T

ESTOSTERONE (T) may increase skeletal muscle volume by promoting hypertrophy of myofibers (1). T appears to cause similar effects in younger and older men (2), highlighting that skeletal muscle retains a high degree of plasticity even into older age. T levels in men begin to decrease by approximately 1% per year after the fourth decade of life (3) in parallel with the reduction in skeletal muscle mass and strength (4). This waning of anabolic maintenance may be one factor implicated in the atrophy of skeletal muscle with old age (sarcopenia). T replacement therapy may therefore delay or minimize age-related muscle atrophy, thus providing the rationale for T therapy in combating the effects of sarcopenia and frailty (5).

Changes in muscle size and structure can be detected by using ultrasound to assess the geometric arrangement of its fibers, termed muscle architecture (6). Muscle thickness (MT; Figure 1) is a simple index of lean body mass (of
which skeletal muscle is the largest single component (7) and is defined as the distance between the deep and superficial aponeuroses (8). Fascicle length ($L_f$), measured as the distance between the insertions of the fascicle into the deep and superficial aponeuroses (8), is proportional to the number of sarcomeres arranged in series and the excursion range of the muscle fiber (9) (Figure 1). Pennation angle ($\theta$, the angle of fascicular insertion into the deep aponeurosis of the muscle (8)) is proportional to the number of sarcomeres packed in parallel along the aponeurosis and is closely related to the force-generating capacity of the muscle (Figure 1). $L_f$ and $\theta$ are reduced in aging (9) and are typical features of sarcopenia (9). However, changes in muscle architecture in relation to variations in T are largely unknown.

Assessment of skeletal muscle using ultrasonography has been validated by comparison with direct anatomical measurement on cadaveric specimens (8). This has been shown to be a sensitive tool for assessing changes in muscle structure and size (for review, see (10)). However, there is little evidence on the use of ultrasound to investigate changes in skeletal muscle architecture in response to androgen administration, particularly when given as physiological doses in older men.

Hence, the aim of this study was to investigate the effects of T replacement on muscle architecture in intermediate-frail and frail (11) elderly men with low to borderline-low T levels using ultrasonography.

**Participants**

This investigation was a substudy of a larger clinical trial (12). Participants were community-dwelling men aged ≥65 years with one or more of Fried’s frailty criteria (11) and morning (before 11.00 AM) total T ≤ 12 nmol/L (345 ng/dL) or calculated free T ≤ 250 pmol/L (7.2 ng/dL). Among the 272 men randomized into the main study (12), 30 intermediate-frail and frail men (mean age 72.6 ± 5.7 years) were enrolled and underwent ultrasound assessments of the gastrocnemius medialis (GM) muscle. The substudy was approved by the Central Manchester Research Ethics Committee as a substantial amendment to the main study (reference number 03/CM/632) (12). Separate written informed consent for this substudy was obtained from each participant.

**Intervention**

Men in the active group received transdermal T gel (Testogel 1%; Bayer Schering Pharma, Berlin, Germany) at a dose of 50 mg/day for 6 months, and those in the control group received a matched placebo gel. The dose of the gel was adjusted to 75 or 25 mg/day according to serum T at Day 10 and 3 months. Dose adjustment was undertaken if T levels remained outside the target range (18–30 nmol/L); the placebo group therefore received the maximum “dose,” and double blinding was preserved.

**Analysis of Blood Samples**

T and sex hormone–binding globulin levels were measured by chemiluminescent immunoassay (Roche Elecsys E170 platform, Roche Diagnostics GmBH, Mannheim, Germany) at baseline, Day 10, 3 months, and 6 months. Inter- and intra-assay coefficient of variation for T was 1.1% and 3.7%, respectively, and sex hormone–binding globulin

**Materials and Methods**

**Design**

This was a single-center, randomized, double-blind placebo-controlled trial.
1.7% and 3.2%, respectively. Free T was calculated using the Vermeulen equation (13). Safety monitoring was performed as described in the main study (12).

Analysis of Muscle Architecture by Ultrasound

MT, Lf, and \( \theta \) (as described earlier) of the dominant GM muscle in each participant were assessed at baseline and at the end of treatment (6-month time point). The participant lay prone on an examination bed, with the feet positioned over the end of the bed at an angle of \( \sim 90^\circ \) to the shin. A water-soluble transmission gel was placed over the head of the probe to increase acoustic coupling. The head of the probe was held against the skin surface to provide an image of both the superficial and the deep aponeuroses of GM and of a number of clearly visible fascicles (Figure 1). Static ultrasound images (Koninklijke Philips Electronics N.V., Amsterdam, The Netherlands) were obtained along the mid-sagittal line of the GM muscle, midway between the proximal and distal tendon insertions of the muscle. Images were recorded on video home system (VHS) and later digitized for analysis using image-processing software (ImageJ; U.S. National Institutes of Health, Bethesda, MD). Care was taken to align the ultrasound probe with the orientation of the GM fascicles and to exert minimal pressure to the probe to avoid compression of the underlying muscle. All measurements were made in triplicate on one scan and measured again on the same recorded image on a separate day by the same observer (R.A.A.). The mean of these six values was used for analysis.

Statistical Analysis

As there were no previous data on which to base a formal power calculation, 30 participants were selected and consecutively enrolled into this exploratory substudy. The trial blinding meant that it was not possible to guarantee equal numbers in the two treatment arms.

All participants providing baseline and 6-month data were included in the analysis. Following the main trial analysis plan, an analysis of covariance model adjusting for baseline frailty status (1 or >1 frailty criteria) was used to assess the effect of T treatment (compared with placebo) on GM muscle architecture. A simpler covariate adjustment scheme was specified here due to the smaller sample size and the fact that the participants in this substudy were recruited over a relatively short time period. Paired \( t \) tests were used to compare changes in T over time. Analyses were performed in Stata (StataCorp, College Station, TX). Significance was set at \( p \leq .05 \).

Repeatability and Reliability of Muscle Measurements by Ultrasound

To determine repeatability of the measurement of MT, Lf, and \( \theta \), two measurements were taken on each of two separate

<table>
<thead>
<tr>
<th>Table 1. Mean (SD) Population Characteristics at Baseline for Both Testosterone and Placebo Groups</th>
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<tbody>
<tr>
<td>Testosterone, ( n = 16 )</td>
</tr>
<tr>
<td>Age (years), mean (SD)</td>
</tr>
<tr>
<td>Height (cm), mean (SD)</td>
</tr>
<tr>
<td>Weight (kg), mean (SD)</td>
</tr>
<tr>
<td>Body mass index, mean (SD)</td>
</tr>
<tr>
<td>Number of frailty criteria</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3–4</td>
</tr>
<tr>
<td>Serum (total) T (nmol/L), mean (SD)</td>
</tr>
<tr>
<td>Free T (pmol/L), mean (SD)</td>
</tr>
<tr>
<td>Sex hormone–binding globulin (nmol/L), mean (SD)</td>
</tr>
</tbody>
</table>

scans for 10 different participants. Estimates of typical error, intra-class correlation coefficients, and confidence intervals were calculated.

Results

Of the 30 participants enrolled, 16 participants received T gel and 14 received placebo gel. One participant failed to attend at baseline scan and one at 6 months, giving 28 participants (15 T and 13 placebo) in the main analyses. Participant characteristics were well balanced across the two groups at baseline (Table 1) as were the ultrasound parameters (Table 3). Data for the measurement of muscle architectural parameters showed low typical error values and high intra-class correlation coefficients (Table 2).

Effects of Treatment on Serum T Levels

Mean (±SD) total T increased from 11.6 ± 3.5 to 18.0 ± 8.1 nmol/L in the T-treated group after 10 days (\( p = .002 \)) and was maintained throughout the 6-month treatment period. Similarly, mean (±SD) free T increased from 176 ± 37 to 326 ± 125 pmol/L (\( p < .001 \)). No substantive changes were seen in the placebo group (total T: 10.4 ± 3.4–10.5 ± 3.8 nmol/L and free T: 171–47 to 174 ± 60 pmol/L).

Effects on Muscle Architecture Assessed by Ultrasound

The results of the study for GM muscle architecture are presented in Table 3. There was a significant effect of treatment
on MT at 6 months (effect size 1.4 mm [95% CI = 0.3–2.5 mm; \( p = 0.15 \))]. MT increased by 2.6% in the T group and decreased by 5.4% in the placebo group. \( L_f \) decreased by 0.8% in the T group and 6.7% in the placebo group, with no effect of treatment being demonstrated (effect size 1.9 mm [95% CI = −1.2 to 5.0 mm; \( p = 0.22 \)). No significant effect of T treatment on \( \theta \) was detected following treatment (effect size: 1.2° [95% CI = −1.3 to 3.7°; \( p = 0.32 \)).

**DISCUSSION**

The present results demonstrate that increasing T for 6 months leads to preservation of MT compared with placebo. To our knowledge, this is the first clinical study to show a significant effect of androgens on muscle architecture using ultrasonography in intermediate-frail and frail elderly men. Although there was no significant effect of treatment on \( L_f \) or \( \theta \), the results of this exploratory investigation are encouraging as they indicate that T therapy was beneficial in maintaining muscle size by ameliorating the decrease in MT associated with aging and low T. Typically, marked hypertrophy of a pennate skeletal muscle would be accompanied by an increase in \( \theta \) (14). However, small changes in muscle size such as those encountered in this study are unlikely to lead to modifications in \( \theta \). Therefore, T treatment in this study probably ameliorated the deterioration in muscle architecture but did not cause hypertrophy. This suggests that the accumulation of new contractile tissue along the aponeurosis was not sufficient to lead to geometrical changes in the arrangement of muscle fibers but was effective in preventing a loss of muscle mass by retaining MT. Previously, an age-associated decrease in \( L_f \) has been demonstrated (9), whereas changes in \( L_f \) with hypertrophy are normally associated with resistance-training regimes involving eccentric contractions, which cause muscle stretch (15). Thus, it is not surprising that absolute \( L_f \) did not increase in response to T treatment alone. Although \( L_f \) tended to decline less rapidly in the T group over the course of this study, the treatment effect did not reach significance.

Although it should be emphasized that magnetic resonance imaging or computed tomography remain the most accurate way of calculating whole muscle volume, previous studies employing the ultrasound method to assess muscle size (8,16) have shown the technique to be accurate and cost-effective in detecting hypertrophy or atrophy and is therefore of benefit when magnetic resonance imaging or computed tomography are not available. Furthermore, ultrasound-based muscle size measurements have been shown to be highly correlated with magnetic resonance imaging (17) and dual energy x-ray absorptiometry (7) in quantifying lean body mass. The inclusion of data on muscle fascicle geometry may thus afford clinicians’ more insight into the degree of change in muscle mass rather than relying on MT as the only marker of muscle size.

There is only one other study that used ultrasound to investigate architectural changes in response to androgen administration, but this was coupled with heavy resistance exercise in a group of previously strength-trained young men and involved intramuscular injection of supraphysiological doses of testosterone enanthate (TE) (18). In this small study, there was a significant increase in triceps brachii MT in both groups, which tended to be greater in the TE group (\( n = 5 \)) when compared with the non-TE group (\( n = 4 \); 29.5% increase vs 13.8% increase, respectively): though this interaction was not statistically significant, \( L_f \) was not shown to change significantly over time in response to the training or TE (18). However, \( \theta \) increased to a greater degree in the androgen-treated group, (18) indicating that the training regime caused substantial increases in contractile tissue, which were potentiated by supraphysiological doses of TE. Although this small study failed to demonstrate a treatment effect for MT (18), the trend of the response does corroborate our present results.

In summary, although the present study does not provide evidence of hypertrophy of GM following T therapy, it shows that MT was clearly improved in the T-treated group, without significant reorganization of the fascicular arrangement. This study contributes to the understanding of the changes in muscle architecture induced by T and is consistent with the findings of previous reports which showed androgens to be effective in maintaining lean body mass (12,19–21). This investigation also adds to the list of studies that have found ultrasound to be a useful method of assessing muscle size and architecture (9,15,16,18,22). Due to its non-invasive nature, minimal training, mobility, and economy, muscle ultrasoundography offers many advantages in clinical practice. Future studies involving larger sample sizes, longer treatment duration, and incorporation of resistance training may elucidate the relationship between T, muscle architecture, muscle strength, and frailty in elderly men.

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### Table 3. Mean (SD) Pretreatment (baseline) and Posttreatment (6 months) Values for Muscle Thickness, Fascicle Length, and Pennation Angle

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Treatmenteffect(95%CI)</th>
<th>Placebo</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness</td>
<td>(mm)</td>
<td>n = 15</td>
<td>15.3(2.9)</td>
</tr>
<tr>
<td></td>
<td>Treatment effect (95% CI)</td>
<td>1.4 (0.3–2.5)</td>
<td></td>
</tr>
<tr>
<td>Fascicle length</td>
<td>(mm)</td>
<td>n = 15</td>
<td>45.1 (7.0)</td>
</tr>
<tr>
<td></td>
<td>Treatment effect (95% CI)</td>
<td>1.9 (-1.2 to 5.0)</td>
<td></td>
</tr>
<tr>
<td>Pennation angle</td>
<td>(°)</td>
<td>n = 15</td>
<td>23.5 (3.8)</td>
</tr>
<tr>
<td></td>
<td>Treatment effect (95% CI)</td>
<td>1.2 (-1.3 to 3.7)</td>
<td></td>
</tr>
</tbody>
</table>

Note: CI = confidence interval.
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Conflict of Interest

R.A.A., S.A.R., M.J.C., J.E.A., J.A.O., O.R.S., C.E.H.S., C.N.M., and M.V.N. have nothing to declare. F.C.W.W. consulted for Bayer Schering Healthcare, Germany; Akzo-Nobel (Organon), The Netherlands; Ferring Pharmaceuticals, Denmark; Pierre-Fabre Medicaments, France; Ardana Biosciences, United Kingdom; Procter & Gamble, United States; and Lilly Icos, United States.

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References