Standard values for diagnosis of LOH overseas

In the guidelines approved by the consensus meeting of the Endocrine Society in the United States (2001),[27] the standard value for indication is total T of less than 2 ng/mL. For patients with values of 200 to 400 ng/mL, free T and BAT are recommended as references. In the ISA, ISSAM, and EAU recommendations reported by Lunenfeld *et al.*,[28] the standard value for LOH diagnosis is total T of less than 8 nmol/L (231 ng/dL) and the normal value is not less than 12 nmol/L (346 ng/dL). Therefore, patients with values from 8 nmol/L to not less than 12 nmol/L (231 to less than 346 ng/dL) are defined as borderline. In such patients, determination of calculated free T is recommended, and a diagnosis and treatment algorithm for LOH has been prepared.[27] The diagnostic criteria have been adopted in hospitals and clinics in Europe and the USA.

On the other hand, Hong *et al.*[29] conducted a screening for prostate disease in Korea, using the same free T assay kit used in Japan. The results showed that, in 403 patients (42 to 78 years old) without prostate cancer, the median free T level was 19.4 pg/mL (8.8 to 31.0 pg/mL) in 41 patients aged less than 50 years, 17.1 pg/mL (1.9 to 29.5 pg/mL) in 97 patients aged 51 to 60 years, 12.9 pg/mL (1.9 to 27.8 pg/mL) in 219 patients aged 61 to 70 years, and 9.4 pg/mL (1.5 to 19.7 pg/mL) in 76 patients aged 70 years and above. Although studied in different ethnic groups, the free T levels were higher in Koreans than in Japanese and Canadians. In the Second Japanese–Asian Men's Health & Aging Conference held in Japan in 2007, the diagnostic method for LOH was investigated in participating physicians from Asian countries. It was found that, in China, total T is mainly used, although there are some hospitals where calculated bioavailable testosterone (cBAT) is used. In Korea, total T is used, by setting a standard value of 2.8 ng/mL or 3 ng/mL. In Malaysia, although no guidelines have been established, diagnosis is made according to the criteria by Lunenfeld *et al.* In the Philippines, total T, free T, and BAT levels are set at not more than 2.7 ng/mL, 5.5 pg/mL, and 281.3 pg/mL, respectively. Also in Thailand, although no guidelines have been established, total T is used in many hospitals, and there are some hospitals where free T of less than 90 pg/mL and hospitals where calculated free T of 7.2 ng/dL is used if total T is at the lower normal limit. Thus, the diagnostic methods for LOH in the ISA, ISSAM, and EAU recommendations are not necessarily prevalent among Asian countries.

Reasons for the adoption of free T levels in the diagnosis of LOH in Japan and the validity

The first reason for the adoption of free T levels is that free T is more strongly correlated with cBAT than total T, with a statistically significant difference ($P < 0.005$; Fig. 4; unpublished). Namiki *et al.*[12] and Matsuda *et al.*[13] also reported data showing a favorable correlation. On the other hand, Martinez-Jabaloyas *et al.*[30] reported a relatively low correlation between free T based on the labeled analogs, using enzyme immunoassay and calculated free T, thus casting a doubt for the utility of free T. Although we don't know the exact reason for such difference, free T at least measured using the DPC kit seems to be clinically useful. Internationally, the measurement of BAT and cBAT has been considered. However, routine measurement of BAT is not possible for the following reasons: a complicated high-performance liquid chromatography technique has to be used for the measurement of BAT; although the measurement of BAT by treating SHBG-T with ammonium sulfate precipitation is considered to be the most reliable method that is less

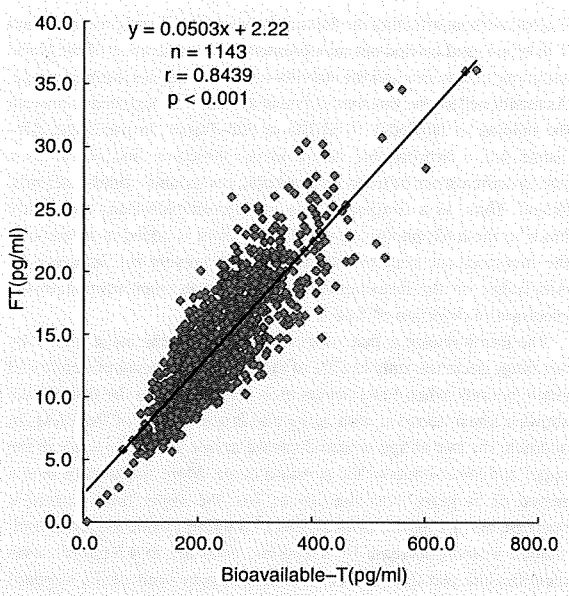

Fig. 4 Calculated bioavailable testosterone (cBAT) was obtained by substituting measured total testosterone as well as the albumin-bound testosterone and sex hormone-binding globulin concentrations into the equation in the literature[11] shown below. The finding of a favorable correlation between BAT and free testosterone means that free testosterone can be used as an indicator to predict BAT levels.

likely to be affected by the effects of other steroids, measurement by ammonium sulfate precipitation requires a very long time, and is difficult to automate. Vermeulen *et al.*[11] have proposed the use of cBAT[4,5] as a substitute for BAT, which can be automatically calculated from measured total T, SHBG, and Alb-Tumin levels using the 'Free & Bioavailable Testosterone Calculator' on the ISSAM website (http://www.issam.ch/freetesto.htm). Unfortunately, however, the measurement of SHBG cannot be performed in the diagnosis of LOH in Japan, because SHBG measurement is not included in the gonadal function tests covered by health insurance and requires additional costs.

The second reason is the finding that the behavior of free T is highly consistent with that of BAT, with free T levels being decreased markedly due to aging, whereas mean total T by age group remains consistent, showing no effects of aging, although the basis of diagnosis of LOH is the measurement of androgens. It is easily understandable that gonadal function decreases with age, from the fact that LH, follicle stimulating hormone, and the carrier protein SHBG levels increase through the negative feedback mechanism, as well as from the effects of testosterone replacement therapy. Although the production of testosterone decreases due to age-related testicular atrophy, there is no clear tendency toward decrease in total T. This is considered attributable to a lowered metabolic rate due to increased SHBG levels. Therefore, it is considered that the decrease in total T occurs at a late stage where LOH has advanced, whereas the decrease in free T occurs at a relatively early stage. From this point of view, free T is considered to be a potentially more sensitive indicator of LOH than total T. In addition, practically, under the current circumstances in Japan, either one of total T or free T has to be chosen as the test covered by health insurance.

The third reason is that, in the measurement of free T by the assay kit from DPC, we employed a method[28] allowing the measurement of free

T alone without affecting the balance between free T and protein-bound T in blood, and carried out measurement in consideration of the blood sampling time points and the stability of serum samples during storage. As mentioned above, this measurement system was designed to prevent the binding of labeled-T to SHBG or Alb-Tumin. In particular, Alb-Tumin has a hydrophobic cavity in the structure, and serves as a non-specific carrier of many hydrophobic compounds (lipids, steroids, drugs). Thus, as a compound (e.g. sulfobromophthalein) that easily binds to the hydrophobic cavity of Alb-Tumin is added in advance in the measurement system, this method can prevent the influence of Alb-Tumin on the measurement, leading to an improvement in the precision of detection of free T.

The fourth reason is that, as mentioned earlier, the mean total T by age range decreases only to 80% of the YAM even during presenile and senile periods, when LOH occurs most frequently, but the free T level shows a linear decrease with aging and drops to 50% of the YAM. In addition, the rate of age-related decrease in free T varied greatly in the upper and lower limits of the standard range. More specifically, over a period of 50 years from the 20s to 70s, the upper limit showed a decrease by 14.1 pg/mL from 27.9 to 13.8 pg/mL, while the lower limit showed a small decrease by 4.0 pg/mL from 8.5 to 4.5 pg/mL. This identifies the fact that, in free T, both the upper limit of the standard range and the mean value decrease greatly with age, but the lower limit is almost unchanged. It means that, for the establishment of a standard value for diagnosis of LOH, it is more reasonable to adopt the approach assessing what magnitude of decrease in the upper limit of the standard range of free T from the peak value in the 20s induces LOH than the approach simply focusing on the lower limit of the standard range for each age group.

For these reasons, free T has been used as an essential test in the diagnosis of LOH in Japan. ISSAM[28] defines LOH as 'a biochemical syndrome associated with advancing age and characterized by a deficiency in blood testosterone levels.' Assuming that free T carries out its role as a bioavailable androgen, a decrease in this hormone means the induction of so-called andropause. Interestingly, from this point of view, our finding that the lower limit of the standard range of free T remains relatively constant regardless of age can be taken as the minimum blood concentration for the maintenance of 'manliness.'

Summary

Determination of androgen levels is essential to the diagnosis of LOH. However, under the current situation, there is no consensus regarding which androgens should be measured for the diagnosis of LOH. The ISA, ISSAM, and EAU recommend measurement of total T levels, which do not necessarily decrease significantly with age, in the diagnosis of LOH. One reason for this may be the difficulty in directly measuring active androgens. Even BAT and calculated BAT are not yet available for routine clinical use due to technical or precision problems. For the reasons described above, it is determined to adopt free T levels in the diagnosis of LOH in Japan. The standard value for diagnosis of LOH is set at a mean–2SD value of 8.5 pg/mL, by calculating the mean value in young adults in their 20s (the YAM). In addition, it has been proposed that patients with values of not less than 8.5 pg/mL and less than 11.8 pg/mL, which is 70% of the mean value for men in their 20s, are to be indicated for ART as LOH borderline cases.

The Japanese Urological Association and the Japanese Association of Men's Health have started verification of the appropriateness of free T as a biological marker of LOH, in consideration of the various clinical symptoms of LOH. In Japan, the use of the YAM of free T in the standard diagnostic criteria for LOH has been proposed. However, it should be verified that free T is clinically applicable as a criterion for determining the LOH cases to be indicated for ART. In addition, it is required to clarify whether normalization of androgen levels by ART can lead to improvement of symptoms.

References

1 Nieshlag E, Swerdloff R, Behre HM *et al.* Investigation, treatment, and monitoring of late-onset hypogonadism in males: ISA, ISSAM, and EAU recommendations. *J. Androl.* 2006; **27**: 135–7.
2 Nieshlag E, Swerdloff R, Behre HM *et al.* International Society of Andrology (ISA); International Society for the Study of the Aging Male (ISSAM); European Association of Urology (EAU): Investigation, treatment, and monitoring of late-onset hypogonadism in males. ISA, ISSAM, and EAU recommendations. *Eur. Urol.* 2005; **48**: 1–47.
3 Nieshlag E, Swerdloff R, Behre HM *et al.* Investigation, treatment, and monitoring of late-onset hypogonadism in males: ISA, ISSAM, and EAU recommendations. *Int. J. Androl.* 2005; **28**: 125–7.
4 Schiavi RC, Schreiner-Engel P, White D *et al.* The relationship between pituitary-gonadal function and sexual behavior in healthy aging men. *Psychosom. Med.* 1991; **53**: 363–74.
5 Wang C, Swedloff RS, Nieschlag E *et al.* International Society for the study of the Aging Male Conference, in Tampa, FL 2008.
6 Namiki M, Akaza H, Shimazui T *et al.* Clinical practice manual for late-onset hypogonadism syndrome. *Int. J. Urol.* 2008; **15**: 377–88.
7 James F, Dunn C, Nisula B *et al.* Transport of steroid hormones: Binding of 21 endogenous steroids to both testosterone-binding globulin and corticosteroid-binding globulin in human plasma. *J. Clin. Endocrinol. Metab.* 1981; **53**: 58–68.
8 Manni A, Pardridge WM, Cefalu W *et al.* Bioavailability of Albumin-Bound Testosterone. *J. Clin. Endocrinol. Metab.* 1985; **61**: 705–10.
9 Cumming DC, Wall SR. Non-sex hormone-binding globulin-bound testosterone as a marker for hyperandrogenism. *J. Clin. Endocrinol. Metab.* 1985; **61**: 873–6.
10 Soudergard R, Bauckstroum T, Shanbhag V *et al.* Calculation of free and bound fractions of testosterone and estradiol-17β to human plasma proteins at body temperature. *J. steroid Biochem.* 1982; **16**: 801–10.
11 Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in Serum. *J. Clin. Endocrinol. Metab.* 1999; **84**: 3666–72.
12 Namiki M, Koh E, Takashima M. [Aging and androgen.] *Hinyokigeka* 2003; **16**: 825–30. (in Japanese).
13 Matsuda T, Tatsumi K. [Diagnosis and therapy of late-onset hypogonadism.] *Hinyokigeka* 2003; **16**: 831–7. (in Japanese).
14 Purifoy FE, Koopmans LH, Mayes DM. Age differences in serum androgen levels in normal adult males. *Hum. Biol.* 1981; **53**: 499–511.
15 Morley JE, Kaiser FE, Perry HM 3rd *et al.* Longitudinal changes in testosterone, luteinizing hormone, and follicle-stimulating hormone in healthy older men. *Metabolism* 1997; **46**: 410–13.
16 Zmuda JM, Cauley JA, Kriska A *et al.* Longitudinal relation between endogenous testosterone and cardiovascular disease risk factors in middle-aged men. A 13-year follow-up of former Multiple Risk Factor Intervention Trial participants. *AM. J. Epidemiol.* 1997; **146**: 609–17.
17 Harman SM, Metter EJ, Tobin JD *et al.* Longitudinal effects of aging on serum total and free testosterone levels in healthy men. *J. Clin. Endocrinol. Metab.* 2001; **86**: 724–31.
18 Feldman HA, Longcope C, Derby CA *et al.* Age trends in the level of serum testosterone and other hormones in middle-aged men: Longitudinal results from the Massachusetts male aging study. *J. Clin. Endocrinol. Metab.* 2002; **87**: 589–98.
19 Jensen TK, Andersson AM, Hjollund NH *et al.* Inhibin B as a serum marker of spermatogenesis: Correlation to differences in sperm concentration and follicle-stimulating hormone levels. A study of 349 Danish men. *J. Clin. Endocrinol. Metab.* 1997; **82**: 4059–63.

20 Iwamoto T, Yanase T, Koh E *et al.* [Reference ranges of total testosterone and free testosterone in Japanese male adults.] *Nippon Hinyokika Gakkai Zasshi* 2005; **95**: 751–60. (in Japanese)
21 Boots LR, Potter S, Potter D *et al.* Measurement of total serum testosterone levels using commercially available kits: High degree of between-kit variability. *Fertil Steril.* 1998; **69**: 286–92.
22 El Shami AS. Patent Bulletin in Japan No.2575338 A Said EL Shami 1985
23 Ooi DS, Innanen VT, Wang D *et al.* Establishing reference intervals for DPC's free testosterone radioimmunoassay. *Clin. Biochem.* 1998; **31**: 115–21.
24 Sato Y, Kato S, Oonishi S *et al.* [Analysis of subjective symptoms and endocrine findings in male patients at PADAM clinics.] *Nippon Hinyokika Gakkai Zasshi.* 2004; **95**: 8–16. (in Japanese).
25 Orimo H, Sugioka Y, Tominaga H *et al.* (Working Committee on diagnostic guidelines for Osteoporosis in The Japanese Society for Bone and Mineral Research). Diagnostic standard of primary osteoporosis (1966 revised ed.) *J. Bone Miner. Metab.* 1997; **14**: 219–33.
26 Orimo H, Hayashi Y, Tominaga H *et al.* (Working Committee on diagnostic guidelines for Osteoporosis in The Japanese Society for Bone and Mineral Research). Diagnostic standard of primary osteoporosis (2000 revised ed.) *J. Bone Miner. Metab.* 2001; **18**: 76–82.
27 Endocrine Society: Summary from the second andropause consensus committee, 2002.
28 Lunenfeld B, Saad F, Hoesl CE. ISA, ISSAM and EAU recommendations for the investigation, treatment and monitoring of late-onset hypogonadism in males: Scientific background and rationale. *Aging Male* 2005; **8**: 59–74. Review.
29 Hong SK, Han BK, Jeong JS *et al.* Serum measurements of testosterone, insulin-like growth factor 1, and insulin-like growth factor binding protein-3 in the diagnosis of prostate cancer among Korean men. *Asian J. Androl.* 2008; **10**: 207–13.
30 Martinez-Jabaloyas JM, Queipo-Zaragoza A *et al.* Evaluation of an immunoassay kit to measure free testosterone. *Actas Urol. Esp.* 2006; 598–601.

Members of Working Committee of the Japanese Urological Association for free testosterone to reestablish the standard values of total T and free T in the serum in adult Japanese males

Katsuyuki Baba MD
Mikihiko Honda MD
Nobuhisa Ishii MD
Tadaichi Kitamura MD
Yukihiro Kondo MD
Ken Marumo MD
Tsuneharu Miki MD
Koichi Nagao MD
Hiroshi Okada MD
Yoshikazu Sato MD
Jintetsu Sou MD
Ryuji Tomiyama, BaSci
Suguru Usui MD
Shinichi Hisasue MD
Shigeo Horie MD
Naoki Itoh MD
Eitetsu Koh MD
Yoshiaki Kumamoto MD
Tadashi Matsuda MD
Kouei Muguruma MD
Hajime Nawata MD
Haruaki Sasaki MD
Taro Shuin MD
Masanobu Tanimura MD
Akira Tsujimura MD
Hiromi Yokoyama MD

IJIR Open

International Journal of Impotence Research (2009) 21, 207–212

0955-9930/09 $32.00
www.nature.com/ijir

ORIGINAL ARTICLE

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

Y Kobori[1], E Koh[1], K Sugimoto[1], K Izumi[1], K Narimoto[1], Y Maeda[1], H Konaka[1], A Mizokami[1], T Matsushita[2], T Iwamoto[3] and M Namiki[1]

[1]*Department of Integrative Cancer Therapy and Urology, Kanazawa University Graduate School of Medical Science, Kanazawa, Ishikawa, Japan;* [2]*Ofuna Chuo Hospital, Ofuna, Kanagawa, Japan and* [3]*IUHW Hospital, Nasushiobara, Tochigi, Japan*

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.
International Journal of Impotence Research (2009) **21,** 207–212; doi:10.1038/ijir.2009.14; published online 7 May 2009

Keywords: testosterone; cortisol; erectile dysfunction; liquid chromatography/tandem mass spectrometry

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).[1] 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.[2] 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.[3] 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.[4] It has been reported that Total-T does not vary, but Free-T decreases gradually with aging.[5]

Cortisol (F), which is an adrenal cortical hormone, does not show changes in level with aging, but is increased by stress.[6] 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

Correspondence: Dr Y Kobori, Department of Integrative Cancer Therapy and Urology, Kanazawa University, 13-1 Takaramachi, Kanazawa, Ishikawa 920-8640, Japan.
E-mail: bori2555@mail.goo.ne.jp
Received 7 November 2008; revised 2 April 2009; accepted 2 April 2009; published online 7 May 2009

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.[7] Here, we developed methods to measure testosterone and cortisol levels in the blood and saliva by liquid chromatography/tandem mass spectrometry (LC/MS-MS).[8] 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 ± 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.[9] 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.[10] 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
Testosterone							
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
Cortisol							
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.[11] 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 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.

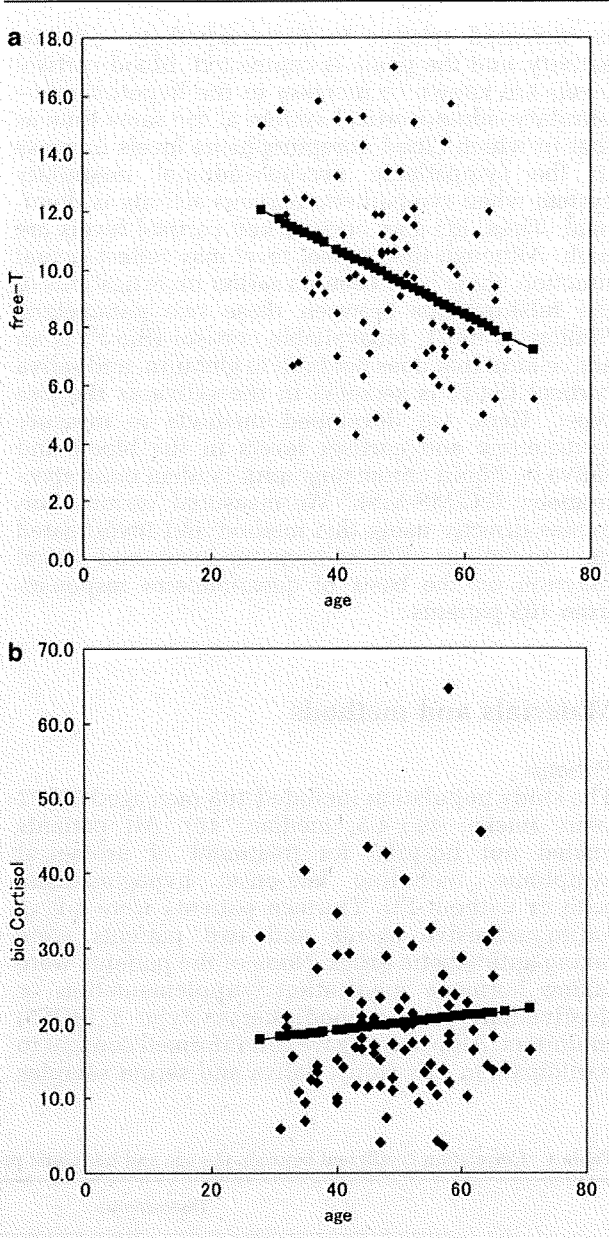

Figure 1 (**a**) Free testosterone showed significant inverse correlations with age ($n=103$, $R=-0.35$, $P<0.001$). (**b**) On the other hand, bioavailable cortisol did not have a meaningful correlation with age ($n=103$, $R=0.10$, $P=0.10$).

Discussion

It has been suggested that androgen insufficiency disrupts cellular signaling pathways and produces pathological alterations in penile tissues, leading to ED.[12] 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.[13,14] The weak relationship between low T and ED is well known and supported by the results of both human and animal studies.[15] 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.[16] 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.[17] 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.[18] 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.[19–21] 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 2 Baseline characteristics and hormonal level (the use of antidepressants vs the non-use of antidepressants)

	Number of patients (%)		
	Not take (n = 39)	*Take (n = 64)*	*P-value*
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 ± s.d.		
Age	48.38 ± 10.11	49.76 ± 9.78	0.52
BMI (kg/m^2)	22.41 ± 2.78	23.77 ± 2.64	0.44
HAD scale			
Depression	8.13 ± 4.16	11.23 ± 4.2	<0.01
Anxiety	7.68 ± 3.61	9.45 ± 4.29	0.02
Total score	15.81 ± 6.8	20.55 ± 7.5	<0.01
Testosterone			
Total (ng ml^{-1})	4.77 ± 1.60	3.86 ± 2.44	0.03
Free (pg ml^{-1})	10.20 ± 2.85	9.27 ± 3.43	0.16
Bio (pg ml^{-1})	965.73 ± 359.81	841.45 ± 383.46	0.11
Saliva (pg ml^{-1})	55.70 ± 23.57	55.91 ± 29.81	0.97
Cortisol			
Total (ng ml^{-1})	108.42 ± 35.05	100.89 ± 42.34	0.35
Bio (ng ml^{-1})	20.58 ± 10.78	19.41 ± 9.95	0.58
Saliva (ng ml^{-1})	2.36 ± 1.57	1.91 ± 1.26	0.13

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*

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

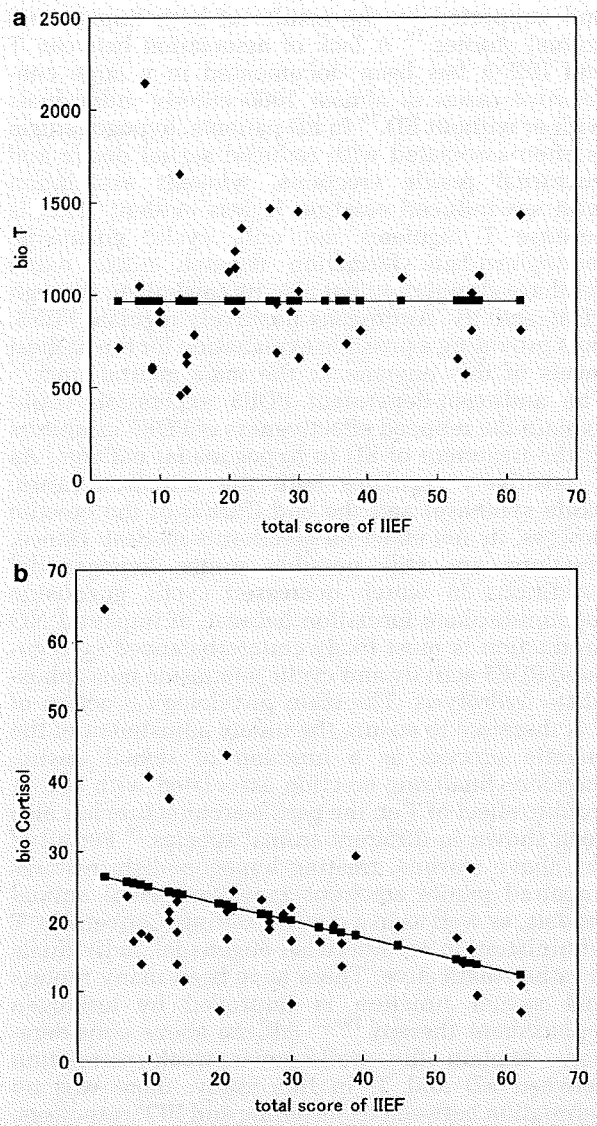

Figure 2 (**a**) Bioavailable testosterone did not show a meaningful correlation with the total score of IIEF ($n=32$, $R=-0.01$, $P=0.98$). (**b**) Bioavailable cortisol showed negative correlations with the total score of IIEF score ($n=32$, $R=-0.43$, $P<0.05$).

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.[22] In a human study, on the other hand, there were no differences in cortisol levels between individuals with and without ED.[23] 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.[24,25] 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 (100–150 ng ml^{-1}). We can analyze cortisol from saliva if we use a high sensitivity analytical procedure such as ELISA or LC-MS/MS.[26] 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.[27] 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.[28] Patients with depression may have elevated cortisol levels.[29] In addition, this type of drug could result in an increase in the prolactin level determining the reduction of T.[30] 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.

References

1 Hwang TIS, Lo HC, Tsai TF, Chiou HY. Association among hypogonadism, quality of life and erectile dysfunction in

middle-aged and aged male in Taiwan. *Int J Impot Res* 2007; **19**: 69–75.
2 Morales A, Lunenfeld B. Standards, guidelines and recommendations of the international society for the study of the aging male (ISSAM). Investigation, treatment and monitoring of late-onset hypogonadism in males. Official recommendations of ISSAM. *Aging Male* 2002; **5**: 74–86.
3 Morley JE. The diagnosis of late life hypogonadism. *Aging Male* 2007; **10**: 217–220.
4 Shirtcliff EA, Granger DA, Likos A. Gender differences in the validity of testosterone measured in saliva by immunoassay. *Horm Behav* 2002; **42**: 62–69.
5 Ellison PT, Bribiescas RG, Bentley GR, Campbell BC, Lipson SF, Panter-Brick C *et al.* Population variation in age-related decline in male salivary testosterone. *Hum Reprod* 2002; **17**: 3251–3253.
6 Kumsta R, Entringer S, Hellhammer DH, Wust S. Cortisol and ACTH responses to psychosocial stress are modulated by corticosteroid binding globulin levels. *Psychoneuroendocrinology* 2007; **32**: 1153–1157.
7 Becker JB, Arnold AP, Berkley KJ, Blaustein JB, Eckel LA, Hampson E *et al.* Strategies and methods for research on sex differences in brain and behavior. *Endocrinology* 2005; **146**: 1650–1673.
8 Honma S, Okuyama M, Kumagai Y. The estimation of bioavailable testosterone in serum using concanavalin A. *Med Sci Digest* 2005; **31**: 99–103.
9 Higashi T. Trace determination of steroids causing age-related diseases using LC/MS combined with detection-oriented derivatization. *Chem Pharm Bull* 2006; **54**: 1479–1485.
10 Rosen RC, Riley A, Wagner G, Osterloh IH, Kirkpatrick J, Mishra A. The international index of erectile function (IIEF): a multidimensional scale for assessment of erectile dysfunction. *Urology* 1997; **49**: 822–830.
11 Rosen RC, Cappellori JC, Smith MD, Lipsky J, Pena BM. Development and evaluation of an abridged, 5-item version of the international index of erectile function (IIEF-5) as a diagnostic tool for erectile dysfunction. *Int J Impot Res* 1999; **11**: 319–326.
12 Traish AM, Goldstein I, Kim NN. Testosterone and erectile function: from basic research to a new clinical paradigm for managing men with androgen insufficiency and erectile dysfunction. *Euro Urol* 2007; **52**: 54–70.
13 Martinez-Jabaloyas JM, Quepo-Zaragoza A, Hernandez-pastor F, Gil-salom M, Chuan-Nuez P. Testosterone levels in men with erectile dysfunction. *BJU Int* 2006; **97**: 1278–1283.
14 Mikhail N. Does testosterone have a role in erectile function? *Am J Med* 2006; **119**: 373–382.
15 Morelli A, Corona G, Filippi S, Ambrosini S, Forti G, Vignozzi L *et al.* Which patients with sexual dysfunction are suitable for testosterone replacement therapy? *J Endocrinol Invest* 2007; **30**: 880–888.
16 Rhoden EL, Teloken C, Sogari PR, Souto CAV. The relationship of serum testosterone to erectile function in normal aging man. *J Urol* 2002; **167**: 1745–1748.
17 Morelli A, Filippi S, Mancina R, Luconi M, Vignozzi L, Marini M *et al.* Androgens regulate phosphodiesterase type 5expression and functional activity in corpora cavernosa. *Endocrinology* 2004; **145**: 2253–2263.
18 Traish AM, Park K, Dhir V, Kim NN, Moreland RB, Goldstein I. Effect of castration and androgen replacement on erectile function in a rabbit model. *Endocrinology* 1999; **140**: 1861–1868.
19 Greco EA, Spera G, Aversa A. Combining testosterone and PDE5 inhibitors in erectile dysfunction: basic rationale and clinical evidence. *Euro Urol* 2006; **50**: 940–947.
20 Shabsigh R. Testosterone therapy in erectile dysfunction and hypogonadism. *J Sex Med* 2005; **2**: 785–792.
21 Shabsigh R, Rajfer J, Aversa A, Traish AM, Yassin A, Kalinchenko SY *et al.* The evolving role of testosterone in the treatment of erectile dysfunction. *Int J Clin Pract* 2006; **60**: 1087–1092.
22 Penson DF, NG C, Rajfer J, Gonzalez-Cadavid NF. Adrenal control of erectile function and nitric oxide synthase in rat penis. *Endocrinology* 1997; **138**: 3925–3932.
23 Derouet H, Lehmann J, Stamm B, Luhl C, Romer D, Georg T *et al.* Age dependent secretion of LH and ACTH in healthy men and patients with erectile dysfunction. *Euro Urol* 2002; **41**: 144–154.
24 Mizuno T, Yotsuyanagi S, Kondo Y, Komatsu K, Ishiura Y, Nakamura Y *et al.* Dehydroepiandrosterone alleviates copulatory disorder induced by social stress in male rats. *J Sex Med* 2006; **3**: 612–618.
25 Niikura O, Yokoyama O, Komatsu K, Yotsuyanagi S, Mizuno T, Namiki M. A causative factor of copulatory disorder in rats following social stress. *J Urol* 2002; **168**: 803–809.
26 Vining RF, Mcginley RA, Maksvytis JJ, Ho KY. Salivary cortisol: a better measure of adrenal cortical function than serum cortisol. *Ann Clin Biochem* 1983; **20**: 329–335.
27 Yamaguchi M, Deguchi M, Wakasugi J, Ono S, Takai N, Higashi T *et al.* Hand-held monitor of sympathetic nervous system using salivary amylase activity and its validation by driver fatigue assessment. *Biosens Bioelectron* 2005; **21**: 1007–1014.
28 Araujo AB, Durante R, Feldman HA, Goldstein I, McKinlay JB. The relationship between depressive symptoms and male erectile dysfunction: cross-sectional results from the Massachusetts Male Aging Study. *Psychosom Med* 1998; **60**: 458–465.
29 Belmaker RH, Agam G. Major depressive disorder. *New Eng J Med* 2008; **358**: 55–68.
30 Corona G, Mannucci E, Fisher AD, Lotti F, Ricca V, Balecia G *et al.* Effect of Hyperprolactinemia in male patients consulting for sexual dysfunction. *J Sex Med* 2007; **4**: 1485–1493.