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Original Article

Cortisol hyporesponsiveness to the low dose ACTH test is a frequent finding in a pediatric population with type 1 diabetes mellitus

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Introduction: In adults with type 1 diabetes mellitus (DM1), a 25% of risk of hypocortisolism has been found through a low dose ACTH test with negative antibodies suggesting other causes of hypothalamic–pituitary–adrenal axis dysfunction.
Aim: To evaluate adrenal function in pediatric patients with DM1 and correlate the results with the frequency of hypoglycemia and metabolic control.
Methods: Sixty-nine patients were enrolled, age 12.3 (5.7–18.1); 50 boys and 19 girls. A 20% had additional autoimmune diseases. Mean hemoglobin A1c (HbA1c) was 8.1% and insulin dose was 1.14 U/kg/d. After an overnight fast, a low dose ACTH test (1 μg) was performed. Basal and stimulated cortisol concentrations, DHEAS, and plasma renin activity (PRA) were measured. A cortisol response post-ACTH below 18 μg/dL was considered abnormal.
Results: 58% of the tested patients had an abnormal response to ACTH test. These patients also had lower DHEAS concentrations, but were not different in diabetes duration, HbA1C, severe hypoglycemia, ACTH, or PRA concentrations compared to those who had a normal cortisol post-ACTH. One patient out of 59, had a positive anti-21-hydroxylase antibody (21OHA) and presented a poor response to ACTH.
Conclusions: We found a significant proportion of our patients having a subnormal cortisol response independent of the presence of anti-adrenal cell antibodies. We did not find a correlation with metabolic control, probably due to the good metabolic control of this group. The absence of 21OHA does not rule out subclinical hypocortisolism in this population. Our results suggest testing adrenal function in children with DM1.

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Primary adrenal insufficiency is a rare condition with a prevalence of 110 cases per million in adults and the majority of cases is of autoimmune origin. In patients with type 1 diabetes mellitus (DM1), adrenal insufficiency is five times more frequent than in the general population, with a reported prevalence of approximately 1.2% (1). If diabetes is associated with other autoimmune diseases this prevalence may increase to 4% (2). Several mechanisms may be underlying adrenal failure in these patients. Inflammatory mediators induced by the immune system have a stimulatory effect on the function of the hypothalamic–pituitary–adrenal (HPA) axis, producing higher basal levels of ACTH. On the other hand, certain cytokines and other inflammatory mediators directly inhibit adrenal function (3). In addition, different studies in patients and animals with diabetes mellitus and poor metabolic control show a glucocorticoid hypersecretion and altered regulation of the HPA axis (4). Some of these changes are partially reversed with insulin therapy and tighter control of blood glucose (5).

Nevertheless, the main risk for the development of adrenal failure is the presence of antibodies against the adrenal cortex, mainly 21-hydroxylase antibodies (21OHAs). Long-term studies monitoring patients with adrenal cortex antibodies show that the increased risk of developing Addison's disease is found in those patients with higher levels of antibodies, in children, males, and those with additional autoimmune diseases such as hypoparathyroidism or candidiasis (6). In patients with different autoimmune diseases, the prevalence of adrenal antibodies is 1–2% (7). Simunkova et al. evaluated adrenal function in patients with DM1, at a median age of 38. They used a low dose ACTH test and found that 25% of the patients had a subnormal response of cortisol and aldosterone. All non-responders (NRs) had negative adrenal antibodies (8).

With this background in mind, and recognizing that there is a lack of this information in children, we decided to assess the adrenal gland function in a population of pediatric patients with DM1. In addition, we evaluated whether the response to ACTH was related to metabolic control, insulin dose, frequency of severe hypoglycemia, and the presence of antibodies anti-adrenal cells.

Methods

Subjects

Consecutive patients under 18 yr with DM1, regularly attending the hospital pediatric diabetes clinic of Clinical San Borja Arriaran Hospital were enrolled. At the time of the study, none of the patients had symptoms suggestive of adrenal insufficiency or were receiving drugs that interfere with the HPA axis. We excluded patients taking corticosteroids and patients with prior diagnosis of adrenal insufficiency. Patients with additional hypothyroidism were under adequate treatment with levothyroxine. All patients had a prefeed (between 08:00 and 09:00 hours) venous blood sample for glycemia, dehydroepiandrosterone (DHEAS), adrenocorticotrophin (ACTH), renin activity, cortisol, and 21OHAs determination. Thereafter 1 μg synacthen (Synacthen Novartis, Frimley, UK) iv was injected and plasma concentration of cortisol was measured after 30 min. The microdose was prepared by taking 0.1 cc dilution of 250 μg of ACTH (Synacthen; Novartis) in 20 cc of saline. We considered a normal cortisol response to ACTH abnormal greater than 18 μg/dL (9).

Results of the HPA assessment of 17 children with idiopathic short stature (8 girls and 9 boys) were used as controls. Mean age at the time of the investigation was 9.2 yr ± 1.2. None of them displayed the features of autoimmune diseases and were not using drugs which could interfere with the test. Metabolic control of patients was assessed by the average of the last year hemoglobin A1c (HbA1c) (DCA Systems, Siemens, NY, USA) and the average dose of insulin used per day. Severe hypoglycemia was defined as those requiring assistance from another individual. Written consent was obtained from all parents or guardians, and protocols and consent forms were approved by local Institutional review board (IRB).

Glucose, 21OHA and hormonal determination

Blood glucose concentration was determined by the glucose oxidase method from Roche (Mannheim, Germany), with intra- and inter-assay coefficients of variations (CVs) <2.5%. Cortisol concentrations were determined by RIA (Siemens Medical Solutions, Los Angeles, CA, USA), the CVs intra- and inter-assay were 3.1 and 5.2%, respectively. Plasma renin activity (PRA) was measured by RIA (DiaSorin, Stillwater, MN, USA). The CV intra- and inter-assay were 6.2 and 7.4%, respectively. The concentrations of ACTH and DHEAS were measured by IRMA and RIA respectively (Diasource, Nivelles, Belgium). The intra-assay CV were 4.9 and 3.5%, respectively, and inter-assay CVs were 6.4 and 5.1%, respectively. The 21OHA were determined by RIA (Kronus Inc., Boise, ID, USA), the CV intra- and inter-assay were 5.9 and 8.1%, respectively.

Statistical analysis

The results are expressed as mean ± SE. The distribution of the data was analyzed with the Kolmogorov test. Differences between groups were calculated using the Student *t*-test or the Mann–Whitney test according to the distribution of data. The differences in each group were calculated using the Student *t*-test or paired Wilcoxon test according to data distribution. All statistical calculations were performed using SPSS 11.5 for Windows™.

Results

A total of 69 patients were enrolled; 50 boys and 19 girls, 35% of these patients were prepubertal (30% male and 47% female). Fourteen patients had additional autoimmune thyroid disease and one patient had celiac disease as well. Clinical and metabolic characteristics of enrolled patients with DM1 are shown in Table 1. Girls were younger and also younger at DM debut. All other clinical and metabolic characteristics were very similar in both genders. Mean baseline and stimulated cortisol were similar in girls and boys. However, girls had lower DHEAS and ACTH concentrations (Table 2). These

Table 1. Clinical and metabolic characteristics of patients with DM1

	All (69)	Boys (50)	Girls (19)
Age (yr)	12.4 ± 0.5	13.3 ± 0.5	10.0 ± 1.0*
BMI (kg/m^2)	20.6 ± 0.5	20.7 ± 0.5	20.6 ± 0.9
Age of onset (yr)	6.8 ± 0.5	7.3 ± 0.5	5.5 ± 0.8**
DM1 duration (yr)	5.3 ± 0.5	5.7 ± 0.5	4.4 ± 1.0
Daily insulin injection	4.6 ± 0.1	4.6 ± 0.1	4.3 ± 0.2
Insulin dose (U/kg/d)	1.14 ± 0.12	1.05 ± 0.4	1.38 ± 0.42
HbA1c (%)	8.1 ± 0.2	8.0 ± 0.2	8.3 ± 0.4

BMI, body mass index; DM1, type 1 diabetes mellitus; HbA1c, hemoglobin A1c.
Data are presented as the mean ± SE. *$p < 0.050$; **$p = 0.05$.

Table 2. Basal and after ACTH concentrations of cortisol and basal DHEAS, plasma renin activity (PRA) and ACTH concentrations

	All (69)	Boys (50)	Girls (19)
Basal cortisol (μg/dL)	14.2 ± 0.6	14.7 ± 0.8	12.7 ± 1.1
Cortisol post-ACTH (mg/dL)	17.0 ± 0.6	17.3 ± 0.8	16.1 ± 1.2
DHEAS (ng/mL)	1505 ± 137	1647 ± 165	1131 ± 226*
ACTH (pg/mL)	14.5 ± 1.2	17.1 ± 1.5	7.8 ± 0.9**
PRA (ng/dL/h)	580 ± 60	600 ± 70	520 ± 90

Data are presented as the average ± SE. *$p < 0.05$; **$p < 0.01$.

Table 3. Clinical, metabolic and hormonal characteristics in non-responders (NR) and responders (R)

	NR (40)	R (29)
Age of onset (yr)	6.4 ± 0.5	7.3 ± 0.5
DM1 duration (yr)	4.8 ± 0.5	6.0 ± 0.8
Insulin doses (μ/kg/d)	1.09 ± 0.04	1.20 ± 0.1
HbA1c (%)	8.0 ± 0.2	8.2 ± 0.2
Severe hypoglycemia	16	14
ACTH (pg/mL)	16.0 ± 1.3	16.7 ± 2.3
PRA (ng/dL/h)	600 ± 90	550 ± 60

DM1, type 1 diabetes mellitus; HbA1c, hemoglobin A1c; PRA, plasma renin activity.
Data are presented as the mean ± SE.

differences were not explained by age or pubertal stage of the patients, because girls had lower DHEAS in prepubertal and pubertal stage in comparison to boys at a similar pubertal stage.

More than half (58%) of the tested patients had an abnormal cortisol response (Table 3). In contrast, the mean baseline cortisol in the group of controls was 10.1 ± 0.9 μg/dL and they achieved an stimulated cortisol level of 21 ± 0.7 μg/dL. Only one girl had a level <18 μg/dL, corresponding to 17 μg/dL.

Gender distribution of the patients was similar to the entire population, with 23% of girls in the NR group.

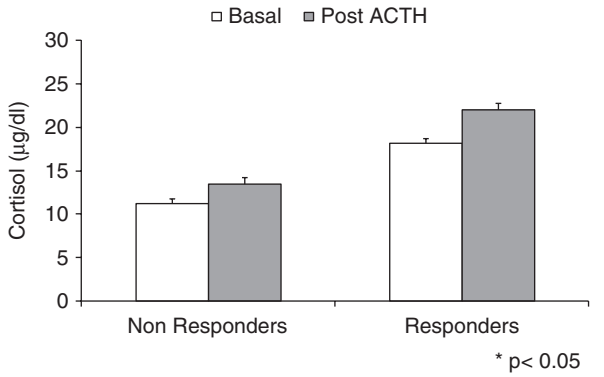

Fig. 1. Concentrations of serum basal cortisol and after ACTH in non-responders and responders. Cortisol (μg/dL). *$p < 0.05$.

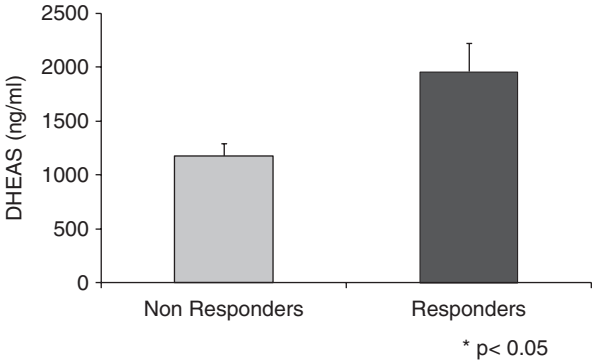

Fig. 2. Concentrations of serum basal DHEAS in non-responders and responders. DHEAS (ng/mL). *$p < 0.05$.

Fasting and poststimulated cortisol levels (Fig. 1) were different between the group of responders (R) and NR). These patients were not different in diabetes duration, mean HbA1C concentrations, frequency or severity of hypoglycemia, ACTH, or PRA concentrations compared to those who had a normal cortisol post-ACTH (Table 3). Nevertheless, the age of diabetes debut of NR is at least a year before. Mean HbA1C was within narrow ranges and showed fairly good metabolic control for the entire group.

We found no correlation in the NR group with the incidence of severe hypoglycemia or fasting glycemia. Mean concentrations of DHEAS, although within normal ranges, were significantly lower in the group of NR (Fig. 2). This difference was maintained after adjusting by age, gender, and Tanner stage.

Five patients (8%) had a stimulated cortisol concentration <10 μg/dL, this value was confirmed by repeating the ACTH test. Only one patient had a positive anti-21OHA and presented a poor response to the ACTH test (baseline cortisol 12.9 μg/dL and post-ACTH 12.9 μg/dL). This patient has have diabetes for 7 yr, had no history of hypoglycemia, and had a good metabolic control. A 20% (14/56) of patients had thyroid antibodies but this finding did not correlate with the lack of response to the ACTH test. The

ACTH test was possible to repeat in 33 of the 40 patients of the NRs group (83%). The lack of response was maintained in 27 patients (75%). Three of the patients who normalized their stimulated cortisol had a very close to normal responses in the first evaluation per dL (17.3, 17.4, and 17.9 μg/dL).

Discussion

This is to our knowledge the first study conducted in pediatric patients with DM1, exploring adrenal cortisol response to an ACTH stimulation. Remarkably, we found a high proportion of our patients having a subnormal cortisol response (58%) without signs of hypocortisolism. In young adults with DM1, Simunkova et al. (8) found that 25% of them had subnormal responses accompanied by low levels of aldosterone and salivary cortisol. Along with this, in low Rs, in women they reported low levels of DHEAS. In our group, we also observed that the NR group, regardless of gender, had significantly lower levels of DHEAS, suggesting the defect occurs in the different adrenal gland zones. Recently, Sayyed (10) recommended the inclusion of DHEA and DHEAS measurements in the laboratory evaluation of HPA function, particularly in subjects who achieve borderline cortisol responses with low dose ACTH test (LDC) stimulation. The impact of these low DHEAS levels in pubertal development and more over in sexual functioning lately has not been tested.

Only one patient from the NR presented had a positive 21OHA, which is not very different from what has been reported in adults with DM1 (7). Indeed, Simunkova et al. (8) found no antibody-positive patients. This is not surprising as several authors have shown that only 1–2% of patients with different autoimmune diseases have antibodies to the adrenal cells (11, 12). We believe that despite the low percentage of positive antibodies in the NR, it cannot be ruled that one of the reasons for the lack of cortisol response is adrenalitis. However, if the defect of the gland was explained only by autoimmune pathogenesis, we would have found a relationship with the presence of other autoimmune diseases (i.e., thyroid disease), a phenomenon that we did not verify in our NR group.

Other pathogenic mechanisms might be possible, it has been demonstrated in humans and animals a close relationship between the immune system and the HPA axis dysfunction (13, 14). Certain inflammatory mediators induced by the process of beta-cell autoimmune destruction are able to stimulate immune function and the HPA axis. This event could explain the increase in basal and stimulated ACTH (15) in patients with autoimmune disease and no signs of hypocortisolism. The increased activity of the HPA axis in diabetic patients may be also attributable to an alteration of the release of ACTH by the corticotroph, in addition, to the action of corticotrophin releasing hormone (CRH) on the adrenal gland independent of the action of ACTH (16). Added to these mechanisms, this hyperactivation of the HPA axis in diabetic patients may be attributable in part to a decrease in the negative glucocorticoid feedback and sensitivity (5). Following glucocorticoid administration, these patients exhibit a greater incidence of non-suppression of pituitary–adrenal activity compared with non-diabetic individuals (17). Studies in Streptozotocin (STZ)-treated rats have also shown that the basal hyperactivation of the HPA axis is associated with further decreased responsiveness of the HPA axis to stress, for example, to insulin-induced hypoglycemia. While basal hyperactivity is secondary to the lack of insulin in STZ-induced diabetes, the reduced response to hypoglycemia is due to the chronic hyperglycemic state (18).

The hyper-mediated adrenocorticotropic inflammatory factors could desensitize the adrenal gland to the ACTH stimulation. In addition, certain cytokines can act directly on the adrenal gland, as stimulatory but also as inhibitory factors (14, 19, 20), More than one half of the ~50 human chemokines have been associated with or implicated in the pathogenesis of DM1, yet their actual expression patterns in the islet environment of type 1 diabetic patients remain, at present, poorly defined (21, 22).

The fact that some of these changes are reversed with insulin therapy and tighter control of blood glucose (23) suggests that there may be relationship between poor response to the test and blood glucose levels prior to its completion. However, we found no significant correlation between glucose concentrations and basal and stimulated cortisol.

In also adults with autoimmune diseases without signs of hypoadrenalism, a compensatory increase in ACTH concentrations has been shown, which would allow maintaining the normal secretion of cortisol, a phenomenon that we did not observe (15). ACTH concentrations and PRA were normal throughout the group, however this does not allow us to rule out that there is a subtle failure of the adrenal gland and that the elevation of ACTH is produced in the final stages of the disease accompanied by low levels of aldosterone and increased plasma renin (24).

We found no relationship with metabolic control between the R group and the NR group. Similar results are observed by Simunkova et al. (8). We believe that these results might be explained by the fair good metabolic control of the entire group. However, it is not possible to rule out that the subnormal cortisol concentrations can be compensated by an increase in the activity of the enzyme 11 beta hydroxysteroid

dehydrogenase type 1, which catalyzes cortisone to cortisol (25).

Surprisingly, we found no correlation with the incidence of severe hypoglycemia and the cortisol response. In contrast, in animals, some studies have shown that in relation to hypoglycemia, the increase in ACTH and corticosterone and other contra-regulation hormones is significantly lower in diabetic rats than in controls (23). A similar situation has been observed in patients with repeated hypoglycemia not related to diabetes, where diminished response of cortisol, possibly due to a central defect with a decrease in the secretion of ACTH by a desensitization of the HPA axis (26). Perhaps our patients, in spite of having diabetes for ~5 yr, with the use of new insulin analogs are exposed to a much lower frequency and severity of hypoglycemia which precludes finding this relationship.

Most symptoms of adrenal insufficiency are non-specific and insidious and autoimmune disease may develop for several years before symptoms appear specifically (24). The first evidence of primary adrenal insufficiency is elevated PRA associated with low normal to low levels of aldosterone. ACTH then increases with a decrease in cortisol response to ACTH test. Finally, we observe low cortisol and high ACTH concentrations, confirming adrenal insufficiency (24). The diagnosis of adrenal insufficiency remains particularly difficult when there is partial deficiency.

One limitation of our study is the use of microdose of ACTH test. There is still some controversy in relation to the best test for assessing subclinical hypocortisolism. The classical dose of ACTH, 250 μg is supraphysiological, since has even been normal in patients with confirmed hypocortisolism (27). In the last decade, several authors have replaced the classic macrodose (250 μg) ACTH test by the microdose (1 μg) ACTH stimulation, since it better resembled the response generated by the hypothalamic pituitary axis (28). The sensitivity and specificity of the test depends on the cutoff used to define a normal response. The dose that we used in this work (1 μg) has been shown useful in assessing the adrenal function in children (28–30), and other authors reported high sensitivity and specificity for the diagnosis of primary adrenal insufficiency in a preclinical stage (31) as well as in the evaluation of secondary adrenal insufficiency (27). Indeed, it has been suggested that a dose of 0.06 μg of ACTH would be enough for evaluation of adrenal function in young individuals (15). In addition, we obtained a high concordance when repeating the test. Furthermore, our controls had a normal cortisol response in 95% of them. As a counterpart, Giordano et al. demonstrated that patients with autoimmune polyglandular syndromes without clinical adrenal insufficiency but with elevated basal levels of ACTH responded poorly to microdose ACTH test and respond normally when using macrodosis (32).

Despite the limitations of this study, we believe that our findings are important to pediatric diabetologist. We found a high proportion of our patients having a subnormal cortisol independent of the presence of anti-adrenal cells antibodies. Indeed, 8% of them showed a very low cortisol concentration (<10 μg/dL) which may place them at high risk of severe hypoglycemia. We believe that our results suggest testing adrenal function in children with DM1. In addition, further research is needed to elucidate the mechanisms and persistence of underlying this insufficient cortisol response.

Conflict of interest

The authors have nothing to disclose.

References

1. Cooper GS, Stroela BC. The epidemiology of autoimmune disease. Autoimmun Rev 2003: 2: 119–125.
2. Barker JM, Ide A, Hostetler C et al. Endocrine and immunogenetic testing in individuals with type 1 diabetes and 21-hydroxylase autoantibodies: Addison's disease in a high-risk population. J Clin Endocrinol Metab 2005: 90: 128–134.
3. Rotondi M, Lazzeri E, Romagnani P, Serio M. Role for interferon-inducible chemokines in endocrine autoimmunity: an expanding field. J Endocrinol Invest 2003: 2: 177–180.
4. Kinsley BT, Simons DC. Evidence for a hypothalamic-pituitary versus adrenal cortisol effect of glycemic control on counterregulatory hormones response to hypoglycemia in insulin-dependent diabetes mellitus. J Clin Endocrinol Metab 1996: 81: 684–691.
5. Chan O, Chan S, Inouye K. Diabetes impairs hypothalamic pituitary adrenal response to hypoglycemia, and insulin treatment normalizes HPA but not epinephrine responses. Diabetes 2002: 51: 1681–1689.
6. Coco G, Dal Pra CH, Presotto F et al. Estimated risk for developing autoimmune Addison's disease in patients with adrenal cortex autoantibodies. J Clin Endocrinol Metab 2006: 91: 1637–1645.
7. Betterle C, Volpato M, Rees Smith B et al. Adrenal cortex and steroid 21 hydrosilase autoantibodies in adult patients with organ specific autoimmune disease markers of low progression to clinical Addison disease. J Clin Endocrinol Metab 1997: 82: 932–938.
8. Simunkova K, Hampl R, Hill M et al. Adrenocortical function in young adults with diabetes mellitus type 1. J Steroid Biochem Mol Biol 2010: 122: 35–41.
9. Laureti S, Arvat E, Candeloro P et al. Low dose (1 mcg) ACTH test en the evaluation of adrenal dysfunction in preclinical Addison disease. Clin Endocrinol (Oxf) 2002: 53: 107–115.
10. Sayyed-Kassem L, El Sibai K, Chaiban J, Dima-Abdelmannan D, Arafah B. Measurements of serum

DHEA and DHEA sulphate levels improve the accuracy of the low-dose cosyntropin test in the diagnosis of central adrenal insufficiency. J Clin Endocrinol Metab 2012: 97: 3655–3662.
11. Laureti S, De Bellis A, Muccitelli VI et al. Levels of adrenocortical autoantibodies correlate with the degree of adrenal dysfunction in subjects with preclinical Addison's disease. J Clin Endocrinol Metab 1998: 83: 3507–3511.
12. Betterle C, Volpato M, Rees Smith B et al. Adrenal cortex and steroid 21-hydroxylase autoantibodies in adult patients with organ-specific autoimmune diseases: markers of low progression to clinical Addison's disease. J Clin Endocrinol Metab 1997: 82: 932–938.
13. Basedovsky HO, Del Rey A. Immune–neuro–endocrine interactions: facts and hypotheses. Endocr Rev 1996: 17: 1764–102.
14. Wick G, Hu Y, Schwarz S, Kroemer G. Immunoendocrine communication via the hypothalamo–pituitary–adrenal axis in autoimmune diseases. Endocr Rev 1993: 14: 539–563.
15. Giordano R, Balbo M, Picu A et al. Corticotrope hypersecretion coupled with cortisol hyporesponsiveness to stimuli is present in patients with autoimmune endocrine diseases: evidence for subclinical primary hypoadrenalism? Eur J Endocrinol 2006: 155: 421–428.
16. Fehm HL, Holl R, Spath-Schwalbe E, Born J, Voigt KH. Ability of corticotropin releasing hormone to stimulate cortisol secretion independent from pituitary drenocorticotropin. Life Sci 1998: 42: 679–686.
17. Hudson JI, Hudson MS, Rothschild AJ, Vignati L, Schatzberg AF, Melby JC. Abnormal results of dexamethasone suppression tests in nondepressed patients with diabetes mellitus. Arch Gen Psychiatry 1984: 41: 1086–1089.
18. Chan O, Inouye K, Riddell MC, Vranic M, Matthews SG. Diabetes and the hypothalamo–pituitary–adrenal (HPA) axis. Minerva Endocrinol 2003: 28: 87–102.
19. Ehrhart-Bornstein M, Hinson JP, Bornstein SR, Scherbaum WA, Vinson GP. Intraadrenal interactions in the regulation of adrenocortical steroidogenesis. Endocr Rev 1998: 19: 101–143.
20. Rotondi M, Lazzeri E, Romagnani P, Serio M. Role for interferon-inducible chemokines in endocrine autoimmunity: an expanding field. J Endocrinol Invest 2003: 2: 177–180.
21. Sarkar SA, Lee CE, Victorino F et al. Expression and regulation of chemokines in murine and human type 1 diabetes. Diabetes 2012: 61: 436–446.
22. Foss-Freitas MC, Foss NT, Rassi DM, Donadi EA, Foss MC. Evaluation of cytokine production from peripheral blood mononuclear cells of type 1 diabetic patients. Ann N Y Acad Sci 2008: 1150: 290–296.
23. Chan O, Chan S, Onouye K. Molecular regulation of the hypothalamic-pituitary –adrenal axis in Streptozotocin induced diabetes: effects of insulin treatment. Endocrinology 2001: 142: 4872–4878.
24. Ten S, New M, Maclaren N. Clinical review 130: Addison disease 2001. J Clin Endocrinol Metabol 2001: 86: 2609–2922.
25. Tomlinson JW, Walker EA, Bujalska IJ et al. 11_-Hydroxysteroid dehydrogenase type 1: a tissue specific regulator of glucocorticoid response. Endocr Rev 2004: 25: 831–866.
26. Hussain K, Hindmarsh P, Aynsley Green A. Neonates with symptomatic hyperinsulinemic hypoglycemia generate inappropriately low serum cortisol counterregulatory hormonal responses. J Clin Endocrinol Metabol 2003: 88: 4342–4347.
27. Oelkers W. Dose–response aspects in the clinical assessment of the hypothalamo-pituitary-adrenal axis, and the low-dose adrenocorticotropin test. Eur J Endocrinol 1996: 135: 27–33.
28. Thaler LM, Blevins LS. The low dose (1ug) adrenocorticotropin stimulation test in the evaluation of patients with suspected central adrenal insufficiency. J Clin Endocrinol Metab 1998: 83: 2726–2729.
29. Mayenknecht J, Diederich S, Bahr V, Plockinger U, Oelkers W. Comparison of low and high dose corticotropin stimulation tests in patients with pituitary disease. J Clin Endocrinol Metab 1998: 83: 1558–1562.
30. Patel L, Clayton PE. Clinical usefulness of the low dose ACTH test. J Endocrinol Invest 1999: 22: 401–404.
31. Laureti S, Arvat E, Candeloro P et al. Low dose (1 ug) ACTH test in the evaluation of adrenal dysfunction in pre-clinical Addison's disease. Clin Endocrinol (Oxf) 2000: 53: 107–115.
32. Giordano R, Pellegrino M, Oleandri S, Baldi S. Adrenal sensitivity to adrenocorticotropin 1–24 Is reduced in patients with autoinmune polyglandular syndrome. J Clin Endocrinol Metab 2004: 89: 675–680.