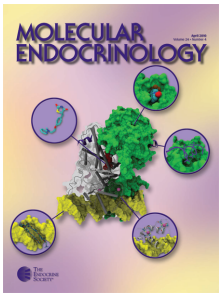
JCEM THE JOURNAL OF CLINICAL ENDOCRINOLOGY & METABOLISM

Plasma Adrenocorticotropin (ACTH) Values and Cortisol Response to 250 and 1 μg ACTH Stimulation in Patients with Hyperthyroidism before and after Carbimazole Therapy: Case-Control Comparative Study

Sunil Kumar Mishra, Nandita Gupta and Ravinder Goswami

J. Clin. Endocrinol. Metab. 2007 92:1693-1696 originally published online Feb 27, 2007; , doi: 10.1210/jc.2006-2090



0021-972X/07/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 92(5):1693–1696

doi: 10.1210/jc.2006-2090

Plasma Adrenocorticotropin (ACTH) Values and Cortisol Response to 250 and 1 μg ACTH Stimulation in Patients with Hyperthyroidism before and after Carbimazole Therapy: Case-Control Comparative Study

Sunil Kumar Mishra, Nandita Gupta, and Ravinder Goswami

Department of Endocrinology and Metabolism, All India Institute of Medical Sciences, New Delhi 110029, India

Context: Although the production and metabolic clearance rate of cortisol is increased during thyrotoxic state, the net effect on adrenocortical reserves is not clear.

Objective: We assessed circulating ACTH levels, cortisol binding globulin (CBG), and adrenocortical reserves in hyperthyroid patients (before and after carbimazole therapy) and healthy controls.

Design and Setting: This was a case-control investigative study in a tertiary care setting.

Patients and Methods: Plasma ACTH and free cortisol index (FCI; serum cortisol/CBG) were measured in 49 consecutive patients with hyperthyroidism and 50 controls. $ACTH_{1–24}$ stimulation tests (250 and 1 μg) were carried out in the first 29 patients and 15 controls. Peak FCI less than the mean −3 SD of healthy controls was considered subnormal. $ACTH_{1–24}$ stimulation tests were repeated in 24 patients in the euthyroid state.

Results: The mean basal plasma ACTH and FCI were higher and CBG was lower in thyrotoxic patients in comparison with controls. The peak cortisol was less than 18 μg/dl in 10 of 29 and 14 of 29 on 250 and 1 μg $ACTH_{1–24}$ stimulation. Peak FCI was subnormal only in three of 27 (11.1%) and two of 21 (7.4%) on 250 and 1 μg $ACTH_{1–24}$ stimulation, respectively. The mean plasma ACTH, basal FCI, and subnormal peak FCI (two of the three) normalized after euthyroidism. Plasma ACTH and FCI did not correlate with severity of thyrotoxicosis.

Conclusions: Up to 11% of thyrotoxics have subnormal peak FCI on $ACTH_{1–24}$ stimulation. Such changes occur despite high basal plasma ACTH and FCI. Use of FCI, rather than total cortisol, is required for the interpretation of cortisol values in thyrotoxicosis due to the variation in CBG. (***J Clin Endocrinol Metab* 92: 1693–1696, 2007**)

DIARRHEA AND WEIGHT loss are prominent clinical features of thyrotoxicosis (1, 2). Similar symptoms are observed in patients with adrenocortical insufficiency (3). Although the metabolic clearance and production rate of the cortisol is enhanced during thyrotoxicosis (4), its net effect on adrenocortical reserves is under investigation (5–8). Currently 250 or 1 μg synthetic $ACTH_{1–24}$ tests are used to assess adrenocortical reserves. There have been three studies in which subnormal adrenal reserves have been reported in 20–30% of thyrotoxic patients after iv $ACTH_{1–24}$ stimulation (5–8).

In thyrotoxicosis, serum cortisol binding globulin (CBG) levels are reduced and return to normal after induction of the euthyroid state (9, 10). Reduction in cortisol reserves in thyrotoxic patients and its normalization after euthyroidism (5–8) could be due to variation related to CBG. In stress, like thyrotoxicosis, circulating ACTH values are expected to increase and more so in presence of adrenal insufficiency. We assessed plasma ACTH and adrenocortical response to 250 and 1 μg of $ACTH_{1–24}$ stimulation in patients with thyrotoxicosis before and after attainment of euthyroidism, taking into consideration changes in CBG levels, in the interpretation of cortisol response.

First Published Online February 27, 2007

Abbreviations: AUC, Area under curve; BMI, body mass index; CBG, cortisol binding globulin; FCI, free cortisol index; TPOAb, thyroid peroxidase antibodies.

Subjects and Methods

Subjects

Study included 49 consecutive patients with hyperthyroidism [39 females; mean ± SD age, 38.0 ± 12.3 yr; body mass index (BMI), 20.1 ± 4.0 kg/m^2; median duration of symptoms, 12 months] attending endocrine clinic of the All India Institute of Medical Sciences and 50 healthy controls. Patients with thyrotoxic crisis and history of recent glucocorticoid intake were excluded. Hyperthyroidism was diagnosed based on the Volpe's criteria with Wayne's score greater than 19, serum total T_4 and TSH in the hyperthyroid range, and high ^{131}I uptake (11). Forty-two patients had diffuse thyromegaly, five had multinodular goiter, and two had solitary toxic nodule on 99mTc pertechnetate thyroid scan. The healthy controls were volunteers (mean age, 35.4 ± 7.6 yr; BMI, 22.8 ± 3.2 kg/m^2) who had normal serum total T_4 and TSH. Ten milliliters of venous blood were drawn from all study subjects at 0800 h for measurement of baseline plasma ACTH, serum total cortisol, T_4, TSH, thyroid peroxidase antibodies (TPOAb), and CBG. Intravenous $ACTH_{1–24}$ (250 and 1 μg; Ciba-Geigy, Basel, Switzerland) stimulation tests were performed in the initial 29 consecutive patients and 15 healthy controls. One microgram $ACTH_{1–24}$ per milliliter saline was prepared afresh before each test by mixing 250 μg $ACTH_{1–24}$ in 250 ml of 0.9% saline in a plastic container (12). One and 250 μg $ACTH_{1–24}$ stimulation tests were performed between 0800 and 0900 h on d 1 and 2, respectively. Blood was drawn for cortisol estimation at −15, 0, +30, +60, and +90 min after ACTH bolus. The free cortisol index (FCI) was calculated by serum cortisol/CBG (nanomoles per milligram).

Definitions of subnormal cortisol response

The mean peak FCI during 250 and 1 μg $ACTH_{1–24}$ stimulation tests in healthy controls was 12.4 ± 1.4 and 9.9 ± 1.04 nmol/mg, respectively. Peak FCI in 250 and 1 μg $ACTH_{1–24}$ stimulation tests were considered subnormal in thyrotoxicosis if the value was less than the mean −3 SD of healthy controls (<8.2 and 6.8 nmol/mg, respectively).

Treatment

Patients were treated with carbimazole (10–15 mg, three times a day). Serum total T_4 was measured at an interval of every 2 months. The mean duration to achieve euthyroidism was 4 ± 1 months. Twenty-four patients were reassessed after clinical (BMI, 22.5 ± 3.8 kg/m^2) and biochemical euthyroidism (mean interval 7 ± 1 months), and repeat tests were performed including fasting serum CBG, plasma ACTH, and $ACTH_{1–24}$ stimulation. The Institutional Ethics Committee of All India Institute of Medical Sciences approved the study protocol, and written informed consent was obtained from all subjects.

Hormone assays

Serum and plasma samples were stored at −20 C and assays were performed together in multiple batches by electrochemiluminescence (Elecsys 2010; Roche, Mannheim, Germany). CBG and plasma ACTH were measured by RIA (Biosource Technologies, Inc., Nivelles, Belgium) and immunoradiometric kit (DiaSorin, Stillwater, MN). The normal range for plasma ACTH, 0800 h serum cortisol, total T_4, TSH, TPOAb, and CBG were 6.0–56.7 pg/ml, 6.2–19.4 μg/dl, 5.1–14.1 μg/dl, 0.3–4.2 μIU/ml, less than 34.0 IU/ml, and 22–154 mg/liter, respectively. The intra- and interassay coefficients of variation for serum cortisol was 1.3–1.6%; CBG, 3.9–5.5%; and 4.7–8.7% for TPOAb and other hormones.

Statistical analysis

SPSS software (version 11.5; SPSS, Inc., Chicago, IL) was used for statistical analyses. The data are given as mean ± SD. ANOVA followed by Bonferroni *post hoc* test was used to compare mean of various variables in different groups. Paired *t* test was used to compare differences in the mean of variables before and after carbimazole therapy. Fisher's exact or χ^2 tests with Yates' correction were used to assess the differences in the frequency of nonparametric variables. Pearson's correlation coefficients were used for correlation analysis. The area under curve (AUC) for FCI was calculated by the trapezoidal rule. $P < 0.05$ was considered significant.

Results

Table 1 shows the clinical characteristics, mean plasma ACTH, serum CBG, total cortisol, and FCI at baseline and after ACTH stimulation in thyrotoxic (before and after treatment) and the control groups.

Serum CBG, plasma ACTH, and FCI in thyrotoxic state

The mean serum CBG, basal plasma ACTH value (median, 32.0 pg/ml; range 11.0–400.0 pg/ml), and frequency of supranormal plasma ACTH values were higher in thyrotoxic patients than healthy controls (Table 1). Although the mean basal cortisol was comparable between thyrotoxic patients and the controls, the mean basal FCI was significantly higher in thyrotoxic patients. All nine thyrotoxic patients and two healthy controls with supranormal plasma ACTH had nor-

TABLE 1. Comparison of baseline characteristics, CBG, and FCI in patients with thyrotoxicosis before and after carbimazole therapy and healthy controls (ANOVA)

Parameters	Thyrotoxicosis before treatment	Thyrotoxicosis after treatment	Controls
n (males:females)	49 (10:39)	24 (5:19)	50 (26:24)
Mean age (yr)	38.0 ± 12.3	NA	35.4 ± 7.6
Mean total T_4 (μg/dl)	20.2 ± 7.0^a	10.2 ± 3.7^b	8.1 ± 1.8^b
Mean TSH (μIU/ml)†	0.03 ± 0.03^a	1.51 ± 2.47^b	2.53 ± 1.65^c
Mean serum CBG (mg/liter)*	42.0 ± 14.3 (n = 40)^a	60.1 ± 11.6^b	54.2 ± 7.3^b
Mean basal cortisol (μg/dl)	11.5 ± 5.0	12.8 ± 5.6	11.1 ± 4.0
Mean basal FCI (nmol/mg)	7.9 ± 3.3^a	6.0 ± 2.7^b	5.9 ± 2.1^b
Mean basal ACTH (pg/ml)†	48.8 ± 58.5^a	31.8 ± 20.0^{a,b}	28.7 ± 12.0^b
Supranormal ACTH (%)	9/49 (18.4%)^a	1/24 (4.2%)^{a,b}	2/50 (4.0%)^b
250 μg ACTH stimulation test	n = 27	n = 21	n = 12
Mean peak FCI (nmol/mg)	13.6 ± 4.1	13.1 ± 2.6	12.4 ± 1.4
Mean timing of peak FCI (min)	78.6 ± 21.8	82.5 ± 16.0	87.5 ± 8.7
Mean FCI +30 min (nmol/mg)	11.3 ± 3.6	11.1 ± 2.1	10.2 ± 1.3
Mean FCI +60 min (nmol/mg)	12.5 ± 4.0	12.3 ± 2.5	11.7 ± 1.5
Mean FCI +90 min (nmol/mg)	13.3 ± 4.3	13.0 ± 2.8	12.4 ± 1.4
Mean AUC for FCI (nmol·min/mg)	1024 ± 321	990 ± 189	917 ± 199
Peak FCI < 3 SDS	3/27 (11.1%)	1/21 (4.8%)	0/12 (0.0%)
Peak cortisol < 18.0 μg/dl (%)**	10/29 (34.5%)^a	1/24 (4.2%)^b	0/12 (0.0%)^b
1 μg ACTH stimulation test	n = 27	n = 21	n = 15
Mean peak FCI (nmol/mg)	11.5 ± 4.3	10.5 ± 2.0	9.9 ± 1.0
Mean timing of peak FCI (min)	40.3 ± 18.4	30.0 ± 0.0	36.0 ± 12.4
Mean FCI +30 min (nmol/mg)	11.1 ± 3.9	10.5 ± 2.0	9.8 ± 1.1
Mean FCI +60 min (nmol/mg)	8.8 ± 3.8	8.6 ± 1.9	8.3 ± 1.3
Mean FCI +90 min (nmol/mg)	6.8 ± 4.5	7.1 ± 1.6	6.6 ± 1.5
Mean AUC for FCI (nmol·min/mg)	817 ± 301	766 ± 152	724 ± 93
Peak FCI < 3 SDS	2/27 (7.4%)	1/21 (4.8%)	0/12 (0.0%)
Peak cortisol < 18.0 μg/dl (%)**	14/29 (48.3%)	5/24 (20.8%)	3/15 (20.0%)

Data in the same row with *different superscript letters* are significantly different from each other. Basal cortisol and basal FCI are the mean of 0800 h serum cortisol values on the days of 1 and 250 μg ACTH stimulation. SDS, SD score. FCI = cortisol/CBG. SI unit conversion factors: cortisol, 1 μg/dl = 27.59 nmol/liter; ACTH, 1 pg/ml = 0.2202 pmol/liter.

† *P* values were calculated after log transformation.

* and **, n is different from the whole group.

mal basal serum cortisol. Eight of nine patients with supranormal plasma ACTH had Graves' disease with TPOAb titers greater than 100 IU/ml. The mean basal FCI was comparable in thyrotoxic patients with and without supranormal plasma ACTH.

$ACTH_{1–24}$ stimulation test in patients with thyrotoxicosis and controls

$ACTH_{1–24}$ stimulation test (250 μg). The mean FCI at +30, +60, and +90 min, peak response, and AUC for FCI was comparable in patients with thyrotoxicosis and healthy controls (Fig. 1). Although peak cortisol response was less than 18.0 μg/dl in 10 of 29 thyrotoxics, only three had subnormal peak FCI. All three subjects with subnormal peak FCI had peak cortisol values less than 18.0 μg/dl. Two of these three also had supranormal ACTH.

$ACTH_{1–24}$ stimulation test (1 μg). The pattern of adrenocortical response was similar to that observed with 250 μg $ACTH_{1–24}$ stimulation. However, the mean FCI response including its peak and AUC were higher after 250 μg $ACTH_{1–24}$ in comparison with 1 μg $ACTH_{1–24}$ stimulation in patient and the control groups (Table 1 and Fig. 1). Although 14 of 29 of thyrotoxics and three of 15 healthy controls had peak cortisol less than 18.0 μg/dl, subnormal peak FCI was observed in only two thyrotoxic patients. Both these patients also had subnormal peak FCI on 250 μg $ACTH_{1–24}$ stimulation.

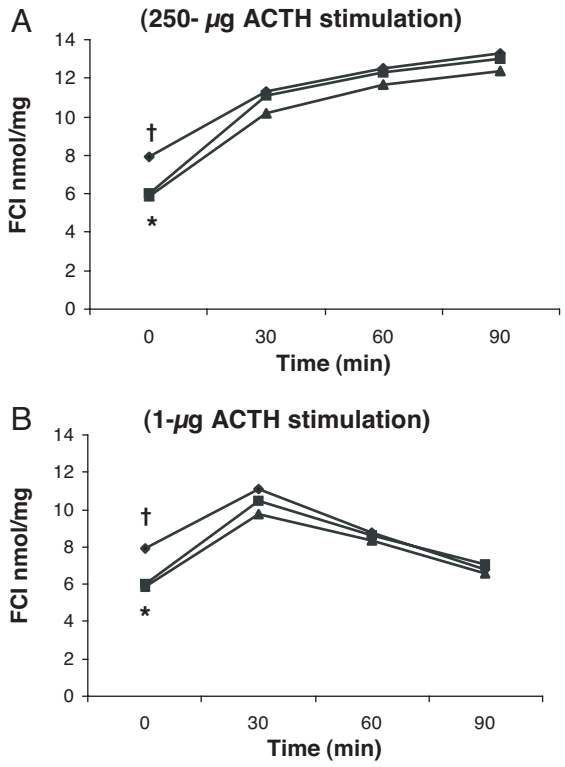


FIG. 1. Mean FCI at baseline (0 min) and at +30, +60, and +90 min. $ACTH_{1–24}$ stimulation tests during 250 μg (A) and 1 μg (B) in thyrotoxic subjects (♦), euthyroid state (■), and healthy controls (▲). * and †, Significant difference ($P < 0.05$) in baseline serum FCI in thyrotoxic *vs.* euthyroid state and healthy controls.

Changes in serum CBG, plasma ACTH, and FCI after carbimazole therapy

The mean plasma ACTH and basal FCI decreased and mean serum CBG significantly increased after attainment of euthyroidism. The mean CBG, plasma ACTH, and basal FCI in thyrotoxic patients after euthyroidism were not significantly different from that in healthy controls (Table 1). The mean FCI response including its peak and AUC after 250 and 1 μg $ACTH_{1–24}$ stimulation were comparable before and after treatment. Two of the three thyrotoxics with subnormal peak FCI on 250 μg $ACTH_{1–24}$ test showed normal plasma ACTH and peak FCI response after euthyroidism. The third patient with subnormal peak FCI and elevated plasma ACTH during the euthyroid state at 6 months showed normal plasma ACTH at 10 months of follow-up.

Severity of hyperthyroidism and adrenocortical reserves

All 49 patients were stratified in two groups at a median serum total T_4 level of 22.2 μg/dl. The median plasma ACTH [28.0 pg/ml, range (11–110 pg/ml) *vs.* 34.0 pg/ml (range 11–400 pg/ml), $P = 0.18$], serum CBG (47.3 ± 17.1 *vs.* 44.1 ± 12.7 mg/liter, $P = 0.59$), and basal FCI (7.4 ± 3.3 *vs.* 8.7 ± 4.0 nmol/mg, $P = 0.35$) were comparable in the two groups. Similarly mean FCI at +30, +60, and +90 min, peak and AUC, and the frequency of subnormal peak FCI after 250 and 1 μg $ACTH_{1–24}$ stimulation were comparable in the two groups. Pearson's test showed no relationship among total T_4, plasma ACTH, and basal and peak FCI in the thyrotoxic state.

Discussion

Holst in 1935 first reported increase in the adrenal size on autopsies of thyrotoxic patients (13). Adrenal glands were moderately enlarged on computerized tomography in Graves' disease (7). Although increased circulating ACTH in thyrotoxicosis is linked to skin hyperpigmentation, there are limited data on plasma ACTH levels in them. Hilton *et al.* (14) reported increased ACTH-like activity in thyrotoxicosis. Subsequently Yamakita *et al.* (8) and Gao *et al.* (15) reported mild elevation of plasma ACTH in Graves' disease that normalized after antithyroid drugs. In the current study, the mean plasma ACTH and FCI values were higher in patients with thyrotoxicosis than that in healthy controls and normalized after attainment of euthyroidism. Presence of adrenal enlargement and supranormal plasma ACTH coupled with significantly higher basal FCI suggest that pituitary-adrenal axis is stimulated in thyrotoxic state.

On the other hand, there are reports of subnormal peak cortisol on $ACTH_{1–24}$ stimulation tests in thyrotoxic subjects (5–8). Peak cortisol less than 18 μg/dl during $ACTH_{1–24}$ stimulation is considered subnormal (16). In the current study, the peak cortisol was also less than 18.0 μg/dl in 34.5 and 48.3% of thyrotoxic patients during 250 and 1 μg $ACTH_{1–24}$ stimulation tests, respectively.

Several investigators suggested adjustment for variation in serum CBG in interpretation of cortisol values (17–19). Measurement of free cortisol would exclude effects related to alteration in serum CBG. Ratio of total serum cortisol and

CBG, *i.e.* FCI, has also been shown to correlate with free cortisol levels (18). Recently le Roux *et al.* (19) compared peak FCI and peak total cortisol values in 31 subjects during the perioperative period of major surgeries. Peak FCI was normal in all seven subjects in whom peak cortisol was less than 18.0 μg/dl (19). There is no study in which adrenocortical reserves have been assessed using FCI in $ACTH_{1-24}$ stimulation tests in thyrotoxic patients. The observation of the current study showing significant reduction in CBG in thyrotoxic patients and its normalization after euthyroidism is in accordance with the previous studies (9, 10). Interestingly, majority of the patients who had peak cortisol values less than 18 μg/dl on 250 and 1 μg $ACTH_{1-24}$ stimulation showed normal peak FCI response. Using peak FCI less than 3 SD score of the healthy controls as the definition of subnormal peak FCI during $ACTH_{1-24}$ stimulation test, only three of 27 thyrotoxic patients (11%) had subnormal response on 250 μg $ACTH_{1-24}$ stimulation.

Overall, the observations of high circulating ACTH and basal FCI in thyrotoxicosis but impaired peak FCI response in 11% support adrenal hyperactivity with the possibility of adrenal exhaustion in a subset of them. Abnormalities observed in the pituitary-adrenal axis were reversible after attainment of euthyroidism. Coexistent autoimmune adrenalitis is reported in up to 5% of patients with Graves' disease (20). Reversible nature of abnormalities in peak FCI suggests autoimmune adrenalitis as the unlikely cause of subnormal reserves.

Thus, up to 11% of thyrotoxics have subnormal peak FCI on $ACTH_{1-24}$ stimulation. Such changes occur despite high basal plasma ACTH and FCI. Use of FCI, rather than total cortisol, is required for the interpretation of cortisol values in thyrotoxicosis due to the variation in CBG. Further studies involving direct measurement of free cortisol would be helpful in assessing adrenocortical reserves in patients with thyrotoxicosis.

Acknowledgments

Received September 25, 2006. Accepted February 20, 2007.

Address all correspondence and requests for reprints to: Ravinder Goswami, M.D., D.M., Associate Professor, Department of Endocrinology and Metabolism, All India Institute of Medical Sciences, New Delhi, India 110029. E-mail: gosravinder@hotmail.com.

Disclosure Statement: There is no conflict of interest and the authors have nothing to disclose.

References

1. **Safer JD, Wasan S, Sellin JH, Vassilopoulou-Selin R** 2005 The skin in thyrotoxicosis and the gastrointestinal tract and liver in thyrotoxicosis. In: Braverman LE, Utiger RD, eds. Werner and Ingbar's the thyroid—a fundamental and clinical textbook. 9th ed. Philadelphia: Lippincott Williams, Wilkins; 553–558 and 589–594
2. **Goswami R, Tandon RK, Dudha A, Kochupillai N** 1998 Prevalence and significance of steatorrhea in patients with active Graves' disease. Am J Gastroenterol 93:1122–1125
3. **Stewart PM** 2003 The adrenal cortex. In: Larsen PR, Kronenberg HM, Melmed S, Polonsky KS, eds. Williams textbook of endocrinology. 10th ed. Philadelphia: Saunders; 525–530
4. **Hoshiro M, Ohno Y, Masaki H, Iwase H, Aoki N** 2006 Comprehensive study of urinary cortisol metabolites in hyperthyroid and hypothyroid patients. Clin Endocrinol (Oxf) 64:37–45
5. **Goswami R, Gupta N, Bal CS, Kochupillai N** 1998 Cortisol reserve and adrenal size in patients with Graves' disease. J Assoc Physicians India 46: 1008–1011
6. **Tsatsoulis A, Johnson EO, Kalogera CH** 2000 The effect of thyrotoxicosis on adrenocortical reserve. Eur J Endocrinol 142:231–235
7. **Goswami R, Kochupillai N** 2001 Adrenocortical reserves in patients with Graves' disease. Eur J Endocrinol 144:85
8. **Yamakita N, Murai T, Kokubo Y, Hayashi M, Akai A, Yasuda K** 2001 Dehydroepiandrosterone sulphate is increased and dehydroepiandrosterone-response to corticotrophin-releasing hormone is decreased in the hyperthyroid state compared with the euthyroid state. Clin Endocrinol (Oxf) 55:797–803
9. **Caron Ph, Bennet A, Barousse Ch, Nisula BC, Louvet JP** 1989 Effects of hyperthyroidism on binding proteins for steroid hormones. Clin Endocrinol (Oxf) 31:219–224
10. **Dumoulin SC, Bertrand PP, Bennet AP, Caron PJ** 1995 Opposite effects of thyroid hormone on binding proteins for steroid hormones (sex hormone-binding globulin and corticosteroid-binding globulin) in humans. Eur J Endocrinol 132:594–598
11. **Volpe R** 1991 Graves' disease. In: Braverman LE, Utiger RD, eds. Werner's and Ingbar's the thyroid—a fundamental and clinical text. 6th ed. Philadelphia: Lippincott Williams, Wilkins; 648–657
12. **Mayenknecht J, Diederich S, Bahr V, Plockinger U, Oelkers W** 1998 Comparison of low and high dose corticotropin stimulation test in pituitary disease. J Clin Endocrinol Metab 83:1558–1562
13. **Holst J** 1935 Pathologische anatomie der orange ausser der Schiddrüse bei der Basedowschen krankheit. Zweite internationale Kropfkonferenz, Bern (1933), Verhandlungbericht; 62
14. **Hilton JG, Black WC, Athos W, McHugh B, Westermann CD** 1962 Increased ACTH-like activity in plasma of patients with thyrotoxicosis. J Clin Endocrinol 22:900–905
15. **Gao Y, Zhang C, Li X** 1996 Effect of methimazole and dexamethasone on leucocyte glucocorticoid receptor, plasma ACTH, and cortisol levels in Graves' disease. Hua Xi Yi Ke Da Xue Xue Bao 27:75–78 (Abstract) (Chinese)
16. **Dorin RI, Qualis CR, Crapo LM** 2003 Diagnosis of adrenal insufficiency. Ann Intern Med 139:194–204
17. **Dhillo WS, Kong WM, le Roux CW, Alaghband-Zadeh J, Jones J, Carter G, Mendoza N, Meeran K, O'Shea D** 2002 Cortisol-binding globulin is important in the interpretation of dynamic test of the hypothalamic-pituitary-adrenal axis. Eur J Endocrinol 146:231–235
18. **le Roux CW, Sivakumaran S, Alaghband-Zadeh J, Dhillo W, Kong WM, Wheeler M** 2002 Free cortisol index as surrogate marker for serum free cortisol. Ann Clin Biochem 39:406–408
19. **le Roux CW, Chapman GA, Kong WM, Dhillo WS, Jones J, Alaghband-Zadeh J** 2003 Free cortisol index is better than serum total cortisol in determining hypothalamic-pituitary-adrenal status in patients undergoing surgery. J Clin Endocrinol Metab 88:2045–2048
20. **Scherbaum WA, Berg PA** 1982 Development of adrenocortical failure in non-Addisonian patients with antibodies to adrenal cortex. A clinical follow-up study. Clin Endocrinol (Oxf) 16:345–352