

## ORIGINAL ARTICLE

# The relationship of serum and salivary cortisol levels to male sexual dysfunction as measured by the International Index of Erectile Function

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To evaluate the biomarkers of sexual function, we investigated the relationship between questionnaire responses and biological hormones such as testosterone (T) and cortisol (F) in serum and saliva. The study population included 105 men aged 30–72 years (mean: 49 ± 4.5, median: 49). Levels of all serum hormones (Total-T, Free-T, Bioavailable-T, Total-F and Bioavailable-F) and salivary hormones (Saliva-T and Saliva-F) were measured directly by liquid chromatography/tandem mass spectrometry. The International Index of Erectile Function (IIEF) was used as a questionnaire to evaluate sexual dysfunction. Free-T and Bioavailable-T showed significant inverse correlations with age ( $P < 0.01$ ). In the group not taking antidepressants, the levels of Bioavailable-F and Saliva-F showed significant inverse correlations with a portion of the IIEF score ( $P < 0.05$ ). However, reductions in Bioavailable-T and Saliva-T showed no association with the IIEF score. In the group taking antidepressants, these hormone levels showed no correlation with IIEF.

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## Introduction

Male sexual hormones such as androgens are generally considered to be associated with sexual function. Among the androgens, testosterone (T) is a useful marker that is frequently used to evaluate male hypogonadism. Androgen production decreases with aging, and decreased testosterone is thought to induce late-onset hypogonadism, one of the most common symptoms of which is erectile dysfunction (ED).<sup>1</sup> Late-onset hypogonadism is defined as a biochemical syndrome associated with advancing age and characterized by a deficiency in serum androgen levels with or without decreased genomic sensitivity to androgens. It may result in significant alterations in the quality of life and

adversely affect the function of multiple organ systems.<sup>2</sup> In Japan, measurement of free testosterone is recommended to diagnose late-onset hypogonadism.

Serum total testosterone (Total-T) consists of testosterone that binds strongly to sex hormone-binding globulin (SHBG), the testosterone that loosely binds to albumin and free testosterone (Free-T). The latter two are designated collectively as bioavailable-T (Bio-T), and its measurement is considered to be an index of androgen activity.<sup>3</sup> In addition, the T of saliva (Sa-T) is regarded as free testosterone, and its measurement is thought to reflect blood Free-T and Bio-T.<sup>4</sup> It has been reported that Total-T does not vary, but Free-T decreases gradually with aging.<sup>5</sup>

Cortisol (F), which is an adrenal cortical hormone, does not show changes in level with aging, but is increased by stress.<sup>6</sup> Cortisol remains constant after increasing during the period of sexual maturation at the same time as T. Cortisol is known to raise blood pressure and blood sugar levels as well as cause sterility and immune dysfunction. When we feel stress, fear or tension, sympathetic nervous activity

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is increased relative to parasympathetic nervous activity, and the penis is contracted. Blood cortisol levels are known to increase in the hypothalamic–pituitary–adrenocortical system at the same time as that in which blood norepinephrine levels increase in the sympathetic nervous–adrenal medullary system when sympathetic nervous activity is dominant. Thus, ED may occur when cortisol levels are high. Although the cortisol level may reflect sexual function, there have been no earlier investigations of the relationships between these two parameters. Similar to Bio-T, bioavailable cortisol (Bio-F) is an active form that does not bind to globulin, and saliva cortisol (Sa-F) is secreted in the saliva as the free type.<sup>7</sup> Here, we developed methods to measure testosterone and cortisol levels in the blood and saliva by liquid chromatography/tandem mass spectrometry (LC/MS-MS).<sup>8</sup> We measured various hormones directly using this method and investigated the correlations of these hormone levels and sexual function on the basis of questionnaire responses from 105 patients.

## Materials and methods

### Patients

The study population included 103 men aged 32–72 years (mean:  $49 \pm 4.5$ , median: 49). All patients visited our hospital for treatment of urological symptoms, including late-onset hypogonadism, with or without ED. Thirteen patients were taking antihypertensive agents and two patients were taking antidiabetic drugs. None of the patients were taking hormone medication (supplementation or deprivation) or phosphodiesterase type 5 (PDE5) inhibitors. All patients gave full informed consent to participate in this study. Saliva and serum samples

were collected in the morning (between 0900 and 1100 hours). In addition, we investigated their sexual function and examined whether the patients had taken any psychotropic drugs. Each patient also completed validated questionnaires exploring anxiety and depression (Hospital Anxiety and Depression Scale [HAD scale]). This study was approved by the Internal Review Board of Kanazawa University, and was supported by a medical research fund from Kanazawa University Hospital (Kanazawa, Japan).

### LC-MS/MS

Levels of all serum hormones (Total-T, Free-T, Bio-T, Total-F and Bio-F) and salivary hormones (Sa-T and Sa-F) were measured directly by LC/MS-MS. LC/MS-MS is effective for determining fixed quantities of very small amounts of material in biological samples.<sup>9</sup> The LC/MS-MS apparatus used was an API4000 (Applied Biosystems, Kanazawa, Ishikawa, Japan) mass spectrometer and an HP1100 (Hewlett-Packard, Kanazawa, Ishikawa, Japan) liquid chromatograph. Bioavailable T (Bio-T) and F (Bio-F) were determined after separation of the SHBG-bound steroid.

Conventionally, Bio-T was calculated on the basis of albumin, SHBG and Total-T, using the method proposed by the International Society for the Study of the Aging Male. However, Bio-T and Bio-F were measured directly as levels of non-SHBG-bound T and F using LC/MS-MS in our study.

### Definition of erectile function

To evaluate sexual dysfunction, the International Index of Erectile Function (IIEF) was used as a questionnaire study.<sup>10</sup> IIEF is divided into five domains (erectile function, orgasmic function, sexual desire, intercourse satisfaction and overall

**Table 1** Correlation coefficient between the age and hormonal profile

	Testosterone				Cortisol		
	Total	Free	Bio	Saliva	Total	Bio	Saliva
Age	0.01	-0.35***	-0.30**	-0.19*	0.07	0.10	0.17
<i>Testosterone</i>							
Total	—	0.52****	0.65****	0.47****	0.02	0.06	0.08
Free	—	—	0.77****	0.67****	0.18*	0.15	0.16
Bio	—	—	—	0.71****	0.05	0.14	0.08
Saliva	—	—	—	—	0.06	0.13	0.12
<i>Cortisol</i>							
Total	—	—	—	—	—	0.91****	0.80****
Bio	—	—	—	—	—	—	0.79****
Saliva	—	—	—	—	—	—	—

$n = 103$ .

\* $P < 0.10$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$ .

Speaman's rank test.

satisfaction) and IIEF5 was evaluated simultaneously.<sup>11</sup> Men who did not complete the questionnaire were excluded from statistical analysis.

### Statistical analysis

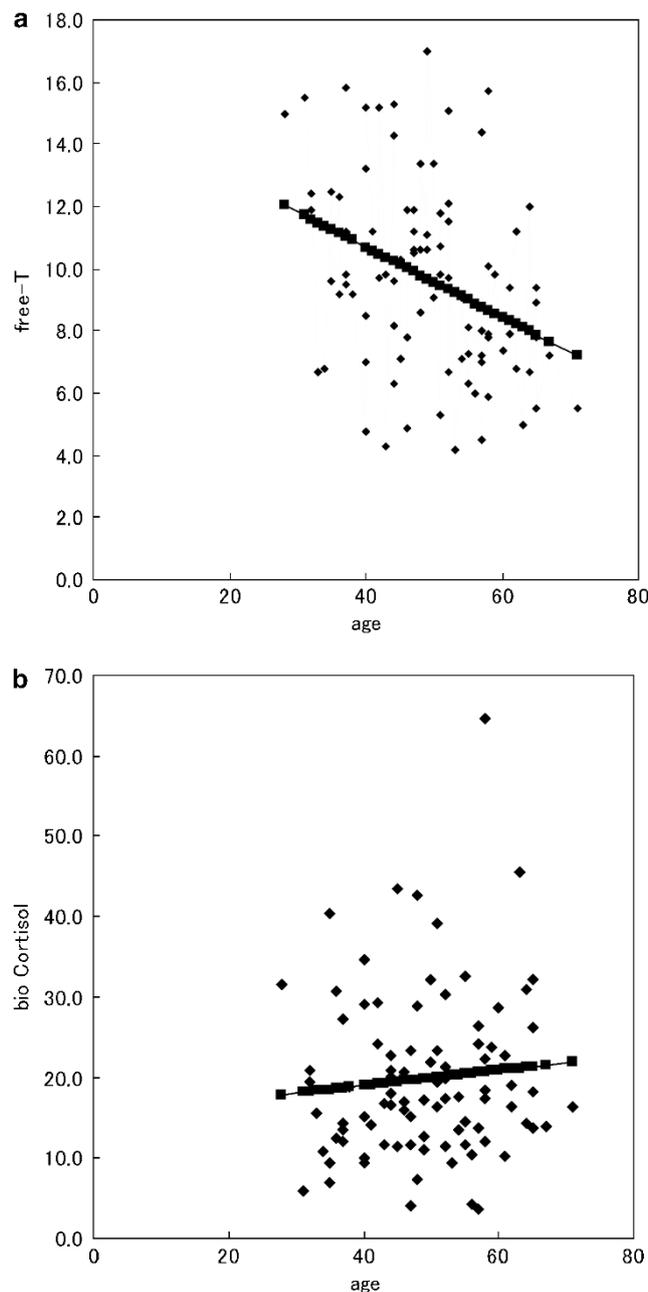
Data from completed questionnaires were input into a Microsoft Excel spreadsheet. All analyses were performed with a SAS package (version 8.1) and differences were considered significant at  $P < 0.05$ . The hormonal values were subjected to logarithmic transformation and controlled for age. Thereafter, we examined correlations between IIEF responses and hormonal levels. Spearman's rank test was used to test the correlations between age and hormonal profile, IIEF score and hormonal profile.

## Results

The relationships of testosterone and cortisol to age are shown in Table 1. Free-T and Bio-T showed significant inverse correlations with age ( $P < 0.01$ ). Sa-T showed a tendency toward a negative correlation, but the relation was not significant. Cortisol did not have a meaningful correlation with age (Figure 1). In addition, the levels of each type of testosterone (Total-T, Free-T, Bio-T and Sa-T) showed very strong correlations to the other forms of testosterone and there were also correlations among cortisol levels (Total-F, Bio-F and Sa-F).

Table 2 shows the baseline characteristics and the levels of each hormone in men who did and did not take psychotropic drugs. Sixty-four of the 103 patients included in the investigation (62%) took psychotropic drugs. The HAD scale was significantly high in the group taking psychotropic drugs. It was suggested that populations of men who took psychotropic drugs suffered from anxiety and depression. With regard to different types of psychotropic drugs, 15 patients took only selective serotonin reuptake inhibitors (SSRI), 14 took serotonin and norepinephrine reuptake inhibitors (SNRI), 9 took tricyclic antidepressants, 10 took minor tranquilizers and 16 patients took antidepressants and minor tranquilizers. Men who did not take psychotropic drugs showed significantly lower Total-T levels. There were no significant differences in levels of hormones other than those of Total-T.

When we examined the correlation between hormonal level and IIEF response, the hormonal levels were controlled for age, as it is well known that testosterone shows a significant inverse correlation with age. Table 3 shows the correlation coefficients for each domain of IIEF (erectile function, orgasmic function, sexual desire, intercourse satisfaction and overall satisfaction), IIEF5 and total score of IIEF, with various hormones in men who did not take psychotropic drugs. Bio-F showed negative correlations with erectile function, sexual



**Figure 1** (a) Free testosterone showed significant inverse correlations with age ( $n = 103$ ,  $R = -0.35$ ,  $P < 0.001$ ). (b) On the other hand, bioavailable cortisol did not have a meaningful correlation with age ( $n = 103$ ,  $R = 0.10$ ,  $P = 0.10$ ).

desire, IIEF5 and total IIEF score ( $P < 0.05$ ). In addition, Sa-F showed negative correlations with intercourse satisfaction and total IIEF score ( $P < 0.05$ ). However, testosterone did not show a meaningful correlation with any of the IIEF domains (Figure 2).

There were no significant correlations between the various hormones and IIEF in this group who took psychotropic drugs.

## Discussion

It has been suggested that androgen insufficiency disrupts cellular signaling pathways and produces pathological alterations in penile tissues, leading to ED.<sup>12</sup> Although there is evidence suggesting that testosterone plays an important role in erectile function, testosterone levels below the normal lower limit may still be sufficient to retain a normal erectile function in most men.<sup>13,14</sup> The weak relationship between low T and ED is well known

**Table 2** Baseline characteristics and hormonal level (the use of antidepressants vs the non-use of antidepressants)

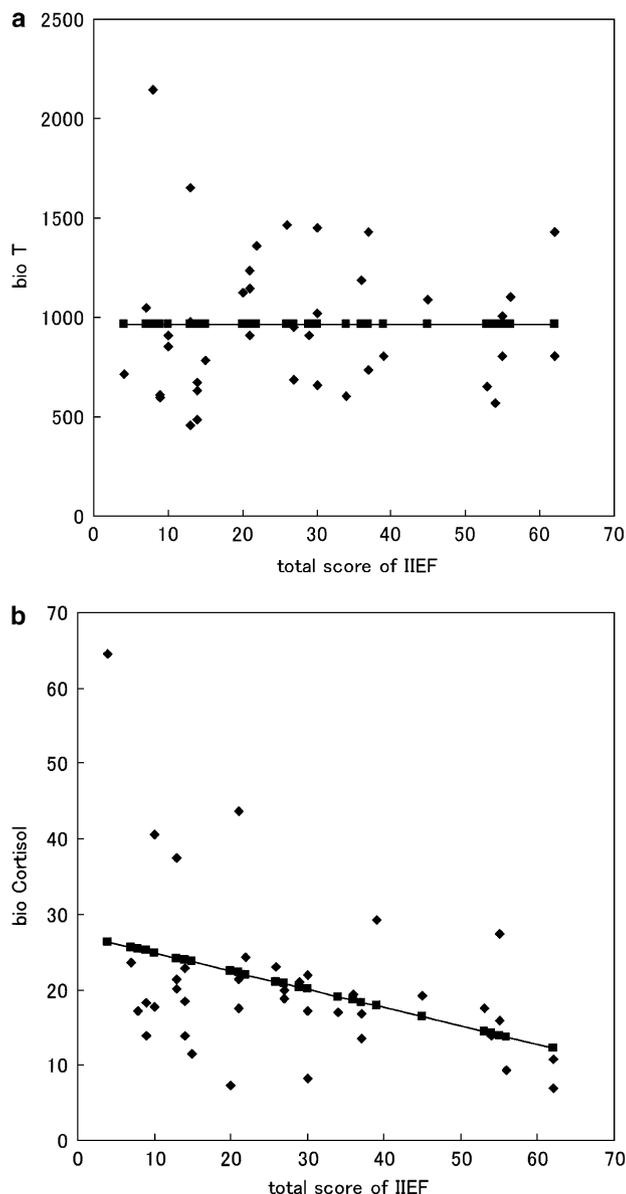
	Number of patients (%)		P-value
	Not take (n = 39)	Take (n = 64)	
High blood pressure	6 (15.4)	8 (12.5)	0.67
Diabetes mellitus	2 (5.1)	5 (7.8)	0.59
Cardiac treatment	2 (5.1)	2 (3.1)	0.61
Benign prostatic hyperplasia	3 (7.6)	5 (7.8)	0.98
	Mean $\pm$ s.d.		
Age	48.38 $\pm$ 10.11	49.76 $\pm$ 9.78	0.52
BMI (kg/m <sup>2</sup> )	22.41 $\pm$ 2.78	23.77 $\pm$ 2.64	0.44
<i>HAD scale</i>			
Depression	8.13 $\pm$ 4.16	11.23 $\pm$ 4.2	<0.01
Anxiety	7.68 $\pm$ 3.61	9.45 $\pm$ 4.29	0.02
Total score	15.81 $\pm$ 6.8	20.55 $\pm$ 7.5	<0.01
<i>Testosterone</i>			
Total (ng ml <sup>-1</sup> )	4.77 $\pm$ 1.60	3.86 $\pm$ 2.44	0.03
Free (pg ml <sup>-1</sup> )	10.20 $\pm$ 2.85	9.27 $\pm$ 3.43	0.16
Bio (pg ml <sup>-1</sup> )	965.73 $\pm$ 359.81	841.45 $\pm$ 383.46	0.11
Saliva (pg ml <sup>-1</sup> )	55.70 $\pm$ 23.57	55.91 $\pm$ 29.81	0.97
<i>Cortisol</i>			
Total (ng ml <sup>-1</sup> )	108.42 $\pm$ 35.05	100.89 $\pm$ 42.34	0.35
Bio (ng ml <sup>-1</sup> )	20.58 $\pm$ 10.78	19.41 $\pm$ 9.95	0.58
Saliva (ng ml <sup>-1</sup> )	2.36 $\pm$ 1.57	1.91 $\pm$ 1.26	0.13

and supported by the results of both human and animal studies.<sup>15</sup> A lack of association between T and IIEF-5 has been documented in a large consecutive series of almost 1000 elderly individuals with or without ED.<sup>16</sup> In ED patients, hypogonadism is often associated with reduced sexual desire and nocturnal penile erections, whereas association with sex-induced erection is less evident. This is because T regulates not only cyclic guanosine monophosphate formation through nitric oxide synthase stimulation but also its catabolism through PDE5 activity. Androgens positively regulate PDE5, thus providing a possible explanation for the highest levels of this enzyme in the male genital tract.<sup>17</sup> The androgen-dependent PDE5 expression could explain the reduced effectiveness of PDE5 inhibitors in the treatment of ED in hypogonadal patients. As T positively regulates both the initiation (nitric oxide synthase) and the end (PDE5) of the erectile process, its net effect on erection is modest. Hence, erections are still possible under hypogonadal conditions in which decreased cyclic guanosine monophosphate formation because of impaired NO production is most likely counterbalanced by regulated PDE5 activity and cyclic guanosine monophosphate hydrolysis. The main physiological action of T is therefore to ensure the timely adjustment of the erectile process as a function of sexual desire, therefore finalizing erection associated with sex. A trophic effect of T on the penile architecture has also been shown in different animal species.<sup>18</sup> For all of the above reasons, treating hypogonadism restores impaired penile erections in experimental animal models, as well as in a clinical setting. Conversely, T administration to otherwise eugonadal individuals is rather ineffective. There have been many reports that sexual function is improved by androgen replacement therapy.<sup>19-21</sup> All the above considerations explain the well-known weak correlation between ED and T. In this study, there was no correlation between testosterone and IIEF responses. That is, testosterone is not an appropriate biomarker for an evaluation of sexual function.

**Table 3** Correlation coefficient between the IIEF score and hormonal profile (The group who did not take psychotropic drugs)

	Testosterone				Cortisol		
	Total	Free	Bio	Saliva	Total	Bio	Saliva
Erectile function	-0.26	-0.07	-0.01	-0.13	-0.28	-0.39**	-0.30
Orgasmic function	-0.10	-0.06	0.05	0.04	-0.20	-0.27	-0.19
Sexual desire	-0.08	-0.05	-0.02	-0.20	-0.30	-0.44**	-0.35*
Intercourse satisfaction	-0.23	-0.12	0.00	-0.11	-0.26	-0.33*	-0.37**
Overall satisfaction	-0.07	-0.33*	-0.16	-0.17	-0.19	-0.29	-0.32*
Total score of IIEF	-0.24	-0.12	-0.01	-0.13	-0.31*	-0.43**	-0.37**
IIEF5	-0.25	-0.08	0.00	-0.15	-0.29	-0.38**	-0.30*

\* $P < 0.10$ , \*\* $P < 0.05$ .  
Spearman's rank test.  
 $n = 32$ .



**Figure 2** (a) Bioavailable testosterone did not show a meaningful correlation with the total score of IIEF ( $n=32$ ,  $R=-0.01$ ,  $P=0.98$ ). (b) Bioavailable cortisol showed negative correlations with the total score of IIEF score ( $n=32$ ,  $R=-0.43$ ,  $P<0.05$ ).

Bio-F and Sa-F showed significant negative correlations with some domains of IIEF in this study. In a rat model of adrenal insufficiency, it was shown earlier that the rat adrenal gland contributes to the maintenance of the erectile mechanism and may affect the neuronal nitric oxide synthase content in the penis.<sup>22</sup> In a human study, on the other hand, there were no differences in cortisol levels between individuals with and without ED.<sup>23</sup> These studies did not include detailed differential counts of cortisol. However, in our study, Bio-F and Sa-F (not Total-F) showed negative correlations with the IIEF score. This suggested that Bio-F and Sa-F, which are active forms of cortisol, likely decrease

sexual function. One reason why ED occurred in patients with high levels of cortisol is because cortisol is increased by stress. It is known that the blood cortisol level increases at the same time as the blood norepinephrine level when sympathetic nervous activity is dominant. Sympathetic nervous activity has a restraining effect on erection, and sexual function is thought to be reduced under stress.<sup>24,25</sup> Our results indicate that increases in plasma and salivary cortisol may play causative roles in ED induced by social stress.

In studies of stress, cortisol and norepinephrine have been overused as standard stress markers. Cortisol level is an index of the endocrine response to stress and shows comparatively high normal values in blood ( $100\text{--}150\text{ ng ml}^{-1}$ ). We can analyze cortisol from saliva if we use a high sensitivity analytical procedure such as ELISA or LC-MS/MS.<sup>26</sup> Measuring cortisol in saliva may become a useful index for evaluating the sexual function non-invasively. In addition, saliva-based measurement methods have attracted attention for measuring amylase in saliva as a marker of the sympathetic nervous system response to stress.<sup>27</sup> Further studies in this field are needed.

In the men who took antidepressants in this study, there were no significant correlations between hormone values and IIEF. Neither testosterone nor cortisol values played an important role in erectile function in the group taking antidepressants. This was thought to be because ED and ejaculation disorder are induced by the actions of antidepressant medication. Furthermore, psychiatric disorder in itself may be a cause of ED.<sup>28</sup> Patients with depression may have elevated cortisol levels.<sup>29</sup> In addition, this type of drug could result in an increase in the prolactin level determining the reduction of T.<sup>30</sup> Therefore, we could not obtain meaningful data from the patients taking psychotropic drugs.

## Conclusions

The active forms of cortisol (Bio-F and Sa-F) showed negative correlations with sexual function in men who did not take psychotropic drugs, although there was no such correlation for testosterone. ED is thought to occur in patients with high levels of cortisol because of the relations between cortisol and stress. Cortisol may thus become a useful index for the evaluation of sexual function.

## Conflict of interest

The authors declare no conflict of interest.

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