CLINICAL STUDY

Cortisol correlates with metabolic disturbances in a population study of type 2 diabetic patients

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Abstract

Objective: The prevalence of type 2 diabetes mellitus is increasing rapidly in industrialized countries, and adrenal glucocorticoids may intensify this disease. We sought to assess the relationship between diabetes-associated metabolic disturbances and cortisol concentrations in patients with type 2 diabetes.

Design: We investigated 190 type 2 diabetic patients who volunteered from a population study of 12 430 people in Luebeck and its suburbs. The target population comprised men and women born between 1939 and 1958 who initially received a postal questionnaire about their health status. We identified 346 subjects with confirmed diabetes mellitus and 216 patients participated in the study. Patients with type 1 diabetes were excluded.

Methods: Five salivary cortisol samples were collected before and after lunch, in the evening and then the next morning before and after standing. Clinical variables associated with diabetes were measured and correlated with cortisol concentrations.

Results: None of the cohort had salivary cortisol concentrations that exceeded the normally accepted range. Based on cortisol samples collected just prior to a standard lunch, the cohort was divided into tertiles. Cortisol was positively related to: fasting blood, urinary and postprandial glucose; glycosylated hemoglobin; and systolic and diastolic blood pressures (all $P < 0.05$). Cortisol concentrations also correlated with the relative abdominal mass ($P < 0.05$) when patients with marked glucosuria were excluded.

Conclusions: The degree of severity of several clinical measures of type 2 diabetes correlates with cortisol concentrations. Moreover, the results provide evidence for a positive relationship between metabolic disturbances and cortisol concentrations that are within the accepted normal range.

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Introduction

Over the last decades, an increasing standard of living in industrialized countries has been paralleled by a continuous rise in the prevalence of metabolic dysfunction, particularly type 2 diabetes (1–3). These diseases are linked by insulin resistance (4). The most serious of the clinical metabolic disturbances – i.e. visceral obesity, hypertension and dyslipidemia – are concurrent risk factors for type 2 diabetes (5, 6). In recent years, alterations in cortisol metabolism have been suggested to play a pathogenic role in metabolic disturbances (7), and some perturbations of the hypothalamic–pituitary–adrenal (HPA) axis have been found in diabetic patients (8, 9). However, a direct correlation

between the severity of diabetes-associated metabolic disturbances and cortisol concentrations has not been demonstrated. This may result from the methodological difficulties of evaluating function in the HPA axis during its circadian variation. In this study, we assessed salivary cortisol profiles during a 24-h period on an outpatient basis.

The clinical picture of type 2 diabetes appears as increases in various measures of circulating glucose, glycosylated hemoglobin, glucosuria, serum insulin and obesity; therefore, we hypothesized that the severity of these disturbances would correlate directly with salivary cortisol concentrations. We also examined the relationships between cortisol and blood pressure and indices of lipid metabolism. The data were collected

in a cross-sectional, population-based study including 12 430 individuals (NORDIA, North German Diabetes Study).

Subjects and methods

Participants

The patients with diabetes mellitus were recruited in a survey of all gainfully employed insurants of the Landesversicherungsanstalt Schleswig-Holstein (regional pension fund for blue collar workers, $n = 12\,430$) in Luebeck and its suburbs, Germany. The target population comprised men and women born between 1939 and 1958 who initially received a postal questionnaire about their health status; 6987 subjects returned the completed questionnaire and 371 of them declared that they had diabetes. Of 235 subjects who had been uncertain about diabetes, 22 subjects had pathologic fasting glucose levels (≥ 7.0 mmol/l, as recommended for use in epidemiological studies) (10). All subjects with confirmed diabetes mellitus ($n = 346$) were invited for a medical examination and 216 patients participated. Of these, 190 had type 2 diabetes. To determine the type of diabetes, the diagnosis was assessed independently by two diabetologists. Each participant gave written informed consent and the study was approved by the local ethics committee.

Structured medical interview and examination

The examination began at 0800 h after an overnight fast. Blood samples were taken for determination of plasma glucose, serum insulin, c-peptide, total cholesterol, high- and low-density lipoprotein (HDL and LDL respectively)-cholesterol, triglycerides, creatinine and glycosylated hemoglobin. Afterwards, volunteers were given a standardized breakfast followed by a second determination of plasma glucose and c-peptide levels as well as urinary glucose and albumin concentrations 1 h after the meal. Between breakfast and the second sampling, a structured medical interview was performed including diabetes-specific medical history. Chronic stress was evaluated by TICS (Trier Inventory of Chronic Stress) (11) and physical activity was scored from 1 (hardly any activity) to 3 (very active). The subsequent physical examination involved measurement of height and body weight, and the resulting body mass index (BMI) was calculated. The relationship of sagittal diameter to body weight was used as an index of central obesity. Sagittal abdominal diameter was measured by abdominal caliper in a lying position on a level with the umbilicus after the patients had exhaled. The relative abdominal mass was estimated by assuming the shape of the abdomen to be approximated by a sphere:

$$V = \frac{4}{3} \pi r^3$$

where V = volume and r = sagittal diameter/2. The specific weight of the abdominal tissue was approximated by the known constant, γ_{abdomen} , of 0.9 g/cm^3 (primarily fat). After inclusion of body weight as an additional parameter, the following equation was derived:

$$\text{Relative abdominal mass} = \gamma_{\text{abdomen}} \frac{\pi \text{ sagittal diameter}^3}{6 \text{ body weight}}$$

Measurement of sagittal diameter contributes more to the prediction of various clinical features such as total cholesterol, glucose and blood pressure than other measures of central obesity as previously demonstrated (12). Blood pressure was defined by the mean of three standardized automatic measurements. Finally, the patients received a standard lunch (light balanced diet: 600 kcal, 35% protein, 30% fat, 35% carbohydrates).

Assays

Cortisol was collected by Salivette (Sarstedt, Rommelsdorf, Germany) and determined by RIA (DPC Biermann GmbH, Bad Nauheim, Germany; inter-assay coefficient of variation (CV) $< 5.2\%$, intra-assay CV $< 4.8\%$). The participants were asked to take samples directly prior to the standard lunch and 45 min later in the course of the study. Three further samples were taken at home: the first sample just before bedtime, the second one the next morning before arising and the third one 30 min later (prior to breakfast).

All blood samples were immediately centrifuged and the supernatants stored at -24°C until assay. Concentrations of plasma glucose were measured using the glucose oxidase method (Glucose Analyser; Beckman Coulter, Inc. Munich, Germany; inter-assay CV $< 2.6\%$, intra-assay CV $< 1.8\%$). An enzyme-linked immunosorbent assay (ELISA) was used for determination of serum insulin (DAKO Ltd, Cambridgeshire, UK; inter-assay CV $< 8.9\%$, intra-assay CV $< 7.5\%$) and c-peptide (DAKO Ltd; inter-assay CV $< 5.7\%$, intra-assay CV $< 5.1\%$). HPLC was used for measurement of glycosylated hemoglobin (MONO S-column; Pharmacia Biotech, Erlangen, Germany; range = $5.4\text{--}7.6\%$, between-run s.d. 0.07% , CV 1%). Total cholesterol, LDL/HDL-cholesterol, triglycerides, creatinine and urinary albumin were determined by routine clinical methods.

Statistical analysis

The participants were divided into three groups (low, medium and high cortisol) based on their pre-lunch salivary cortisol concentrations. Of the five samples of

the cortisol diurnal profile collected, the pre-lunch sample was chosen to discriminate tertiles because all study participants had had the same standard schedule for at least the 4 preceding hours. Therefore, the pre-lunch sample was the single sample during the day that would be least biased by other external influences such as time of waking, food intake or physical activity. Subsequent to discrimination of the tertiles, the area under the five-sample cortisol curves was calculated to determine whether overall differences in cortisol among the tertiles distinguished by the pre-lunch sample were observed.

Values are presented as means $\pm$ S.E.M. ANOVA, adjusting for age and sex, was performed to determine the differences in the measured parameters between subjects of the different cortisol tertiles. To avoid interference between relative abdominal mass (energy storage) and glucosuria (energy loss), differences in relative abdominal mass across tertiles were also analysed excluding data from patients with marked glucosuria (>10 g/l). We also tested the influence of cortisol on other clinical measures (Table 1) by ANOVA. P values <0.05 were considered significant.

Results

Descriptive statistics of the subjects

The three groups were equal with respect to age, gender, duration of diabetes, diabetes-specific therapy, occurrence of hypoglycemic episodes, body weight, BMI, urinary albumin, exercise, alcohol consumption,

smoking, stress, antihypertensive drugs, serum insulin and c-peptide concentrations (Table 1).

Diurnal cortisol profiles

Fig. 1A shows the diurnal cortisol profile in the whole study population of patients with type 2 diabetes ($n = 190$) demonstrating two distinct peaks: the first, half an hour after arising in the morning (14.90 ± 0.55 nmol/l); and the second, 1 h after lunch (6.62 ± 0.28 nmol/l). After division into tertiles according to the pre-lunch salivary cortisol values, the three profiles were explicitly separated (Fig. 1B). The differences in cortisol concentrations between the three groups were significant at the time after awakening in the morning and 1 h after lunch ($P < 0.01$). Strikingly, the curve of the high-cortisol group merely shows one peak in the morning, there was no lunch-related peak at noon, which may possibly reflect the higher overall cortisol levels in the higher cortisol group. Comparison of the area under the curves among the three groups was also significant ($P = 0.001$, Fig. 1C).

Comparisons among the three cortisol groups and their clinical features

Subjects with high cortisol concentrations had higher levels of fasting and urinary glucose ($P = 0.001$ for both; Fig. 2A and B respectively), postprandial glucose ($P = 0.008$; Fig. 2C), glycosylated hemoglobin ($P = 0.012$; Fig. 2D, Table 1), and systolic and diastolic blood pressures ($P = 0.029$ and $P = 0.015$ respectively;

Table 1 Group comparison of clinical features.

	Low cortisol ($n = 63$)	Medium cortisol ($n = 64$)	High cortisol ($n = 63$)	P value
Age (years)	52.2 $\pm$ 0.8	51.9 $\pm$ 0.6	53.9 $\pm$ 0.6	0.083
Gender: female (%)	36.5	21.9	20.6	0.079
Duration of diabetes (years)	5.9 $\pm$ 0.7	6.0 $\pm$ 0.9	6.2 $\pm$ 0.7	0.951
Glycosylated hemoglobin (%)	7.35 $\pm$ 0.15	7.72 $\pm$ 0.21	8.12 $\pm$ 0.22	0.012
Diabetes-specific therapy (%)				
Diet	31 $\pm$ 5	28 $\pm$ 5	20 $\pm$ 5	0.258
Oral drugs	42 $\pm$ 6	54 $\pm$ 6	53 $\pm$ 6	0.213
Insulin	25 $\pm$ 5	17 $\pm$ 4	25 $\pm$ 5	0.691
Occurrence of hypoglycemic episodes during the past 12 months (%)	16 $\pm$ 5	17 $\pm$ 5	25 $\pm$ 6	0.344
Body weight (kg)	93.5 $\pm$ 2.4	92.8 $\pm$ 1.8	92.8 $\pm$ 1.9	0.775
BMI (kg/m ²)	31.2 $\pm$ 0.7	31.5 $\pm$ 0.6	31.2 $\pm$ 0.7	0.777
Urinary albumin (mg/l)	38.5 $\pm$ 14.7	89.5 $\pm$ 29.4	53.6 $\pm$ 11.8	0.174
Activity score	2.4 $\pm$ 0.07	2.3 $\pm$ 0.08	2.4 $\pm$ 0.08	0.623
Packyear	26.9 $\pm$ 3.5	23.5 $\pm$ 3.0	20.1 $\pm$ 0.5	0.090
Alcohol consumption (units/month)	17.5 $\pm$ 3.9	23.2 $\pm$ 7.2	13.4 $\pm$ 3.1	0.323
Stress score	94.4 $\pm$ 2.2	89.7 $\pm$ 2.2	87.5 $\pm$ 2.3	0.253
Antihypertensive drugs (%)	49.2	43.8	50.0	0.316
Fasting insulin (pmol/l)	98.9 $\pm$ 11.1	103.8 $\pm$ 11.9	114.1 $\pm$ 8.9	0.405
Insulin postprandial (pmol/l)	272.7 $\pm$ 22.7	251.0 $\pm$ 20.1	251.2 $\pm$ 16.5	0.747
Fasting c-peptide (nmol/l)	1.00 $\pm$ 0.06	1.03 $\pm$ 0.07	1.07 $\pm$ 0.06	0.607
c-Peptide postprandial (nmol/l)	1.78 $\pm$ 0.11	1.86 $\pm$ 0.12	1.74 $\pm$ 0.11	0.726

Values are means $\pm$ S.E.M.

One 'pack year' is equivalent to 20 cigarettes per day during one year.

Figure 1 Diurnal cortisol profiles of (A) the whole study population and (B) after division into tertiles. (C) Area under the curve (AUC) for the three cortisol groups (means $\pm$ S.E.M.).

Fig. 2E). Relative abdominal mass was not significantly different among the cortisol tertiles (Fig. 3) but became different in the low- ($n = 46$), medium- ($n = 42$) and high- ($n = 34$) cortisol group when subjects with marked glucosuria (> 10 g/l) were excluded ($P < 0.05$; insert in Fig. 3). These subjects were excluded because glucosuria was found to interfere with the relative abdominal mass ($\beta = -0.230$; $P = 0.023$ (β = standardised coefficient of correlation)). Differences among the three groups were not detected with respect to triglycerides, total cholesterol, and HDL- or LDL-cholesterol (Table 2).

Discussion

Our results demonstrate that in patients with type 2 diabetes, those with the highest cortisol profiles have

highest fasting, postprandial and urinary glucose, glycosylated hemoglobin and systolic/diastolic blood pressures. Relative abdominal mass correlates with cortisol concentrations when patients with marked glycosuria (> 10 g/l) are excluded. Because these clinical features are well-known consequences of hypercortisolism, it is important to point out that the results described here were found in diabetic patients in the absence of cortisol excess; cortisol concentrations were within the accepted normal range (13). However, even smaller increases in serum cortisol may contribute to the abnormal glucose metabolism known to occur in metabolic abnormalities and consequently type 2 diabetes (14). Masuzaki *et al.* (15) reported that transgenic mice overexpressing 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1), the enzyme activating the inactive form of glucocorticoids, show (in combination with several metabolic abnormalities) increased adipose levels of corticosterone although circulating glucocorticoid levels were normal. Possibly, our patients exhibited a cortisol excess in adipose tissue as well. This hypothesis is supported by findings demonstrating that 11 β -HSD1 elevation in adipose tissue of obese humans is associated with features of the metabolic syndrome (16, 17) although there was no difference in plasma cortisol (16). However, it should be considered that alterations in circulating cortisol levels could equally arise as a result of impaired metabolism or enhanced regeneration from cortisone via 11 β -HSD isoforms.

It seems likely that our positive finding of a relationship between increasing cortisol concentrations and metabolic disturbances in type 2 diabetes was, in large part, a consequence of the design of the timing used for collecting cortisol samples. The activity and caloric intake of the subjects were controlled by participating in the study for 4 h before and during the collection periods of the first two samples. The cortisol concentrations in the remaining three samples were probably dominated primarily by the circadian and postural importance of the collection times: before sleep and on waking and subsequent standing. Others have shown less convincing evidence for an effect of cortisol on both type 2 diabetes (9) and the metabolic syndrome (18–21) in non-hospitalized patients, who were studied on an outpatient basis.

Our results show that within the range of physiological normal, increasing cortisol is strongly associated with increasing diabetic pathophysiology, particularly in the variables that depend on hyperglycemia. It seems probable that the stimulatory effect of cortisol on these variables is a consequence of the direct action of cortisol on hepatic gluconeogenesis. The effects of high-dose cortisol infusions on glucose production have been determined to be a consequence of gluconeogenesis in volunteers studied under conditions in which other hormones were clamped (14). Moreover, in a clinical research center study, Tayek's group (22)

Figure 2 Fasting (A), urinary (B) and postprandial glucose (C), glycosylated hemoglobin (D), systolic and diastolic blood pressure (E) in the low- ($n = 63$), medium- ($n = 64$) and high- ($n = 63$) cortisol tertiles (means $\pm$ S.E.M.). ANOVA adjusted for age and sex.

also suggested that type 2 diabetic patients might have a mild form of injury response, based on their findings that both type 2 diabetics and cancer patients had: significantly elevated circulating concentrations of cortisol, glucose, insulin, c-peptide; depressed triiodothyronine; and elevated glucose production rates. Given the comparability of the results from our cross-sectional community studies and those of Tayek, performed in a clinical research center study, as well as the well-known effects of cortisol on mobilization of peripheral substrate and its use for hepatic gluconeogenesis, the effects we have observed do point to a dose-related effect of cortisol on gluconeogenesis as an explanation for our results. The results also suggest that these type 2 diabetic patients were increasingly insulin resistant.

Within this physiological concentration range of cortisol, there are also significant effects on abdominal fat mass and arterial blood pressure, but little, if any effect on either lipid metabolism or insulin secretion. It is, of course, well known under conditions of clinically recognized marked hypercortisolemia, such as Cushing's

Figure 3 Relative abdominal mass in the low- ($n = 63$), medium- ($n = 64$) and high- ($n = 63$) cortisol tertiles (means $\pm$ S.E.M.). ANOVA adjusted for age and sex. Inset, relative abdominal mass when subjects with marked glucosuria (> 10 g/l) were excluded.

Table 2 Group comparison of lipid metabolism.

	Low cortisol (n = 63)	Medium cortisol (n = 64)	High cortisol (n = 63)	P value
Serum triglycerides (mmol/l)	2.33±0.20	2.71±0.25	3.67±0.75	0.060
Total cholesterol (mmol/l)	5.97±0.12	5.99±0.11	6.33±0.23	0.126
HDL-cholesterol (mmol/l)	1.23±0.04	1.25±0.04	1.22±0.03	0.692
LDL-cholesterol (mmol/l)	3.83±0.11	3.67±0.11	3.74±0.15	0.795

Results are means±S.E.M.

syndrome, that lipid metabolism, insulin secretion and control of blood pressure are dysregulated and that redistribution of energy stores occurs, to a much greater extent than the changes observed here (23). Nonetheless, the effects of the small increases in cortisol observed had pronounced effects in the type 2 diabetics studied.

The range of the integrated increase in salivary cortisol in our study is narrow, but it should be remembered that it is the protein-unbound, biologically active, free concentrations of the steroid that were measured. It seems clear from our results that over this range the changes in mean steroid concentrations are biologically effective. Similarly, Samuels and McDaniel (24) have shown that the slight rise in cortisol that occurs during the trough of the circadian rhythm during fasting was sufficient, when mimicked by cortisol infusion in fed individuals, to inhibit the thyrotropin secretion observed in fasted man (24).

The increased cortisol concentrations suggest, in agreement with Richardson and Tayek (22), that individuals with type 2 diabetes may be under minor stress. Increased cortisol secretion is probably also accompanied by increased sympatho-adrenal tone. In patients with type 2 diabetes, counter-regulation is known to start at normoglycemic thresholds, indicating elevated sympathetic neural outflow (25, 26). In addition, Reaven *et al.* (2) suggested that the sympathetic nervous system is the link between insulin and blood pressure, and the cardiovascular system is the best-studied system of physiological responses to stress (27). In our subjects, blood pressure was increased specifically in the high-cortisol group, although it remains to be clarified whether any part of the increase is cortisol dependent. From the animal literature, it is known that baroreflex activity is altered by circulating corticosterone concentrations (28), and that corticosterone or a glucocorticoid-receptor antagonist (RU486) implanted over the nucleus of the tractus solitarius directly alter baroreflexes (29), providing a potential mechanism for the increased blood pressure in our high-cortisol group. Because insulin concentrations were equal across groups, insulin does not appear to be linked to blood pressure.

An important *post hoc* finding was that the relative abdominal mass was only different among the cortisol tertiles after exclusion of data from patients with

marked glucosuria (>10 g/l). Glucosuria ranged between 0 and 80 g/l in the present study. Presuming that the caloric equivalent of 1 g glucose is 4 kcal and that the urine volume of a diabetic patient is between 1 and 4 l/day, the caloric loss of the patients ranges between 0 and 1280 kcal/day. These differences in caloric loss apparently exert a significant effect on energy homeostasis, thereby masking the link between hypercortisolemia and increased body weight measures including relative abdominal mass. This finding agrees with the observations that insulin resistance, causing hyperglycemia and thereby glucosuria, is associated with a reduced risk of weight gain in non-diabetic subjects, and that, once people develop diabetes, they tend to lose weight (30).

Our findings suggest that HPA axis activity may play a role in the development of type 2 diabetes-associated metabolic disturbances. A link between a rise in cortisol and metabolic disturbances has been suspected for a long time. This study was cross-sectional and no long-term trends have been assessed yet. Therefore, the present data do not allow a conclusion to be drawn regarding cause and effect relationships. However, we presume that glucocorticoid actions fall into different categories, depending on the physiological endpoint assessed, with mediating effects in some instances and with suppressive or preparative effects in others, and with all effects probably regulated by the concentration of the hormone (31). Our results are in line with a new paradigm recently published that suggests the function of activated stress systems is to serve as an underpinning for supplying energy to the brain (32). Based on our findings here, further investigations, focusing on HPA activity, are indicated with the aim of developing prevention and treatment of type 2 diabetes.

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