

Peer Review File

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Referee A

In the current study, the authors describe cortisol levels from the corpora cavernosa compared to the systemic circulation during the stages of arousal. The manuscript is easy to read and well-written. I have a number of concerns regarding the data and the conclusions:

Major

The assertion that cortisol might act as an antagonist is not supported by the data. Correlation does not suggest causality.

How does the impaired bloodflow alone during an erection change cortisol irrespective of its biologic effect? It seems like this could be significant, particularly given the differences in arterial inflow and venous outflow in men with ED compared to men without ED.

Is there data to suggest that cortisol levels fluctuate wildly within a time period on the order of seconds to minutes? The hypothesis of this paper would require such an assertion to be plausible, and citations showing this should be provided

Doesn't the sexual downsides in Cushing patients primarily stem from their hypogonadism? Were other hormones such as Testosterone checked in this study?

Ref 12 does not appear to discuss exogenous administration of ACTH whatsoever

The statistics do not provide p-values in the text, and it is unclear if the values in the parentheses represent – are they standard deviations?

The text states that cortisol levels changed significantly with arousal, but the data does not support this as it appears statistically unchanged.

Despite being potentially statistically significant, can the authors comment on whether the changes seen are also clinically significant?

If I perform a straightforward t-test between the values labeled as significant in Table 1, using the provided mean and standard deviations, the p-values are 0.6 to 0.9 instead of the <0.05 as claimed. Statistical review recommended.

Table 2 does not provide p-values or comparisons

Minor

-Line 90 is grammatically incorrect

-Were the variables presented with means all normally distributed? Was this checked?

-How do refs 16 and 17 relate to visually explicit material?

Reply:

It is the referee's general impression that the manuscript is well-written and easy to read.

It is the referee's point of view that the correlation presented in the manuscript between the courses of cortisol in the cavernous and systemic blood through different stages of sexual arousal in patients suffering from erectile dysfunction (ED) in comparison to a cohort of healthy males does not necessarily suggest causality. The referee may have overlooked that this fact has been addressed in the **Discussion** section. It reads: *One can speculate as to whether the immanent*

absence of a decline in the course of cortisol in the systemic circulation might contribute to the impairment in erectile capability.

The referee has brought up the question as to how the impaired blood flow alone during an erection does change cortisol irrespective of its biologic effect?

In the phase of flaccidity, arterial blood flow to the corpus cavernosum is extremely low (impaired). Due to the pronounced increase in penile blood flow during rigidity (up to 8-fold, not impaired, as stated by the referee) (this has been outlined comprehensively elsewhere [Batra & Lue, In: Kirby RS, Carson C, Webster GD (Eds): *Impotence: Diagnosis and Management of Male Erectile Dysfunction*. Butterworth-Heinemann, Oxford 1991, Chapter 2, pp 19-26; Shirai *et al*, *Thohoku J Exp Med* 120: 377-83, 1976], one would expect an adjustment increase in cortisol in the corpus cavernosum to occur if cortisol failed to decline in the systemic circulation with the beginning of sexual arousal (when the penis becomes rigid).

The referee has raised the question whether there are data available suggesting that cortisol levels fluctuate within a time period on the order of seconds to minutes? This suggestion is supported by the references [14] and [15] cited in the **Introduction** and also by studies describing significant short-term changes (increase or decline) in the concentrations of hypothalamic or pituitary hormones (such as human growth hormone) or adrenal transmitters of the sympathetic system (adrenaline, noradrenaline) in the systemic circulation (Becker AJ, et al. *J Urol* 164: 2138-2142, 2000; Wiedeking C, et al. *Psychosom Med* 39: 143-148, 1977; Becker AJ, et al. *J Urol* 164: 573-577, 2000)

The referee has raised the question as to whether the sexual downsides in the Cushing patients primarily originate from their hypogonadism? He also questioned whether other hormones such as testosterone were checked in the study?

It seems that the referee's remark on the Cushing patients is due to a misreading of the data presented in the manuscript, since no patients diagnosed with symptoms of Cushing Syndrome were enrolled into the study. As to the question regarding other hormones, it is submitted that, in fact, the courses of testosterone, human growth hormone (hGH), serotonin, β -endorphin and oxytocin through different stages of sexual arousal were evaluated in the same cohorts (healthy males and/or patients with ED, respectively). The results have been published in high-rated, peer-reviewed journals (Becker AJ, et al., *UROLOGY* 58: 435-440, 2001; Becker AJ, et al. *UROLOGY* 59: 609-614, 2002; Ückert S, et al. *Urol Res* 31: 55-60, 2003; Ückert S, et al. *World J. Urol.* 20: 323-326, 2003; Ückert S, et al. *ANDROLOGIA* 50: e13049, doi: 10.1111/and.13049, 2018)

According to the comment given by the referee, the citations by Derouet et al (2002) and Isidori et al (1984) have been rearranged in the **Introduction** as well as in the list of references.

p-values have been provided in the **Results** section. In the **Materials & Methods**, the format of the data presented has been clarified (Mean \pm SD).

The referee has pointed out that the data do not support the statement that cortisol levels changed significantly with the beginning of sexual arousal (in the stage of penile tumescence).

It is submitted that, in the healthy males, cortisol significantly dropped in the systemic circulation (from 14.8 to 13.2) and the cavernous compartment (from 15.8 to 13.3) after the on-set of sexual arousal, from the stage of tumescence to rigid erection. It has been more clearly outlined in the **Results** that no (significant) drop in cortisol levels was registered at penile tumescence.

Comments on the potential clinical significance of the findings have been implemented into the **Discussion** section.

The referee recommends a statistical review, that is performing a straightforward t-test between the values labeled as significant in **Table 1**, using the provided mean and standard deviations.

It is submitted the statistical analysis of the data was assisted and supervised by the Department of Biomathematics of the Hannover Medical School. Analysis was conducted in accordance with the customary examples based on normal distributions, the estimation of sample size and power for comparing two means (power of *t*-Test = 80%, alpha =/ \leq 5%) as outlined in **Fundamentals of Biostatistics** (by Bernard Rosner). The parameters included the level of significance, the standard deviation (SD) in a population/cohort, as well as the SD of the difference, if applicable.

Table 2 does not provide p-values since there are no significant statistical differences in the readings summarized in the table.

Reference [17] cited in the manuscript does not specifically relate to visually explicit material but in general to the methodology how to withdraw blood from both the systemic circulation and the penile cavernous space. The position of the reference within the **Material & Methods** has been converted.

Referee B

The authors report on cortisol measurements in systemic circulation (cubital vein measurement) and within the corpora at 4 timepoints before, during, and after sexual arousal in men with ED and healthy controls. The authors should be commended on this idea and enrolling a significant number on men into the study. There are a few major concerns I have regarding the methodology and presentation of results which should be addressed before publication is considered.

Major Concerns:

1. Introduction: The authors state that there are conflicting results in prior studies on cortisol levels during sexual arousal. It is not clear in the introduction how the methods or study design of this project are different from than prior studies. Therefore it is unclear to the reader how the results presented in this paper build on or overcome limitations of prior studies.

2. Method: It is not clear how patients were enrolled, were they screened from a urology clinic, recruited specifically for the research study etc. This is important for understanding the study design.

3. Methods: Patients were enrolled with organic and psychogenic ED. I would suspect patients with psychogenic ED would be more likely to have higher cortisol levels compared to patients with organic ED due to higher levels of stress associated with sexual arousal. The authors found no difference in cortisol levels between those with organic and psychogenic ED. However, it is not clear how organic ED was defined? Were vascular parameters on doppler used to identify men with vasculogenic ED? A lot of ED is likely multifactorial with psychogenic ED playing a component in many men. The definition used to classify men as organic or psychogenic should be provided as well as the number of men within each classification.

4. Methods: How was tumescence, rigidity, and detumescence defined and identified? Also, in the men with ED, was it assessed whether the quality of erection they obtained during the study was the same as they achieve at home? If not, this could be the result of higher stress level within the experimental setting leading to falsely elevated cortisol levels.

5. Results: There are long spaces between the numbers within parentheses, I believe this should be a plus minus sign to note standard deviation. However, the presentation of the results needs to be cleaned up to make easily readable to the audience. Also, p values should be presented in the results, tables, and figures rather than just using an * without stating the specific value.

6. Results: An explanation for why number of lab draws for detumescence and flaccidity is lower? I would expect these to be the easiest times to get samples.

7. Discussion: Needs a discussion of limitations of this study.

Minor Concerns:

1. Last sentence in introduction should be a statement in the methods

2. Numerous run-on sentences: First sentence in abstract background section; Sentence line 88-90; First sentence in discussion; Sentence from line 145-148.

3. Introduction and discussion should be broken down into paragraphs.

4. Methods subsection heading not necessary since only 1 paragraph

Reply

[1] The referee has commended to enroll a significant number of men into the study.

It is submitted that the estimation of sample size was assisted by the Department of Biomathematics of the Hannover Medical School and conducted in accordance with the customary examples based on normal distributions and power for comparing two means (power of t -Test = 80%, $\alpha = /< 5\%$) The parameters applied included the estimated level of significance, the estimated standard deviation (SD) in a population/cohort as well as the SD of the difference between the cohorts.

[2] The referee has stated that, in the **Introduction**, it is unclear how the methods or design of the study are different from prior studies and whether or not the results presented in the paper are build on or overcome limitations of prior studies.

It is submitted that this is the first study to determine and compare the courses of cortisol in the both the systemic and cavernous blood through different stages of sexual arousal in healthy males and patients with erectile dysfunction (ED). This is emphasized in the last sentence of the **Introduction** (It reads: *Our study aimed to investigate through the different stages of the sexual arousal cycle, exemplified by different functional states of the penis, in healthy men and patients with ED the levels of cortisol in the cavernous blood and systemic circulation.*)

[3] In order to make it easier for the non-expertized readers to understand the study design, the **Methods** section has been supplemented with information how patients were enrolled/screened.

[4] The definitions used to classify men with ED as either as organic or psychogenic as well as the number of men with each classification has been provided in the **Methods** section.

[5] Information on how the different functional conditions of the penis through the sexual arousal process were defined and assessed has been added to the **Methods** section, the potential association between stress level of the individuals and the secretion of cortisol has been addressed in the **Discussion**.

[6] In order to comply with the referee's comment, p-values have been provided in the **Results** section. In the **Materials & Methods**, the format of the data presented has been clarified (Mean \pm SD).

[7] An explanation for why the number of lab draws for detumescence and flaccidity is lower has been provided in the **Material & Methods**.

In order to conduct accurately comparison of paired samples, only plasma levels of cortisol assayed in said samples were statistically evaluated. This is in accordance with the customary examples based on normal distributions.

[8] Potential limitations of the study have been addressed in the **Discussion**. It now reads: *In the group of patients, individuals were of older age (mean: 52 years vs. 25 years in the group of healthy males) and exhibited some age-related comorbidities, such as hypertension, atherosclerosis, obesity, diabetes. With aging, the activity of the neurohypophyseal and adrenocorticotrophic axis may alter (Tamma G, et al., 2015).* The list of references has been supplemented by referring to the article by Tamma G, et al., 2015.

Minor Concerns

[1] In order to avoid run-on sentences, the **Introduction** and **Discussion** of the manuscript have been rephrased to a certain degree.

[2] The **Introduction** and **Discussion** have been subdivided into paragraphs.

[3] In the **Methods**, the subsections have been removed.