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ORIGINAL ARTICLE

Male Aging

Effects of long-term androgen replacement therapy on the physical and mental statuses of aging males with late-onset hypogonadism: a multicenter, randomized controlled trial in Japan (EARTH Study)

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Androgen replacement therapy (ART) efficacy on late-onset hypogonadism (LOH) has been widely investigated in Western countries; however, it remains controversial whether ART can improve health and prolong active lifestyles. We prospectively assessed long-term ART effects on the physical and mental statuses of aging men with LOH in Japan. The primary endpoint was health-related quality of life assessed by questionnaires. Secondary endpoints included glycemic control, lipid parameters, blood pressure, waist circumference, body composition, muscular strength, International Prostate Symptom Scores (IPSS), International Index of Erectile Function-5 (IIEF-5) scores, and serum prostate-specific antigen levels. Of the 1637 eligible volunteers, 334 patients > 40 years with LOH were randomly assigned to either the ART (n = 169) or control groups (n = 165). Fifty-two weeks after the initial treatment, ART significantly affected the role physical subdomain of the short form-36 health survey (SF-36) scale (P = 0.0318). ART was also associated with significant decreases in waist circumstance (P = 0.002) and serum triglyceride (TG) (P = 0.013) and with significant increases in whole-body and leg muscle mass volumes (P = 0.071 and 0.0108, respectively), serum hemoglobin (P < 0.001), IPSS voiding subscore (P = 0.0418), and the second question on IIEF-5 (P = 0.0049). There was no significant difference between the groups in terms of severe adverse events. In conclusion, in patients with LOH, long-term ART exerted beneficial effects on Role Physical subdomain of the SF-36 scale, serum TG, waist circumstance, muscle mass volume, voiding subscore of IPSS, and the second question of IIEF-5. We hope our study will contribute to the future development of this area.

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INTRODUCTION

In men, the serum testosterone (T) levels remain stable until approximately 40 years of age, after which the circulating total T and biologically active free T levels decrease annually by 1%–2% and 2%–3%, respectively.[1,2] The Baltimore Longitudinal Study of Aging also observed low total T and free T index levels in approximately 20% and 35% of men aged 60–69 years, respectively.[3] The reduced total T levels appear to stabilize at approximately 70 years of age. In aging men, changes in the proximal part of the hypothalamic-pituitary-testicular axis lead to reduced T production;[2] therefore, hypogonadism, defined as a serum T level below the reference range for adult men, is common in older men. Symptomatic hypogonadism prevalence increases with age, particularly after the age 70 years.[4] However, most men > 40 years begin to exhibit more severe decreases in T levels, resulting in physical, psychological, and sexual symptoms.

Recently, the term late-onset hypogonadism (LOH) has been coined to describe a condition involving decreased T levels and hypogonadal symptoms; this clinical and biochemical syndrome is associated with advancing age and characterized by certain symptoms resulting from deficient serum T levels.[5] LOH recognition has been supported by a recent consensus among professional societies. Biochemical criteria to define LOH have been proposed by different international societies, including the European Academy of Andrology, International Society of Andrology, International Society for the Study of the Aging Male, European Association of Urology, American Society of Andrology, International Society for the Sexual Medicine, Endocrine Society, American Association of Clinical Endocrinologists.[6–9] According to

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these guidelines, the total T level has been used to diagnose biochemical hypogonadism. However, in Japan, it has been decided to recommend the free T level measurement as a LOH diagnostic examination, as specified in the "Clinical Practice Manual for LOH Syndrome;"[10] therefore, free T level measurements are widely used in Japan.[11,12]

Late-onset hypogonadism symptoms are logically associated with age-related T deficiencies, and therefore can be reversed by T administration. Such an androgen replacement therapy (ART) can be offered to men with LOH unless there are contraindications such as unstable cardiovascular disease, prostate cancer (PCa), or high hemoglobin levels; however, long-term treatment effects are uncertain. In recent decades, hypogonadism was thought to affect the health-related quality of life (HRQoL) in aging men but was considered unlikely to affect morbidity or mortality.[13] However, recent studies revealed that hypogonadism predicts the future development of type 2 diabetes mellitus (DM),[14] metabolic syndrome (MetS),[15] cardiovascular events,[16–19] mobility limitation,[20] frailty,[21] and mortality.[16–19] Although several studies regarding ART efficacy for LOH have been conducted in Western countries, most were small-scale and short-term surveys. Only a few randomized controlled trials (RCTs) involving > 100 patients and durations > 12 months have investigated ART efficacy for LOH.[22,23] Accordingly, ART is widely used to treat LOH, but evidence-based information regarding its real benefits and short- and long-term risks is not yet available, and therefore whether ART improves HRQoL and conserves daily living activities remains controversial. In addition, no large-scale RCT has investigated ART efficacy for LOH in Asian populations. Because ethnicity may affect serum T levels,[24–28] the results of a larger multicenter, long-term RCT conducted in Asia would be of great interest.

The present large-scale study was intended to assess the effects of a 52 weeks ART course with T enanthate on HRQoL in a Japanese population. We examined ART effects on MetS, body composition, bone mineral density (BMD), muscular strength, lower urinary tract symptoms (LUTS), and sexual functioning in addition to the safety and tolerability of a 52 weeks ART course in this population.

PATIENTS AND METHODS

Study design

This large-scale, multicenter, parallel-group, open-label, RCT was intended to determine ART effects on physical and mental health in patients with LOH. The primary study endpoint was ART efficacy for improving HRQoL, assessed by the short form-36 health survey (SF-36) and aging males symptoms (AMS) scores. Secondary endpoints included glycemic control, lipid parameters, blood pressure (BP), waist circumference, body mass index (BMI), body composition, muscular strength, International Prostate Symptom Scores (IPSS), International Index of Erectile Function-5 (IIEF-5) scores, BMD, and serum prostate-specific antigen (PSA) levels. Safety endpoints included vital signs, adverse events, and standard biochemical and hematologic profiles.

This study was approved by the Ethical Committee of the Kanazawa University Graduate School of Medical Science and was conducted in accordance with the Declaration of Helsinki and International Conference on Harmonization guidelines. The documents given to all study participants clearly stated that participation would be on a voluntarily basis and refusal to participate in the study would not result in any adverse consequences. The documents further stated that data would be statistically processed without identifying the individuals, individual privacy would be protected, and the results would be presented in this study (Trial registration: UMIN 000001946).

Study populations

Hypogonadal men (free T < 11.8 pg ml^{-1} with ages ranging from 40 to 90 years, performance statuses of 0–2, according to the Eastern Cooperative Oncology Group, and the ability to make hospital visits were included in the study. We excluded men with already-known prostate disease (cancer, disease requiring further treatment, or a serum PSA level ≥ 2.0 ng ml^{-1}); any unstable, uncontrolled, or severe chronic medical disease, including infection or extensive bone or skin disease, requiring treatment; sleep apnea syndrome; myocardial infarction history within the previous year; severe hypertension (systolic BP ≥ 180 mmHg); polycythemia (hemoglobin ≥ 18.0 g dl^{-1}); drug abuse or addiction history; mental illness history requiring regular psychotropic medication; or disallowed medications use that interfered with sex steroid action or bone mass.

Procedures

Volunteers were recruited through advertisements and provided written informed consent before undergoing initial serum PSA and free T level screening. At the baseline visit, eligible participants were assessed via interviews to determine their current medications and medical illnesses as well as physical examinations, blood sample tests, questionnaires, and anthropometric, body composition, and bone densitometric measurements. After completing the baseline assessments, the enrolled participants were randomly assigned (1:1) to the intervention or control group according to a minimization method to ensure balanced stratification. The intervention group was administered T enanthate (250 mg; Enarmon Depot®; ASKA Pharmaceutical Co., Ltd., Tokyo, Japan) via intramuscular injection every 4 weeks for a total of 52 weeks; the matched control group received no treatment. Lipid and glucose tolerance abnormalities were considered adjustment factors during the dynamic allocation.

The waist circumference, triglyceride (TG), high-density lipoprotein cholesterol (HDL-Chol), BP, and fasting blood glucose level were examined as MetS markers and were evaluated before and after ART in both the groups. Men who discontinued participation during the study was required to complete an end-of-study visit as soon as possible after the withdrawal and recovery visits.

Questionnaires

We administered two types of questionnaires, the SF-36 scale as a generic measure and AMS score as a disease-specific measure, at the baseline and after 52 weeks to evaluate and compare HRQoL. SF-36 assesses the following eight measures of functioning: (1) physical functioning; (2) social functioning; (3) role limitations because of health problems (physical role); (4) role limitations because of emotional problems (emotional role); (5) mental health; (6) vitality; (7) bodily pain; and (8) general health perception. The raw scores were transformed to a standardized scale ranging from 0 to 100, with a higher score indicating a better status.[29] The AMS score comprises 17 items scored on a five-point scale. The 17 items are distributed over three subdomains: five, seven, five questions address psychological factors, physical factors, and sexual function factors, respectively. The total AMS score defines symptom severity as "no/little," (17–26 points) "mild," (27–36 points) "moderate," (37–49 points) or "severe" (≥ 50 points).[30]

Moreover, IPSS for LUTS and IIEF-5 for sexual function were also administered. IPSS contains seven symptom questions that assess weak urine stream, intermittency, and straining (voiding symptoms), frequency, urgency, and nocturia (storage symptoms), and incomplete bladder emptying feeling (postvoiding symptom). The response options range from "not at all" (0 points) to "almost always" (5 points), with

a maximum total of 35 points.[31] The IIEF-5 score comprises only five questions, and each item is scored on a five-point ordinal scale wherein lower values indicate a poorer sexual function. The possible scores for IIEF-5 range from 1 to 25 points (1 question can receive a score from 1 to 5; all others can receive scores from 0 to 5); a score > 21 points is considered normal erectile function, whereas scores at or below this cut-off indicate erectile dysfunction.[32]

Bone densitometry and body composition

The lumbar vertebral (L2-L4 individually and together) BMD was measured by dual-energy X-ray absorptiometry at the baseline and 52 weeks visits with a Lunar Prodigy instrument (GE Healthcare, Madison, WI, USA). Scanning was performed according to the manufacturer's instructions. The absorptiometry instrument was subjected to routine quality assurance maintenance, including calibration, every morning using the standard provided by the manufacturer.

The total body composition was assessed using the BodyPlanner™ DF800 body fat monitoring system (Yamato Biospace Technology, Hyogo, Japan), which uses bioelectrical impedance analysis to systemically evaluate the body fat percentage and muscle volume. The segmental body muscle volume (whole body, arms, and legs) was measured in eight phases from level 0 to 7 (large volume).

Anthropometry

Anthropometric measurements were assessed at the baseline and after 16, 28, and 52 weeks. The body weight was measured to the nearest 0.1 kg after removing coats, sweaters, and shoes and the height were measured to the nearest 0.1 cm using a fixed stadiometer. BMI was calculated as the weight in kilograms divided by the square of the height in meters. Waist circumference was measured midway between the lower rib and iliac crest after normal expiration using a nonstretch measuring tape without placing pressure on the skin. All measurements were performed in duplicate, and the average was used as the value for each circumference; the results were rounded to the nearest 0.1 cm. The systolic and diastolic BPs were measured in duplicate with an automated and calibrated oscillometric device on the left arm with the participant in a seated position after 5 min of rest. The mean systolic and diastolic BPs were then calculated.

The isometric hand-grip strength was measured in both the dominant and nondominant hands using a Smedley-type dynamometer (Smedley's Dynamometer TTM, Tokyo, Japan). The participants were instructed to stand with their shoulders adducted and neutrally rotated, their arm in a vertical position and their wrist in a neutral position. Subsequently, each participant squeezed the grip with maximal strength while alternating between the left and right hands. Each test was repeated at least 3 times until no further improvements were observed. The best value for each side was used for the analysis; the values were reported in kilogram-force units.

Laboratory assays

Fasting blood samples were collected for biochemical and hematologic profiling before administering T enanthate and between 8:30 and 10:30 AM to minimize diurnal variations; the samples were analyzed on the same day using commercially available routine autoanalyzer methods. Fasting serum samples were batch-frozen and stored at −20°C to be used in additional assays in the future after completing the present study. The serum free T levels were measured by radioimmunoassay (RIA) with a DPC's free-T kit (Mitsubishi Kagaku, Tokyo, Japan). The serum PSA levels were measured by RIA with a minimum detectable concentration of 0.008 ng ml^{-1}.

Adverse events and safety

Androgen replacement therapy safety was assessed according to the PSA level, liver and kidney functioning, and hematological parameters. The serum PSA levels were evaluated at the baseline and after 28 and 52 weeks. An increased serum PSA level (≥ 2.0 ng ml^{-1}) was considered an adverse event manifestation. Sera were also subjected to liver function (aspartate aminotransferase [AST], alanine aminotransferase [ALT], and γ-glutamyl transpeptidase [γ-GTP]), kidney function (creatinine), and hematological assays (hemoglobin and hematocrit) according to standard autoanalyzer methods at the baseline and at 16, 28, and 52 weeks during visits conducted by nonblinded study personnel. During the study, patients with a hemoglobin level of ≥ 18.0 g dl^{-1}, hematocrit level of ≥ 51.0%, liver function values > 3-fold of the upper limit of normal (AST: 13–33 IU l^{-1}; ALT: 8–42 IU l^{-1}; γ-GTP: 10–47 IU l^{-1}), or creatinine levels of ≥ 2.0 mg dl^{-1} were subjected to an additional blood evaluation after 1 week. If the values remained too high, study participation was discontinued.

An adverse event was defined as any unfavorable and unintended sign (e.g. abnormal laboratory finding), symptom, or disease temporally associated with medication use, regardless whether the event was considered medication-related. Information regarding adverse events was obtained by questioning or examining the participants at each visit during the treatment period.

Statistical analysis

We analyzed data according to an intention-to-treat analysis, which was defined as including participants who had been randomly assigned, had received at least one dose of medication, and had at least one efficacy value. To compare efficacy outcomes between the two groups, the changes in each parameter between the baseline and week 52 were calculated. Normally distributed data were assessed using Student's *t*-test, whereas nonnormally distributed data were assessed using the Wilcoxon test for the baseline characteristics and between-group comparisons. Data were expressed as means (standard deviations) or medians (25^{th}–75^{th} percentiles). Safety was evaluated through documentation and adverse event reporting. All statistical analyses were conducted using the JMP software package (version 9.0; SAS Institute, Cary, NC, USA). Statistical significance was defined as $P < 0.05$ for all analyses.

Role of the funding source

The Health Labor Sciences Research Grants for Comprehensive Research on Aging and Health provided funding support as the study sponsor, including study monitoring, but had no influence on the study design, data analysis, interpretation of the findings, or decision to submit the manuscript for publication, all of which were solely the responsibility of the investigators. The authors designed the study, had unrestricted access to the data, performed and interpreted all data analysis, and wrote the manuscript independently of the sponsor.

RESULTS

Participant flow and characteristics

This study was conducted between December 2007 and October 2010. The study participant recruitment and enrollment flow is shown in **Figure 1**. The recruitment process identified 1637 volunteers. After excluding 1303 ineligible individuals (500 did not meet the inclusion criteria; 803 refused to participate), 334 Japanese men were randomly assigned to either receive 250 mg of T enanthate (intramuscular injection) every 4 weeks or undergo

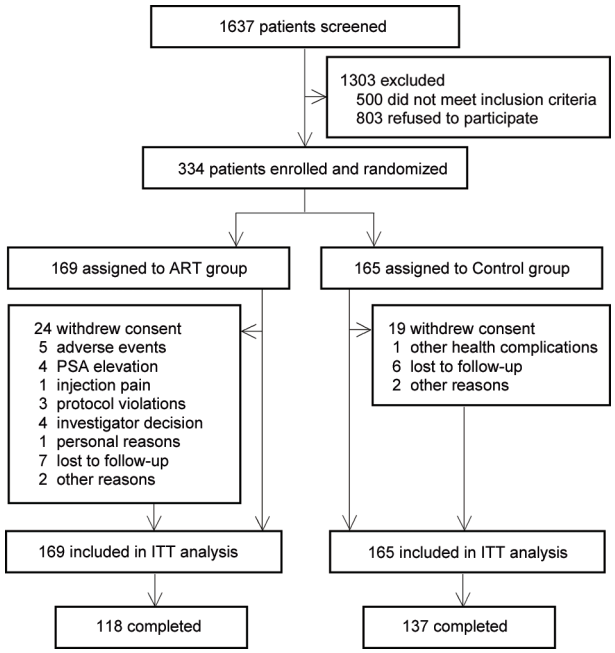

Figure 1: Flow chart of the recruited participants in the EARTH study.

observation alone (ART group, n = 169; control group, n = 165). There were no significant differences in the baseline clinical and biological characteristics between the two groups except for the serum LDH and albumin levels (**Table 1**). The baseline median SF-36 scale, AMS, IPSS, and IIEF-5 scores were also comparable between the groups, except the SF-36 scale physical functioning subdomain (**Table 2**).

Overall, 255 of the 334 men (76.35%) completed the 52 weeks study treatment period; 118 (69.82%) received T enanthate and 137 (83.03%) received no treatment. Discontinuations were due to withdrawal of consent (24 ART, 19 control), adverse events (5 ART), PSA elevation (4 ART), injection pain (1 ART), protocol violations (3 ART), investigator decisions (4 ART), personal reasons (1 ART), loss to follow-up (7 ART, 6 control), other health complications (1 control), or other reasons (2 ART, 2 control).

Health-related quality of life by short form-36 health survey and aging males symptoms

Regarding the primary endpoint, there was a small but significant between-group difference with respect to the change from the baseline in the SF-36 scale role physical subdomain (**Table 3**); specifically, ART was associated with a significantly greater (P = 0.0318). ART had a marginal positive effect on the role emotional subdomain (P = 0.0727). No significant differences were observed in any of the other six subdomains of the SF-36 scale or AMS total scores and subscores.

The secondary endpoints

Comparison of changes in the following secondary endpoints between the two groups is shown in **Table 4**.

Anthropometry

Androgen replacement therapy was associated with a significant decrease in the waist circumference from the baseline compared with the control (P = 0.002); however, no significant difference in the BMI was observed. Moreover, there were no significant differences in weight and height. Finally, there was also no significant difference regarding changes in the isometric handgrip strength on either side.

Table 1: Baseline clinical and biological characteristics of the ART and control groups

Variable	*Mean±s.d.*		*P*
	ART (n=169)	*Control (n=165)*	
Age (year)	65.65±9.01	67.55±9.36	ns
Anthropometry			
Weight (kg)	64.63±11.18	65.41±10.35	ns
Height (cm)	165.99±6.45	165.48±6.07	ns
Body composition			
Waist circumstance (cm)	86.27±9.39	87.39±9.01	ns
BMI (kg m^{-2})	23.33±3.17	23.92±3.31	ns
Body fat (%)	22.74±6.83	23.53±7.11	ns
Bone mineral density (%)	96.0±22.8	98.0±27.5	ns
Muscle volume			
Whole body (level 0–7)	4.0±4.0	4.0±4.0	ns
Arm (level 0–7)	5.0±3.5	4.0±3.0	ns
Leg (level 0–7)	4.0±4.0	3.0±4.0	ns
Muscle strength			
Right-hand grip (kgf)	36.04±7.41	34.90±8.88	ns
Left-hand grip (kgf)	34.92±7.48	33.43±8.56	ns
BP			
Systolic BP (mmHg)	130.99±13.79	133.44±16.26	ns
Diastolic BP (mmHg)	77.56±11.04	77.32±10.99	ns
Free testosterone (pg ml^{-1})	7.1±3.2	6.7±3.5	ns
PSA (ng ml^{-1})	0.77±0.63	0.75±0.71	ns
Glycemic control			
Fasting blood sugar (mg dl^{-1})	134.67±40.70	125.83±36.19	ns
Hemoglobin A1c (%)	6.19±0.96	6.10±0.93	ns
Lipid parameters			
Total cholesterol (mg dl^{-1})	185.79±27.21	187.98±30.68	ns
Triglycerides (mg dl^{-1})	106.0±86.5	103.0±69.0	ns
HDL-cholesterol (mg dl^{-1})	56.34±14.14	54.32±13.79	ns
Complete blood count			
White blood cell (×10^3 μl^{-1})	5.75±1.49	5.84±1.64	ns
Red blood cell (×10^4 μl^{-1})	457.78±43.15	448.10±45.91	ns
Hemoglobin (g dl^{-1})	14.33±1.34	14.03±1.46	ns
Hematocrit (%)	42.60±3.74	41.80±3.98	ns
Platelet (×10^4 μl^{-1})	21.46±4.86	21.85±7.38	ns
Other chemicals			
Blood urea nitrogen (mg dl^{-1})	15.49±3.84	16.44±4.39	ns
Creatinine (mg dl^{-1})	0.85±0.16	0.87±0.20	ns
Sodium (mEq l^{-1})	141.04±2.48	141.22±2.66	ns
Potassium (mEq l^{-1})	4.40±0.39	4.37±0.40	ns
Chlorine (mEq l^{-1})	103.80±2.80	104.31±2.64	ns
Calcium (mg dl^{-1})	8.51±1.98	8.31±2.11	ns
Phosphorus (mg dl^{-1})	3.23±0.53	3.21±0.63	ns
Alkaline phosphatase (IU l^{-1})	228.08±68.64	226.64±76.68	ns
γ-glutamyl transpeptidase (IU l^{-1})	40.92±26.76	36.22±29.29	ns
Aspartate aminotransferase (IU l^{-1})	24.70±8.98	25.15±12.71	ns
Alanine aminotransferase (IU l^{-1})	26.11±13.69	24.19±13.38	ns
Lactate dehydrogenase (IU l^{-1})	181.71±31.31	191.11±34.83	0.0197
Total bilirubin (mg dl^{-1})	0.83±0.33	0.79±0.28	ns
Total protein (g dl^{-1})	7.13±0.39	7.12±0.43	ns
Albumin (g dl^{-1})	4.34±0.32	4.26±0.31	0.0310

HDL: high-density lipoprotein; ns: not significant; BMI: body mass index; ART: androgen replacement therapy; s.d.: standard deviation; BP: blood pressure; PSA: prostate-specific antigen

Table 2: Baseline median scores of the eligible participants on questionnaires assessing SF-36, AMS, IPSS, and IIEF-5

Variable	*Median±IQR*		*P*
	ART (n=169)	*Control (n=165)*	
SF-36			
Physical functioning	95.0±10.0	90.0±15.6	0.0028
Role physical	100.0±12.5	100.0±25.0	ns
Bodily pain	74.0±38.0	74.0±48.0	ns
General health perceptions	57.0±15.0	57.0±23.9	ns
Vitality	68.8±31.3	68.8±31.3	ns
Social functioning	100.0±25.0	100.0±37.5	ns
Role emotional	100.0±16.7	100.0±25.0	ns
Mental health	80.0±27.5	80.0±25.0	ns
AMS			
Total score	34±15	37±14	ns
Psychological subscore	8±5	7±6	ns
Somatovegetative subscore	14±7	15±7	ns
Sexual subscore	14±6	14±5	ns
IPSS			
Total score	8±13	7±11	ns
Storage subscore	3±4	3±5	ns
Voiding subscore	3±7	3±7	ns
Postvoiding subscore	1±2	1±2	ns
IIEF-5			
Total score	10±13	8±12	ns
Question 1	2±2	2±3	ns
Question 2	2±2	2±2	ns
Question 3	2±2	2±3	ns
Question 4	2±3	2±4	ns
Question 5	2±2	2±3	ns

SF-36: short form-36 health survey; AMS: aging males symptoms; IPSS: International Prostate Symptom Scores; IIEF-5: International Index of Erectile Function-5; IQR: Inter-quartile range; ns: not significant; ART: androgen replacement therapy

Table 3: Comparison of changes in the primary endpoints from the baseline to week 52 between the two groups

Variable	*ART*		*Control*		*P*
	Mean±s.d.	*n*	*Mean±s.d.*	*n*	
SF-36					
Physical functioning	–0.06±7.18	98	–0.39±10.78	122	0.7870
Role physical	2.08±14.92	99	–3.63±20.34	123	0.0318
Bodily pain	0.07±22.43	100	0.71±21.96	120	0.9701
General health perceptions	2.42±14.06	100	–0.39±14.39	122	0.1415
Vitality	3.06±18.37	100	–0.74±17.79	120	0.4626
Social functioning	1.00±22.24	99	0.10±27.24	121	0.9244
Role emotional	2.94±16.07	100	–1.99±17.87	121	0.0727
Mental health	2.90±17.58	100	0.26±16.45	122	0.5310
AMS					
Total score	–0.96±7.56	100	–0.99±7.66	122	0.9170
Psychological subscore	–0.49±2.63	100	–0.44±2.67	122	0.4802
Somatovegetative subscore	–0.16±3.76	100	–0.43±3.68	122	0.7588
Sexual subscore	–0.31±2.98	100	–0.12±3.74	122	0.5577

SF-36: short form-36 health survey; AMS: aging males symptoms; s.d.: standard deviation; ART: androgen replacement therapy

Bone densitometry and body composition

No significant difference was observed between the groups regarding change in the lumbar spine BMD from the baseline to 52 weeks.

The whole-body and leg muscle mass volumes significantly increased in the ART group relative to the control group ($P = 0.0108$ and $P = 0.0071$, respectively). There was also a tendency toward a greater decrease in the body fat percentage in the ART group relative to the control group, although this difference did not reach statistical significance.

Table 4: Comparison of changes in the secondary endpoints between the two groups

Variable	*ART*		*Control*		*P*
	Mean±s.d.	*n*	*Mean±s.d.*	*n*	
Body composition					
Waist circumstance	–0.75±3.27	116	0.66±3.76	132	0.0020
BMI	0.10±0.93	116	0.06±1.46	132	0.7890
Body fat	–0.43±4.66	115	0.67±4.50	131	0.0603
Muscle volume					
Whole body	0.22±1.45	114	–0.21±1.54	131	0.0108
Legs	0.27±1.19	114	–0.11±1.37	131	0.0071
Arms	0.01±1.74	114	–0.41±1.86	131	0.0913
Muscle strength					
Right-hand grip	1.40±4.48	114	0.85±3.47	130	0.2800
Left-hand grip	1.08±4.83	114	0.72±4.24	131	0.5258
BP					
Systolic BP	1.83±13.55	106	–0.55±16.98	126	0.2442
Diastolic BP	1.31±10.89	104	0.47±11.61	126	0.5709
Bone mineral density	3.87±5.13	112	3.97±7.75	123	0.9086
PSA	0.19±0.42	109	0.21±0.74	126	0.1665
Glycemic control					
Fasting blood sugar	1.28±44.88	107	8.36±40.86	125	0.2101
Hemoglobin A1c	0.01±0.47	113	0.03±0.42	130	0.7548
Lipid parameters					
Triglycerides	–12.10±80.45	115	4.07±42.72	130	0.0103
Total cholesterol	–0.48±22.36	115	–3.50±27.67	130	0.3508
HDL-cholesterol	–1.70±8.01	115	–1.36±7.28	129	0.7289
Complete blood count					
Red blood cell	27.01±27.09	114	–1.99±23.87	130	<0.0001
Hemoglobin	0.79±0.97	114	–0.11±0.88	130	<0.0001
Hematocrit	2.52±2.75	114	–0.22±2.29	130	<0.0001
Platelet	–0.30±2.99	114	–0.57±2.91	130	0.4789
Other chemicals					
Aspartate aminotransferase	0.18±8.34	115	–0.97±9.45	130	0.3157
Alanine aminotransferase	2.46±20.41	115	0.03±11.98	129	0.2518
γ-glutamyl transpeptidase	3.63±28.72	115	–2.53±18.16	128	0.8183
Creatinine	0.022±0.096	115	0.004±0.136	130	0.2424
Albumin	0.001±0.280	110	0.001±0.268	122	0.9980
Calcium	0.71±1.57	110	1.07±1.84	127	0.1056
IPSS					
Total score	–0.56±4.66	100	0.88±6.24	120	0.0950
Storage subscore	–0.07±2.23	100	0.45±2.80	120	0.1450
Voiding subscore	–0.43±2.84	100	0.44±3.69	120	0.0418
Postvoiding subscore	–0.06±1.29	100	–0.02±1.25	120	0.6257
IIEF-5					
Total score	0.16±5.15	97	–0.96±5.19	115	0.2623
Question 1	0.05±1.11	95	–0.17±1.24	114	0.4432
Question 2	0.20±1.17	93	–0.29±1.24	111	0.0049
Question 3	0.08±1.18	89	–0.22±1.16	110	0.2347
Question 4	–0.03±1.27	92	–0.23±1.40	111	0.6447
Question 5	0.04±1.16	92	–0.15±1.28	111	0.4132

HDL: high-density lipoprotein; IPSS: International Prostate Symptom Scores; IIEF-5: International Index of Erectile Function-5; s.d.: standard deviation; ART: androgen replacement therapy; BP: blood pressure; PSA: prostate-specific antigen; BMI: body mass index

Laboratory assessments

Androgen replacement therapy exerted a good partial effect on the lipid profile. Significant reductions were observed with respect to TG in the ART group relative to the control group ($P = 0.0103$), which was consistent with the greater decrease in waist circumference observed in the ART group ($P = 0.002$). However, there were no significant differences regarding changes in the total cholesterol (T-Chol) and HDL-Chol between the two groups. For glycemic control, no significant differences were observed between the groups in terms of changes in the levels of the MetS markers hemoglobin A1c or fasting glucose. Similarly, no significant differences were observed with respect to changes in the systolic or diastolic BP. However, marked increases in RBC, hematocrit, and hemoglobin were observed in the ART group at 52 weeks relative to the control group ($P < 0.0001$ for all). There was no significant difference between the two groups in terms of changes in the routine biochemical parameters indicative of liver and renal function.

International Prostate Symptom Scores and International Index of Erectile Function-5

Androgen replacement therapy was associated with significant improvements from the baseline in terms of the IPSS voiding subscore ($P = 0.0418$), whereas no significant differences were observed in the total score, the storage subscore, or the postvoiding subscore. Regarding sexual function, ART was associated with significant improvements from the baseline with respect to the second question of IIEF-5 ($P = 0.0049$) but not the total score or the four other questions of IIEF-5.

Safety

A total of 27 men in the ART group and nine in the control group discontinued the study protocol. Among these men, 10 in the ART group experienced adverse events that were considered related to the study medication. The details of the adverse events are as follows: four exhibited an elevated serum PSA level at the 28 weeks visit, one experienced injection pain at the 16 weeks visit, two reported sleep apnea syndrome at either the 16 and 28 weeks visit, one exhibited polycythemia at the 28 weeks visit, one exhibited mild liver dysfunction at the 16 weeks visit, and one exhibited mild bradycardia at the 28 weeks visit. In one case involving serum PSA elevation, the serum PSA level had increased to 8.719 ng ml^{-1} (from a baseline of 1.368 ng ml^{-1}) at the 28 weeks visit but rapidly decreased to 1.350 ng ml^{-1} at 6 weeks after discontinuing ART. In the remaining three cases involving serum PSA elevation (baseline to discontinuation ranges of 0.559–2.444, 1.720–2.245, and 0.838–2.929 ng ml^{-1}), prostate needle biopsy was not required because the serum PSA levels remained stable at levels < 3.0 ng ml^{-1}.

Discontinuation due to protocol violations (1 subject each with a high PSA value at enrollment, previous PCa history, or nonscheduled visit), personal reasons (1 subject moved), and other reasons (1 subject each with IgG4-related disease, inguinal hernia operation, anxiolytic use, or anti-phospholipid antibody syndrome and 2 patients with cerebral infarction) were reported in the ART group. In addition, discontinuations due to other reasons (1 subject each with lumbar spinal canal stenosis, colon cancer, or benign colon polyps) were reported in the control group. Seven and six men in the ART and control groups, respectively, were lost to follow-up during this study.

DISCUSSION

In this randomized, nonplacebo-controlled, parallel-group, multicenter clinical trial, we demonstrated the effects of a 52 weeks course of ART involving the intramuscular T enanthate administration every 4 weeks on HRQoL in a Japanese population. Moreover, we revealed ART effects on MetS, body composition, BMD, muscular strength, LUTS, and erectile function. To the best of our knowledge, this is the largest Asian parallel-group RCT-based study on this subject. It is very important to examine whether in an Asian population, ART would exert LOH effects similar to those observed European and American populations because both ethnicity and body composition may affect the serum T levels.[24–28]

The uncertainty surrounding LOH has resulted in the formulation of several recommendations for its diagnosis and treatment by panels of experts.[7,9] The biochemical criteria for LOH definition vary somewhat according to the different international societies. In Japan, the Japanese Urological Association and Japanese Association of Men's Health recommend measuring the serum free T levels when diagnosing LOH.[10] Many studies conducted in Western countries did report age-related decreases in the serum total T level;[1–4] in contrast, three major studies have shown that the total T levels do not decrease with age in the Japanese population.[11,12,33] Therefore, the total T level cannot be used as a marker in Japan, despite its recommended use in European and American populations. Conversely, there is considerable controversy regarding the best free T measurement method. Although equilibrium dialysis is widely accepted as the "gold standard" for measuring free T,[9] it is considered laborious, slow, and expensive.[34] The analog-based free T measurement is a suitable and convenient biochemical LOH syndrome determinant in Japanese men.[12] Consequently, in the Japanese clinical practice manual, ART is recommended as the absolute first-line treatment when the free T level is < 8.5 pg ml^{-1} and should also be considered for men with free T levels < 11.8 pg ml^{-1}.

Androgen replacement therapy is thought to exert multifarious effects on the mood, energy, and health. As such, there are many tools for evaluating HRQoL in LOH-affected populations, including generic measures: the SF-36 and the shorter version SF-12; the psychological general well-being index; the sickness impact profile; the 10-point visual analogue quality of life scale; and the Endicott quality of life enjoyment and satisfaction questionnaire (Q-LES-Q), and disease-specific measures: AMS scale; St. Louis University androgen deficiency in aging males scale; and age-related hormone deficiency dependent quality of life questionnaire.[13] Although several RCTs have focused on ART effects on HRQoL, three trials demonstrated significant ART effects on QoL measures; one RCT incorporated the Q-LES-Q[35] and the others used the SF-36 scale.[36,37] However, other trials have not shown significant ART effect on HRQoL, and therefore, these results remain controversial and imprecise.[38–41] In a previous RCT that assessed ART effects on HRQoL and subthreshold depression (dysthymia or minor depression), Shores *et al*. reported that negative ART effects with respect to HRQoL may have been due to the use of insensitive HRQoL measures (SF-36 and Q-LES-Q).[41] A recent study also found no significant differences in the SF-36 and Questions on Life Satisfaction Module scores with the exception of an improvement in one hormone-related QoL section.[42] In contrast to the studies reporting negative ART effects on SF-36, our present study showed a moderate but significant improvement in the role physical subdomain of the SF-36 scale and a tendency toward improvement in the role emotional subdomain.

The AMS scale was designed to measure the severity of aging symptoms and their impact on HRQoL;[30] however, the relevance of this scale for LOH diagnosis remained unclear.[22] Most studies have failed to find an association between the total AMS scores and T levels,[43–45] and only weak correlations have been reported between the AMS scale subdomain scores (psychological, somatovegetative, sexual) and T levels.[45,46] Moreover, the AMS scale validity, as an instrument

for measuring clinical efficacy, is unknown because this tool has not been evaluated in an LOH-specific RCT, although the efficacy has been empirically demonstrated in open-label studies of truly hypogonadal men.[47,48] In contrast, a recent double-blind RCT demonstrated that long-acting T treatment significantly improved the total AMS scores and psychological and somatovegetative domain scores.[49] In our study, no significant improvement in the ART group was found in total AMS score as well as three AMS sub-score when compared with the control group, which may be partially interpreted by the results of a previous cross-sectional study showing no association between AMS score and circulating T levels.[43] In our study population, the average AMS score at baseline ranged from 34 to 37 points and therefore indicated only "mild-to-moderate" severity. For this reason, there was little room for symptom improvement during treatment and again, the treatment response may have been better in patients with more severe symptoms.

A number of clinical studies on ART effects on MetS and type 2 DM has increased, and a few RCTs were recently conducted to evaluate ART effectiveness in this regard.[50–52] Moreover, two meta-analyses of these RCTs suggested that ART improves both metabolic glycemic control and visceral obesity.[53,54] However, no conclusions regarding significant improvements in MetS and type 2 DM could be drawn in studies involving small numbers of patients and short treatment durations. More recently, three cumulative registry studies suggested that long-term T treatment in the elder men with hypogonadism ameliorated type 2 DM and MetS components,[55–57] although these were not RCTs, but uncontrolled studies showing positive T effects without scientific reliable candor and rigor. Our RCT, which included 334 study patients and a treatment duration of 52 weeks, demonstrated that ART significantly reduced TG levels with a concomitant and significant reduction in the waist circumstance, whereas no significant differences were observed in the changes in the T-Chol and HDL-Chol levels between the two groups. In contrast to the positive results reported by previous RCTs, we did not observe improvements in either the hemoglobin A1c or fasting glucose levels in the ART group. It is possible that the reason for this discrepancy in glycemic control may be the characteristics of the patients enrolled in this study; most had a type 2 DM level that required treatment at our university hospital and would therefore have more severe disease compared with patients in previous RCTs.

A recent meta-analysis revealed beneficial ART effects on the body composition, specifically a significant increase in the lean body mass (2.7 kg; 95% confidence interval [CI], 1.6, 3.7) and a significant decrease in the fat mass (−2.0 kg; 95% CI, −3.1, −0.8) without a change in body weight.[7] This agrees with our finding that the whole-body muscle mass volume was significantly increased in the ART group. Furthermore, body fat percentage tended to decrease in the ART group, although this difference did not reach statistical significance. As both fat loss and muscle gain occurred, there was no change in BMI. However, there was a significant decrease in the waist circumstance in the ART group, indicating reduced visceral obesity,[58] and the different BMI and waist circumstance outcomes are supported by previous studies in which visceral obesity, relative to other obesity subtypes, was more strongly and inversely related to the total and free T levels.[59,60] Among obese men (BMI > 30), 20%–64% have T levels in the hypogonadal range, which mostly reflects the influence of visceral obesity.[2,61] ART, however, reduces the waist–hip ratio and weight,[62] and therefore appears to counteract some components of the MetS phenotype. In contrast, a cumulative registry study of 255 men with hypogonadism suggested long-term T treatment produced a significant decrease in the body weight, waist circumstance, and BMI.[63]

According to various reports, ART effects on muscle strength remain controversial. Several studies have reported greater improvements in grip strength with ART than with placebo, whereas other studies have reported no significant changes in grip strength.[64–66] Changes in the lower-extremity muscle strength and physical function measures have only been reported in a few studies with inconsistent results. Recent cross-sectional studies have shown that in aging men, positive correlations exist between the T level and upper and lower extremity muscle strength parameters as measured by the leg extensor and isometric hand grip strength.[67] Moreover, in a clinical review, Cunningham and Toma also mentioned that T effects on muscle strength were heterogeneous and tended toward improvements only in the leg/knee extension and dominant arm handgrip.[68] However, in the present study, there was no difference in the handgrip changes between the sides. One ART inclusion criteria in our study was a free-T level < 11.8 pg ml^{-1}; dissimilarities in grip strength could be partly explained by differences in the baseline T levels in each study.

Little is known regarding whether or not ART has an effect on improvements in LUTS, mainly due to benign prostate hypertrophy (BPH) in aging men. Few studies have investigated the relationship between T treatment and LUTS to date. One study identified hypogonadism in approximately 20% of elderly men with LUTS, with no impact on the symptom status.[69] Another found a correlation between symptoms of LUTS and plasma T concentrations.[70] If the severity of LUTS was dependent on declining T levels, ART could alleviate LUTS in men with LOH. Previously, we showed that ART improved LUTS in hypogonadal men, with mild BPH.[71] Recently, Yassin *et al*.[72] reported that T replacement was associated with amelioration of LUTS in men with LOH, showing a significant decrease in the mean IPSS with time following the initiation of exogenous T. In our study, the IPSS voiding subscore consisting of 3 questions was also significantly improved after 52 weeks of ART, suggesting an increase in the bladder muscle contractility and compliance by T administration.

Several RCTs have shown the benefits of ART for hypogonadal men with erectile dysfunction.[73] Recently, two meta-analyses reported that hypogonadal men received some benefits from ART, although the clinical effects were greater on sexual desire than on erectile function and highlighted the requirement for large-scale and long-term RCTs to clarify the ART effects on erectile dysfunction in hypogonadal men.[74,75] In the present study, although the difference in the IIEF-5 total score change did not reach statistical significance, a significant difference in the change of the IIEF-5 second question score was observed. One reason for the insignificant change in the IIEF-5 total score may be that our study included men with nonsymptomatic hypogonadism who did not always present with erectile dysfunction. Similarly, there was no significant improvement in the AMS sexual subscore in our study. Recently, several clinical studies on ART in hypogonadal men demonstrated no significant improvements in the AMS sexual subscore, in agreement with our study.[22,49,76,77]

Prostate cancer incidence is among the greatest ART-related concerns. The prostate is an androgen-sensitive organ, and T administration has been reported to accelerate metastatic PCa progression.[78] Although T supports the growth of existing PCa, previous clinical studies have shown no increased PCa risk from ART.[79–81] However, recent meta-analyses have reported that the man-years of exposure were too limited to allow a reliable risk assessment.[82,83] Recently, no correlation or association between T therapy and increased PCa risk, or increased aggressiveness of PCa at diagnosis has been reported in a large cohort studies.[84,85] Moreover, in a population-based observational study of T therapy in men, with

a history of PCa, treatment was not colligated with increased overall or cancer-specific mortality.[86] Consequently, there is no clinically significant adverse impact on the PCa incidence and exacerbation by ART. In our 52 weeks study of 334 hypogonadal men, four men in the ART group discontinued T replacement because of rapid PSA elevation; however, we performed no prostate needle biopsies because the serum PSA levels remained within the safe range or decreased after discontinuation. No PCa was observed during our study.

We observed a trend toward a greater overall PSA increase in the ART group versus the control group at 28 weeks, although the difference was not significant; the level appeared to decrease in the ART group at 52 weeks. This transient PSA elevation may be explained by the prostate saturation theory,[87] which suggests that PSA and prostate tissue growth are sensitive to changes in serum T only if the serum T levels are low. At low levels, the prostate androgen receptor (AR) lacks bound T and is inactive, causing prostate shrinkage and decreased PSA levels. Conversely, at normal T levels, all available ARs are saturated and active, and further increases in T have no effect on the prostate size or PSA levels. A recent clinical study involving the prostate saturation theory compared serum T and PSA levels during a 12 months ART course in hypogonadal men with varying baseline T levels and found that the greatest PSA level was observed after 1 month of treatment and decreased thereafter, which agreed with our transient overall PSA elevation at 28 weeks.[88]

The potential association between ART and cardiovascular events is of concern. Initially, ART was thought to increase cardiovascular disease risks. However, most epidemiological studies have reported no significant difference in the prevalence of cardiovascular events between hypogonadal and eugonadal men.[89] A recent population-based cohort study suggested that low T predicts cardiovascular disease mortality but not death due to other causes.[21] In a prospective population-based study of elderly Swedish men, higher serum T levels were associated with a reduced 5 years cardiovascular events risk.[90] Moreover, two meta-analyses showed that the cardiovascular event risk did not significantly differ between the ART and control groups.[82,83] In our study, two men in the ART group had cardiovascular events during treatment. Because our study was not sufficiently powered to assess the cardiovascular event risk, this observation should be cautiously interpreted. Our short study duration precludes the drawing of conclusions regarding the cardiovascular event risk with ART. Therefore, large, multicenter, and long-term (several years) clinical studies are required to assess the potential cardiovascular event risk.

The limitations of the present study are as follows: (1) participants with LOH were not always symptomatic and most were undergoing medical treatment for type 2 DM; (2) the study design was not a double-blinded, placebo-controlled but a open-labeled, parallel-controlled RCT because no placebo injections correspond to T enanthate and the alternative long-term intramuscular injection of physiological saline was not permitted for ethical reasons; and (3) this RCT was not powered to assess cardiovascular risk debated in recent years. Given these limitations, we could draw conclusions regarding ART effect on Japanese LOH patients, including asymptomatic hypogonadal patients or DM patients receiving treatment, without serious difficulties.

CONCLUSION

In this study, long-term T enanthate-based ART exerted beneficial effects on the physical role subdomain of the SF-36 scale, TG, waist circumstance, whole-body and leg muscle mass volumes, IPSS voiding subscore, and the second question of IIEF-5 in patients with LOH. However, there were no significant effects of ART on the other possible androgen-dependent parameters investigated, especially glycemic control, in the present study. Further studies are required to examine the psychological characteristics of patients with LOH, and we hope that our study will contribute to future developments in this area.

AUTHOR CONTRIBUTIONS

HK, HS, TI, KS, MI, EK, and MN planned, coordinated, and conducted the study. HK, TT, YT, EK, and MN provided medical care. HO provided randomization and supervised the statistical analysis. TI, TT, YT, EK, and MN took part in conducting the study. The scientific program was planned by HK, HS, HO, EK, and MN and conducted by MN. HK and MN received research grants from the Health Labor Sciences in affiliation with the Japanese government. All authors read and approved the final manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

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