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Research Article

Lessons from ACTH Tolerance Testing: Androgens and Hypertension

Fred Chasalow[1,2]*

[1]*IOMA LLC, California, USA*

[2]*Visiting Professor, Department of Laboratory Medicine, VAMC, San Francisco, CA, USA*

***Corresponding Author**: Fred Chasalow, IOMA LLC, California, USA. .

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Abstract

The 17α-hydroxylase-17,20-lyase enzyme has a key role in adrenal steroid synthesis. The same protein catalyzes both functions. The lyase is needed for synthesis of adrenal androgens while the 17α-hydroxylase is needed for synthesis of cortisol. However, there is little understanding of the regulatory process that determines how [a] adrenal androgen production (DHEA-sulfate) is initiated and/or [b] what controls whether the adrenal produces cortisol or corticosterone.

This article describes a method to evaluate adrenal function and illustrates the results obtained in different disorders. Of particular importance, was the result in children with growth hormone deficiency. These children oversecreted corticosterone in response to ACTH and the response reverted to normal after 3 days of GH replacement therapy. The control group was children with short stature but who were not GH deficient. In that group, the 3 day course of GH therapy, did not change the corticosterone response to ACTH. We concluded that GH stimulated 17α-hydroxylase-17,20-lyase activity and GH deficient individuals would be expected to have low DHEA-S levels, low cortisol levels and elevated corticosterone levels. In fact, many studies have shown that GH secretion decreases as we grow older and that DHEA-S serum levels decrease with age. We propose that these are not independent observations but are related as cause and consequence.

Although the function of DHEA-S remains unknown, the same is not true for corticosterone because it functions as a mineralocorticoid. Individuals with GH deficiency have elevated corticosterone levels. This initiates the mineralocorticoid receptor cascade: [a] synthesis of epithelial sodium channels, [b] hypokalemia, [c] spiral steroid synthesis, [d] Na-K-ATPase regulation and [e] hypertension. Prior to the discovery of the spiral steroids (step c) and their function, when other causes of hypertension were eliminated, patients were diagnosed as having essential hypertension or, perhaps, low renin hypertension. We knew all along there had to be a cause of essential hypertension. Potentially, we can now diagnose and monitor essential hypertension by measuring spiral steroids, the missing link in the endocrine cascade. Thus, one cause of essential hypertension may be inadequate 17α-hydroxylase due to GH deficiency..

Keywords: *ACTH; Premature Adrenarche; Corticosterone; PTSD; Hypertension; Spiral Steroids; GH Deficiency*

Abbreviations

17OHP: 17α-hydroxyprogesterone; 21-OHP: 21-hydroxyprogeterone; DHEA: Dehydroepiandrosterone; ACTH: Adrenocorticotropin; PA: Premature Adrenarche

Introduction

The major site of synthesis for many steroids is the adrenal. Most steroids are synthesized and secreted episodically with a major episode as part of the 'dawn' phenomenon. Consequently, a single serum sample is seldom adequate for evaluation without knowledge of the last episode. As a child develops, evaluation of adrenal function is complicated by advancing adrenarche and puberty. As adults age, the synthesis of adrenal androgens decline. To evaluate both processes, endocrinologists rely on measuring steroids secreted after an ACTH challenge, typically after overnight dexamethasone suppression. The purpose of the dexamethasone is to suppress the dawn phenomenon, thus avoiding potential confusion caused by carryover secretion rather than from the external ACTH bolus [1].

The disorders can be separated into several categories:

- Syndromes: (symptoms without knowledge of pathogenesis):
    - Premature adrenarche in boys [1].
    - Premature adrenarche in girls [1].
    - Risk of 2nd Miscarriage [2].
- Inherited diseases: (mutations in a known gene)
    - 21-hydroxylase deficiency (congenital adrenal hyperplasia) [3].
    - 7-dehydrosterol reductase deficiency (Smith-Lemli-Opitz syndrome [4].
- IGF deficiency secondary to acquired growth hormone deficiency [5]:
    - PTSD [6-8] (hypothalamic hyperfunction).
    - Essential hypertension (perhaps low renin hypertension) [9].

Methods and Procedures

Clinical: The night before the test, 1 mg of dexamethasone should be administered at 2300. After the overnight fast, an in-dwelling iv line should be inserted; baseline serum samples should be collected every 15 minutes. After two baseline samples are obtained, an iv bolus of 0.25 mg of synthetic ACTH (Cortrosyn™) should be administered. Serum samples should be collected every 15 minutes for an hour after the ACTH bolus. The two baseline samples and the samples collected at 30 and 45 minutes after the ACTH bolus should be analyzed. The other two samples (15 minute and 60 minutes) need only be analyzed if the results of the primary samples are discordant [1].

Laboratory: Serum samples should be assayed for cortisol, corticosterone, DHEA, DHEA-Sulfate, 17-OHP and androstenedione. Testosterone should also be analyzed in samples from boys.

Results and Discussion

One of the first uses of an ACTH tolerance test was to confirm the diagnosis of 21-hydroxylase deficiency (Type 3 response) and to identify carrier status in family members (Type 2 response) [3]. With the development of gene sequencing, this application is no longer necessary. Initially, the responses seemed to be a continuum, but clinical correlation distinguished three different types of 17-OHP response (Figure 1 and table 1) and two different types of response for glucocorticoids based on the corticosterone response (Figure 2 and 3 and table 2).

Group	Type 1 Responders	Type 2 Responders	Total
Girls			
Normal girls and women	13	1	14
Girls with PA	1	9	10
Total	14	10	24
Boys			
Normal boys	12	0	12
Boys with PA	5	0	5
Total	17	0	17

Table 1: *Type of response to ACTH in children with PA.*
The difference between girls with PA and normal girls was evaluated with the Wilcoxon rank sum test.
This test was chosen because it does not assume a normal distribution. The result was significant at the P < 0.01 level [1].

		Normal	GH deficient
n		30	13
IGF1 (SmC)	u/ml	0.86 ± 0.07	0.22 ± 0.05
Glucose (nadir)	mg/dl	36 ± 3	31 ± 3
Cortisol			
(Baseline)	pg/d1	23 ± 1	19 ± 1
(Stimulated)	µg/dl	23 ± 1	25 ± 2
Corticosterone			
(Baseline)	µg/dl	1.6 ± 0.2	1.7 ± 0.3
(Stimulated)	µg/dl	1.9 ± 0.2	3.6 ± 0.4*

Table 2: *Response to insulin induced hypoglycemia [5].*
Notes:
Stimulated samples - 40 min after insulin bolus.
Values reported as mean + SEM.
**p < 0.001 compared to normal (red box).*

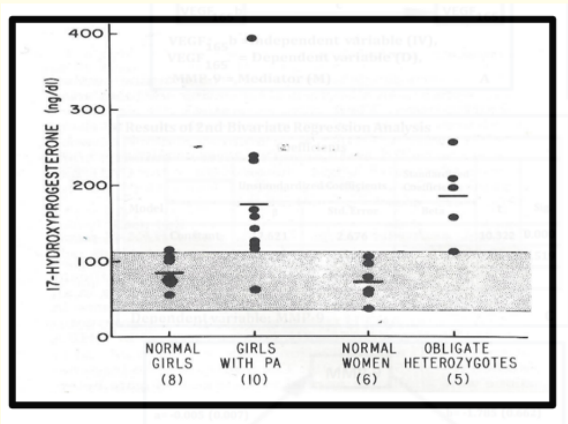

Figure 1: *Serum 17-OHP response to ACTH after pretreatment with dexamethasone.*
Each value represents the mean 17-OHP concentration in serum obtained 20 and 45 minutes after ACTH infusion.
The average 17-OHP concentration in each group is represented by the bar. The shaded area shows the normal range in 14 normal women and 26 normal men.

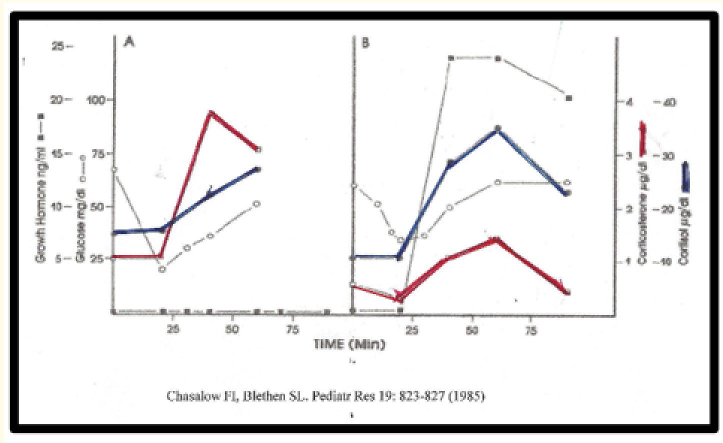

Figure 2: *Response to insulin-induced hypoglycemia.*
Panel A: A child with GH deficiency. Panel B: A short child without GH deficiency.
At time ‘0’ each subject received 0.1 unit/pk of regular insulin as an iv bolus.
Serum samples were taken at the indicated times. Blue lines - Cortisol. Red lines - Corticosterone.

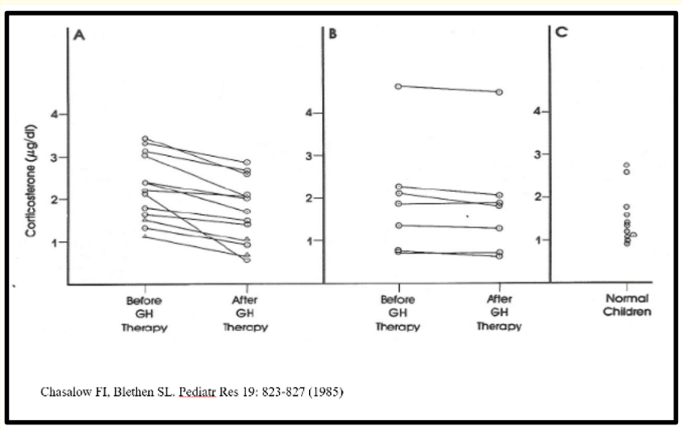

Figure 3: *Effect of GH on corticosterone response to ACTH after dexamethasone suppression. Each of the values shown is the average of the samples taken 30 and 45 minutes after administration of ACTH. A shows the results in children with GH deficiency. B shows the results in short children without GH deficiency. C shows the corticosterone secretory response to a single injection of ACTH after dexamethasone suppression in children of normal stature.*

Characterization of the 17-OHP response to ACTH after dexamethasone suppression

- Type 1 response - Average 17-OHP response less than 100 ng/dl.
    - Wild type for 21-hydroxylase - both gene copies are functional.

- Type 2 response - average 17-OHP response between 100 ng/dl and 300 ng/dl.
    - Pattern originally characterized as carrier for 21-hydroxylase deficiency.
    - One copy of the gene is functional and one copy is non-functional
    - In addition to the gene for 21-hydroxylase, 7-dehydrosterol reductase deficiency carriers have a Type 2 response and there may be other genes with the same response.
- Type 3 response - average 17-OHP response > 400 ng/dl - 21-hydroxylase deficiency
    - Pattern characteristic of classical 21-hydroxylase deficiency or one of the variants. Neither copy of the gene is fully functional.

Syndromes and diseases with a type 2 response:

- Heterozygotes for 21-hydroxylase deficiency.
- Heterozygotes for 7-dehydrosterol reductase deficiency - Smith-Lemli-Opitz syndrome. We observed this response before the underlying biochemical defect had been discovered.
- Girls (but not boys) with premature adrenarche.
    - Although the girls with PA have a similar response to that of the heterozygotes, that is not the explanation for the finding. Similarly, it is not the explanation in boys.
- Girls with pseudo-precocious puberty due to autonomous ovarian cysts [10].
    - For these girls, overnight dexamethasone doesn't suppress baseline 17-OHP levels.
- Women with polycystic ovarian disease [11].
- Women with a history of miscarriage [2].
    - There seem to be other, unidentified, autosomal mutations that cause a Type 2 response in this group.

Although the mechanism for the initiation of PA remains unknown, an elevated 17-OHP (Type 2 response) that is not suppressed by overnight dexamethasone confirms androgens are being made but the cause is not heterozygotes for 21-hydroxylase deficiency.

Characterization of the glucocorticoid response to ACTH after dexamethasone suppression:

- Type A response - GH deficient [5]
    - Cortisol < 23 µg/dl.
    - Corticosterone > 3.6 µg/dl.
    - Ratio < 8.

- Type B response (wild type)
    - Cortisol > 25 μg/dl.
    - Corticosterone < 1.6 μg/dl.
    - Ratio > 12.

Syndromes and diseases with a type A response

- Children with growth hormone deficiency [5]:
    - This is the prototype. A 3-day course of rhGH partially restored the Type B response.
    - GH deficient patients have low IGF levels (both IGF1 and IGF2).
    - IGF levels increase with rhGH therapy.
    - All responses to hGH (except sweating) are mediated by IGF [12].
- Patients with post-traumatic stress disease (PTSD) [6,7].

A review of the clinical data for patients with PTSD noted most patients have a sleep disorder and do not experience REM sleep [6]. As most GH secretory episodes occur during REM sleep, PTSD patients would be expected to be GH deficient and, in fact, IGF levels were clearly below normal for age [8]. However, the IGF levels of combat soldiers were indistinguishable from non-combat soldiers in the same country. The investigators assumed that the non-combat soldiers would have adequate GH secretion and, therefore, both groups did not have GH deficiency. However, the correct conclusion might be that all soldiers in country had sleep disorders and were GH deficient. Whether the sleep disorder was caused by normal military activities or PTSD, specifically, could not be determined.

Yehuda, with the same ACTH protocol test that described here, tested patients with PTSD. The patients had a Type A, below normal, cortisol response. However, corticosterone was not measured. To account for the response, Yehuda proposed that PTSD patients had a super-response to cortisol and, thus, did not need as much cortisol [7]. In summary, Yehuda's PTSD patients were probably GH deficient and had an ACTH response similar to the GH deficient children.

Overall, PTSD patients have a multi-step cascade: [1] sleep disorders, [2] inadequate hGH secretion, [3] low IGF levels, [4] inadequate 17α-hydroxylase, [5] probably with elevated corticosterone levels, [6] hypokalemia, [7] synthesis of potassium sparing, spiral steroids and [8] hypertension. As long as the cascade remains intact, PTSD patients will probably continue to have symptoms.

Patients with essential hypertension [9]

The endocrine cascade in patients with essential hypertension is similar to that of PTSD patients but has a different initiation point. In PTSD, GH deficiency occurs because of disordered REM sleep. In patients with essential hypertension, it occurs because of the normal, age-dependent decrease, in GH secretory episodes during REM sleep. The known age-dependent decrease in DHEA-sulfate confirms the decreased activity of the 17α-hydroxylase-17,20-lyase enzyme. In summary, PTSD and normal aging share a common endocrine cascade: low IGF levels, inadequate 17α-hydroxylase activity, corticosterone elevation, hypokalemia, hyperprolinemia and hypertension. They differ by a different initiating site.

Conclusion

With the discovery of spiral steroids and of their function as potassium recovery hormones, we have a new view of essential hypertension [13]. Few physicians have considered GH deficiency as a concern in older patients. After all, growth is complete. Why do we need it? The ACTH tests described here in Figure 2 and 3, show the role of 17α-hydroxylase. We need it to make cortisol, not corticosterone.

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Conflict of Interest

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Bibliography

1. Granoff A., *et al*. "17-Hydroxyprogesterone Responses to Adrenocorticotropin in Children with Premature Adrenarche". *The Journal of Clinical Endocrinology and Metabolism* 60 (1985): 409-415.

2. Lieber E., *et al.* "Multiple malformation syndrome suggestive of Smith-Lemli-Opitz syndrome with holoprosencephaly in a 46,XY stillborn infant". *American Journal of Human Genetics* 39 (1986): 200A.

3. Lee P and Gareis F. "Evidence for partial 21-hydroxylase deficiency among heterozygote carriers of congenital adrenal hyperplasia". *The Journal of Clinical Endocrinology and Metabolism* 41.2 (1975): 415-418. doi.org/10.1210/jcem-41-2-415.

4. Chasalow F., *et al*. "Possible abnormalities of steroid secretion in children with Smith-Lemli-Opitz syndrome and their parents". *Steroids* 46 (1985): 827-843. doi: 10.1016/0039-128x(85)90032-7.

5. Chasalow F and Blethen S. "Modulation of Glucocorticoid Secretion by Growth Hormone". *Pediatric Research* 19 (1985): 823-827. doi: 10.1203/00006450-198508000-00009.

6. Chasalow F and Blethen S. "New Concept: Endocrine Dysfunction in Post-Traumatic Stress Disorder (PTSD): The Role of Ionotropine". *EC Pediatrics* 8.10 (2019): 1104-1110. Doi: 10.31080/ecpe.2019.08.00560.

7. Yehuda R. "Biology of Posttraumatic Stress Disorder". *The Journal of Clinical Psychiatry* 62.17 (2001): 41-46.

8. Chanson P., *et al*. "Reference Values for IGF-1 serum concentrations: Comparison of Six Immunoassays". *The Journal of Clinical Endocrinology and Metabolism* 101 (2016): 3450-3458. Doi: 10.1210/jc.2016-1257.

9. Carretero O and Oparil S. "Essential Hypertension, Part I: Definition and Etiology". *Circulation* 101 (2000): 329-335. Doi.org/10.1161/01.CIR.101.3.329.

10. Chasalow F., *et al*. "Adrenal Steroid Secretion in Girls with Pseudo- precocious Puberty due to Autonomous Ovarian Cysts". *The Journal of Clinical Endocrinology and Metabolism* 63 (1986): 828-834.

11. Cinar N., *et al*. "Adrenocortical steroid response to ACTH in different phenotypes of non-obese polycystic ovary syndrome 5 (2012): 42. Doi:10.1186/1757-2215-S-42.

12. Hasan W Cowen T., *et al*. "The Sweating Apparatus in Growth Hormone Deficiency, Following Treatment with r-hGH and in Acromegaly". *Autonomic Neuroscience* 89 (2001): 100-109. Doi: 10.1016/S1566-0702(01)00257-0.

13. Chasalow F. "An Introduction to Spiral Steroids". *EC Paediatrics* 9.4 (2020): 83-87. Doi: 10.31080/ecpe.2020.09.00694.

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