

Cortisol Secretion in Patients With Type 2 Diabetes

Relationship with chronic complications

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OBJECTIVE — The presence of an enhanced cortisol secretion in patients with type 2 diabetes is debated. In type 2 diabetic subjects, cortisol secretion was found to be associated with the complications and metabolic control of diabetes. We evaluated cortisol secretion in 170 type 2 diabetic subjects and in 71 sex-, age-, and BMI-matched nondiabetic subjects.

RESEARCH DESIGN AND METHODS — In all subjects, we evaluated ACTH at 8:00 A.M. in basal conditions and serum cortisol levels at 12:00 P.M. (F24) and at 9:00 A.M. after a 1-mg overnight dexamethasone suppression test and 24-h urinary free cortisol (UFC). In diabetic patients, we evaluated the presence of chronic complications (incipient nephropathy, asymptomatic neuropathy, background retinopathy, and silent macroangiopathy). Patients were subdivided according to the absence (group 1, $n = 53$) or presence (group 2, $n = 117$) of diabetes complications.

RESULTS — In group 2, UFC (125.2 ± 4.6 nmol/24 h) and F24 (120.6 ± 4.1 nmol/l) were higher than in group 1 (109.2 ± 6.8 nmol/24 h, $P = 0.057$, and 99.7 ± 6.1 nmol/l, $P = 0.005$, respectively) and in nondiabetic patients (101.7 ± 5.9 nmol/24 h, $P = 0.002$, and 100.3 ± 5.3 nmol/l, $P = 0.003$, respectively). In diabetic patients, the number of complications was associated with F24 ($R = 0.345$; $P < 0.0001$) and diabetes duration ($R = 0.39$; $P < 0.0001$). Logistic regression analysis showed that the presence of diabetes complications was significantly associated with F24, sex, duration of diabetes, and glycated hemoglobin.

CONCLUSIONS — In type 2 diabetic subjects, hypothalamic-pituitary-adrenal activity is enhanced in patients with diabetes complications and the degree of cortisol secretion is related to the presence and number of diabetes complications.

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In patients with type 2 diabetes, glucocorticoid secretion has been suggested to be a possible link between insulin resistance and the features of the metabolic syndrome (hypertension, obesity, coronary heart disease, hyperlipidemia, and type 2 diabetes) (1–4). In fact, while glucocorticoid excess (overt or sub-

clinical) has been demonstrated to lead to diabetes or to worsen metabolic control (5–7), the relationship between cortisol levels, insulin resistance, and chronic complications in type 2 diabetic patients without hypercortisolism is still a matter of debate.

In past years, the hypothalamic-pituitary-adrenal (HPA) axis secretion in patients with type 2 diabetes has been extensively investigated (8–14). In particular, some studies reported in these subjects an elevation of ACTH (10,12), basal (9–11) and after dexamethasone test serum cortisol (13,14), and late-night salivary cortisol levels (15). In contrast, other previous studies (16–17) did not show any alteration of pituitary-adrenal axis secretion. The presence of chronic complications of type 2 diabetes (i.e., macroangiopathy, retinopathy, and neuropathy) has been associated to with HPA axis activity (9,18–23), and an association between the degree of severity of several clinical measures of diabetes and cortisol secretion in type 2 diabetic subjects with normal HPA activity has been recently reported (24).

To further investigate this topic, we evaluated the HPA activity in a sample of type 2 diabetic patients with and without chronic complications and in a sample of nondiabetic patients.

RESEARCH DESIGN AND METHODS

Subjects were recruited from January 2003 to January 2004 at San Giuseppe-Fatebenefratelli Hospital in Milan, Italy. In 294 consecutive type 2 diabetic inpatients enrolled in a previous study designed to evaluate the prevalence of subclinical hypercortisolism in diabetes (7), biochemical parameters of HPA axis secretion and the presence of diabetes complications were evaluated. Selection criteria were as follows: age at diagnosis >30 years, BMI ≥ 19 and ≤ 40 kg/m², no need of insulin therapy in the first 2 years of disease, no history of ketoacidosis, no hypoglycemia in the last 6 months, no signs or symptoms of hypercortisolism (including moon facies, striae rubrae, hypertrichosis, skin atrophy, and buffalo

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Abbreviations: F24, serum cortisol levels at 12:00 P.M.; F-Dex, dexamethasone suppression test; HOMA-IR; homeostasis model assessment of insulin resistance; HPA, hypothalamic-pituitary-adrenal; UFC, urinary free cortisol.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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hump), hyperandrogenism, chronic renal failure, acute illnesses, alteration of sleep-wake cycle, depression, alcoholism, and no present glucocorticoid therapy or intake of drugs known to interfere with HPA axis or with autonomic nervous system (i.e., β -blockers, α -blockers, and cholinergic agonists and antagonists). Diabetic subjects with disease duration <2 years were included if they were never treated with insulin.

To investigate the relationship between chronic complications of diabetes and cortisol levels in type 2 diabetic subjects with normal HPA activity, subjects with ascertained or suspected hypercortisolism were excluded (7). Moreover, since proliferative or laser-treated retinopathy and painful neuropathy have been described to be associated to HPA hyperactivity (21,22), patients with these severe complications were excluded, as well as patients with overt nephropathy (microalbumin excretion >200 mg/l) and/or severe macroangiopathy (carotid and/or peripheral arterial stenosis >70%, coronary heart disease, and/or intermittent claudication). Eventually, 170 of 294 patients were included in the study.

Seventy-one consecutive sex-, age-, and BMI-matched nondiabetic (fasting blood glucose <6.05 mmol/l and a normal oral glucose tolerance test [25]) inpatients were selected as control subjects according to the above-mentioned selection criteria (control group). The control subjects were admitted to the hospital for multinodular goiter with normal thyroid function ($n = 21$) or obesity ($n = 50$) to exclude the presence of cardiorespiratory complications of their diseases.

In all subjects, the following determinations were performed: serum cortisol levels at 12:00 P.M. (F24) (normal values <207.0 nmol/l), 24-h urinary free cortisol (UFC) (normal values 22.1–165.6 nmol/24 h), and plasma ACTH at 8:00 A.M. (normal values 2.2–11.0 pmol/l); after F24, UFC, and ACTH determinations, serum cortisol levels were measured at 9:00 A.M. after a 1-mg overnight dexamethasone suppression test (F-Dex) (cutoff value <50.0 nmol/l, according to previous studies [5]).

In type 2 diabetic patients, the presence of chronic complications of diabetes was investigated. Patients were subdivided into two groups based on the absence (group 1) or the presence (group 2) of chronic complications (somatic or autonomic neuropathy, macroangiopathy, background retinopathy, and incipient

nephropathy). In non-insulin-treated type 2 diabetic subjects, homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as previously reported (26); in all type 2 diabetic patients, waist circumference was measured and data concerning A1C levels and use of oral hypoglycemic agents and/or insulin treatment were also collected.

Subjects with systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg and/or on antihypertensive treatment were defined as hypertensive (27). Dyslipidemia was defined as triglycerides ≥ 1.7 mmol/l or HDL cholesterol <1.03 mmol/l in men and <1.3 mmol/l in women (27,28). Subjects were also considered dyslipidemic if an antidiabetic treatment was given. Type 2 diabetic patients were considered affected by abdominal obesity if waist circumference was >102 cm in men and >88 cm in women and affected by metabolic syndrome based on the Adult Treatment Panel III criteria (27).

On the day of admission, all individuals had a catheter inserted in the forearm vein to avoid stress-related HPA axis activation due to venipuncture; all biochemical determinations were made no earlier than the 2nd day after admission. Serum cortisol and UFC (after dichloromethane extraction) levels were determined immunometrically by chemiluminescence (Immulite; Diagnostic Products, Los Angeles, CA, and TDX-FLX Abbott Diagnostika kit; Wiesbaden-Delkenheim, Germany); plasma ACTH levels (mean of three determinations at 20-min intervals) were measured immunometrically by chemiluminescence (Immulite).

In non-insulin-treated type 2 diabetic patients, a fasting blood specimen was obtained for determination of plasma glucose and serum insulin (enzyme immunoassay [Eurogenetics Tasah, Hampton, U.K.]) to calculate HOMA-IR (26).

In all type 2 diabetic subjects, the presence of somatic neuropathy was evaluated by measuring the diabetic neuropathy score (29). The presence of autonomic neuropathy was investigated performing deep-breathing, lying-to-standing, and postural hypotension tests, as described elsewhere (23). The presence of diabetic macroangiopathy was ascertained by ultrasonography measuring carotid flow; patients were defined as being affected by this complication if the reduction of arterial flow at the level of common internal and external carotid arteries was >50% (30). Background reti-

nopathy was ascertained by fundus oculi examination (31). Subjects with microalbumin levels between 20 and 200 mg/l (in at least three consecutive samples) were considered to be affected by incipient diabetic nephropathy (32).

Statistical analysis

For each variable, normality of distribution was tested by the waist circumference “WC statistic” of the Shapiro-Wilk test. The results are expressed as means \pm SD or median (range) for non-normally distributed variables if not otherwise reported. A conventional score of diabetes complications has been attributed to each type 2 diabetic subject as the sum of chronic complications (considering the value of 1 if present and 0 if absent for each diabetes complication).

Data from control and patient groups as a whole were compared by *t* test or Mann-Whitney *U* test as appropriate; χ^2 test was performed for categorical variables. General linear modeling was used to compare data from the control group, group 1, and group 2 after adjusting for age and sex. The associations between variables were tested by either Pearson correlation coefficient or Spearman’s rank correlation test, as appropriate.

In type 2 diabetic patients, logistic regression analysis was performed to evaluate in type 2 diabetic patients the association between the presence of chronic complications of diabetes and cortisol secretion after adjusting for possible confounding factors such as age, sex, BMI, presence of hypertension, duration of disease, and metabolic control.

Statistical analysis was performed by using SPSS version 12.0 statistical package software (SPSS, Chicago, IL). *P* < 0.05 was considered significant.

RESULTS— The clinical characteristics and biochemical parameters of HPA axis function from the whole diabetic group and from the control group are summarized in Table 1. Age, BMI, waist circumference, and sex were comparable between the all diabetic patients and control subjects. In the diabetic group, F24, UFC, and ACTH levels were above the normal range in 6, 28, and 2 subjects, respectively; no patient showed F-Dex levels above the cutoff value. UFC, F-Dex, F24, and the prevalence of hypertensive subjects were higher in the type 2 diabetic groups with respect to the control group (Table 1).

HPA axis activity parameters of dia-

Table 1—Clinical characteristics and HPA axis activity in type 2 diabetic patients and control subjects

	Control subjects	All diabetic subjects	Group 1 diabetic subjects	Group 2 diabetic subjects
<i>n</i>	71	170	53	117
Age (years)	62.3 ± 12.5 (40–86)	61.1 ± 11.0 (34–82)	60.1 ± 11.9 (34–82)	61.5 ± 10.5 (38–81)
Female/male (%)	44/27 (62.0/38.0)	102/68 (60.0/40.0)	39/14 (73.6/26.4)	63/54 (53.8/46.2)*
BMI (kg/m ²)	29.6 ± 0.5 (22–39)	30.4 ± 0.3 (21.2–40.0)	31.0 ± 0.6 (21.2–40.3)	30.2 ± 0.4 (21.9–39.0)
UFC (nmol/24 h)	101.7 ± 5.9 (36.4–265.0)	120.1 ± 3.9 (33.1–258.9)†	109.2 ± 6.8 (33.1–220.8)	125.2 ± 4.6 (31.7–258.9)‡§
ACTH (pmol/l)	4.1 (2.2–8.4)	4.6 (2.2–12.7)	4.3 (2.2–10.8)	4.7 (2.2–12.7)
F-Dex (nmol/l)	27.6 (5.5–38.6)	30.4 (13.8–49.7)	27.6 (13.8–49.7)	30.4 (13.8–49.7)¶
F24 (nmol/l)	100.3 ± 5.3 (30.4–179.4)	115.8 ± 3.9 (27.6–369.8)	99.7 ± 6.1 (27.6–171.1)	120.6 ± 4.1 (30.4–369.8)##**
Dyslipidemia	21 (29.6)	64 (37.6)	17 (32.1)	47 (40.2)
Hypertension	34 (47.9)	108 (63.5)††	24 (45.3)	84 (71.8)‡‡§§

Data are means ± SD (range) for the normally distributed variables age and BMI. Data are means ± SE (range) for UFC and F24. Data are median (range) for the non-normally distributed variables ACTH and F-Dex. Data are *n* (%) for dyslipidemia and hypertension. Reported results were confirmed after logarithmic transformation of these variables and after adjustment for age and sex. Normal values for UFC, ACTH, and F24 are 22–165.5 nmol/24 h, 2.2–11.0 pmol/l, and <207 nmol/l, respectively. Cutoff value for F-Dex <50 nmol/l. * $\chi^2 = 6.03$, $P = 0.05$ versus group 1 by χ^2 test. † $P = 0.011$. ‡ $P = 0.057$. § $P = 0.002$. || $P = 0.025$ versus control group by *t* test. ¶ $P = 0.011$. # $P = 0.003$ versus control group by general linear modelling. ** $P = 0.005$ versus group 1 by general linear modelling. †† $\chi^2 = 5.1$, $P = 0.024$ versus control group by χ^2 test. ‡‡ $\chi^2 = 10.81$, $P = 0.001$. §§ $\chi^2 = 11.07$, $P = 0.001$.

betic patients subdivided according to the absence (group 1) or the presence (group 2) of chronic complications are reported in Table 1 and clinical characteristics of patients from the two groups in Table 2. Age and BMI were comparable among group 1, group 2, and the control group. The parameters of HPA axis function were higher in group 2 than in group 1 and the control group (Table 1). Compared with group 1, group 2 showed a higher prevalence of male subjects, insulin-treated and hypertensive patients, higher A1C level, and a longer duration of diabetes, whereas the prevalence of metabolic syndrome, waist circumference, and HOMA-IR levels was comparable between the two groups (Table 2).

No correlation was found between any parameter of HPA axis function and A1C, disease duration, waist circumference, HOMA-IR, and number of compo-

nents of the metabolic syndrome. In type 2 diabetic patients, the number of diabetes complications was significantly associated with F24 ($R = 0.345$; $P < 0.0001$) and duration of diabetes ($R = 0.39$; $P < 0.0001$). The other HPA axis parameters (i.e., UFC, F-Dex, and ACTH) were not associated with diabetes complications.

Logistic regression analysis data are reported in Table 3 and showed that F24 levels were significantly ($P = 0.039$) related to the presence of diabetes complications after adjusting for the other covariates; among the latter, sex ($P = 0.041$), duration of diabetes ($P = 0.006$), and A1C ($P = 0.044$) were significantly related to the presence of diabetes complications. The other parameters of HPA axis entered separately in the logistic regression analysis instead of F24 were not associated with the presence of diabetes complications (data not shown).

Taking into account each diabetes complication, the logistic regression analysis showed that F24 was significantly related to retinopathy ($P = 0.010$) and neuropathy ($P = 0.039$); these latter complications were also significantly linked to duration of diabetes ($P < 0.0001$ and $P = 0.016$, respectively). Diabetic macroangiopathy was found to be related to age ($P = 0.004$) and nephropathy to sex ($P = 0.028$). The other parameters of HPA axis entered separately in the logistic regression analysis instead of F24 were not associated with the presence of any diabetes complication (data not shown).

CONCLUSIONS— Glucocorticoid secretion has been suggested to be a possible link between insulin resistance and the features of metabolic syndrome (1–4). Several previous studies concerning the HPA axis function in type 2 diabetic

Table 2—Clinical characteristics of type 2 diabetic patients from group 1 and group 2

	All diabetic subjects	Group 1 diabetic subjects	Group 2 diabetic subjects
<i>n</i>	170	53	117
Type 2 diabetes duration (years)	7 (0–40)	4 (0–40)	10 (0–39)*
A1C (%)	9.9 ± 2.5 (5.0–16.0)	9.3 ± 2.4 (5.0–16.0)	10.2 ± 2.5 (5.0–15.0)†
HOMA-IR‡	3.6 ± 2.0 (0.3–9.8)	3.6 ± 2.2 (0.5–9.8)	3.6 ± 2.0 (0.3–9.5)
Waist circumference (cm)	100.5 ± 10.7 (72.0–135.0)	101.5 ± 12.9 (72.0–129.0)	100.3 ± 9.6 (72.0–135.0)
Metabolic syndrome	103 (60.6)	29 (54.7)	74 (63.2)
Macroangiopathy	46 (27.1)		46 (39.3)
Retinopathy	50 (29.4)		50 (42.7)
Neuropathy	69 (40.6)		69 (59.0)
Nephropathy	44 (25.8)		44 (37.6)
Insulin therapy	32 (18.8)	6 (11.3)	26 (22.2)§

Data are means ± SD, median (range), or median (%). Metabolic syndrome defined on the basis of the Adult Treatment Panel III criteria (27). * $P < 0.0001$ versus group 1. † $P = 0.028$ versus group 1. ‡Non-insulin-treated diabetic patients; *n* = 138, 47, and 91 for all diabetic subjects, group 1, and group 2, respectively. § $P = 0.067$ versus group 1.

Table 3—Odds ratios, 95% CIs, and P values for detecting chronic diabetes complications for potential risk factors using multivariable logistic regression models

	All complications	Macroangiopathy	Retinopathy	Neuropathy	Nephropathy
F24					
OR	1.31	1.20	1.36	1.24	1.17
95% CI	1.01–1.70	0.97–1.49	1.08–1.72	1.01–1.52	0.95–1.44
P value	0.039	0.089	0.010	0.039	0.135
Age (years)					
OR	0.997	1.07	0.97	0.968	1.01
95% CI	0.96–1.04	1.02–1.12	0.93–1.01	0.93–1.00	0.970–1.05
P value	0.887	0.004	0.186	0.069	0.745
Sex (male vs. female)					
OR	2.33	0.53	1.23	0.82	2.32
95% CI	1.03–5.35	0.24–1.19	0.54–2.80	0.41–1.63	1.10–4.92
P value	0.041	0.126	0.620	0.571	0.028
A1C (1% increase)					
OR	1.18	1.11	1.16	1.08	1.01
95% CI	1.01–1.39	0.94–1.31	0.98–1.38	0.94–1.24	0.87–1.18
P value	0.044	0.212	0.076	0.31	0.872
Type 2 diabetes duration (years)					
OR	1.09	1.029	1.111	1.049	1.02
95% CI	1.02–1.15	0.99–1.07	1.06–1.16	1.01–1.09	0.98–1.07
P value	0.006	0.175	<0.0001	0.016	0.289
Hypertension (presence vs. absence)					
OR	2.14	2.01	1.37	1.50	1.404
95% CI	0.92–4.93	0.75–5.38	0.53–3.52	0.68–3.30	0.57–3.45
P value	0.075	0.164	0.510	0.318	0.459

F24, age, sex, duration of diabetes, A1C, and presence of hypertension were included as independent variables. OR, odds ratio.

patients gave conflicting results, with some authors finding an enhanced HPA axis activity (9–15) and others failing to show any alteration (16,17).

In the present study, we evaluated HPA activity in a sample of type 2 diabetic subjects with and without chronic complications and in a sample of nondiabetic patients. We found that in diabetic subjects without chronic complications, HPA axis activity was comparable with that of nondiabetic patients, whereas in diabetic subjects with chronic complications, cortisol level was increased in respect to both diabetic subjects and control subjects. The absence of ACTH reduction in the presence of a mildly higher cortisol secretion seems to confirm that in type 2 diabetic subjects with chronic complications, a “central” alteration of HPA axis is present, as suggested by previous studies (9–14).

Our results are in line with those from a previous study by Liu et al. (15), who found increased late salivary cortisol levels in elderly and obese type 2 diabetic subjects. These findings are also in keeping with a previous study of Roy et al. (18) in which a trend for higher cortisol secretion in patients with either diabetic retinopathy or cardiovascular complications

was found (18). On the contrary, some previous studies (16,17) failed to show any alteration of HPA axis secretion in type 2 diabetes. In our opinion, these conflicting results may be explained at least partially by the inclusion in the same sample study of patients with and without diabetes complications. This, according to our data, could have masked the effect of HPA activation in diabetic patients with chronic complications.

Another important finding of the present study is that in our diabetic subjects, the degree of cortisol secretion as reflected by F24 is directly associated with both the presence and number of type 2 diabetes complications. Conversely, the other parameters of cortisol secretion were not significantly associated with the presence or number of diabetes complications. In our opinion, these seemingly discordant results may be explainable considering that F24 is highly accurate for the diagnosis of hypercortisolism (5,15) and possibly better than UFC and F-Dex (33).

Our diabetic subjects with chronic complications were more frequently men and showed higher A1C levels and longer duration of disease with respect to dia-

betic patients without chronic complications. Since age, A1C, and duration of diabetes have been recently reported (15,24,34) to be directly associated with cortisol secretion in type 2 diabetic subjects with normal HPA activity, we hypothesize that these variables might have been responsible for our finding of enhanced cortisol secretion in diabetic subjects with chronic complications. Nevertheless, as indicated by logistic regression analysis, in our series cortisol levels were associated with the presence of chronic diabetes complications after correction for age, sex, presence of hypertension, and duration and metabolic control of their disease (Table 3).

The design of our study does not allow us to draw conclusions about causality. From these data, indeed, we are not able to discern a possible causative effect of cortisol secretion on the pathogenesis of chronic diabetes complications. Given the recognized deleterious role of glucocorticoids on glucose metabolism (1–7), it is possible to speculate that an increased cortisol secretion may contribute to worsening the metabolic control of diabetes and insulin sensitivity, consequently, inducing a higher prevalence of

chronic diabetes complications. However, in our sample, the parameters of insulin resistance studied (i.e., waist circumference and HOMA-IR) were comparable between diabetic subjects with and without complications and not associated with the parameters of HPA axis. These findings seem to exclude that the possible effect of increased cortisol secretion on the pathogenesis of chronic diabetes complications could be mediated by insulin resistance. Conversely, as suggested by Richardson and Tayek (35), diabetic subjects with chronic complications may be exposed to a chronic stress, which, in turn, may increase cortisol secretion. In this regard, it is important to note, however, that in our study, type 2 diabetic subjects with severe complications (i.e., painful or severe macroangiopathy, coronary heart disease and/or neuropathy, proliferative or laser-treated retinopathy, and overt nephropathy) were excluded. In addition, since hospitalization may lead to a condition of enhanced HPA activity (7), we enrolled nondiabetic inpatients as control subjects, and the significant difference in cortisol secretion observed between the diabetic and control subjects suggests that hospitalization did not play a major role.

Looking at a single chronic diabetes complication, in our patients, cortisol level was significantly associated with diabetic retinopathy and neuropathy and tended to be associated with macroangiopathy after correction for age, sex, presence of hypertension, duration of diabetes, and A1C (Table 3). This is in keeping with previous data suggesting that in type 2 diabetic subjects the presence of retinopathy (21,32), neuropathy (22,23), and macroangiopathy (18–20) is directly correlated with cortisol secretion. Although the design of our study does not allow us to look for a cause-effect relationship, a higher level of cortisol, even in the normal range (either due to a constitutive HPA axis activation or secondary to a chronic stress condition), may predispose to the development of diabetic retinopathy, neuropathy, and macroangiopathy. In our diabetic patients, an association between the presence of diabetic nephropathy and cortisol secretion was not found. In 80% of patients with Cushing's syndrome, an increased albumin excretion, which was reversed after successful treatment of hypercortisolism, has been described (36). However, in our sample, cortisol secretion was normal, thus we cannot compare our results to those ob-

served in patients with overt hypercortisolism. The underlying pathogenesis of diabetic nephropathy is, in fact, still unclear, although genetic factors are thought to play a role (37).

In conclusion, our data show that in type 2 diabetic subjects, HPA activity is enhanced only in patients with chronic complications and the degree of cortisol secretion is directly associated with the presence and the number of diabetes complications. Further studies are needed to investigate the possible causative role for cortisol secretion in the development of chronic complications of diabetes.

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