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The Male Contraceptive Regimen of Testosterone and Levonorgestrel Significantly Increases Lean Mass in Healthy Young Men in 4 Weeks, but Attenuates a Decrease in Fat Mass Induced by Testosterone Alone

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In hypogonadal men, testosterone (T) in replacement dosages is known to increase fat-free mass (lean mass) and decrease fat mass. In young eugonadal men, similar dosages of T increase lean mass, but much higher dosages of T are required to decrease total body fat mass. Current T-based male hormonal contraceptive regimens include a second agent, such as a progestin, to maximize inhibition of pituitary gonadotropins and improve efficacy. To study the effect of such combinations on body composition, we randomized healthy, young, eugonadal men into four combinations of exogenous T and the progestin, levonorgestrel (LNG): 1) 100 mg T enanthate, im, weekly plus 125 μg LNG, orally, daily (T+LNG); 2) T plus placebo LNG (T alone); 3) placebo T plus LNG (LNG alone); and 4) placebo T plus placebo LNG (placebo). We then analyzed body composition by dual energy x-ray absorptiometry after 4 and 8 wk of treatment. T+LNG significantly increased total lean mass after 4 and 8 wk of treatment (3.5 ± 0.9% and 4.2 ± 1.2%, respectively; $P < 0.05$) and truncal lean mass after 4 and 8 wk of treatment (4.7 ± 0.9% and 5.0 ± 0.9%, respectively; $P < 0.05$) compared with baseline and placebo. T alone also increased total and truncal lean mass significantly compared with placebo after 4 wk of treatment, but not compared with baseline (3.3 ± 1.4% and 3.2 ± 2.3%, respectively; $P < 0.05$ *vs.* placebo), suggesting an additive effect of T and LNG to increase lean mass. Fat mass significantly decreased in the abdomen in men administered T alone compared with LNG alone (−4.9 ± 2.8%; $P < 0.05$). Fat mass significantly increased in the abdomen with LNG alone (4.1 ± 1.0%; $P < 0.05$) compared with baseline and was unchanged with the combination of T+LNG, suggesting that LNG attenuates the decrease in fat mass seen with T alone. There was no change in weight or body mass index in any group during the study. This study shows that in young eugonadal men 1) T alone rapidly increases lean mass and decreases fat mass in 4–8 wk; 2) T+LNG rapidly increases lean mass, but has no effect on fat mass; and 3) LNG alone increases fat mass. The favorable profile on body composition by T is, therefore, partially attenuated by the progestin, LNG. These findings suggest that androgen-based male hormonal contraceptives might have favorable effects on body composition. The impact of these changes on cardiovascular risk in normal men needs further study. (*J Clin Endocrinol Metab* 88: 1167–1173, 2003)

THE COMBINATION OF testosterone (T) and a progestational agent has been used in male hormonal contraceptive regimens, effectively suppressing gonadotropins and spermatogenesis in healthy young men (1–3). In these studies the usual dosage for T administration is 100 mg, im, weekly of T enanthate or T cypionate, regimens that normalize serum T in hypogonadal men (4–6). T is known to increase lean mass and decrease fat mass when analyzed by dual energy x-ray absorptiometry (DEXA) in hypogonadal men at replacement doses (7–9). Bhasin *et al.* (10) demonstrated increases in lean mass by DEXA after 20 wk with 125 mg T, im, weekly, but a decrease in total body fat mass required much higher doses of T (600 mg weekly for 20 wk).

Levonorgestrel (LNG) is an androgenic progestin that is frequently combined with T in hormonal male contraceptive regimens (3, 11). We hypothesized that changes in body lean and fat mass with the combination of T plus LNG would be greater and occur earlier than with T alone because of the androgenicity of LNG (12, 13). In this study we examined the effects of T plus LNG, T alone, LNG alone, or placebo for 8 wk on the body composition of normal young men.

Abbreviations: BMI, Body mass index; CT, computed tomography; DEXA, dual energy x-ray absorptiometry; E_2, estradiol; LNG, levonorgestrel; MRI, magnetic resonance imaging; T, testosterone.

Subjects and Methods

Subjects

Men between the ages of 18 and 45 yr were recruited by flyers, newspaper ads, and radio advertisement and through the University of Washington Clinical Research Study Internet Site. After informed consent, subjects were accepted into the study during the control phase with a normal medical history and physical examination, normal serum levels of T, estradiol (E_2), FSH, and LH. Exclusion criteria were chronic systemic disease, regular medication use, tobacco use, and use of steroid hormones in the past 12 months, including androstenedione and dehydroepiandrosterone. The University of Washington Human Subjects Committee Institutional Review Board and the V.A. Research and Development Committee approved this study.

Protocol

The study consisted of a 4-wk control phase, an 8-wk treatment phase, and an 8-wk recovery phase. Nurses and investigators were blinded to treatment conditions. Thirty-seven healthy young men (average age, 32.7 ± 1.4 yr) who met screening criteria were randomized into one of

four groups for an 8-wk treatment phase: 1) T+LNG: 100 mg T enanthate (Delatestryl, manufactured for BTG Pharmaceuticals Co. by Bristol-Myers Squibb Co., Princeton, NJ), im, weekly plus 125 μg LNG, orally, daily (formulated as a white powder in a clear capsule; Wyeth Ayerst, Philadelphia, PA); 2) T alone: 100 mg T enanthate, im, weekly and oral placebo (lactose as a white powder in a clear capsule) daily; 3) LNG alone: placebo T (sterile sesame seed oil), im, weekly plus 125 μg LNG, orally, daily; and 4) placebo: placebo T, im, weekly plus placebo LNG, orally, daily. Assignment for each consecutively enrolled subject was made by a research pharmacist using a predetermined assignment sheet created using a random number generator.

Subjects were asked to consume an average of one or fewer alcoholic beverage per day and to use an approved contraceptive throughout the study. Subjects were also asked not to change their diet or exercise patterns during the study. Dietary and exercise habits were reviewed at each study visit.

Three subjects discontinued the study after two or fewer T injections, one because of mood changes, one due to scheduling conflicts, and one because of noncompliance. Thirty-four subjects completed the treatment phase of the study. Two subjects did not complete the recovery period because they moved out of the state. One subject in the placebo group completed the study, but was eliminated from analysis because of a body mass index (BMI, 35.8 kg/m^2) that was 3 SD above that in the rest of the study participants (25.2 $\pm$ 3.5 kg/m^2). This subject was not obese, but had a very muscular body habitus. Exclusion of data from this subject did not change any of the statistically significant results.

Hormone assays

Serum hormone levels were obtained between 1700–1900 h. T, FSH, and LH levels were measured by immunofluorometric assay (Delfia, Wallac, Inc., Turku, Finland). Peak levels (24 h after injection) and trough levels (immediately before next injection) of hormones were measured during treatment wk 8. The assay sensitivity for T was 0.35 nmol/liter; the intraassay coefficient of variation was 4.5%, and the interassay coefficient of variation was 9.5% for a mean low range pooled T value of 6 nmol/liter, a mean midrange pooled T value of 11.4 nmol/liter, and a mean high range pooled T value of 24 nmol/liter. E_2 was measured with a standard DSL-39100 kit (Diagnostic Systems Laboratories, Inc., Webster, TX) with 0.75 pg/ml sensitivity and 6.0% coefficient of variation for interassay variability for a pooled low range value of 29 pmol/liter and 4.6% coefficient of variation for intraassay variability for a midrange pooled value of 110 pmol/liter. The sensitivities of the assay for FSH and LH were 0.016 and 0.019 IU/liter, respectively. The intraassay coefficient of variation was 12.0%, and the interassay coefficient of variation was 22.3% for a low range pooled value of FSH of 0.045 IU/liter; these values were 2.9% and 6.1%, respectively, for a midrange pooled value of 0.96 IU/liter. The intraassay coefficient of variation was 6.5%, and the interassay coefficient of variation was 17.7% for a low range pooled LH value of 0.074 IU/liter, and 3.2% and 12.5% for a midrange pooled value of 1.15 IU/liter. Samples from each participant were run in duplicate in the same assay to avoid interassay variability.

Body composition

Weight using the same scale and BMI were monitored throughout the study. During the last week of the control period, all subjects had a DEXA scan to assess body composition. The DEXA scans were performed on a QDR-4500A (Hologic, Inc., Waltham, MA) using Hologic software version 9.03 with a precision error of less than 1% for regional and total body scans. One technician with certification from the International Society for Clinical Densitometry was assigned to this project and verified the accuracy of each scan. The standard whole body DEXA examination included total body and regional measurements. Soft tissue regions measured outside of bone included two of the trunk, one of the pelvis, one of the abdomen, and one for each limb. The window of measurement for the total abdomen by DEXA was from lumbar vertebrae 1 (L1) through to the pelvic sacrum. The DEXA scan was repeated at wk 4 and 8 during treatment and once during the recovery phase. Abdominal and limb soft tissue mass were measured during the control period and treatment wk 8 only.

General laboratories

Blood sampling for determinations of complete blood count and differential, electrolytes and glucose (chemistry 7), calcium, lactate dehydrogenase, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, albumin, bilirubin, total protein, creatinine, calcium, uric acid, phosphate, nonfasting total cholesterol, triglycerides, high density lipoprotein, and low density lipoprotein as well as urinalysis were performed three times during the control period, once during treatment, and once during recovery. Laboratory values were analyzed by Dynacare Laboratories (Seattle, WA).

Statistics

Data are presented as the mean $\pm$ SEM. Subjects who did not complete both treatment phase DEXA scans have been excluded from the analysis to present data at each time point from the same individuals. There originally were eight subjects in the placebo and T alone groups, seven subjects in the LNG alone group, and nine subjects in the T+LNG group. Excluding one subject in the placebo group, three subjects in the T alone group, and two subjects in the T+LNG group did not change the statistical significance of any of the reported changes in hormones or body composition. Changes from baseline within a group were analyzed with an unpaired one-sample *t* test. Differences between groups were assessed by ANOVA and verified by Kruskal-Wallis test. Differences between treatment groups were further compared using a paired two-tailed *t* test. We used Duncan's comparison measure as our *post hoc* test.

Results

All data presented are from individuals that completed the baseline and treatment phase DEXA scans (see *Subjects and Methods*).

Hormones

T. Trough levels of T did not increase significantly in the placebo or T+LNG group, but were significantly higher in the T alone group than baseline values and significantly lower in the LNG alone group than baseline or placebo group values ($P < 0.05$; Table 1). Peak levels of T significantly decreased in the LNG alone group *vs.* baseline and the placebo group ($P < 0.05$; Fig. 1). Peak levels of T increased significantly in the T alone and T+LNG groups compared with baseline and the placebo group ($P < 0.05$; Table 1). Trough and peak T levels did not significantly differ between the T alone and T+LNG groups. There were no significant differences in T levels among groups at baseline or during the recovery period.

E_2. There was a significant increase in the trough, but not the peak, level of E_2 from baseline in the placebo group. Trough, but not peak, E_2 levels significantly decreased in the LNG alone group compared with the placebo group ($P < 0.05$; Fig. 1). Both trough and peak levels of E_2 significantly increased from baseline in the T alone group, and peak, but not trough, E_2 levels were significantly higher than those in the placebo group. Trough levels of E_2 were not significantly elevated in the T+LNG group; however, peak levels in this group were significantly elevated above baseline ($P < 0.05$). There were no significant differences in E_2 trough or peak levels between the T alone and T+LNG groups. There were no significant differences in E_2 levels between groups at baseline or during the recovery period.

Gonadotropins. Serum gonadotropins were measured during treatment wk 8 at the same time as a trough level of T was

TABLE 1. Circulating hormone levels at baseline, peak, and trough levels at 8 wk during treatment and weight throughout the study in the following groups: placebo (n = 7), LNG alone (n = 7), T alone (n = 5), and T + LNG (n = 7)

Groups	Study phase			
	Baseline	Week 8 trough	Week 8 peak	Recovery
Testosterone (nmol/liter)				
Placebo	25.9 ± 2.9	27.4 ± 3.8	28.9 ± 2.8	24.0 ± 1.8
LNG alone	17.5 ± 1.5	11.9 ± 1.8[a,b]	13.3 ± 1.5[a,b]	19.8 ± 0.9
T alone	19.8 ± 2.3	25.5 ± 1.3[a,c]	41.5 ± 3.9[a,b,c]	18.9 ± 1.4
T + LNG	16.6 ± 1.5	20.5 ± 1.5	32.5 ± 1.9[a,b,c]	20.4 ± 1.6
Estradiol (pmol/liter)				
Placebo	123.4 ± 16.6	145.9 ± 21.3[a]	143.9 ± 21.1	133.8 ± 21.2
LNG alone	110.3 ± 9.1	100.7 ± 14.6[b]	99.6 ± 8.8	126.0 ± 9.0
T alone	109.4 ± 5.2	131.6 ± 10.1[a]	152.3 ± 10.8[a,b,c]	124.1 ± 8.1
T + LNG	107.8 ± 8.3	124.8 ± 8.4	146.0 ± 10.5[a,c]	124.1 ± 11.7
FSH (IU/liter)				
Placebo	4.4 ± 0.7	4.4 ± 0.6	ND	4.3 ± 0.7
LNG alone	2.7 ± 0.3	2.0 ± 0.2[a,b]	ND	2.7 ± 0.3
T alone	3.7 ± 0.6	0.2 ± 0.1[a,b,c]	ND	3.4 ± 0.5
T + LNG	3.7 ± 0.6	0.09 ± 0.03[a,b]	ND	3.2 ± 0.6
LH (IU/liter)				
Placebo	4.8 ± 0.4	4.8 ± 0.6	ND	4.6 ± 0.6
LNG alone	3.9 ± 0.5	2.9 ± 0.5	ND	3.8 ± 0.9
T alone	4.2 ± 0.7	0.2 ± 0.1[a,b,c]	ND	4.0 ± 0.5
T + LNG	3.5 ± 0.6	0.05 ± 0.01[a,b]	ND	3.2 ± 0.6
	Baseline	Wk 4	Wk 8	Recovery
Weight (kg)				
Placebo	81.1 ± 5.7	81.0 ± 5.8	82.0 ± 5.7	81.3 ± 5.6
LNG alone	83.4 ± 2.6	82.7 ± 2.4	82.5 ± 2.7	83.3 ± 2.4
T alone	80.2 ± 4.8	80.5 ± 5.3	81.1 ± 5.3	80.3 ± 5.5
T + LNG	76.0 ± 2.6	76.3 ± 2.7	76.7 ± 2.9	77.4 ± 3.1
BMI (kg/m^2)				
Placebo	24.4 ± 1.5	24.6 ± 1.5	24.4 ± 1.5	24.5 ± 1.5
LNG alone	26.5 ± 1.1	26.3 ± 1.1	26.2 ± 1.2	26.5 ± 1.1
T alone	24.6 ± 1.2	24.7 ± 1.4	24.9 ± 1.4	24.6 ± 1.4
T + LNG	24.2 ± 0.8	24.3 ± 0.8	24.4 ± 0.8	24.9 ± 0.8

T and E_2 peak levels were 24 h after T injection at 8 wk during the treatment phase. LH and FSH levels were measured during the time of trough levels of T (immediately before T injection) at 8 wk during the treatment phase. ND, Not determined.
[a] $P < 0.05$ *vs.* baseline; [b] $P < 0.05$ *vs.* placebo; [c] $P < 0.05$ *vs.* LNG alone.

measured. FSH did not change significantly in the placebo group. FSH decreased significantly in the LNG alone, T alone, and T+LNG groups compared with baseline and placebo group values ($P < 0.05$; Table 1 and Fig. 1).

LH did not change significantly in the placebo or LNG alone group, although LH tended to decrease in the LNG alone group *vs.* baseline ($P = 0.07$). LH decreased significantly in the T alone and T+LNG groups compared with baseline and the placebo groups ($P < 0.05$; Table 1 and Fig. 1).

There were no significant differences in gonadotropin levels between the T alone group and the T+LNG group. There were no significant differences in gonadotropin levels between groups at baseline or during the recovery period.

Body composition

Total lean mass increased significantly by 3.5 ± 0.9% (1.8 ± 0.4 kg; $P < 0.05$) above baseline in the T+LNG group after 4 wk of drug administration (Table 2 and Fig. 2). Total lean mass remained significantly elevated to 4.2 ± 1.2% (1.9 ± 0.6 kg; $P < 0.05$) above baseline after 8 wk of drug and returned to baseline values during the recovery phase. The increase in total lean mass at 4 and 8 wk of treatment in the T+LNG group was significantly higher than that in the placebo group ($P < 0.05$). T alone tended to increase total lean mass above baseline after 4 and 8 wk ($P = 0.06$ and 0.09, respectively; Table 1 and Fig. 2). The increase in total lean mass in the T alone group was significant *vs.* that in the placebo group at 4 wk of treatment ($P < 0.05$). There were no significant changes in total lean mass in the placebo group or LNG alone group during treatment or the recovery phase. There were no significant differences in total lean mass between groups at baseline or during the recovery period.

Truncal lean mass increased significantly in the T+LNG group to 4.7 ± 0.9% (1.2 ± 0.2 kg; $P < 0.05$) and 5.0 ± 0.9% (1.3 ± 0.2 kg; $P < 0.05$) above baseline and compared with placebo after 4 and 8 wk of drug administration, respectively, and remained significantly elevated compared with that in the placebo group (3.0 ± 1.4%; $P < 0.05$) during the recovery period. Truncal lean mass increased significantly in the T alone group to 3.2 ± 2.3% (0.8 ± 0.5 kg; $P < 0.05$) above baseline and compared with placebo at 4 wk and remained elevated to 3.3 ± 1.7% (0.8 ± 0.4 kg) above baseline compared with that in the placebo group, but did not reach significance at 8 wk of treatment ($P = 0.50$). There was no significant change in truncal lean mass in the placebo group or LNG alone group during the treatment or recovery phase. There were no significant differences in truncal lean mass between any of the groups at baseline or for the placebo, LNG alone, or T alone groups during the recovery period.

T+LNG and T alone significantly increased limb lean mass

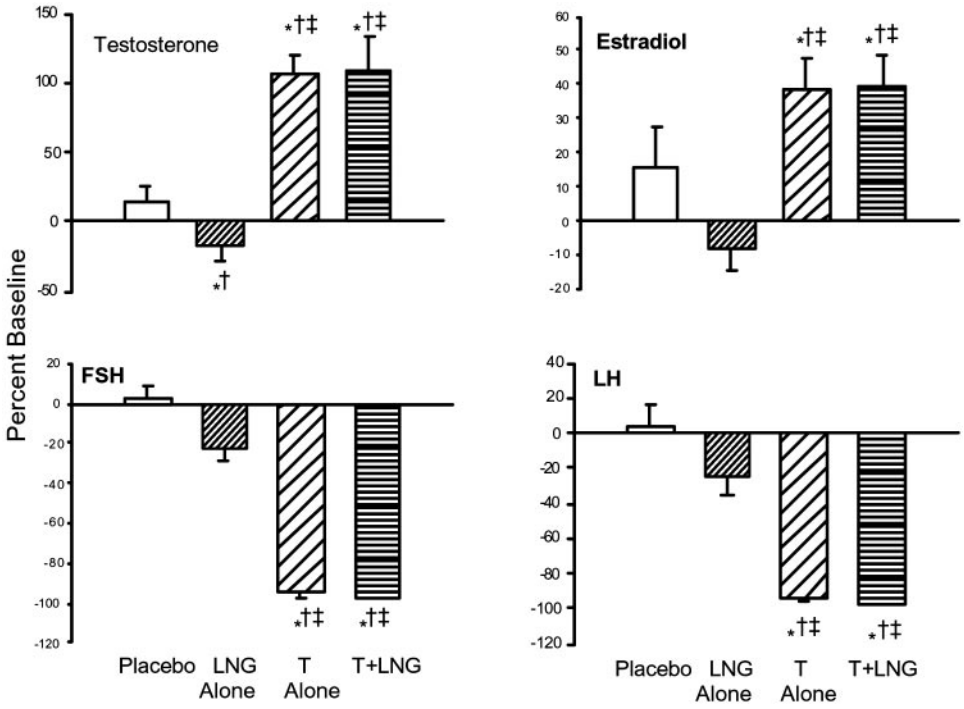

FIG. 1. Change in serum hormone levels. Data are the percent baseline (±SEM) changes in T, E_2, FSH, and LH levels at 8 wk of treatment. T and E_2 are peak levels. □, Placebo; ▨, LNG alone; ▨, T alone; ▤, T+LNG. *, $P < 0.05$ *vs.* baseline; †, $P < 0.05$ *vs.* placebo; ‡, $P < 0.05$ *vs.* LNG alone.

TABLE 2. Body composition (by DEXA scan) at baseline, at 4 and 8 wk during treatment, and after 8 wks of recovery in the following groups: placebo (n = 7), LNG alone (n = 7), T alone (n = 5), and T + LNG (n = 7)

Groups	Study phase				%Δ from baseline	
	Baseline	Week 4	Week 8	Recovery	Week 4	Week 8
Total lean mass (kg)						
Placebo	56.7 ± 2.5	56.1 ± 2.3	56.7 ± 2.6	56.4 ± 2.1	1.0 ± 0.8	0.04 ± 0.8
LNG alone	60.1 ± 2.2	60.1 ± 2.4	60.1 ± 2.4	61.1 ± 2.4	−0.1 ± 1.1	−0.06 ± 1.4
T alone	54.8 ± 3.5	56.6 ± 3.9[b]	56.1 ± 3.7	55.8 ± 3.9	3.3 ± 1.4[b]	2.5 ± 1.4
T + LNG	56.2 ± 1.9	58.1 ± 1.8[a,b,c]	58.2 ± 1.9[a,b,c]	58.9 ± 2.0	3.5 ± 0.9[a,b,c]	4.2 ± 1.2[a,b,c]
Trunk lean mass (kg)						
Placebo	26.9 ± 1.1	26.3 ± 1.1	27.1 ± 1.2	26.3 ± 0.9	−2.3 ± 1.1	0.87 ± 0.85
LNG alone	28.5 ± 1.0	28.23 ± 1.1	28.4 ± 1.1	28.9 ± 1.0	−0.9 ± 1.1	−0.4 ± 1.8
T alone	26.1 ± 1.7	26.9 ± 1.7[b]	27.0 ± 1.6	26.8 ± 1.8	3.2 ± 2.3[b]	3.3 ± 1.7
T + LNG	25.9 ± 0.9	27.1 ± 0.8[a,b,c]	27.2 ± 0.9[a,b,c]	27.0 ± 1.1[b]	4.7 ± 0.9[a,b,c]	5.0 ± 0.9[a,b,c]
Limb lean mass (kg)						
Placebo	13.7 ± 0.8	ND	13.7 ± 0.7	ND	ND	−1.6 ± 2.1
LNG alone	14.3 ± 0.6	ND	14.3 ± 0.7	ND	ND	0.3 ± 2.0
T alone	13.5 ± 0.9	ND	14.4 ± 0.9[a]	ND	ND	3.3 ± 1.0[a]
T + LNG	13.6 ± 0.4	ND	14.0 ± 0.4[a]	ND	ND	3.2 ± 1.2[a]
Total fat (%)						
Placebo	17.3 ± 2.5	17.51 ± 2.7	17.4 ± 2.5	17.6 ± 2.5	0.3 ± 1.7	0.02 ± 2.5
LNG alone	20.6 ± 2.1	20.6 ± 2.0	21.0 ± 2.0	20.6 ± 1.8	0.2 ± 2.2	2.4 ± 1.4
T alone	18.7 ± 2.3	17.64 ± 2.2	17.6 ± 2.3[a,c]	17.6 ± 2.1	−5.4 ± 1.8	−6.1 ± 2.2[a,c]
T + LNG	17.4 ± 1.5	17.07 ± 1.4	16.9 ± 1.4	1.4 ± 1.8	−1.8 ± 2.6	−2.1 ± 4.2
Abdominal fat mass (kg)						
Placebo	3.9 ± 0.76	ND	4.0 ± 0.6	ND	ND	4.4 ± 4.6
LNG alone	5.2 ± 0.6	ND	5.37 ± 0.6[a]	ND	ND	4.1 ± 1.0[a]
T alone	5.1 ± 0.7	ND	4.8 ± 0.6[c]	ND	ND	−4.9 ± 2.8[c]
T + LNG	3.9 ± 0.4	ND	4.1 ± 0.5	ND	ND	4.2 ± 3.6

Abdominal fat mass and limb (right arm and leg) lean mass were measured at baseline and 8 wk of treatment only. ND, Not determined.
[a] $P < 0.05$ *vs.* baseline; [b] $P < 0.05$ *vs.* placebo; [c] $P < 0.05$ *vs.* LNG alone.

(right arm and leg) after 8 wk of treatment (3.2 ± 1.2% and 3.3 ± 1.0% increase *vs.* baseline, respectively; $P < 0.05$) after 8 wk of treatment. There was no significant change in lean mass in the limbs in the placebo or LNG alone groups. There were no significant differences in lean mass in the limbs between groups at baseline or during the recovery period.

There was no significant change from baseline or between groups for total percent fat mass in the placebo, LNG alone, or T+LNG groups over the 8-wk treatment period. T alone significantly decreased total percent fat mass *vs.* baseline at wk 8 of treatment (−5.6 ± 2.0%; $P < 0.05$; Table 2).

T+LNG did not affect abdominal fat mass. T alone sig-

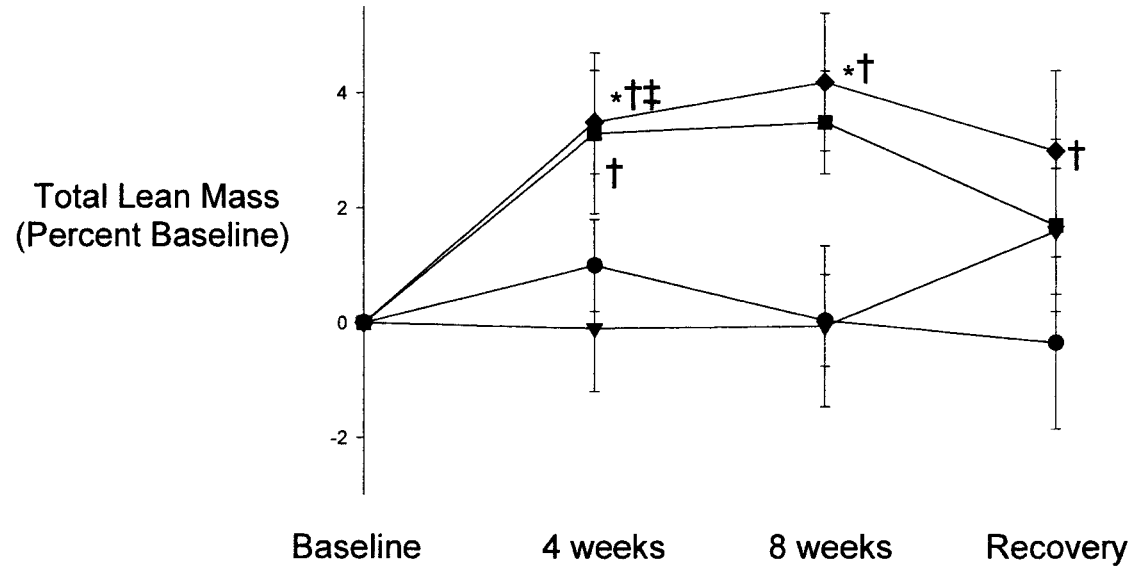

FIG. 2. Change in total lean mass. T increases total lean mass in 4 wk in healthy young men. Data are the percentage of baseline ± SEM. ●, Placebo; ▼, LNG alone; ■, T alone; ◆, T+LNG. *, $P < 0.05$ *vs.* baseline; †, $P < 0.05$ *vs.* placebo; ‡, $P < 0.05$ *vs.* LNG alone.

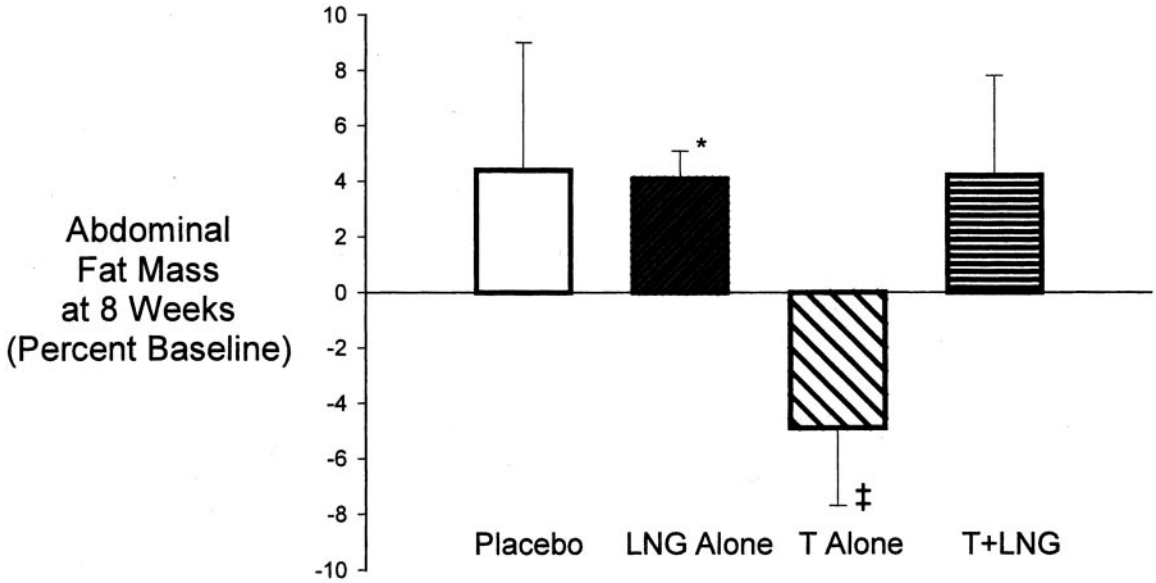

FIG. 3. Change in abdominal fat mass. T decreases, LNG increases, but T+LNG does not affect abdominal fat mass in 8 wk in healthy young men. □, Placebo; ■, LNG alone; ▨, T alone; ▤, T+LNG. Data are the percentage of baseline ± SEM. *, $P < 0.05$ *vs.* baseline; ‡, $P < 0.05$ *vs.* LNG alone.

nificantly decreased abdominal fat mass after 8 wk of treatment compared with LNG alone (−4.9 ± 2.8%; $P < 0.05$; Table 2) and tended to decrease abdominal fat mass compared with baseline and placebo ($P = 0.09$). Treatment with LNG alone significantly increased abdominal fat mass *vs.* baseline (4.1 ± 1.0%; $P < 0.05$; Fig. 3). There was no change in abdominal fat mass in the placebo group during the treatment or recovery phase.

There was no significant change in weight or BMI during the study for any group compared with baseline or each other (Table 1).

Laboratory tests, and physical and vital signs

There were no changes in the routine laboratory tests monitored throughout the study (see *Materials and Methods*), including hematocrit. There were no changes in heart rate, blood pressure, gynecomastia, or testicular size throughout the study (data not shown).

Discussion

T when administered with LNG to healthy young men significantly increased total lean mass and regional lean mass

in the trunk and limbs in only 4 wk. The rapidity of the change suggests a direct and potent link between T and body composition in men.

T alone tended to increase total lean mass and significantly increased limb lean mass within 4–8 wk. That the increase in total lean mass in the T alone group had only a trend to significance *vs.* baseline might reflect the small number of subjects (n = 5) in this group. Alternatively, the T+LNG group might have had a greater androgen stimulus to increase lean mass than the T alone group. LNG is a T-derived progestin known to bind with high affinity to the androgen receptor. Although it has less affinity for the androgen receptor than does dihydrotestosterone (13), LNG binds with greater affinity to the androgen receptor than do other progestational agents (12). The ability of LNG to bind to the androgen receptor suggests that the effect of LNG on lean body mass would be additive to that of T. LNG alone, however, did not have any effect on lean mass; therefore, although LNG might potentiate the effects of T on lean mass, T is the more potent stimulus to increasing lean mass.

Exogenous T+LNG and T alone also significantly increased truncal lean mass by DEXA. A number of other studies have also monitored changes in truncal lean mass with various interventions (14, 15). DEXA has been verified by computed tomography (CT) as an accurate measure of total and regional lean mass in the limbs (16–18), but there are no studies verifying measurements of truncal soft tissue mass by DEXA with either CT or magnetic resonance imaging (MRI) even though almost half of the lean mass in the body is found in the trunk (19). Verification of the changes seen in truncal lean mass in our study by CT or MRI is needed.

The increase in lean mass with T administration seen in our study could be explained by an increase in the water content of this tissue, as one of the side-effects of T administration is fluid retention (4). However, it is likely that the observed changes in lean mass reflect increases in muscle mass, not fluid shifts. In a study similar to ours, Bhasin *et al.* (10) administered T to healthy young men at doses ranging from 25–600 mg. Total lean mass increased 2.9 ± 0.8% after the administration of 125 mg T, im, for 20 wk, as measured by DEXA and underwater weighing. To determine whether the increase in lean mass was secondary to water retention, total body water was measured by nuclear magnetic spectroscopy after the men ingested deuterium hydroxide. The ratio of total body water to fat-free mass by underwater weighing did not significantly change for any treatment group.

Measurement of abdominal fat mass by DEXA has been demonstrated to correlate highly with abdominal fat measured by MRI and CT (20, 21). In 17 eugonadal men, the average central abdominal fat between L1 and L3 was 1.13 ± 0.34 kg by DEXA and 0.89 ± 0.2 kg by MRI, with a correlation of $r^2 = 0.87$ ($P < 0.001$) (20). There was also a strong relationship between abdominal fat percentage and volumes of fat measured between L1 and L4 by DEXA and CT ($r^2 = 0.92$; $P < 0.001$). We measured a window of fat from L1 to just above the pelvic sacrum. This region of measurement is slightly larger than that in the previous studies and includes the suprapubic area of fat to ensure that the complete abdomen was included in the window of measurement.

Total and abdominal fat mass in our study decreased significantly in the T alone group in 8 wk. Interestingly, although T decreased abdominal fat mass, abdominal fat mass increased in the group administered LNG alone. T+LNG did not affect abdominal fat mass, consistent with the opposite effect of LNG on fat mass than T. The individual effects of the hormones on fat mass are therefore cancelled when both hormones are administered together, suggesting that LNG differentially affects lean and fat mass.

Another explanation for the increase in fat mass in the LNG alone group could be that gonadotropin and T levels were significantly suppressed by LNG. However, if LNG also bound to and suppressed sex hormone-binding globulin similar to androgens (22, 23), increasing free T levels, fat mass would be expected to decrease similar to levels in the T alone group. Free T levels were not measured in this study.

The increase in lean mass and decrease in fat mass by T in this study are consistent with reports in hypogonadal men (7–9), eugonadal young (10), and older men (24). Muscle strength has been demonstrated to increase after im T administration to men in as little as 1 month of treatment (4, 25, 26). It is interesting that the rapid increase in lean mass was offset by a similar rapid decrease in fat mass, so that weight and BMI did not change. There was an increase in absolute lean mass by DEXA in the T+LNG group, suggesting that a longer study would have demonstrated the increase in weight as changes in lean mass accrued, as weight gain is clearly associated with T administration to eugonadal and hypogonadal men at these doses (8, 27, 28).

As T is metabolized to E_2 by aromatase, and E_2 levels were increased by approximately 39% in the groups administered T, the question arises as to what role E_2 has in determining body composition in men administered T. Estrogen receptors are found in adipocytes in male rats (29) and male estrogen receptor-α knockout mice have increased white adipose tissue as well as adipocyte size and amount (30), suggesting that the elevated E_2 levels in men administered T might play a role in decreasing fat mass. Analysis of body composition when T is administered with and without an aromatase inhibitor would help decipher the role E_2 plays in body composition in men given T.

Exogenous T plus a progestin such as LNG is a promising combination for a male contraceptive regimen. One of the major side-effects of exogenous T and progestin is decreased serum high density lipoproteins and possibly increased low density lipoprotein particle concentrations (11, 31–33). These lipid alterations associated with a T plus progestin combination might confer a higher cardiovascular risk (33). The favorable changes on lean and fat mass after an androgen-based contraceptive such as T+LNG might offset the effects of the unfavorable effects on lipid parameters.

Further investigations must be performed of the effects of androgens and progestins on fat mass and lean body mass to fully understand the risks and benefits of androgen-based contraceptives.

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References

1. **Wu FCW, Balasubramanian R, Mulders TM, Coelingh-Bennik HJ** 1999 Oral progestogen combined with testosterone as a potential male contraceptive: additive effects between desogestrel and testosterone enanthate in suppression of spermatogenesis, pituitary-testicular axis, and lipid metabolism. J Clin Endocrinol Metab 84:112–122
2. **Meriggiola MC, Bremner WJ** 1997 Progestogen-androgen combination regimens for male contraception. J Androl 18:240–243
3. **Bebb RA, Anawalt BD, Christensen RB, Paulsen CA, Bremner WJ, Matsumoto AM** 1996 Combined administration of levonorgestrel and testosterone induces more rapid and effective suppression of spermatogenesis than testosterone alone: a promising male contraceptive approach. J Clin Endocrinol Metab 81:757–762
4. **Bhasin S** 1992 Androgen treatment of hypogonadal men. J Clin Endocrinol Metab 74:1221–1225
5. **Sokol RZ, Palacios A, Campfield LA, Saul C, Swerdloff RS** 1982 Comparison of the kinetics of injectable testosterone in eugonadal and hypogonadal men. Fertil Steril 37:425–430
6. **Snyder PJ, Lawrence DA** 1980 Treatment of male hypogonadism with testosterone enanthate. J Clin Endocrinol Metab 51:1335–1339
7. **Wang C, Swedloff RS, Iranmanesh A, Dobs A, Snyder PJ, Cunningham G, Matsumoto AM, Weber T, Berman N** 2000 Transdermal testosterone gel improves sexual function, mood, muscle strength, and body composition parameters in hypogonadal men. Testosterone Gel Study Group. J Clin Endocrinol Metab 85:2839–53
8. **Bhasin S, Storer TW, Berman N, Yarasheski KE, Clevenger B, Phillips J, Lee WP, Bunnell TJ, Casaburi R** 1997 Testosterone replacement increases fat-free mass and muscle size in hypogonadal men. J Clin Endocrinol Metab 82: 407–413
9. **Katznelson L, Finkelstein JS, Schoenfeld DA, Rosenthal DI, Anderson EJ, Klibanski A** 1996 Increase in bone density and lean body mass during testosterone administration in men with acquired hypogoandism. J Clin Endocrinol Metab 81:4358–4365
10. **Bhasin S, Woodhouse L, Casaburi R, Singh AB, Bhasin D, Berman N, Xhen X, Yarasheski KE, Magliano L, Dzekov J, Bross R, Phillips J, Sinha-Hikim I, Shen R, Storer TW** 2001 Testosterone dose-response relationships in healthy young men. Am J Physiol 281:E1172–E1181
11. **Anawalt BD, Amory JK** 2001 Advances in male hormonal contraception. Ann Med 33:587–595
12. **Collins D** 1993 Selectivity information on desogestrel. Am J Obstet Gynecol 168:1010–1016
13. **Lemus AE, Vilchis F, Damsky R, Chavez BA, Garcia GA, Grillasca I, Perez-Palacios G** 1992 Mechanism of action of levonorgestrel: in vitro metabolism and specific interactions with steroid receptors in target organs. J Steroid Biochem Mol Biol 41:881–890
14. **Treuth MS, Ryan AS, Pratley RE, Rubin MA, Miller JP, Nicklas BJ, Sorkin J, Harman SM, Goldberg AP, Hurley BF** 1994 Effects of strength training on total and regional composition in older men. J Appl Physiol 77:614–620
15. **Douchi T, Kuwahata R, Yamasaki H, Yamamoto S, Oki T, Nakae M, Nagata Y** 2002 Inverse relationship between the changes in trunk lean and fat mass during gonadotropin-releasing hormone agonist therapy. Maturitas 42:31–35
16. **Levine JA, Abboud L, Barry M, Reed JE, Sheedy PF, Jensen MD** 2000 Measuring leg muscle and fat mass in humans: comparison of CT and dual-energy x-ray absorptiometry. J Appl Physiol 88:452–456
17. **Visser M, Fuerst T, Lang T, Salamone L, Harris TB, for the Health, Aging, and Body Composition Study-Dual-Energy X-Ray Absorptiometry and Body Composition Working Group** 1999 Validity of fan-beam dual-energy x-ray absorptiometry for measuring fat-free mass and leg muscle mass. J Appl Physiol 87:1513–1520
18. **Wang W, Wang Z, Faith MS, Kotler D, Shih R, Heymsfield SB** 1999 Regional skeletal muscle measurement: evaluation of new dual-energy x-ray absorptiometry model. J Appl Physiol 87:1163–1171
19. **Pritchard JE, Nowson CA, Strauss BJ, Carlson JS, Kayamakci B, Wark JD** 1992 Evaluation of dual energy x-ray absorptiometry as a method of measurement of body fat. Eur J Clin Nutr 47:216–228
20. **Kamel EG, McNeill G, Han TS, Smith FW, Avenell A, Davidson L, Tothill P** 1999 Measurement of abdominal fat by magnetic resonance imaging, dual-energy x-ray absorptiometry and anthropometry in non-obese men and women. Int J Obes 23:686–692
21. **Svendsen OL, Hassager C, Bergmann I, Christiansen C** 1993 Measurement of abdominal and intra-abdominal fat in postmenopausal women by dual energy x-ray absorptiometry: comparison with computerized tomography. Int J Obes 17:45–51
22. **Ganzalo IT, Swerdloff RS, Nelson AL, Clevenger B, Garcia R, Berman N, Wang C** 2002 Levonorgestrel implants (Norplant II) for male contraception clinical trials: combination with transdermal and injectable testosterone. J Clin Endocrinol Metab 87:3562–3572
23. **Nilsson B, von Scholtz B** 1989 Binding of levonorgestrel, norethisterone and desogestrel to human sex hormone binding globulin and influence on free testosterone levels. Gynecol Obstet Invest 27:151–154
24. **Snyder PJ, Peachey H, Hannoush P, Berlin JA, Loh L, Lenrow DA, Holmes JH, Dlewati A, Santanna J, Rosen CJ, Strom BJ** 1999 Effect of testosterone treatment on body composition and muscle strength in men over 65 years of age. J Clin Endocrinol Metab 84:2647–2653
25. **Sih R, Morley JE, Kaiser FE, Perry III HM, Patrick P, Ross C** 1997 Testosterone replacement in older hypogonadal men: a 12-month randomized controlled trial. J Clin Endocrinol Metab 82:1661–1667
26. **Urban RJ, Bodenburg YH, Gilkison C, Foxworth J, Coggan AR, Wolfe RR, Ferrando A** 1995 Testosterone administration to elderly men increases skeletal muscle strength and protein synthesis. Am J Physiol 269:E820–E826
27. **Anawalt BD, Herbst KL, Matsumoto AM, Mulders TMT, Coelingh-Bennink HJT, Bremner WJ** 2000 Desogestrel plus testosterone effectively suppresses spermatogenesis but also causes modest weight gain and high-density lipoprotein suppression. Fertil Steril 74:707–714
28. **Bagatell CJ, Herman JR, Matsumoto AM, Rivier JE, Bremner WJ** 1994 Metabolic and behavioral effects of high-dose, exogenous testosterone in healthy men. J Clin Endocrinol Metab 79:561–567
29. **Pederson SB, Borglum JD, Erikson EF, Richelsen B** Nuclear estradiol binding in rat adipocytes. Regional variations and regulatory influences of hormones. Biochim Biophys Acta 1093:80–86
30. **Cooke PS, Heine PA, Taylor JA, Lubahn DB** 2001 The role of estrogen receptor-α in male adipose tissue. Mol Cell Endocrinol 178:147–154
31. **Tan KCB, Shiu SWM, Kung AWC** 1999 Alterations in hepatic lipase and lipoprotein subfractions with transdermal testosterone replacement therapy. Clin Endocrinol (Oxf) 51:765–769
32. **Herbst KL, Deeb SS, Bremner WJ, Amory JK,** Testosterone (T) significantly increases hepatic lipase activity (HLA) and decreases high-density lipoprotein cholesterol (HDL-C) in three weeks in elderly men. Proc of the 84th Annual Meeting of The Endocrine Society, San Francisco, CA, 2002, Abstract P2-653.
33. **Bagatell CJ, Bremner WJ** 1995 Androgen and progestagen effects on plasma lipids. Prog Cardiovasc Dis 38:255–271