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Effects of transdermal testosterone gel or an aromatase inhibitor on serum concentration and pulsatility of growth hormone in older men with age-related low testosterone*

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Abstract

Background—Growth hormone is the major regulator of growth and body composition. Pulsatile GH secretion declines exponentially with age. Testosterone replacement is being increasingly offered to older men with age-related low testosterone. Testosterone administration has been shown to stimulate GH secretion. However, little is known about the effect of testosterone aromatization to estradiol on GH pulsatility and its impact on IGF-1 in older men.

Objective—This randomized controlled proof-of-concept trial investigated the relative effects of testosterone and estradiol on GH pulsatility and IGF-1 in older men with low testosterone.

Design—Thirty-seven men, 65 years with total testosterone <350 ng/dL were randomized to 5 g transdermal testosterone gel (TT), 1 mg oral aromatase inhibitor (AI) or placebo daily for 12 months. Primary outcome was deconvolution and approximate entropy analyses of pulsatile including basal and entropic modes of secretion performed at baseline and 3 months. Secondary outcomes included IGF-1 evaluated at baseline, 3 and 6 months.

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Authors Contributions

JPD collected the data, conducted blood sample analysis and statistical analysis and wrote the manuscript; JDV analyzed the data and reviewed the manuscript; OC conducted blood sample analysis; MDS reviewed statistical analysis and wrote manuscript; DM assigned nurse and clinical coordinator of the study; CWC contributed to clinical aspect of the trial; JME designed the study, wrote and reviewed the manuscript; SB designed the study, wrote and reviewed the manuscript.

Disclosure Statement

Dr. Basaria has previously received investigator initiated research grants from Abbvie Pharmaceuticals (previously Solvay Pharmaceuticals) and has previously consulted for Eli Lilly, and Takeda Pharmaceuticals.

Conflict of Interest

All the other authors declare that they have no conflict of interest.

Results—At 3 months, mean GH and in IGF-1 were similar between the three groups. At 6 months, IGF-1 significantly increased by 15.3 ± 10.3 ng/ml in the TT-group compared to placebo ($P = 0.03$). Both intervention groups significantly increased GH pulse frequency (TT-group, $P = 0.04$; AI-group, $P = 0.05$) compared to placebo. The GH secretory-burst mode (duration) significantly decreased in the TT-group ($P = 0.0018$) compared to placebo while it remained unchanged in the AI-group ($P = 0.059$).

Conclusions—In older men, testosterone increases GH pulse frequency while the aromatization to estradiol is involved in the rise of IGF-1 levels.

Keywords

Aromatase inhibitor; Testosterone replacement; Growth hormone; Insulin-like growth factor-1

1. Introduction

Growth hormone (GH) is a regulator of growth, metabolism and body composition. Similar to the age-related decline in gonadotropin-releasing hormone and serum testosterone levels, aging is also associated with decreased synthesis and secretion of hypothalamic growth hormone releasing hormone (GHRH), resulting in a decrease in serum GH concentration [1]. Decreases in these anabolic hormones have been implicated in the age-related decline in lean mass, muscle strength and physical function [2]. Interestingly, GH secretion is dependent on gonadal steroids; exogenous testosterone administration stimulates GH secretion and IGF-1 levels in boys, hypogonadal adult men and healthy older men [3–7]. Some evidence suggests that stimulation of GH secretion by testosterone requires aromatization to estradiol, and inhibition of production or action of estradiol attenuates GH secretion [8]. Similarly, dihydrotestosterone, a non-aromatizable androgen, also does not stimulate GH secretion [4].

The use of aromatase inhibitors (AI) is being considered in older men with age-related low serum testosterone levels [9–11]. Administration of AI increase serum testosterone levels by stimulating gonadotropin secretion. Although previous mechanistic studies have investigated the role of AI on GH secretion in older men using exogenous testosterone clamp [12], no trial in older men exists that directly compares the long term effects of an aromatase inhibitor (AI) with transdermal testosterone (TT) on GH axis. Hence, we evaluated differential effects of TT, AI and placebo on GH levels and pulsatility over 3 months in older men with age-related low serum testosterone levels. Serum IGF-1 levels were also evaluated over 6 months duration.

2. Methods

2.1. Trial Participants and Protocol

This was a double-blind, randomized, placebo-controlled trial of 12 months duration conducted at the National Institute on Aging (NIA) Intramural Research Unit (MedStar Harbor Hospital, Baltimore) (NCT00104572). Men aged ≥ 65 years with fasting morning (7–10 am) total T levels <350 ng/dl were enrolled. Subjects were randomized to 3 groups: transdermal testosterone (TT) gel 5 g/day and placebo tablet (TT-group, $N = 13$); oral

aromatase inhibitor (Anastrozole, AstraZeneca) 1 mg/day and placebo gel (AI-group, N = 13); placebo tablet and placebo gel daily (Placebo, N = 9). The primary outcome of the trial was change in bone density over the course of 1-year intervention [11]. Evaluation of GH axis was one of the secondary outcomes (determined *a priori*); GH secretion and pulsatility were measured at baseline and 3 months; IGF-1 was measured at baseline, 3 and 6 months. The eligibility criteria of the trial have been published previously [11].

2.2. 8 h GH Sampling Protocol

Subjects slept overnight at the research unit from 10 pm to 6 am. An intravenous catheter was placed in the forearm vein and kept open to allow infusion of saline solution. Blood sampling for GH was performed at 0 min and then every 20 min for 8 h.

2.3. Hormone Measurements

Serum total testosterone and estradiol levels were measured using liquid chromatography tandem mass spectrometry [11]. Luteinizing and follicle-stimulating hormone were measured by ELISA (Millipore, Chicago, IL) with a sensitivity of 0.01 ± 0.02 mIU/mL. GH was measured using ultrasensitive ELISA (Alpco, Salem, NH) with a sensitivity of 0.0115 ng/mL (intra-assay CV: 3.65%; inter-assay CV: 5.98%). IGF-1 was measured by ELISA (R&D Systems, Minneapolis, MN) with a sensitivity of 0.094 ng/mL (intra-assay CV: 3.5%; inter-assay CV: 8.1%).

2.4. Deconvolution Analysis

Each 8 h GH concentration time series was analyzed with a variable-waveform deconvolution method using Matlab-based algorithm [13]. The goal is to decompose the simple hormone concentration *versus* time plot into a combination of secretory bursts, of individual amplitude or mass (area under the burst), superimposed upon a stable time-invariant baseline secretion rate while estimating the hormone half-life of removal. The algorithm detrends the data and normalizes concentrations to the unit interval [0,1] [7]. The deconvolution parameters evaluated: basal, pulsatile and total secretion rate (concentration units/time), secretory burst mode (time for maximal secretion within each burst), number of pulses per time (pulse frequency), secretory burst mass, mass secreted per burst (concentration units), regularity of interpulse intervals (γ), weibull density (λ) that allows for lesser variability, and ApEn used as a scale- and model-independent regularity statistic to quantify the orderliness [7].

2.5. Statistical Analysis

Mean GH concentration determined from the 8-h pulsatile GH secretion was the primary outcome; IGF-1 was the secondary outcome. Mean change from baseline was calculated for each time point and expressed as mean $\pm$ SEM. Baseline characteristics were compared across the three groups using F tests from one way ANOVA (Table 1). Changes from baseline were regressed on the baseline value of the endpoint, treatment group, time, and treatment group-by-time interactions using baseline-adjusted two-way random-effects ANOVA for repeated measures (excluding GH pulsatility, which was only assessed at one follow-up visit). Following deconvolution analysis, changes between the baseline and 3

month measurements and differences in changes between groups were assessed by paired and unpaired-t-tests, respectively. P-values (p) <0.05 were considered statistically significant (SAS, version 9.3).

3. Results

3.1. Study Participants

Baseline characteristics were similar across three groups (Table 1). The mean age of the participants was 71 years. Serum concentrations of gonadal steroids and gonadotropins were similar between the three groups. There was no difference in mean GH or IGF-1 concentration between the groups and the summary data for 8 h GH time series were also similar between the groups.

3.2. Changes in Gonadal Steroids, Mean GH (8 h) and IGF-1

In both the intervention groups, total testosterone levels significantly increased from baseline into the *a priori* determined target range of 500–1000 ng/dL. Serum testosterone levels significantly increased from baseline in both the treatment groups while there was no change in the placebo group. Serum total estradiol levels significantly increased in the TT-group and decreased in the AI-group. Gonadotropin levels were suppressed in the TT-group whereas they increased in the AI-group [10].

At 3 months, mean change from baseline in mean GH and IGF-1 levels were similar between the three groups (Table 1), however at 6 months, IGF-1 significantly increased (15.3 ± 10.3 ng/ml.) in the TT-group compared to placebo ($P = 0.03$) but not compared to AI-group ($P = 0.17$) (Table 1).

3.3. Changes in GH Secretory Dynamics

Based on the deconvolution analysis at 3 months, neither TT nor AI significantly changed basal, pulsatile or total GH secretion compared to placebo (Fig. 1A, B, C). The GH secretory-burst mode (duration) significantly decreased in the TT-group ($P = 0.0018$) compared to placebo while the change in the AI-group was marginal ($P = 0.059$) (Fig. 1D). Both the intervention groups increased GH pulse frequency (TT-group, $P = 0.04$; AI-group, $P = 0.052$) compared to placebo (Fig. 1E). Changes in GH secretory-burst mass were not significant in both intervention groups compared to placebo (Fig. 1F). The GH interpulse interval (min) and the ApEn remained similar compared to placebo (Fig. 1G, H, I).

4. Discussion

This is the first proof of concept trial to evaluate the differential effects of TT and AI on GH pulsatility and IGF-1 in older men with age-related low serum testosterone levels. We conclude: 1) neither intervention altered basal or total GH secretion 2) testosterone is primarily responsible for increasing GH pulse frequency, and 3) testosterone increases IGF-1 levels via aromatization to estradiol. Consistent with the literature [1], we found trends of an increase in pulsatile and total GH secretion in TT-group with some decrease in the AI-group,

suggesting the role of aromatization. The GH pulse frequency increased with both interventions, suggesting a direct influence of androgens.

Prior studies have shown potentiation of GH pulsatility with estradiol [14–16]. Indeed, in the current study, estrogen stimulated GHRH drive in the TT-group but not in the AI-group, as a significant increase in IGF-1 levels was seen at 6 months in the TT-group. Since an increase in serum IGF-1 levels is associated with an increase in muscle and bone mass [2,11], it seems that aromatization of testosterone is important for this outcome.

Limitations of the study include small sample size and short-term evaluation; however, both the sample size and duration of the trial are similar to previous mechanistic studies [17].

In summary, testosterone and estradiol have differential effects on GH secretory dynamics and aromatization of testosterone is essential in increasing serum IGF-1 levels. These findings should be considered while evaluating the role of AI in the treatment of age-related low testosterone.

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Fig. 1.

Changes in GH secretory dynamics (A) basal secretion rate, (B) pulsatile secretion rate, (C) total secretion rate, (D) secretory burst mode, (E) number of pulses per time, (F) secretory burst mass, (G) regularity of interpulse intervals (γ), (H) weibull density (λ) that allows for lesser variability, and (I) ApEn after 3 months of treatment. Data are presented as mean $\pm$ SEM. Statistical analysis: Deconvolution analysis and between group's comparisons were assessed by paired and unpaired-t-tests.

Table 1

Hormonal parameters at baseline and post-intervention.

Parameters	Placebo (n = 9)	TT-group (n = 13)	AI-group (n = 13)	P value
Demographics				
Age (yrs)	72 ± 1	72 ± 1	70 ± 1	0.61
BMI (kg/m ²)	27.6 ± 1.2	30.1 ± 1.1	27.8 ± 1.2	0.27
Sex hormones				
Testosterone (ng/dL)	303.8 ± 16.6	300.1 ± 13.4	271.6 ± 12.8	0.40
Testosterone (ng/dL)-3 month	44.1 ± 30.6	292.2 ± 122.9 ^{***,+++}	248.2 ± 36.8 ⁺⁺	0.12
Testosterone (ng/dL)-6 month	40.1 ± 25.9	267.9 ± 101.8 ^{***,+++}	256.2 ± 37.3 ⁺⁺	
Estradiol (pg/mL)	16.0 ± 1.6	20.0 ± 2.0	15.0 ± 2.0	0.16
Estradiol (pg/mL)-3 month	1.2 ± 0.67	11.2 ± 3.0 ^{***,+++}	-3.2 ± 2.3 ^{†††}	0.09
Estradiol (pg/mL)-6 month	0.2 ± 1.7	4.6 ± 5.6 [*]	-6.4 ± 1.5 ^{†††}	
SHBG (nmol/L)	58.5 ± 7.1	43.4 ± 6.2	39.9 ± 5.6	0.13
SHBG (nmol/L)-3 month	-0.3 ± 2.6	-3.6 ± 1.6	-4.5 ± 2.7 [*]	0.84
SHBG (nmol/L)-6 month	-0.48 ± 2.8	-2.2 ± 1.1	-1.6 ± 2.0	
LH (mIU/mL)	12.2 ± 3.4	11.4 ± 2.3	6.4 ± 0.8	0.17
LH (mIU/mL)-3 month	1.7 ± 2.1	-6.9 ± 1.4 ^{***,+++}	4.1 ± 1.4 ^{+++,†††}	0.001
LH (mIU/mL)-6 month	4.2 ± 2.2	-7.6 ± 2.0 ^{***,+++}	4.7 ± 1.0 ^{+++,†††}	
FSH(mIU/mL)	8.2 ± 3.6	8.0 ± 1.8	6.5 ± 1.6	0.85
FSH(mIU/mL)-3 month	0.8 ± 0.9	-4.1 ± 0.7 ^{***,+++}	1.6 ± 0.6 ^{+++,†††}	0.001
FSH(mIU/mL)-6 month	1.4 ± 0.4	-5.4 ± 1.6 ^{***,+++}	3.5 ± 1.0 ^{+++,†††}	
IGF-1				
IGF-1 (µg/L)	112.1 ± 11.2	112.1 ± 10.5	109.2 ± 9.8	0.97
IGF-1 (µg/L) - 3 month	-1.5 ± 5.3	5.3 ± 5.8	-1.1 ± 4.5	0.001
IGF-1(µg/L) - 6 month	-11.7 ± 9.9	15.3 ± 10.3 [*]	0.3 ± 11.1	
8-h GH time series (baseline)				
Basal secretion (ng/ml/8 h)	3.7 ± 1.7	3.5 ± 1.4	2.2 ± 0.7	0.23
Pulsatile secretion (ng/ml/8 h)	3.4 ± 0.7	3.9 ± 1.1	6.8 ± 1.6	0.43
Total hormone secretion (ng/ml/8 h)	7.7 ± 2.2	5.8 ± 1.4	10.0 ± 2.2	0.57
Secretory-burst mode (min)	10.8 ± 0.6	14.8 ± 1.3	13.1 ± 1.5	0.97
Number pulses (per 8 h)	4.5 ± 0.5	3.2 ± 0.4	3.5 ± 0.5	0.76
Mass per burst (ng/ml)	0.7 ± 0.1	1.2 ± 0.3	2.2 ± 0.4	0.13
γ (regularity)	43.4 ± 40.4	91.7 ± 46.2	77.4 ± 40.6	0.70
λ (Weibull parameter)	15.2 ± 1.6	10.9 ± 1.9	12.9 ± 2.1	0.35
ApEn	0.8 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.18

Data presented as mean ± SEM. Groups were compared by linear regression, ANOVA, paired and unpaired t-test.

*
p 0.05.**
p 0.01.

p 0.001 compared to placebo.

++
p 0.01.

+++
p 0.001 compared to baseline.

+++
p 0.001 compared to testosterone.

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