ORIGINAL RESEARCH—ENDOCRINOLOGY

Profile of Serum Testosterone Levels after Application of Testosterone Ointment (Glowmin) and Its Clinical Efficacy in Late-Onset Hypogonadism Patients

Toshiyasu Amano, MD,* Tetsuya Imao, MD,* Katsurou Takemae, MD,* Teruaki Iwamoto, MD,†‡ Katsunori Yamakawa, MD,‡ Katsuyuki Baba, MD,‡ Mariko Nakanome, BSc,‡ Hiroki Sugimori, MD,§ Toshiaki Tanaka, MD,§ Katsumi Yoshida, MD,§ Takuyuki Katabami, MD,¶ and Masami Tanaka, MD**

*Department of Urology, Nagano Red Cross Hospital, Nagano, Japan; †Center for Infertility and IVF, International University of Health and Welfare, Nasu, Tochigi, Japan; ‡Department of Urology, St. Marianna University, Kawasaki, Japan; §Department of Preventive Medicine, St. Marianna University, Kawasaki, Japan; ¶Department of Metabolism and Endocrinology, St. Marianna University, Kawasaki, Japan; **Department of Pharmacology, St. Marianna University, Kawasaki, Japan

DOI: 10.1111/j.1743-6109.2007.00689.x

ABSTRACT

Introduction. Testosterone replacement therapy has been applied to alleviate the various symptoms of late-onset hypogonadism (LOH) patients. Several routes are available for the administration of testosterone to LOH patients, and transdermal delivery is an attractive method above all.

Aim. The aim of this article was to clarify the profile of serum total testosterone (TT) and free testosterone (FT) levels after application of testosterone ointment (Glowmin [GL], Daito Pharmaceutical Co. Ltd., Tokyo, Japan) and its clinical efficacy in LOH patients.

Methods. Serum TT and FT levels were examined in healthy male volunteers and LOH patients after application of 3 mg of GL. Then, 50 LOH patients received 3 mg of GL twice daily on scrotal skin (6 mg/day) for 12 weeks. Subsequently, TT and FT levels immediately prior to GL application were compared with those at 1 hour after GL treatment. Furthermore, the clinical effects of GL in the aforementioned 50 LOH patients were estimated after 12 weeks of GL treatment.

Main Outcome Measures. Hormonal effects of GL were evaluated by serum TT and FT levels. Aging males symptoms (AMS), international index of erectile function (IIEF-5), and MOS 36-item short form Healthy Survey (SF-36) questionnaire were used to assess the clinical efficacy of GL for LOH patients.

Results. Maximum TT and FT values, which were detected 1–2 hours after application of a 2-cm line of GL (3 mg of testosterone) to scrotal skin, were not elevated beyond physiological levels; subsequently, these levels returned to circadian rhythm after 4 hours in four healthy male volunteers. The highest TT and FT levels were also obtained after 1–2 hours in four LOH patients involving identical administration methods; moreover, these levels were maintained within a normal range for 6 hours. After 12 weeks of GL treatment in 50 LOH patients, TT and FT levels demonstrated the same satisfactory response as that of the initial GL administration without GL accumulation effects. GL accumulation after 1 week in healthy men and after 12 weeks in LOH patients was not observed. Furthermore, AMS score decreased markedly; IIEF-5 and four domains of the SF-36 score were elevated significantly following GL application. Severe adverse reactions were not observed.

Conclusions. Accordingly, GL, which is a short-acting testosterone ointment eliciting physiological elevation of TT and FT, appears to be suitable for LOH treatment. **Amano T, Imao T, Takemae K, Iwamoto T, Yamakawa K, Baba K, Nakanome M, Sugimori H, Tanaka T, Yoshida K, Katabami T, and Tanaka M. Profile of serum testosterone levels after application of testosterone ointment (Glowmin) and its clinical efficacy in late-onset hypogonadism patients. J Sex Med 2008;5:1727–1736.**

Key Words. Testosterone Ointment; Serum Testosterone Level; Low-onset Hypogonadism

Introduction

According to the recommendation of the International Society for the Study of the Aging Male (ISSAM), late-onset hypogonadism (LOH) is defined as a clinical and biochemical syndrome associated with advancing age characterized by typical symptoms and deficiency in serum testosterone levels [1]. Testosterone replacement therapy (TRT) has been applied in order to alleviate the various symptoms of LOH patients [2–6]. Several testosterone formulations are available, including injectable, oral, buccal, transdermal, and subcutaneous preparations [2]. However, Japanese andrologists typically administer only testosterone enanthate injection for the treatment of LOH; additionally, newer testosterone agents are not permitted for current clinical use under Japanese regulations of the Ministry of Labor and Public Health. Under these difficult circumstances, we discovered a testosterone ointment over the counter (OTC); this preparation was approved by the Ministry of Public Health in 1965. This ointment (Glowmin [GL], Daito Pharmaceutical Co. Ltd., Tokyo, Japan) contains 100 mg of testosterone per 10 g of matrix (1%).

The objective of this study was to clarify the profile of serum total testosterone (TT) and free testosterone (FT) levels following GL application in healthy male volunteers and LOH patients. Furthermore, the clinical efficacy of GL in the treatment of LOH was investigated employing the aging males symptoms (AMS) scale [7], the developed international index of erectile function (IIEF-5) [8], and the MOS 36-item short form Healthy Survey (SF-36) [9] questionnaire.

Methods

According to the manufacturer's instructions, GL testosterone ointment (Daito Pharmaceutical Co. Ltd.) contains 100 mg of testosterone per 10 g of matrix (1%). A 2-cm line of GL applied to the skin contains 3 mg of testosterone.

The following four designed studies were evaluated:

1. Serum TT and FT levels were measured in four healthy male volunteers aged 40, 47, 49, and 59 years. Blood samples were obtained at 10:00 AM, 10:30 AM, 11:00 AM, 12:00 PM, 2:00 PM, 6:00 PM, 9:00 PM, 9:30 PM, 10:00 PM, and 6:00 AM; circadian rhythms of TT and FT were examined. On the following day, serum TT and FT levels were estimated in the four subjects following application of a 2-cm line of GL (3 mg of testosterone) to scrotal skin twice daily (immediately after the blood sampling at 10:00 AM and 9:00 PM); subsequently, these data were compared with the respective circadian rhythm. In addition, the profiles of serum TT and FT levels were observed after 1 week of consecutive administration of GL in these four men.
2. The criteria of LOH were defined as serum TT level was <2.7 ng/mL, and FT level was <10.0 pg/mL according to recent Japanese LOH guideline [10]. The profiles of TT and FT were assessed in four LOH patients 120–360 minutes following a single application of 3 mg of GL to scrotal skin.
3. Fifty LOH patients received 3 mg of GL twice daily on scrotal skin (6 mg/day) for 12 weeks. Subsequently, TT and FT levels immediately prior to GL application were compared with those at 1 hour after GL treatment. Blood samples were obtained from all patients in the morning. Patient ages ranged from 34 years to 81 years (mean 55.5 ± 11.4); serum TT level was <2.7 ng/mL and FT level was <10.0 pg/mL. Patients displaying prostate specific antigen (PSA) levels exceeding 4.0 ng/mL were excluded from this study. Any adverse reactions were reported. Serum TT and FT levels were measured via radioimmuno assay methods employing the Diagnostic Products Corporation (DPC)/testosterone kit for TT and the DPC/FT kit for FT (Mitsubishi Chemical Yatoron; Tokyo, Japan).

The blood sampling tests, such as luteinizing hormone (LH), follicle-stimulating hormone, PSA, liver function (glutamic–oxaloacetic transaminase, glutamic–pyruvic transaminase), lipid (cholesterol, triglyceride), and hemoglobin, were measured before and 12 weeks after GL treatments.

Laboratory reference values for testosterone vary widely in a U.S. study [11]. And there are some technical problems in these methods to determine serum levels of TT and FT [12]. However, these are the only available methods to measure TT and FT levels right now in Japanese medical systems. Calculated FT decided by the ISSAM formula and FT measured by these methods were correlated significantly [10]. Precision on FT determined by these methods is confirmed [10], and coefficient of variation is 11.6% [13]. In addition, it is almost impossible to measure dihydrotestosterone (DHT) levels, because of medical costs and technical problems under

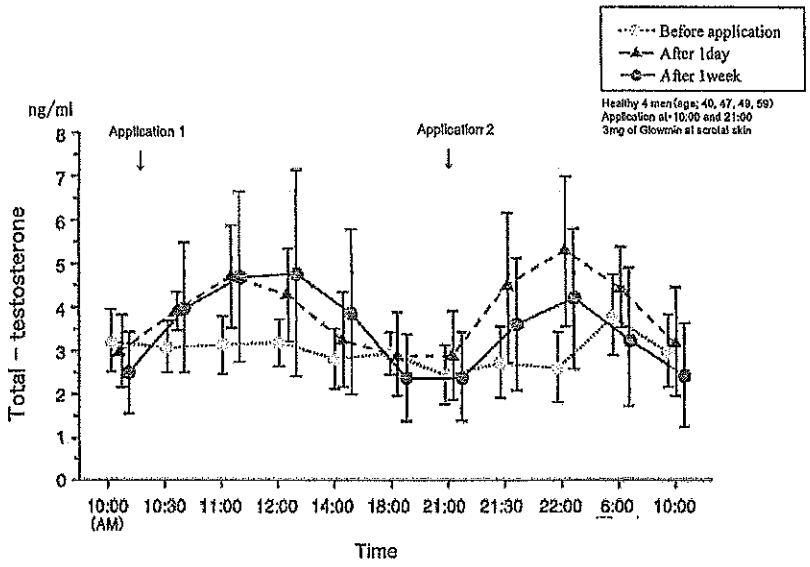

Figure 1 Circadian rhythm of serum total testosterone levels and profile following application of 3 mg of Glowmin to scrotal skin in four healthy men.

Japanese regulations of the Ministry of Labor and Public Health.

4. The clinical effects of GL in the aforementioned 50 LOH patients were estimated by AMS [7], IIEF-5 [8], and the SF-36 [9] questionnaire after 12 weeks of GL treatment.

Statistical analysis was performed by paired *t*-test.

This study was approved by the Institutional Review Board from Nagano Red Cross Hospital and St. Marianna University. Informed consent was obtained from all participants in the four studies.

Results

1. Serum TT and FT levels in healthy male volunteers, which were high in the morning, diminished in the evening. The four healthy male volunteers were aged 40, 47, 49, and 59 years. These data revealed that testosterone circadian rhythms were maintained in middle-aged men. However, their circadian rhythms greatly blunted compared with young men, and the data obtained from these four men are compatible with recent data that testosterone levels in older men are stable throughout the morning and early afternoon, declining only modestly thereafter [14].

Peak TT (Figure 1) and FT (Figure 2) levels, which were observed after 60–120 minutes, were not elevated beyond physiological levels. Subsequently, TT and FT levels returned to circadian rhythm after 4 hours in healthy men. The profiles of serum TT and FT levels were almost the same

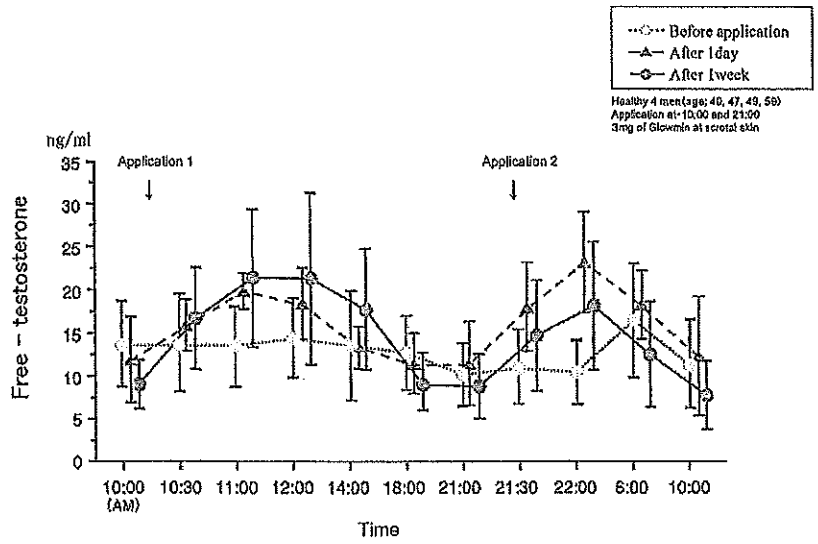

Figure 2 Circadian rhythm of serum free testosterone levels and profile following application of 3 mg of Glowmin to scrotal skin in four healthy men.

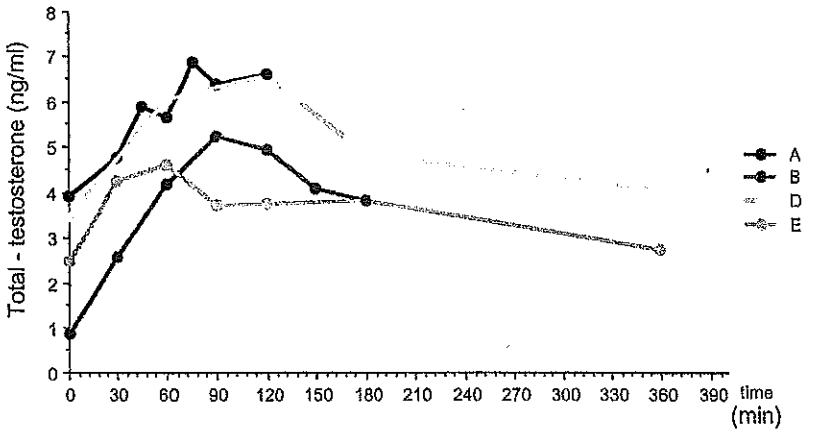

Figure 3 Serum total testosterone profile in four late-onset hypogonadism patients following application of 3 mg of Glowmin.

patterns after 1 week of consecutive GL application for scrotal skin (Figures 1 and 2).

Based on our data, we calculated that C_{max} was 4.84 ng/mL and C_{ave} was 3.41 ng/mL, respectively. However, individual variations were rather large. Thus, we also calculated $T_{1/2}$. The results were that $T_{1/2}$ was 8.46 hours at the first administration of GL, and 8.24 at 1 week after GL administration. These data were very similar, and these were interpreted that there was no accumulation of GL when GL was administered every 11–13 hours.

2. In four LOH patients, peak TT (Figure 3) and FT (Figure 4) levels also occurred after 60–120 minutes. TT and FT levels were maintained within the normal range for 6 hours.
3. In 50 LOH patients following 12 weeks of application of 3 mg of GL twice daily (total, 6 mg testosterone/day) to scrotal skin, TT and FT levels immediately prior to GL treatment were 2.5 ± 1.1 ng/mL and 8.1 ± 4.3 pg/mL, respectively; these values were not significantly different from pretreatment data. However, TT and FT readings at 1 hour after GL application were 5.5 ± 2.4 ng/mL and 13.3 ± 6.1 pg/mL, respectively (Table 1). These data demonstrated that serum TT and FT levels were markedly elevated 1 hour after GL administration relative to those levels observed prior to treatment (paired *t*-test) (Figure 5). And these elevations revealed the same good responses as the initial GL administration. These findings were indicative of the remote possibility of GL accumulation in the body. The results of other blood sampling tests before and 12 weeks after GL administrations are shown in Table 2. No serious abnormal data were observed, and there are no significant differences between pre- and post-GL treatment, except significant decline of LH levels ($P = 0.0048$).
4. In 50 LOH patients treated with GL for 12 weeks, the AMS score, including mental factor, physical factors, and sexual function factor, decreased significantly (Figure 6). The IIEF-5 score increased markedly following the 12-week GL regime (Figure 7). In addition, SF-36 domain scores of role physical (RP),

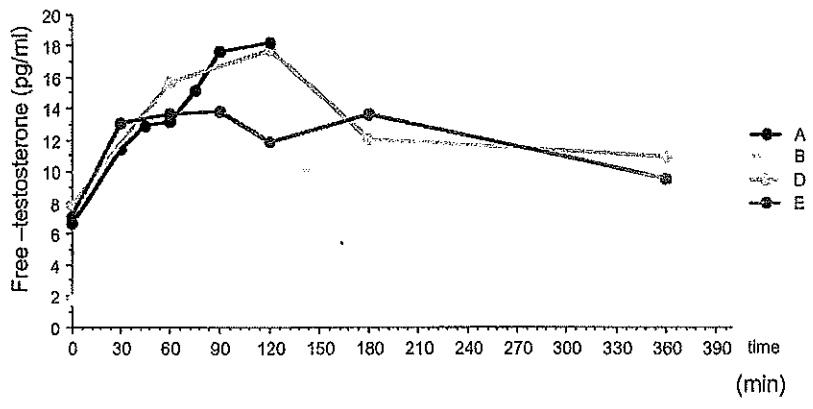

Figure 4 Serum free testosterone profile in four late-onset hypogonadism patients following application of 3 mg of Glowmin.

Table 1 Serum total testosterone (TT) and free testosterone (FT) levels before and 12 weeks after application of Glowmin (GL) in hypogonadism (late-onset hypogonadism) patients

		Mean ± SD	$n = 50$
		12 weeks after GL treatment	
	Before GL treatment Before application	Before application	1 hour after application
TT (ng/mL)	2.7 ± 1.0	2.5 ± 1.1	5.5 ± 2.4*
FT (pg/mL)	7.4 ± 2.5	8.1 ± 4.3	13.3 ± 6.1**

*$P \leq 0.005$ (before treatment vs. 1 hour after application).
**$P \leq 0.0001$ (before treatment vs. 1 hour after application).

social functioning (SF), role emotional (RE), and mental health (MH) improved substantially (Figure 8) after 12 weeks of GL treatment. The three questionnaires (AMS, IIEF-5, and SF-36) are validated internationally. These results indicated that several symptoms of LOH, such as physical, psychological, and sexual symptoms, were relieved by application of GL.

Three subjects reported adverse reactions: two complained regarding difficulties associated with urination and one displayed seborrhea. Adverse reactions were not serious. Nearly all participants described a rather smooth sensation and not a sticky sensation upon application of GL to scrotal skin.

Discussion

LOH patients exhibit various symptoms, which include physical, psychological, and sexual aspects. To alleviate these symptoms, palliative treatments, including Chinese herbal medicine [3], selective

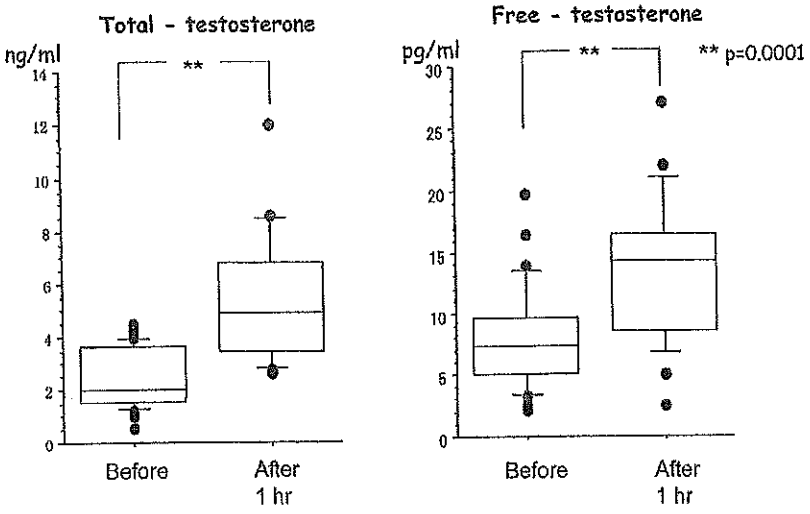

Figure 5 Serum total testosterone and free testosterone levels before and 1 hour after application of Glowmin in 50 late-onset hypogonadism patients during 12-week Glowmin regime.

Table 2 Blood sampling tests before and 12 weeks after application of Glowmin in Hypogonadism (late-onset hypogonadism) patients

	Before	12 weeks after	
LH (mIU/mL)	4.53 ± 3.63	2.57 ± 1.90	$P = 0.0048$
FSH (mIU/mL)	9.39 ± 6.67	6.64 ± 7.47	NS
PSA (ng/mL)	1.10 ± 0.95	1.22 ± 0.99	NS
GOT (IU/L)	21.6 ± 6.4	23.0 ± 5.7	NS
GPT (IU/L)	28.0 ± 20.7	24.3 ± 9.3	NS
Cholesterol (mg/dL)	196.4 ± 66.9	200.3 ± 26.9	NS
TG (mg/dL)	137.2 ± 74.6	174.1 ± 118.9	NS
Hemoglobin (g/dL)	14.8 ± 1.2	14.8 ± 1.0	NS

LH, luteinizing hormone; FSH, follicle-stimulating hormone; PSA, prostate specific antigen; GOT, glutamic–oxaloacetic transaminase; GPT, glutamic–pyruvic transaminase; TG, triglyceride; NS, not significant.

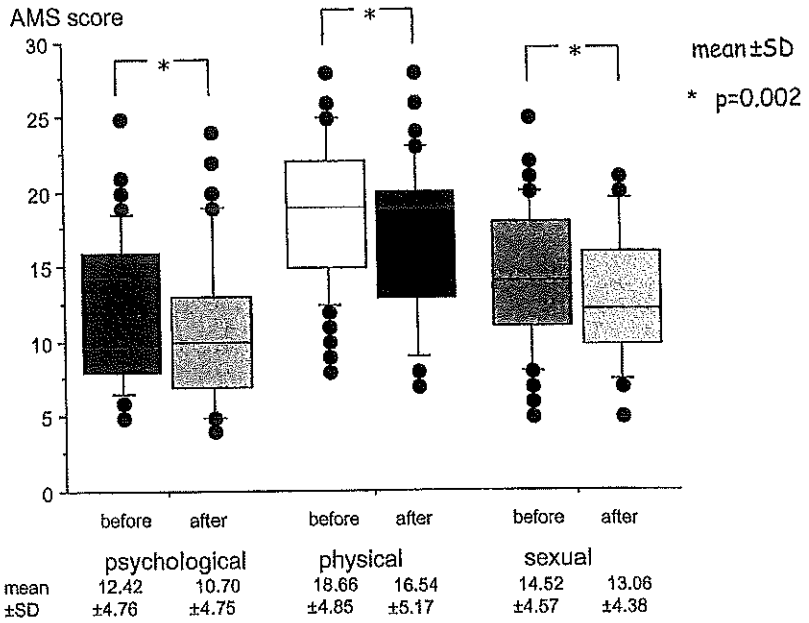

Figure 6 Aging males symptoms (AMS) score levels prior to and after 12-week Glowmin regime in 50 late-onset hypogonadism patients. Each domain including psychological, physical, and sexual improved following Glowmin treatment.

serotonin reabsorption inhibitor for depression [4], phosphodiesterase type 5 (PDE 5) inhibitors for erectile dysfunction (ED) [5,6], etc., have been applied. However, the basic essential treatment for LOH involves TRT. Although hormonal therapy employing human chorionic gonadotropin (hCG) is also available [15], TRT is an important and indispensable treatment for LOH [16–19]. The following criteria were proposed as desirable characteristics of the ideal hormone replacement therapy [19]: (i) it should produce levels consistent with physiological testosterone in the blood; (ii) it should restore circadian variation; and (iii) it should produce normal ratios of DHT/testosterone and E_2/testosterone.

In our first study, circadian rhythms of TT and FT were observed even in healthy middle-aged men; however, their circadian rhythms greatly blunted compared with young men [11]. GL is a proved short-acting agent, which influences serum TT and FT on the order of 4–6 hours. Furthermore, GL accumulation after 1 week in healthy men and after 12 weeks in LOH patients was not evident in this investigation.

Several routes are available for the administration of testosterone, e.g., injection, oral, buccal, transdermal, and subcutaneous [20]. The most convenient, tolerable route is likely the oral approach. Methyltestosterone (Enarmon) is available. However, absorption of methyltestosterone (Enarmon) in the small intestine is not efficient, and side effects associated with liver dysfunction are rather common; thus, this drug is not utilized for LOH [20]. Testosterone undecanoate (Andriol) is a promising oral agent [21], which induces normal serum testosterone levels for 8 hours [20] with less liver dysfunction (Nebido (Schering) data book). However, testosterone undecanoate (Andriol) is not available in Japan at present. Accordingly, intramuscular injection of testosterone enanthate (Enarmon depot) is widely indicated for treatment of LOH in Japan. The profile of serum testosterone levels following injection of testosterone enanthate (Enarmon depot) is much

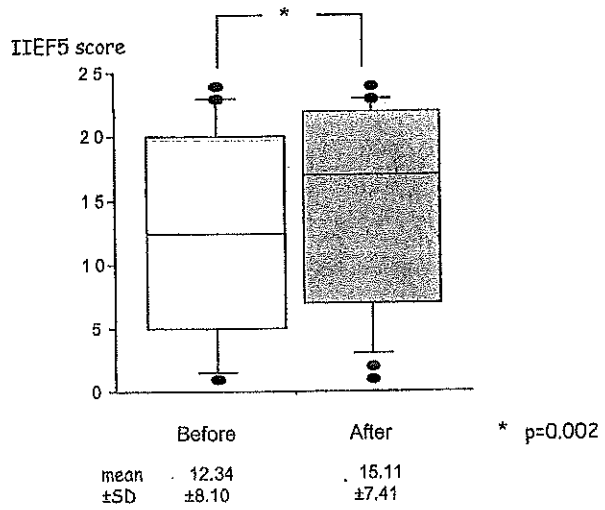

Figure 7 International index of erectile function (IIEF-5) score levels prior to and after 12-week Glowmin regime in 50 late-onset hypogonadism patients. IIEF-5 scores also improved following Glowmin treatment.

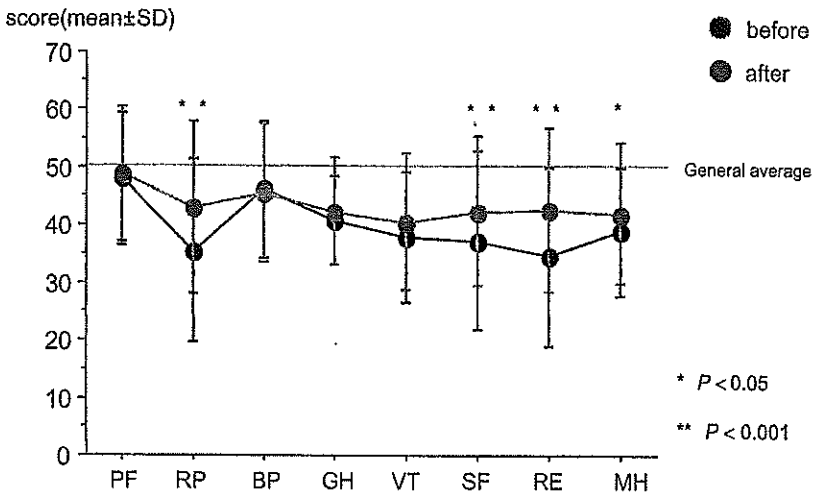

Figure 8 Short form-36 score levels prior to and after 12-week Glowmin regime in 50 late-onset hypogonadism patients. Four domains (RP, SF, RE, and MH) improved following Glowmin treatment. PF, physical functioning; RP, role physical; BP, bodily pain; GH, general health; VT, vitality; SF, social functioning; RE, role emotional; MH, mental health.

higher than the normal range; moreover, these levels decrease quickly, returning to the original low level within 2–3 weeks [22]. Testosterone undecanoate (Nebido) is a long-acting intramuscular injectable drug [20], which maintains normal serum testosterone levels 12 weeks after injection (Nebido (Schering) data book). Testosterone undecanoate is rather well-tolerated by LOH patients. However, the use of testosterone undecanoate is not permitted in Japan.

Transdermal delivery is an attractive method for administration of testosterone to LOH patients. Two main forms (gel and patch) are available [20,23,24]; furthermore, serum testosterone levels are maintained within the physiological range by daily usage (Testogel (Schering) data book). Adverse effects, including skin eruption, contact dermatitis, and itching, are observed on occasion; however, transdermal application is less invasive and bodes well with respect to patient tolerance. However, these gel and patch testosterone preparations are not available in Japan either. Consequently, intramuscular injection of testosterone enanthate (Enarmon depot) for TRT in LOH patients is the only avenue available to Japanese urologists and andrologists. Under these difficult circumstances, we discovered the existence of testosterone ointment (GL) in the form of an OTC (no prescription requirement) preparation, which was approved by the Japanese Ministry of Public Health in 1965. The indications for GL are erectile dysfunction, ejaculation disturbance, hypogonadism, decreased libido, etc., attributable to low serum testosterone level. Our preliminary reports suggested that GL appeared to be a short-acting testosterone ointment [25], which might possibly treat LOH [26,27].

Miwa et al. reported that LOH symptoms were not significantly related to serum levels of TT and FT [28]. However, in the current study, elevation of serum testosterone levels and improvement of AMS (mental factor, physical factors, and sexual function factor), IIEF-5, and SF-36 scores (bodily pain, SF, RE, and MH) were observed after 12 weeks of treatment with GL. Adverse reactions were mild; two patients complained of difficulties associated with urination and one exhibited seborrhea. The characteristics of GL were assessed in healthy men and LOH patients in detail in this investigation. Our data indicated that GL is a promising agent for LOH patients despite the restricted clinical use of similar agents in Japan. At the onset of this research, GL was applied twice daily to scrotal skin per the original GL instructions. Our results revealed that GL induced mild elevation of serum testosterone level for 4–6 hours. Although their circadian rhythms greatly blunted compared with young men [14], their circadian rhythms of serum TT and FT were also detected in healthy middle-aged men. However, the physiological functions of these circadian rhythms are not clear.

The skin site facilitating the highest rate of absorption of steroid ointments appears to be the scrotum [29]. However, a larger volume of GL will be required for once daily application; additionally, the application area of scrotal skin is limited. Moreover, the issue of transfer of ointment to clothing must be resolved [30]. Consequently, we are currently examining the profile of serum TT and FT levels following GL application to other skin sites with respect to transfer to clothing.

Reference ranges of serum TT and FT in Japanese adult males were reported in 2004 [13]. Based

on these data, the reference range of TT was established as 2.01–7.50 ng/mL; moreover, that of FT was classified for every decade subgroup as the mean ± 2 SD because of the strong influence of aging on FT [13]. In addition, the 80% and 70% values of the young adult mean (males 20–39 years of age) were 12.4 pg/mL and 10.9 pg/mL, respectively; these values had been proposed as a guideline for TRT in Japan [13]. In this study, LOH patients characterized by serum TT level <2.7 ng/mL or FT level <10.0 pg/mL were treated. The current experimental protocol was designed prior to the determination of these criteria. The values are not identical; however, no significant difference is evident. Consequently, the selection criteria for our LOH patients are believed to be adequate.

During the 12-week GL regime, two of the 50 LOH patients complained of difficulties associated with urination and one exhibited seborrhea. These adverse reactions were not serious. The results of blood sampling tests reveal no abnormal data either no significant changes except decline of LH levels 12 weeks after GL treatments, including PSA, liver and lipid, function, and polycythemia. To conduct TRT in LOH patients, several adverse effects must be considered, including liver dysfunction, polycythemia, sleep apnea, lipid metabolic abnormality, prostate cancer, etc. The aforementioned untoward effects were not observed in the present study. The observation period was 12 weeks. At the very least, no serious side effects related to GL were evident during this short interval. However, late-onset adverse reactions, particularly the risk of prostate cancer, are very important. Reduction of testosterone levels in prostate cancer patients leads to regression of cancer; thus, TRT in hypogonadal men in the presence of suspected or confirmed prostate cancer is contraindicated [31]. The relationship between TRT and risk of prostate cancer is a contradiction. Several articles noted that the level of PSA was not significantly altered after 1 year of TRT [32], and that the prevalence rate of TRT patients in terms of prostate cancer was similar to that of the general population for 6-month to 36-month follow-up periods [33]. On the other hand, other reports demonstrated that prostate cancer might become clinically apparent within months to a few years following the initiation of TRT [34]. Although recent review article indicated that there has not been a scientific basis for the belief that testosterone causes prostate cancer to grow [35], PSA measurement appears to be recommended for LOH patients receiving TRT over a duration of several years at present.

Conclusion

The current data demonstrated that GL is a short-acting testosterone ointment capable of moderate elevation of TT and FT. Treatment of LOH patients with GL with respect to the circadian rhythms of serum TT and FT levels appears to be beneficial. The clinical effects of GL observed in LOH patients include mental factor, physical factors, and sexual function factor in AMS; erectile dysfunction in IIEF-5; and RP, SF, RE, and MH in the SF-36 questionnaire. Untoward effects were not serious. Thus, GL is believed to be an efficacious, safe agent for TRT in LOH patients.

Corresponding Author: Toshiyasu Amano, MD, Department of Urology, Nagano Red Cross Hospital, Wakasato 5-22-1, Nagano, 380-8582, Japan. Tel: +81-26-226-4131; Fax: +81-26-224-1065; E-mail: amanot@nagano-med.jrc.or.jp

Conflict of Interest: None declared.

Statement of Authorship

Category 1

(a) **Conception and Design**
Toshiyasu Amano; Teruaki Iwamoto

(b) **Acquisition of Data**
Toshiyasu Amano; Tetsuya Imao; Tetsurou Takemae; Katsunori Yamakawa; Katsuyuki Baba

(c) **Analysis and Interpretation of Data**
Toshiyasu Amano; Mariko Nakanome; Hiroki Sugimori; Toshiaki Tanaka; Katsumi Yoshida; Takuyuki Katabami; Masami Tanaka

Category 2

(a) **Drafting the Article**
Toshiyasu Amano

(b) **Revising It for Intellectual Content**
Toshiyasu Amano; Teruaki Iwamoto; Katsumi Yoshida; Takuyuki Katabami; Masami Tanaka

Category 3

(a) **Final Approval of the Completed Article**
Toshiyasu Amano; Teruaki Iwamoto

References

1 Nieschlag E, Swerdloff R, Behre HM, Gooren LJ, Kaufman JM, Legros JJ, Lunenfeld B, Morley JE, Schulman C, Wang C, Weidner W, Wu FCW.

Investigation, treatment and monitoring of late-onset hypogonadism in males. Aging Male 2005; 8:56–8.
2 Lunenfeld B, Saad F, Hoesl CE. ISA, ISSAM and EAU recommendation for the investigation, treatment and monitoring of late-onset hypogonadism in males: Scientific background and rationale. Aging Male 2005;8:59–74.
3 Amano T, Kobori Y, Matsushita T, Takemae K. The role of Chinese herbal medicine in the treatment of partial androgen deficiency I aged male. Jpn J Impotence Res 2003;18:307–11.
4 Seidman SN, Rabkin JG. Testosterone replacement therapy for hypogonadal men with SSRI-refractory depression. J Affect Disord 1998;48:157–61.
5 Shamioui R, Ghanem H, Fahmy I, El-Meleigy A, Ashoor S, Elnashaar A, Kamei I. Testosterone therapy can enhance erectile function response to sildenafil in patients with PADAM: a pilot study. J Sex Med 2005;2:559–64.
6 Aversa A, Isidori AM, Greco EA, Giannetta E, Gianfrilli D, Spera E, Fabbri A. Hormonal supplementation and erectile dysfunction. Eur Urol 2004; 45:535–8.
7 Heinemann LAJ, Zimmermann T, Vermeulen A, Thiel C, Hummel W. A new "aging males' symptoms" rating scale. Aging Male 1999;2:105–14.
8 Rosen RC, Cappelleri 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;11:319–26.
9 Fukuhara S, Ware JE, Kosinski M, Wada S, Gandek B. Psychometric and clinical tests of validity of the Japanese SF-36 healthy survey. J Clin Epidemiol 1998;51:1045–53.
10 Iwamoto T, Baba K. Diagnosis. In: Japanese Urological Association and Japanese Men's Health Association, eds. Guideline for LOH symptoms. Tokyo, Japan: Jiho Co.; 2007:51–8.
11 Lazarou S, Reyes-Vallejo L, Morgentaler A. Wide variability in laboratory reference values for serum testosterone. J Sex Med 2006;3:1085–9.
12 Rosner W, Auchus RJ, Sluss PM, Raff H. Position statement: Utility, limitations, and pitfalls in measuring testosterone: An endocrine society position statement. J Clin Endocrinol Metab 2007;92:405–13.
13 Iwamoto T, Yanase T, Koh E, Horie H, Baba K, Namiki M, Nawata H. Reference ranges of serum total and free testosterone in Japanese male adults. Nippon Hinyokika Gakkai Zasshi 2004;95:751–60.
14 Crawford ED, Barqawi AB, O'donnel C, Morgentaler A. The association of time of day and serum testosterone concentration in large screening population. BJU Int 2007;100:509–13.
15 Tsujimura A, Matsumiya K, Takao T, Miyagawa Y, Takada S, Koga M, Iwasa A, Takeyama M, Okuyama A. Treatment with human chorionic gonadotropin for PADAM: A preliminary report. Aging Male 2005;8:175–9.
16 Basaria S, Dobs AS. Hypogonadism and androgen replacement therapy in elderly men. Am J Med 2001;110:563–72.
17 Morales A, Tenover JL. Androgen deficiency in the aging male: When, who, and how to investigate and treat. Urol Clin North Am 2002;29:975–82.
18 Tenover JL. Testosterone replacement therapy in older adult men. Int J Androl 1999;22:300–6.
19 Comhaire FH. Andropause: Hormone replacement therapy in the aging male. Eur Urol 2000;38:655–62.
20 Amano T. Androgen replacement therapy up-to-date ~current status in Japan and abroad~. Gender Sex Special Med (Jpn) 2006;3:65–70.
21 Kohn FM, Schill WB. A new oral testosterone undecanoate formulation. World J Urol 2003;21: 311–5.
22 Juul A, Skakkebaek NE. Androgens and the aging. Hum Reprod Update 2002;7:423–33.
23 An NM, Kim DD, Shin YH, Lee CH. Development of novel soft hydrogel for the transdermal delivery of testosterone. Drug Dev Ind Pharm 2003;29:99–105.
24 Mazer N, Bell D, Wu J, Fisher J, Cosgrove M, Eilers B. Comparison of the steady-state pharmacokinetics, metabolism, and variability of a transdermal testosterone patch versus a transdermal testosterone gel in hypogonadal men. J Sex Med 2005;2:213–26.
25 Amano T, Takemae K, Baba K, Nakazawa R, Nakanome M, Iwamoto T, Tanaka M, Yoshida K, Sugimori H, Tanaka T, Katabami T. Circadian rhythm and pharmacokinetic profiles of free testosterone after application of testosterone ointment in healthy men. Jpn J Impotence Res 2005;20:13–8.
26 Baba K, Nakazawa R, Nakanome M, Iwamoto T, Amano T, Tanaka T, Sugimori H, Yoshida K, Tanaka M, Katabami T. The effects of transdermal testosterone for hypogonadal men. Jpn J Impotence Res 2005;20:19–24.
27 Amano T, Imao T, Takemae K, Iwamoto T, Baba K, Yamakawa K, Nakazawa R, Yoshida K, Sugimori H, Tanaka T, Katabami T, Tanaka M. The efficacy of testosterone ointment (Glowmin) treatment for late-onset-hypogonadism (LOH) patients according to LOH severity. Hinyokika Kiyo 2007;53:25–9.
28 Miwa Y, Kaneda T, Yokoyama O. Correlation between the aging male's symptoms scale and sex steroids, gonadotropins, dehydroepiandrosterone sulfate, and growth hormone levels in ambulatory men. J Sex Med 2006;3:723–6.
29 Feldmann RJ, Maibach HI. Regional variation in percutaneous penetration of ^{14}C cortisol in man. J Invest Dermatol 1967;48:181–3.
30 Mazer N, Fisher D, Fisher J, Cosgrove M, Bell D, Eilers B. Transfer of transdermally applied testoster-

one to clothing: A comparison of a testosterone patch versus a testosterone gel. J Sex Med 2005; 2:227–34.
31 Kirby R, Gould D. Testosterone replacement therapy in hypogonadal men and prostate cancer risk. BJU Int 2005;96:471–2.
32 El-Sakka AI, Hassoba HM, Elbakry AM, Hassan HA. Prostate specific antigen in patients with hypogonadism: Effect of testosterone replacement. J Sex Med 2005;2:235–40.
33 Rhoden EL, Morgentaler A. Risk of testosterone-replacement therapy and recommendations for monitoring. N Engl J Med 2004;350:482–92.
34 Gaylis FD, Lin DW, Ignatoff JM, Amling CL, Tutrone R, Cosgrove DJ. Prostate cancer in men using testosterone supplementation. J Urol 2005; 174:534–8.
35 Morgentaler A. Testosterone and prostate cancer: A historical perspective on a modern myth. Eur Urol 2006;50:935–9.