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Adrenocortical function in young adults with diabetes mellitus type 1☆

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ABSTRACT

In 75 young adults with diabetes mellitus type 1 (DM 1) we have performed a cross-sectional study to gain more information about their adrenocortical function. We have found in a surprisingly large portion of patients (25%) a subnormal response (<500 nmol/L, low responders) of the serum cortisol during low-dose Synacthen test, accompanied by significantly decreased stimulated values of aldosterone and salivary cortisol. Basal serum cortisol, aldosterone, and dehydroepiandrosterone sulphate (in women only) were significantly reduced in low responders as well, while ACTH, cortisol binding globulin, plasma renin activity, urinary free cortisol/24 h, and salivary cortisol did not differ. The results indicate that the disorder of adrenocortical function in low responders occurs in all adrenocortical zones. The patients with the highest risk in respect to revealed hypocorticalism were DM 1 with autoimmune thyroiditis, 13 out of 36 in contrast to 5 out of 39 suffered from isolated form of DM 1, with onset around 30 years, independently on sex. The biorhythm of salivary cortisol in low responders under real-life conditions did not significantly differ from normal responders, except of the decreased values in the morning. Antibodies against 21-hydroxylase and adrenal cortex were negative in the entire group of diabetics studied. In conclusion, this is the first study to demonstrate in as much as 25% of young adults with DM 1 patients without any signs of adrenal autoimmunity decreased both basal and stimulated serum cortisol and aldosterone levels, implying existence of subclinical primary hypocorticalism.

1. Introduction

Adrenocortical function in diabetes type 1 (DM 1) can be influenced by several mechanisms. First of all, autoimmune DM 1 is often associated with other autoimmune endocrinopathies, including Addison's disease (AD). The occurrence of manifested AD in DM 1 patients is estimated between 0.5 and 1%, associated autoimmune thyroiditis (AIT) increases the risk of AD to 4% [1,2]. The main risk factor for development of autoimmune adrenalitis is the presence of autoantibodies against adrenal cortex (ACA), in particular of antibody against 21-hydroxylase (21OH Ab) [3–5].

Adrenocortical function and hypothalamo–pituitary–adrenal (HPA) axis are influenced in autoimmune diseases independently of the existence of adrenal autoantibodies. Pro-inflammatory cytokines affect HPA axis mostly as stimulants, however, inhibitory effects were described as well [6,7]. Reduced adrenocortical sensitivity to low adrenocorticotropin (ACTH) dose coupled with cortisol (F) hypo-responsiveness was found in patients with different types of autoimmune diseases, including autoimmune polyglandular syndrome 2 (APS 2) with/without DM 1 [8].

According to some reports, an insufficient metabolic control in diabetic patients is often accompanied by changes in hypothalamo–pituitary–adrenal (HPA) axis regulation. The function and sensitivity of the feed-back mechanism loop is further negatively influenced by hypo/hyperinsulinemia, hypo/hyperglycaemia and/or hypoleptinemia [9,10]. Young adults with DM 1, chosen for the study, are characterized, among other, by a marked tendency towards the manifestation of additional autoimmune diseases after the onset of diabetes [11–13]. In our previous study we have found very high incidence of AIT in these patients [14]. Taking into account described association between autoimmune polyglandular syndrome (APS) and disorders of adrenocortical function, respectively. HPA axis dysregulation, we supposed an increased

Abbreviations: 21OH Ab, antibodies against 21-hydroxylase, aldo aldosterone; AIT, autoimmune thyroiditis; ACTH, adrenocorticotropin; ACA, adrenal cortex autoantibodies; APS, autoimmune polyglandular syndrome; AD, Addison's disease; BMI, body mass index; COC, combined oral contraception; CBG, cortisol binding globulin; DM 1, diabetes mellitus type 1; DM 1+AIT, diabetes mellitus type 1 with autoimmune thyroiditis; DHEA-S, dehydroepiandrosterone sulphate; F, cortisol; HbA1C, glycosylated hemoglobin; H, healthy subjects; HPA axis, hypothalamo–pituitary–adrenal axis; LDST, low-dose Synacthen test; LR, low responders; NR, normal responders; PRA, plasma renin activity; RIA, radioimmunoassay; TSH, thyrotropin; fT_4, free tyroxine; UFC, urinary free cortisol.

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occurrence of pathological findings in this group. That is why we have performed a cross-sectional study on a clinically as well as biochemically homogeneous group of young adults with DM 1, to gain more detailed data about their adrenocortical function, in this subgroup of DM 1 so far not published.

2. Subjects and methods

2.1. Subjects

The studied group consisted of 75 young adult with DM 1, 47 men and 28 women. An isolated form of DM 1 was found in 39 patients, combination of DM 1 with AIT was diagnosed in 36 patients.

Further characteristics of the group of patients: age range 20–50 years, age of diabetes onset 25 ± 7 (mean ± SD) years, duration of diabetes 12 ± 7 years. Body mass index (BMI) at the time of investigation was 24 ± 3 kg/m^2, and glycosylated hemoglobin (HbA1C) 7.2 ± 1.8%.

When investigated, the patients did not display any clinical symptoms of adrenal function impairment and did not use any drug which could interfere with HPA axis. Patients who used drugs with glucocorticoids in any form and indication were not included in the study. Patients using angiotensin-converting enzyme inhibitors discontinued the use of medication 14 days prior to the Synacthen test. In all patients thyroid gland state was evaluated by determination of anti-thyreoglobulin and anti-thyroid peroxidase autoantibodies, thyrotropin (TSH) and free thyroxine (fT_4) and by thyroid ultrasonography examination [14,15]. Women included in the study did not use any contraceptives or their use was discontinued at least 3 months before the investigation. In no case of DM 1 patients was found proteinuria or persisting microalbuminuria.

Simultaneously, 30 healthy subjects (H), 15 men and 15 women were investigated. Their age at the time of investigation was 27 ± 4 years, BMI 23 ± 2 kg/m^2. None of them displayed clinical features of autoimmune disease such as positive titres of organ or systemic autoantibodies and did not use drugs which could intefere with the tests. The patients as well as healthy subjects signed an informed consent before entering the study, which was approved by the Ethical Committee of the Institute of Endocrinology.

2.2. Examination of adrenal function

Adrenocortical function was evaluated by basal and stimulated values of F in plasma and saliva and aldosterone (aldo) during low-dose Synacthen test (LDST), by determination of urinary free cortisol (UFC) during 24 h and by estimation of diurnal rhythm of salivary F. Occurrence of adrenal autoimmunity was evaluated by detection of specific autoantibodies ACA and 21OH Ab.

2.3. Performance of low-dose Synacthen test

The content of the ampoule with 250 μg/1 mL ACTH (Synacthen, tetracosactide. 250 μg, Novartis Pharma GmbH, Nuernberg, Germany) was added to 249 mL sterile saline. Each patient received 1 mL of the solution containing 1 μg Synacthen i.v. Individual doses were prepared 10 min before administration. The LDST was carried out in a specialized Laboratory for Functional Tests, after overnight fasting at 9 a.m. in recumbence position. Sixty minutes before the test the subjects were not allowed to smoke, drink liquids and brush their teeth. The patients applied the last dose of insulin the evening before the investigation. After 30 min of rest in bed with a cannula introduced into the cubital vein, blood and saliva were collected (time 0), and then 1 μg ACTH was administered intravenously. The next blood and saliva samples were collected at 20th, 30th, 40th and 60th min after ACTH administration. Thirty minutes after withdrawal the blood samples were centrifuged at 3000 rpm for 15 min, and serum was left frozen in plastic tubes and stored at −20 °C until analyzed. Saliva was collected by spitting into plastic tubes, the material was frozen at −20 °C and stored at this temperature. No saliva sample was contaminated with blood.

The serum obtained for determination of basal F level at time 0 was further used for measurement of other parameters, namely ACA, 21OH Ab, plasma renin activity (PRA), ACTH, transcortin (CBG), aldo, dehydroepiandrosterone sulfate (DHEA-S), HbA1C, blood glucose, TSH, fT_4, autoantibodies against thyroid peroxidase and thyreoglobulin. Serum and salivary F were determined at all times after ACTH stimulation, while stimulated values of aldo were measured only at times 30 and 60 min. Blood glucose was measured only at the start and completion of the test.

Normal response of serum F to administration of 1 μg ACTH was established as more than 500 nmol/L [16]. We did not record any complication either in diabetic patients or healthy subjects during LDST, in none of them hypoglycaemia or allergic reaction occurred.

2.4. Daily cortisol rhythm

Within 1 week after LDST the diurnal profile of salivary F was examined under real-life conditions in DM 1 patients and H. Saliva was collected at 08:00, 12:00, 17:00 and 22:00 o'clock. The subjects were not allowed to eat, smoke or clean their teeth for 30 min prior to saliva collection. In nine DM patients in which hypoglycaemia appeared during the day of saliva collection, the follow up was cancelled and repeated 2 days later. The collected saliva samples were transported to the laboratory by the second day after collection. The content of the tubes was frozen and after thawing centrifuged at 1000 rpm and then stored in deep freeze (−80 °C) until analyzed. Urine collection for determination of UFC was performed on the day of saliva collection.

2.5. Methods for steroid and hormone determination

Total serum F was determined by non-extraction radioimmunoassay (RIA) with use of rabbit antiserum to cortisol-3-(O-carboxymethyloxime): bovine serumalbumin (BSA) on the solid phase (tube walls), and [^{125}I] cortisol-tyrosine methylester as a tracer, on an automatic analyzer Stratec (Immunotech, Marseille, France). Intra-assay and inter-assay coefficients of variation (c.v.) were 5.2 and 9.8%, physiological range 135–607 nmol/L [17]. Salivary F was determined by the same method, but using 30 μL of saliva instead 1.5 μL of serum. Intra-assay and inter-assay c.v. were 4.0 and 10.2%, physiological range 2.0–29.0 nmol/L. DHEA-S was determined by RIA kit from Immunotech. Intra- and inter-assay c.v. were 4.2 and 7.2%. Physiological range μL: males 15–30 years 7.20–16.1, 30–40 years 6.40–15.0, 40–50 years 5.10–9.0. Females 15–30 years 2.40–14.5, 30–40 years 1.80–9.70, 40–50 years 0.66–7.20.

PRA was estimated by determination of angiotensin I using RIA kit from Immunotech. The detection limit for PRA was 0.1 ng/mL. Intra-assay and inter-assay c.v. were 10.4 and 10.5%, physiological range 0.50–1.90 ng/mL/h.

Plasma ACTH was determined by IRMA kit from Immunotech according to the manufacturer instructions. The detection limit was 1.2 pg/mL, intra-assay and inter-assay c.v. were 9.1 and 9.6%, physiological range 1.0–50.0 ng/L.

Aldo was determined by RIA kit from Immunotech after extraction to dichloromethane. Intra- and inter-assay c.v. were 7.8 and 8.4%, physiological range 0.01–0.50 nmol/L.

CBG was measured by RIA kit MG 130 61 purchased from Immuno-Biological Laboratories (Hamburg, Germany). Intra- and inter-assay c.v. were 3.8 and 5.0%. Physiological range: males 763–904 nmol/L, females 812–919 nmol/L.

UFC was determined by RIA kit from Orion Diagnostica (Finland). Intra-assay and inter-assay c.v. were 3.8 and 4.4%, physiological range 38–208 nmol/24 h.

TSH and fT_4 were measured by ECLIA, Roche Diagnostics GmBH, Mannheim, Germany, using Elecsys system 2010. Intra-assay and inter-assay c.v. for TSH and fT_4 were 3.0 and 2.0% (intra-), and 7.2 and 4.8% (inter-), respectively, physiological range for TSH 0.27–4.20 mIU/L, for fT_4 12.0–22.0 pmol/L.

2.6. Autoimmune markers

21OH Ab was determined by RIA kit from DRG-Diagnostics (Marburg, Germany). The assay detection limit, intra-assay and inter-assay c.v. were 1 U/mL, 5.8 and 7.1%, respectively. ACA were detected by indirect imunofluorescence on cryostat sections of monkey adrenal glands from The Binding Site (Birmingham, UK).

Anti-thyreoglobulin and anti-thyroid peroxidase autoantibodies were determined by Aeskulisa ELISA kits from Aesku Diagnostics (Wendelsheim, Germany) with assay cut-off for normal values 150 and 50 IU/mL, respectively.

2.7. Metabolic parameters

HbA1C was determined by immunochemical methods using quantitative immunoturbidimetry standardized by IFCC (Roche Diagnostics GmbH, Mannheim, Germany) on Integra 400 analyser (Roche Diagnostics GmbH, Mannheim, Germany). Intra-assay and inter-assay c.v. were 5.9 and 3.8%, physiological range below 4.5% according IFCC.

Glucose was measured by glucoso-oxidase methods on Glucose analyser; Beckman, Fullerton, CA, physiological range 3.9–5.6 mmol/L.

2.8. Statistical analysis

The influence of adrenal function on basal steroid levels and their alterations during time-dependent function tests was evaluated using repeated measures ANOVA model with the factors subject (evaluates the inter-individual variability), status (Healthy, DM 1-Response <500, DM 1-Response ≥500) and time of the test (e.g. 0, 20, 30, 40, 60 min) and status × time interaction. ANOVA testing was followed by least significant difference multiple comparisons. Due to non-Gaussian data distribution and non-constant variance in the data and residuals, the original data was transformed to symmetry and homoscedasticity using a power transformation. Eventual non-homogeneities persisting in the data after power transformation were detected using residual analysis. If the absolute values of studentized residuals exceeded the value 3, the respective experimental points were excluded from statistical evaluation. Concerning the elimination of non-homogeneities, their proportion of the total data was 0.76, 0.76, 1.24 and 5.74% for salivary cortisol, serum cortisol, diurnal serum cortisol profile, and aldosterone, respectively. The relationships between dichotomous data were evaluated using Fisher's exact test or by χ^2 test. For evaluation of the differences between two groups of numeric data, Mann–Whitney robust test was applied. When evaluating differences between more than two groups, one-way robust Kruskal–Wallis ANOVA followed by Dunn's multiple comparisons were used.

3. Results

3.1. Low-dose Synacthen test

a) Response of serum F to 1 μg ACTH in the 30th minute higher than 500 nmol/L was found in 56 out of 75 patients with DM 1, i.e. in 75% (normal responders, NR). Response of serum F less than 500 nmol/L was found in 19 patients, i.e. 25% (low responders, LR). The basic clinical and metabolic characteristics of LR and NR are presented in Table 1. The onset of DM 1 was earlier in NR than LR, while age, BMI, duration of DM, daily dose of insulin, blood glucose and HbA1C did not differ.
b) When evaluating sex dependence of the response of serum F, LR occurred in 8 out of 28 women (29%) and in 11 out of 47 men (23%). Using Pearson's Chi-quadrate test, the differences between groups of men and women were not statistically significant ($p = 0.6187$).
c) Taking into account the character of the disease, it appeared that out of 36 DM 1 patients with associated AIT, LR occurred in 13 subjects (36%), while in 39 patients with isolated form of DM 1 there were only five occurrences of LR (13%). The differences were significant by both Fisher's exact and Pearson's Chi-square tests ($p = 0.039$).
d) Fig. 1 shows the sex differences of basal levels DHEA-S in LR, NR and H. In females in the LR group, the basal levels of DHEA-S were significantly lower than in NR and healthy, while no differences were found in males.

3.2. Basal levels of studied hormones and other parameters in LR, NR and H

Basal levels of selected hormones, steroids and other parameters studied in serum and saliva in LR, NR and in H are summarized in Table 2. LR had significantly lower basal serum F than NR. Aldo values were significantly lower in LR than NR or H, while ACTH, PRA, UFC, CBG and salivary F did not differ in all groups

3.3. Stimulated values of studied hormones and other parameters in serum and saliva in LR, NR and H

Fig. 2 shows concentration of serum F during LDST, while Fig. 3 gives concentrations of salivary F. Significantly lower values of serum F in LR than in both NR or H were found at all sampling times, while no differences between NR a H were observed.

Table 1
Basic clinical and metabolic characteristics of low responders (LR) and normal responders (NR).

Variable	LR			NR			Difference Mann–Whitney test
	Median	Lower quartile	Upper quartile	Median	Lower quartile	Upper quartile	
Age (years)	38.0	33.0	43.5	33.5	28.8	43.0	–
BMI (kg/m^2)	26.0	24.1	28.0	24.0	22.5	26.1	–
Duration of DM 1 (years)	11.0	9.0	16.5	11.5	3.8	17.3	–
Onset of DM 1 (years)	29.0	21.0	31.5	22.0	18.0	27.3	$p < 0.05$
Dose of insuline (IU/kg/day)	0.66	0.53	0.78	0.66	0.53	0.74	–
Blood glucose (mmol/L)	8.6	6.5	13.0	8.7	6.7	11.1	–
HbAlC (%)	6.8	6.2	7.6	7.0	5.7	8.5	–

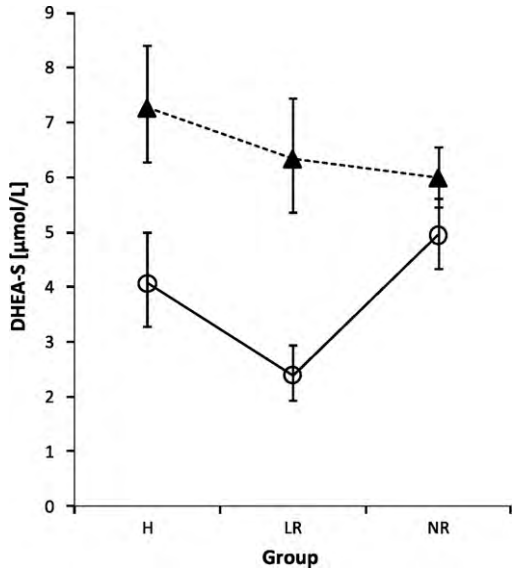

Fig. 1. Concentrations of basal serum DHEA-S and sex differences. Empty circles (women, F) and full triangles (men, M) symbolize the subgroup means (H, NR and LR). The error bars symbolize 95% confidence intervals for the subgroup means as computed using least significant difference multiple comparisons. The subgroups with non-overlapping 95% confidence intervals are statistically significant ($p < 0.05$). The significances of the factors, covariate and between-factor interaction Group × Sex was as follows: Group: $F = 4.71$, $p = 0.01$; Sex: $F = 38.46$, $p < 0.0001$; Age: $F = 3.59$, NS; Group × Sex: $F = 6.77$, $p = 0.002$; F, Fisher's statistics; p, significance level. The levels of mean factors differed as follows ($p < 0.05$): H > LR, LR < NR (patient's status), M > F (Gender).

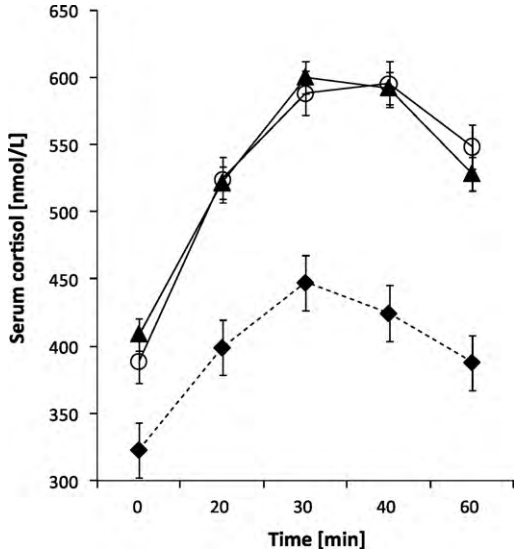

Fig. 2. Concentrations of serum cortisol during low-dose Synacthen test. Empty circles (H), full triangles (NR) and full squares (LR) symbolize the means for individual stages of the test. The error bars symbolize 95% confidence intervals for the subgroup means as computed using least significant difference multiple comparisons. The groups with non-overlapping 95% confidence intervals are statistically significant ($p < 0.05$). The significances of the factors and between-factor interaction were as follows: Group: $F = 165.9$, $p < 0.0001$; Time: $F = 97.9$, $p < 0.0001$; Subject: $F = 5.59$, $p < 0.0001$; Group × Time: $F = 2.77$, $p = 0.01$; F, Fisher's statistics; p, significance level. The levels of mean factors differed as follows ($p < 0.05$): H > LR, LR < NR (patient's status), 0 < 20, 0 < 30, 0 < 40, 0 < 60, 20 < 30, 20 < 40, 30 > 60, 40 > 60 (stages of the test).

Table 2
Basal concentrations of selected hormones in low responders (LR), normal responders (NR) and healthy subjects (H).

Variable	H			LR			NR			Difference Dunn's test
	Median	Lower quartile	Upper quartile	Median	Lower quartile	Upper quartile	Median	Lower quartile	Upper quartile	
ACTH (ng/L)	18.8	10.6	28.7	23.9	9.2	32.6	19.3	15.5	33.7	–
PRA (ng/mL/hour)	0.61	0.57	0.71	0.4	0.34	0.67	0.54	0.25	0.78	–
UFC (nmol/L/day)	114	97	140	141	94	179	102	60	189	–
CBG (nmol/L)	1095	928	1297	959	911	1190	1178	986	1317	–
TSH (mIU/L)	2.12	1.62	2.48	1.35	0.77	1.78	1.78	1.00	2.53	–
fT4 (pmol/L)	16.1	15.2	16.9	16.8	14.8	19.3	15.4	14.4	17.2	–
F salivary (nmol/L)	8.9	4.8	13.0	7.0	4.8	8.7	7.0	5.0	10.9	–
F serum (nmol/L)	378	331	463	322	272	362	405	342	476	LR-NR ($p < 0.05$)
Aldosterone (nmol/L)	0.29	0.18	0.35	0.14	0.12	0.20	0.17	0.12	0.26	LR-H, LR-NR ($p < 0.05$)

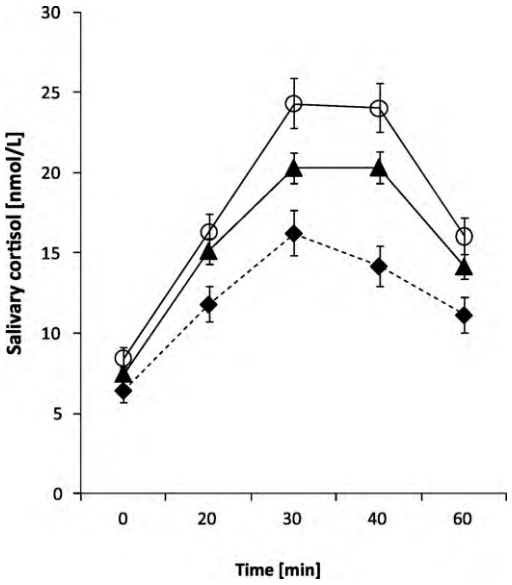

Fig. 3. Concentrations of salivary cortisol during low-dose Synacthen test. The drawings and symbols are the same as for Fig. 2. The significances of the factors and between-factor interaction were as follows: Group: $F = 52.37$, $p < 0.0001$; Time: $F = 155.71$, $p < 0.0001$; Subject: $F = 9.12$, $p < 0.0001$; Group × Time: $F = 1.36$, NS. The levels of mean factors differed as follows ($p < 0.05$): H > LR, H > NR, LR < NR (patient's status), 0 < 20, 0 < 30, 0 < 40, 0 < 60, 20 < 30, 20 < 40, 30 > 60, 40 > 60 (stages of the test).

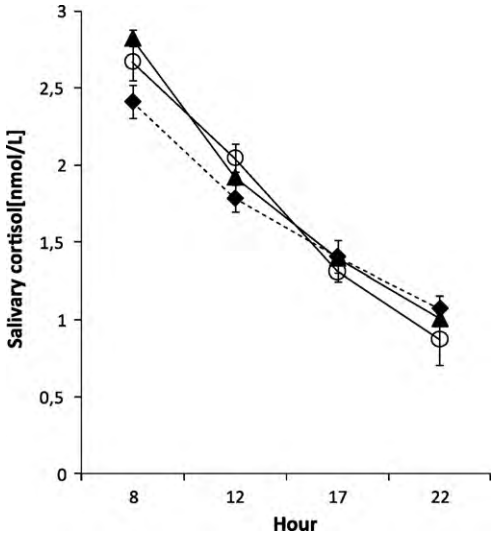

Fig. 5. Daily profile of salivary cortisol concentrations. Empty circles (H), full triangles (NR) and full squares symbolize (LR) the means for individual stages of the test. The error bars symbolize 95% confidence intervals for the means as computed using least significant difference multiple comparisons. The groups with non-overlapping 95% confidence intervals are statistically significant ($p < 0.05$). The significances of the factors and between-factor interaction were as follows: Group: $F = 1.8$, NS; Time: $F = 260.69$, $p < 0.0001$; Subject: $F = 8.8$, $p < 0.0001$; Group × Time: $F = 2.8$, $p = 0.01$; F, Fisher's statistics; p, significance level. The levels of mean factors differed as follows ($p < 0.05$): 8–12, 8–17, 8–22, 12–17, 12–22, 17–22 (daytime).

LR had significantly lower response of salivary F than H or NR in all stages of the test. Significant differences were observed between NR and H in most stages of the test.

Fig. 4 shows concentration of serum aldo during LDST. LR had lower response of serum aldo than H or NR. The differences between NR and H during test were significant regardless of the stage.

Fig. 5 shows daily profile of salivary F in LR, NR and H. Significantly lower values of salivary F were found in LR than in NR at 08:00 a.m. only, while no differences were recorded at other times.

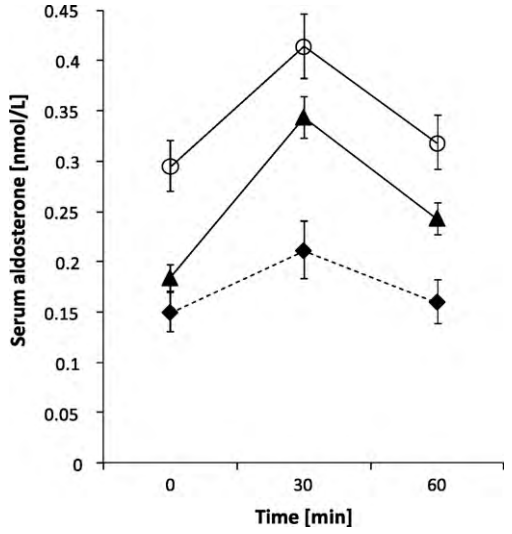

Fig. 4. Concentrations of serum aldosterone during low-dose Synacthen test. The drawings and symbols are the same as for Fig. 2. The significances of the factors and between-factor interaction were as follows: Group: $F = 61.09$, $p < 0.0001$; Time: $F = 31.76$, $p < 0.0001$; Subject: $F = 7.05$, $p < 0.0001$; Group × Time: $F = 2.14$, NS. The levels of mean factors differed as follows ($p < 0.05$): H > LR, H > NR, LR < NR (patient's status), 0 < 30, 0 < 60, 30 > 60 (stages of the test).

4. Discussion

According to general consensus, as sufficient serum F response reached during the LDST we considered 500 nmol/L or more [16,18]. Some authors recommended to take CBG into account for evaluation of adrenocortical function [19]. The fact that CBG concentration did not differ among DM 1 patients and the healthy subjects indicates that the cause of low serum F concentrations in LR patients is a decreased adrenocortical secretion rather than a change in the binding protein level. A few studies reported negative correlation between CBG and the insulin dose in DM 1 [20,21], however, we did not find significant differences of CBG between LR and NR. It could be explained by the fact that both groups did not differ in daily dose of insulin.

DM 1 represents a heterogenous disease with a number of forms differing in their immunogenetic background, pre- clinical as well as clinical course. With respect to these facts we have chosen for our cross-sectional study a homogenous group of young adults, known to possess among other a marked tendency to a common occurrence of other immunoendocrinopathies. The most frequent one is a combination of DM 1 and AIT and this also concerns our group of randomly selected young adult diabetics, in which occurrence of AIT reached as many as 36 out of 75 patients. Giordano et al. [8] found in patients with autoimmune polyglandular syndrome a decreased response of the adrenal cortex to low-dose ACTH stimulation. It is in concert with our finding of a significantly higher prevalence of subnormal serum F response during LDST in DM 1 patients with associated AIT (at 36%), as compared to the isolated form of DM 1 (at 13%). In addition, we have found for the first time that at this form of DM the highest risk with respect to revealed hypocorticalism encounter patients, in which DM 1 is manifested around 30 years of age, independently on sex. Therefore these patients should require an increased attention.

A number of papers point to the advantage of salivary F determination for examination of adrenocortical function. This approach is especially valuable in cases with increased CBG levels either due to oestrogen administration or due to other pathophysiological situ-

ations (obesity, metabolic syndrome, etc.) [22]. Determination of salivary F during LDST revealed considerable differences among stimulated values. In the LR group the median of maximum stimulated concentration of salivary F reached 17 nmol/L, significantly lower values of salivary F were measured also in the 20th, 30th and 60th minute. Maximum stimulated salivary concentrations of F suggested by Contreras [23] for healthy subjects, 20–25 nmol/L, were reached only in H. The NR patients approached this value very closely, while LR diabetic values were substantially lower. Decreased values of salivary F in the LR group further confirm an insufficient response of the adrenal cortex to low Synacthen dose in this group of diabetics. Significantly lower concentrations of basal aldo in LR in comparison with H and NR groups were found. A primary hyporenin hypoaldosteronism described in long-term DM 1 has not yet a clear pathogenesis. The role of angiotensin-converting enzyme inhibitors was considered [24]. In all our patients angiotensin-converting enzyme inhibitors therapy was discontinued long enough before the study, so that its eventual effect on the renin-angiotensin axis was eliminated. In addition, no differences were recorded among DM 1 patients, including LR and H in basal PRA values. Lower maximal stimulated concentrations of aldo were also found in LR in comparison with NR and H. A pronounced decreased secretion of aldo without changes of PRA in LR reminds impaired secretory function of zone glomerulosa, described at the initial stage of autoimmune adrenalitis [25,26].

DHEA-S is also considered to be a helpful parameter in the diagnosis of hypocorticalism [27]. Its levels are age-and sex dependent. Here, significantly lower concentrations of DHEA-S were found in LR in comparison with NR in a group of women only, in contrast to males. This sexual discrepancy is difficult to explain since DHEA-S is believed to be almost exclusively adrenal product. Nevertheless a lower level of DHEA-S in females can serve as further evidence for decreased adrenocortical function in LR diabetics. The results indicate that the disorder of adrenocortical function in LR DM 1 patients occurs in all adrenal zones.

When following the biorhythm of salivary F under real-life conditions, we have observed in LR patients significantly lower values of salivary F at 08:00 a.m. only; other values during the rest of the day did not differ from NR group. The results of the studies concerning F and ACTH biorhythm in subclinical forms of hypocorticalism in non-diabetics may be helpful in explaining this finding demonstrating implies that in the afternoon hours ACTH does not decrease and a sufficient concentrations of serum F are thus maintained [28]. To find out if sufficient levels of daily salivary F found in LR, comparable with NR, are a consequence of the elevated ACTH would require data about the HPA axis' activity in this situation.

According to literature, morning salivary F levels fall into the range 3.5–27.0 nmol/L [30]. Basal concentration of salivary F below 4.4 nmol/L can be used for detection of hypocorticalism with 86% specificity and 91% sensitivity [29]. As much as 36% of LR had their home morning salivary F below the lower limit of this range. As shown in Table 2 and Fig. 4., basal concentrations of salivary F in LR patients tend to be higher when measured in the institute than at home (medians 7.00 nmol/L vs. 5.08 nmol/L). We suppose that increased F secretion in the institute was caused by stress before the Synacthen test, for which the patients had an inserted canulla for 30 min. This experience indicates that collection of salivary F at home will most likely provide lower values than those obtained in a medical institution. This should be taken into account especially in the evaluation of subclinical forms of hypocorticalism.

UFC has been used by some authors in diagnosing disorders of adrenal function and for monitoring substitution treatment in AD. In this study we did not find significant differences among LR and other groups (NR, H). We have thus confirmed previous findings that UFC is not a suitable diagnostic marker of early subclinical hypocorticalism [30,31].

Surprisingly, we have not detected in our study positive titres of antibodies either to 21OH Ab or to ACA in any of 75 diabetics, in spite of the fact that in a great part the diabetes was associated with AIT. According to literature, positive titres of adrenal antibodies could be expected in as much as 1–4% [2,32]. Though we cannot exclude that the subclinical hypocorticalism found in the patients was primarily a consequence of autoimmune adrenalitis which was no longer active at the time of our examination, the high percentage of LR makes this idea unlikely. Nevertheless, the role of polyautoimmunity is supported by a significantly higher occurrence of LR in patients in which DM 1 is associated with AIT.

The basic issue for endocrinologists and diabetologists is a clinical relevance of revealed hypocorticalism during LDST, found in as much as 25% of all diabetic patients studied. Some light upon this problem was thrown recently by Paisley et al. [33], who demonstrated in non-diabetics that lower "suboptimal" peak in F response after another physiological stimulus, here hypoglycaemia, does not equate to a low daily F production rate and should not be an automatic indication for lifelong glucocorticoid substitution therapy. Our finding that no significant differences in metabolic control or daily dose of insulin in LR compared with NR were present indicates that peripheral cortisol metabolism at the time of our study was not significantly altered. Thus a new, so far not addressed question is raised, whether subnormal cortisol response can be compensated by changes of its peripheral metabolism, concretely by the activity of 11β-hydroxysteroid dehydrogense enzyme type 1 (11β-HSD 1), responsible for conversion of inactive cortisone to active cortisol. It was evidenced that local tissue bioavailability of F participates at regulation of many metabolic processes, including glucose metabolism and glycaemia [34,35]. The mechanism enabling to maintain normal adrenal cortisol secretion in immunopathies may be also compensatory increased ACTH stimulation [36]. It may be speculated that ACTH hypersecretion could play in LR a similar role for maintaining practically the same daily profile of salivary F as was found in NR.

The question remains, whether the above-mentioned compensatory mechanisms are sufficiently efficient in low responders under a severe stress, infections, trauma, etc., or the need of hydrocortisone replacement therapy is indicated. These pathogenetic and clinical questions thus remain an important issue for further research.

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