until assayed. Plasma concentrations of AVP were measured by radioimmunoassay (RIA) Serum cortisol, GH, and AVP were evaluated by RIA using commercial kits. The intra-assay coefficients of variation were 2.8% for GH, 4.0% for cortisol, and 6.1% for AVP. The interassay coefficients of variation were 5.6% for GH, 7.0% for cortisol, and 13% for AVP. These coefficients of variations were measured at the following hormone concentrations: 1.8 mIU/L for GH, 360 nmol/L for cortisol, and 2.2 ng/L for AVP. The sensitivity of the RIA methods were 0.04 mIU/L for GH, 16.5 nmol/L for cortisol, and 1.2 ng/L for AVP. SM-C levels were measured by RIA, using kits obtained from Nichols Institute Diagnostics (San Juan Capistrano, CA). The intra-assay coefficient of variation was 5.0% and the interassay coefficient of variation was 10.0%. The sensitivity of the assay was 0.1 mU/mL. The normal range in our laboratory observed in adult male subjects is 0.62 to 0.98 mU/mL.

Plasma osmolality was determined with an advanced osmometer (Osmette S. Sedas s.r.l., Milan, Italy); blood glucose was measured using a glucose oxidase-peroxidase procedure utilizing an IL 918 autoanalyzer (Instrumentation Laboratory, Italy); hematocrit was measured with a microhematocrit (Drummond Scientific, Broomal, CA). HbA_{1c} was determined with reagents obtained from Bio-Rad Laboratories (Hercules, CA) (normal range, 3.9% to 6.1%).

Data were analyzed using the SPSS V60 statistical package for Windows (SPSS, Chicago, IL). The following tests were used as appropriate: Wilcoxon's matched-pair rank sum test, the Mann-Whitney *U* test, 2-way analysis of variance (ANOVA), and Spearman's rank correlation coefficient.

To exclude the possible interference of confounding variables such as age, body mass index, duration of diabetes, and insulin treatment, data were reanalyzed with analysis of covariance (ANCOVA). Data are reported as means ± SE.

RESULTS

Blood glucose levels in diabetic smokers and nonsmokers during exercise are shown in Table 2. No significant differences between groups were found. Blood glucose levels in normal subjects were in the normal range in basal conditions and during exercise. The basal levels of SM-C (diabetic smokers, 0.78 ± 0.09 mU/mL; normal smokers, 0.86 ± 0.08) and FFA (diabetic smokers, 0.8 ± 0.12 mmol/L, normal smokers, 0.67 ± 0.06) were similar in diabetic smokers and normal

Table 2. Blood Glucose Levels (mg/dL) During Exercise in Diabetic Patients

	Time (min)						
	0	+10	+20	+30	+40	+50	+60
Diabetic smokers	174.8 ± 8.9	184.2 ± 8.7	190.7 ± 8.5	199.6 ± 10.8	209.3 ± 11.4	219.4 ± 12.0	225.1 ± 10.3
Diabetic nonsmokers	168 ± 7.5	180.6 ± 9.1	188.6 ± 7.7	198.2 ± 9.4	200.5 ± 9.8	211.9 ± 10.7	218.8 ± 9.9

Table 3. Basal and Peak Values (mean ± SE) of Physiological and Biochemical Variables in Diabetic Smokers and Nonsmokers and in Control Smokers and Nonsmokers

	Diabetic Smokers		Normal Smokers		Diabetic Nonsmokers		Normal Nonsmokers	
	Basal	Peak	Basal	Peak	Basal	Peak	Basal	Peak
Heart rate (beats/min)	78 ± 7	129 ± 8	75 ± 8	126 ± 8	77 ± 4	130 ± 5	73 ± 4	124 ± 7
Mean blood pressure (mm Hg)	98 ± 6	115 ± 9	96 ± 5	109 ± 8	97 ± 7	114 ± 7	98 ± 7	107 ± 8
Respiratory rate (min^{-1})	13 ± 1.2	22.5 ± 2.9	12.8 ± 1.3	23.3 ± 2.2	12.8 ± 1.5	22.9 ± 2.7	12.5 ± 0.9	22.9 ± 2.8
Tidal volume (L)	0.7 ± 0.2	2.2 ± 0.4	0.9 ± 0.2	2.0 ± 0.5	0.7 ± 0.2	2.4 ± 0.4	0.8 ± 0.2	2.1 ± 0.3
Ventilation (L/min)	10.1 ± 0.6	71.1 ± 3.1	10.4 ± 0.6	70.7 ± 2.8	10.4 ± 0.5	69.2 ± 2.9	10.5 ± 0.7	70.9 ± 0.5
V_{O_2} (mL/min)	329 ± 14	2245 ± 150	330 ± 12	2240 ± 145	328 ± 9	2258 ± 140	326 ± 12	2241 ± 146
V_{CO_2} (mL/min)	289 ± 18	2110 ± 168	296 ± 16	2090 ± 165	290 ± 16	2115 ± 165	298 ± 17	2097 ± 159
R	0.87	0.93	0.88	0.93	0.88	0.92	0.89	0.94

smokers. Similar values were observed in diabetic and normal nonsmokers.

Heart rate, mean blood pressure, respiratory rate, ventilation, tidal volume, V_{O_2}, V_{CO_2}, and R in the 4 groups are shown in Table 3. No significant differences between groups were observed.

GH Response to Exercise

Basal GH levels were similar in diabetic smokers (1.9 ± 0.4 mIU/L), diabetic nonsmokers (2.0 ± 0.5 mIU/L), normal smokers (1.5 ± 0.3 mIU/L), and normal nonsmokers (1.4 ± 0.2 mIU/L). In all groups, exercise induced a significant increase in serum GH concentrations, with a peak response at 15 minutes ($P < .001$ *v* time 0 in all groups). The increment was significantly higher in the diabetic smokers and nonsmokers than in the normal smokers and nonsmokers (diabetic nonsmokers *v* normal nonsmokers ANOVA: $F = 7.93$, $P < .002$; ANCOVA: $F = 6.854$, $P < .01$; diabetic smokers *v* normal smokers ANOVA: $F = 11.075$; $P < .001$, ANCOVA: $F = 9.998$, $P < .002$) (Fig 1). In both diabetic smokers and nonsmokers, the GH peak responses were not statistically correlated with basal and 30-minute glucose concentrations and basal HbA_{1C}, FFA, and SM-C levels.

No significant differences were observed between diabetic smokers and nonsmokers. In contrast, the GH response to exercise was significantly lower in normal smokers than in normal nonsmokers (ANOVA: $F = 12.2$, $P < .001$, ANCOVA: $F = 10.5$, $P < 0.002$) (Fig 1).

Cortisol Response to Exercise

Basal cortisol levels were similar in diabetic smokers (368.2 ± 32 nmol/L), diabetic nonsmokers (370.3 ± 36 nmol/L), normal smokers (344.3 ± 30 nmol/L), and normal nonsmokers (357.1 ± 29 nmol/L). In all groups, exercise induced a significant increase in serum cortisol concentrations, with a peak response at 30 minutes ($P < .001$ *v* time 0 in all groups). The increment was significantly higher in the diabetic smokers and nonsmokers than in the normal smokers and nonsmokers (diabetic nonsmokers *v* normal nonsmokers ANOVA: $F = 6.10$, $P < .02$, ANCOVA: $F = 5.022$, $P < .05$; diabetic smokers *v* normal smokers ANOVA: $F = 10.520$; $P < .002$, ANCOVA: $F = 6.498$, $P < .02$) (Fig 1). In both diabetic smokers and nonsmokers, the cortisol peak responses were not statistically correlated with basal and 30-minute glucose concentrations and basal HbA_{1C}, FFA, and SM-C levels.

No significant differences were observed between diabetic smokers and nonsmokers. In contrast the cortisol response to exercise was significantly lower in normal smokers than in normal nonsmokers (ANOVA: $F = 7.36$, $P < .01$; ANCOVA: $F = 6.5$, $P < .02$) (Fig 1).

AVP Response to Exercise

Basal AVP levels were similar in diabetic smokers (2.3 ± 0.4 ng/L), diabetic nonsmokers (2.6 ± 0.5 ng/L), normal smokers (2.1 ± 0.6 ng/L), and normal nonsmokers (2.3 ± 0.3 ng/L). In all groups, exercise induced a significant increase in plasma AVP concentrations, with a peak response at 15 minutes ($P < .001$ *v* time 0 in all groups). The increment was significantly higher in the diabetic smokers and nonsmokers than in the normal smokers and nonsmokers (diabetic nonsmokers *v* normal nonsmokers, ANOVA: $F = 11.370$, $P < .001$, ANCOVA: $F = 10.88$, $P < .002$; diabetic smokers *v* normal smokers, ANOVA: $F = 11.180$; $P < .001$, ANCOVA: $F = 9.980$, $P < .002$) (Fig 1). No significant differences were observed between normal smokers and non smokers and between diabetic smokers and nonsmokers. In both diabetic smokers and nonsmokers, the AVP peak responses were not statistically correlated with basal and 30-minute glucose concentrations and basal HbA_{1C}, FFA, and SM-C levels.

DISCUSSION

The data presented here show lower GH and cortisol responses to physical exercise in normal smokers than in nonsmokers. These results agree with the hypothesis that chronic nicotine consumption may attenuate GH and cortisol responses to releasing stimulations. In both normal groups, we also measured the simultaneous AVP response to physical exercise, because vasopressin is known to exert a releasing activity on ACTH secretion[5] and to mediate the ACTH response to various challenging stimulations.[6] In contrast with cortisol, the AVP rise induced by physical exercise was not inhibited by habitual cigarette smoking. The difference between cortisol and AVP secretory patterns suggests that different neuroendocrine pathways mediate the 2 exercise-induced hormonal responses.

In agreement with our previous findings,[4] the data presented here show higher responses of GH, ACTH/cortisol, and AVP to

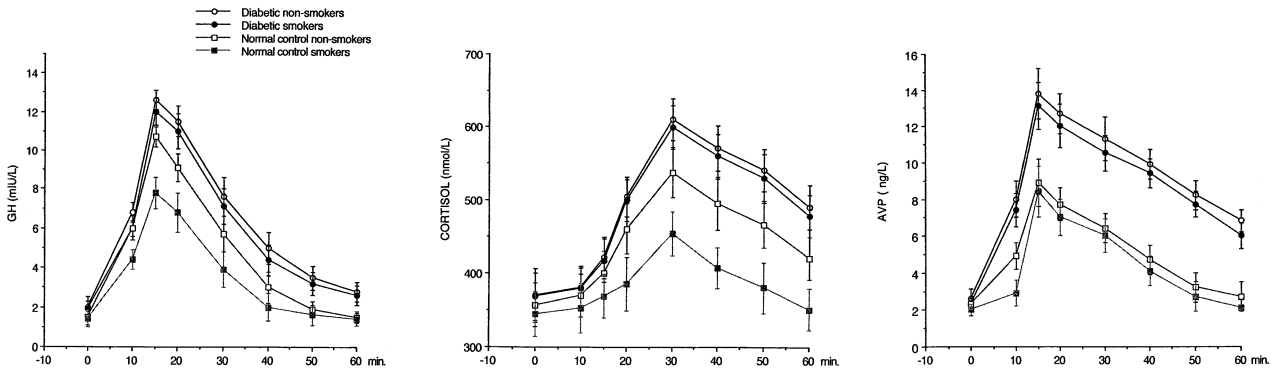

Fig 1. Serum GH, cortisol, and AVP concentrations (basal levels and response to physical exercise) in diabetic smokers and nonsmokers and in normal smokers and nonsmokers. Each point represents the mean ± SE of the observations.

physical exercise in diabetics than in normal subjects. However, in contrast with the above-described observation in normal subjects, habitual cigarette smoking was unable to reduce exercise-induced GH and ACTH/cortisol responses in diabetics. In fact, no significant differences were observed between diabetic smokers and nonsmokers. The different pattern between diabetics and normal subjects cannot be attributed to differences in cardiovascular or respiratory responses, which showed similar values during exercise in all groups. The statistical results and the interpretation of the hormonal data did not change after excluding the effects of confounding variables such as age, body mass index, duration of diabetes, and insulin treatment with ANCOVA.

Possible alterations of the hormonal response induced by the elevated glucose levels in diabetic patients can be excluded, because no significant correlations were observed between the hormone peak response and basal or 30-minute glucose concentrations.

In view of the observation that the GH and cortisol secretory systems in diabetic smokers are hyper-responsive to acute nicotine inhalation with cigarette smoking,[4] interactions between nicotine- and diabetes-induced neuroendocrine alterations may be supposed. Diabetes-induced changes responsible for this phenomenon may be recognized in the enhancement of adrenergic activity, which is known to occur in diabetic patients. In fact, nicotine has been shown to stimulate both ACTH/cortisol[9,10] and GH[8] secretions through catecholaminergic mediation, probably in the brainstem, and hence in the hypothalamic paraventricular nucleus (PVN) for ACTH.

Adrenergic neurons also mediate the GH and ACTH/cortisol responses to physical exercise.[11] Diabetics differ from normal subjects because of adrenergic hyperreactivity. This might explain why in our diabetics chronic nicotine consumption failed to reduce pituitary hormonal responses to exercise. In fact, the hyper-reactive adrenergic control renders the hypothalamic-pituitary axis resistant to changes induced by chronic nicotine consumption.

From a clinical point of view, the changes observed in normal chronic cigarette smokers may be regarded as a positive phenomenon. They may represent adaptation of nicotinic neurotransmission to chronic stimulation and, thus, may be associated with reduction of the negative cerebral effects of long-term nicotine consumption. Smoking is an important risk factor for cardiovascular and renal diseases.[12-14] We suppose that nicotine activity in the brainstem contributes to the pathogenesis of these pathologies through an enhancement of blood pressure, because brainstem catecholaminergic neurons control both ACTH/cortisol secretion from the hypothalamic PVN and cardiovascular centers.[9,15,16] The results of the present study demonstrate that the hypothetical protective reduction of nicotine activity observed in nondiabetic smokers does not occur in diabetic smokers and makes diabetics more susceptible than nondiabetics to smoking-related pathological complications. Further studies are needed to substantiate this hypothesis.

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