No effect of short-term testosterone manipulation on exercise substrate metabolism in men

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Submitted 13 May 2005; accepted in final form 29 June 2005

Braun, Barry, Laura Gerson, Todd Hagobian, Daniel Grow, and Stuart R. Chipkin. No effect of short-term testosterone manipulation on exercise substrate metabolism in men. J Appl Physiol 99: 1930–1937, 2005. First published June 30, 2005; doi:10.1152/japplphysiol.00565.2005.—Compared with women, men use proportionately more carbohydrate and less fat during exercise at the same relative intensity. Estrogen and progesterone have potent effects on substrate use during exercise in women, but the role of testosterone (T) in mediating substrate use is unknown. The purpose of this investigation was to assess how large variations in the concentration of blood T would impact substrate use during exercise in men. Nine healthy, active men were studied in three distinct hormonal conditions: physiological T (no intervention), low T (pharmacological suppression of endogenous T with a gonadotrophin-releasing hormone antagonist), and high T (supplementation with transdermal T). Total carbohydrate oxidation, blood glucose rate of disappearance, and estimated muscle glycogen use were assessed by using stable isotope dilution and indirect calorimetry at rest and while bicycling at ~60% of peak O₂ consumption for 90 min. Relative to the physiological condition (T = 5.5 ± 0.5 ng/ml), total plasma T was considerably suppressed in low T (0.8 ± 0.1) and elevated in high T (10.9 ± 1.1). Despite the large changes in plasma T, carbohydrate oxidation, glucose rate of disappearance, and estimated muscle glycogen use were very similar across the three conditions. There were also no differences in plasma concentrations of insulin, lactate, or free fatty acids. Plasma estradiol (E) concentrations were elevated in high T, but correlations between substrate use and plasma concentrations of T, E, or the T-to-E ratio were very weak (r² < 0.20). In conclusion, unlike the effect of acute elevation in E to constrain carbohydrate use in women, acute changes in circulating T concentrations do not appear to alter substrate use during exercise in men.

androgen; carbohydrate oxidation; fat oxidation; glucose uptake; stable isotope; glycogen

THE MAJORITY OF WELL-CONTROLLED human studies (adequate sample size, men and women matched for appropriate characteristics, exercise intensity below the lactate threshold) show that men oxidize more carbohydrate and less fat than women working at the same relative exercise intensity (9, 15, 18, 19, 32). The mechanisms underlying this male-female difference are inevitably complex (gender-related patterns of body composition, muscle fiber type, blood flow, etc., are likely to play some role), but the prime suspect has been the obvious difference in circulating sex hormones (3, 6, 8, 10, 20, 36, 39). It is clear from strong animal and human studies that the presence of a typically “female” sex hormone environment (i.e., characterized by high concentrations of estrogen and sometimes progesterone) at least partly explains the results (7–9, 11, 13, 14, 23, 25, 33). Acute changes in circulating concentrations of the ovarian hormones have potent effects on substrate utilization in response to metabolic stress (7–9, 11, 13, 14, 23, 25, 33). Every review article written has suggested that, in similar fashion, acute changes in circulating testosterone could mediate substrate use (3, 6, 8, 10, 20, 36, 39). There are few data to support or refute that proposition, however.

The implication, from the aforementioned studies of human sex differences, is that testosterone opposes the effect of estrogen and contributes to the “male” pattern of substrate use by reducing reliance on lipid and increasing use of carbohydrate. Indirect evidence (e.g., lipoprotein lipase activity) from human studies, however, suggests that fat utilization is actually increased with testosterone supplementation (31, 37). In rodents, testosterone treatment has been reported to increase lipid use (42), reduce exercise glycogen use (40), and reverse the elevated rates of glycogenolysis induced by castration (30). Lipolysis in isolated adipocytes was not increased when hypophysectomized rats were given testosterone, however (43), and testosterone treatment actually lowered the rate of lipolysis in human preadipocytes (12) and in brown adipocytes from rats (28). These contradictory data are insufficient to draw firm conclusions about the role of circulating testosterone in regulating human substrate use.

To facilitate the study of metabolic regulation by circulating sex hormones in humans, our laboratory developed a “suppression/replacement” approach to create controlled, reproducible sex hormone environments in healthy individuals (11). Recently, our laboratory used a gonadotrophin-releasing hormone (GnRH) antagonist and exogenous administration of relevant hormones to test substrate kinetics and oxidation in the presence of very low, normal physiological, and high-physiological concentrations of estrogen and/or progesterone in young women (11). We used the same model system in the present study to create three distinct hormonal environments in young men. The purpose of the study was to investigate how low, normal, and high concentrations of blood testosterone affected the balance between blood glucose uptake, estimated glycogen utilization, and lipid oxidation during prolonged, submaximal exercise.

METHODS

Subjects. Subjects for this study were nine healthy, physically active men, who participated in regular aerobic activity at least three times per week (Table 1). All of the subjects were in excellent overall health, free of cardiovascular, metabolic, respiratory, or endocrine diseases, and taking no medications. The American Physiological Society and its Publishing Partner, Elsevier, have entered into a license agreement that allows retractions of this article to be published. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
TESTOSTERONE, EXERCISE, AND SUBSTRATE METABOLISM

Table 1. Subject physical characteristics and demographic data

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
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<tr>
<td>Age, yr</td>
<td>24.8±6.8 (18–39)</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>74.2±6.0 (67–83)</td>
</tr>
<tr>
<td>Height, cm</td>
<td>176.2±6.9 (168–188)</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>16.3±3.6 (11–22)</td>
</tr>
<tr>
<td>Lean mass, kg</td>
<td>59.4±4.3 (55–66)</td>
</tr>
<tr>
<td>(\text{VO}_2\text{max}, \text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1})</td>
<td>48.9±6.8 (38–59)</td>
</tr>
</tbody>
</table>

Values are mean ± SD (with range in parentheses) for 9 subjects. \(\text{VO}_2\text{max}\), maximal oxygen consumption.

health; had no history of cardiovascular, metabolic, or hormonal disorders; and used no medications other than occasional over-the-counter aspirin or ibuprofen. After study procedures were explained verbally, subjects signed a written, informed consent document approved by Institutional Review Boards at both the University of Massachusetts and Baystate Medical Center.

**Pretesting procedures.** Body composition was determined by dual-energy X-ray absorptiometry (Lunar). Maximal oxygen consumption (\(\text{VO}_2\text{max}\)) was measured on an electronically braked cycle ergometer (SensorMedics 800S, Yorba Linda, CA) with an incremental ramp protocol starting at 100 W and increasing by 25 W every 2 min until volitional exhaustion. Oxygen consumption and carbon dioxide production were measured by indirect calorimetry with a TrueMax 2400 metabolic measurement system (Parvo Medics, Sandy, UT).

**Hormonal control.** Subjects were tested in three different hormonal conditions: physiological, low testosterone (LT), and high testosterone (HT). All subjects were tested in the physiological condition first and then in the two altered hormonal conditions: LT and HT. The order of the LT and HT condition testing was balanced across subjects.

To reduce circulating testosterone to concentrations characteristic of hypogonadal men (<1 ng/ml), subcutaneous injections of 3 mg Cetrotide (Serrono, Randolph, MA) were given to suppress endogenous production of GnRH. The injections were given 70–74, 46–50, and 22–26 h before LT testing. To raise circulating testosterone into the upper end of the physiological range for young men (>8 ng/ml), subjects were two Androderm (5-mg transdermal testosterone) patches (WatsonPharma, Corona, CA) per day for 3 days (total of 6 patches) before HT testing. No pharmacological intervention was used before physiological testosterone (PT) testing. Individual subjects were always tested at the same time of day in all three conditions. At least 7 days elapsed between testing sessions.

**Control of diet and activity.** Subjects were regularly reminded to maintain a similar activity level and consistent dietary habits throughout the course of the study. They refrained from exercise for 24 h before testing in all conditions. Although diet was not rigidly controlled throughout the entire study, all subjects consumed the same preexercise meal 12 h (for subjects tested in the morning for all 3 conditions) or 3 h (for subjects tested in the afternoon for all 3 conditions) before each test. The meal comprised 35% of estimated daily energy requirements and was composed of 55% carbohydrate, 15% protein, and 30% fat. Resting metabolic rate was estimated based on standard equations, and this value was multiplied by an activity factor of 1.55 or 1.73 (depending on activity level estimated from training diaries) to calculate daily energy requirements (26). Subjects were instructed to fast after this meal until testing.

**Testing procedures.** Subjects reported to the laboratory, and a 21-gauge catheter was inserted into an antecubital vein for infusion of stable isotope. A second catheter was placed in a forearm or wrist vein of the contralateral arm for blood sampling. A venous blood sample was collected before infusion for determination of background isotopic enrichment, and a priming bolus of 200 mg [6,6-\(^2\)H]glucose in 0.9% sterile saline was then rapidly infused into the venous catheter. To reach and maintain isotopic equilibrium, [6,6-\(^2\)H]glucose was then continuously infused at 2.5 mg/min with a peristaltic infusion pump (Harvard Apparatus, South Natick, MA). Venous blood samples and 5-min collections of expired oxygen and carbon dioxide were taken at rest and 75 and 90 min after the start of the infusion.

Immediately after the last resting measurement, the subject began submaximal cycling exercise. To maintain a steady isotopic enrichment of blood glucose during exercise, the [6,6-\(^2\)H]glucose infusion rate was increased to 6.0 mg/min (11). During the first 15 min of exercise in the PT condition, the intensity was adjusted by manipulating the pedaling resistance until oxygen consumption reached a steady state at ~60% of the previously measured \(\text{VO}_2\text{max}\). The protocol was repeated in exactly the same way for the other two conditions. Blood and breath samples (5 min) were collected at 15, 30, 45, 60, 75, and 90 min of exercise (Fig. 1).

**Biochemical assays.** Samples of venous blood for analysis of glucose, lactate, and glucose isotopic enrichment were collected in heparinized syringes and then transferred to vacutainers containing sodium fluoride (to inhibit glycolysis). Samples for analysis of testosterone, estradiol, free fatty acids, and cortisol were collected in nonheparinized syringes and transferred to vacutainers containing a clotting factor. All samples were immediately centrifuged, and the plasma was transferred to cryogenic vials and frozen at −70°C until analysis. Glucose and lactate concentrations were determined by using a glucose/lactate analyzer (GLS Analyzer, Analox Instruments, Lunenberg, MA). Testosterone and estradiol levels were determined by using radioimmunoassays (Diagnostic Systems Laboratories, Webster, TX). Free fatty acid concentrations were measured by using a standard colorimetric assay (Wako Chemicals, Richmond, VA).

For determination of [6,6-\(^2\)H]glucose enrichment, 0.7 ml of plasma was deproteinized by adding 1.2 ml of 0.3 N Zn(SO)\(_4\) and 1.2 ml of 0.3 N Ba(OH)\(_2\). Tubes were vortexed, incubated in an ice bath for 20 min, and centrifuged at 4°C at 2,500 g for 20 min. After the supernatant was extracted, water was removed from the supernatant via freeze-drying (−50°C, 5 mTorr). The dried samples were reconstituted in 100 µl of a 2:1 acetic anhydride and pyridine mixture, capped, heated in a water bath at 60°C for 60 min, and transferred to clean 13×100-mm borosilicate tubes. Double-distilled water (1.5 ml) and 0.4 ml of dichloromethane were added in that order, and the tubes were centrifuged at 2,000 rpm for 10 min. The dichloromethane phase was transferred to a 1-ml gas chromatography vial and evaporated under nitrogen. Tubes were capped, and 25 µl of ethyl acetate were added by using a gas-tight syringe. A 1-µl sample of the pentaacetate derivative was injected and separated on a gas chromatograph, and spectra were recorded on a mass spectrometer (Hewlett-Packard 6890, Palo Alto, CA). Selected ion monitoring was used to compare the abundance of the unlabeled fragment with that of the enriched isotopomer (Chemstation Software). After correcting for background enrichment, the abundance of the deuterated isotopomer (mass-to-charge ratio = 202) was expressed as percentage of total glucose species (mass-to-charge ratio = 200 + 201 + 202).

**Calculations.** To test the rate at which glucose is taken up from the blood [rate of disappearance (Rd)] and replaced by the liver [rate of appearance (Ra)], equations specifically designed for use with stable isotopes in biological systems were used (41).

![Fig. 1. Schedule to show blood and breath sample collections at 15-min intervals during exercise (Ex).](http://jap.physiology.org/)

J Appl Physiol • VOL 99 • NOVEMBER 2005 • www.jap.org
Glucose $R_d$ and $R_t$ are as follows:

Glucose $R_d$ (mg/min)
\[
= F - V\left[\left(C_1 + C_2\right)\left(I/2\left(I/I_2 - I/I_1\right)\right)\right]
\]

Glucose $R_t$ (mg/min)
\[
= R_d - V\left[\left(C_2 - C_1\right)\left(I/I_2 - I/I_1\right)\right]
\]

where $F$ is the isotope infusion rate; $I_1$ and $I_2$ are enrichments of plasma glucose with isotope label at time $t_1$ and $t_2$, respectively; $C_1$ and $C_2$ are plasma glucose concentrations; and $V$ is the estimated volume of distribution for glucose (180 ml/kg).

\[
\%\text{CHO} = \left[\left(RER - 0.71\right)/0.29\right] \times 100
\]

where %CHO is percent energy from carbohydrate, and RER is the respiratory exchange ratio.

Carbohydrate oxidation rate (mg/min)
\[
= \left[\left(%\text{CHO}/100\right) \times (V_O2 \text{ in l/min}) \times \left(5.05 \text{ kcal/l}\right)\right]/4.0 \text{ g/kcal}
\]

where $V_O2$ is O2 uptake. Estimate of muscle glycogen utilization (EMGU) was determined by:

\[
\text{(total carbohydrate oxidation rate)} - \text{(blood glucose } R_d)\]

This estimate is based on the assumption that 100% of blood glucose taken up from the blood is oxidized, which is unlikely to be true; i.e., the percentage of $R_d$ oxidized is probably 70–90% (15, 24) but may vary across the conditions used in this study. Thus the calculation underestimates glycogen use and is best described as minimal muscle glycogen utilization.

Statistical analysis. Statistical analysis was accomplished by using SAS software (Cary, NC). A mixed-model two-way ANOVA was used to determine the treatment × time interaction by using a compound symmetric covariate structure for all variables. By convention, significant differences were defined as $P < 0.05$. When appropriate, post hoc tests of significance were performed with a Tukey honestly significant difference test. In general, data are presented as the group mean, 95% confidence interval, and exact P value, except where noted. In Tables 1–3, data from the HT and LT conditions are presented as the difference from the physiological condition, the 95% confidence interval of the difference, and the exact P value. Pearson product-moment correlations were used to assess the relationship between circulating testosterone, estrogen, or the testosterone-to-estradiol ratio and carbohydrate oxidation, fat oxidation, glucose kinetics, and estimated muscle glycogen use.

RESULTS

Hormonal environment. The treatments resulted in three very distinct testosterone environments (Fig. 2A). Compared with the physiological condition (PT), the serum concentration of testosterone before exercise was considerably elevated (2-fold greater) in the HT condition and markedly suppressed (reduced by 6-fold) in the LT. During exercise, circulating testosterone concentrations did not change to any appreciable extent relative to preexercise levels. The direction of changes in plasma concentrations of 17β-estradiol generally tracked the plasma testosterone data (Fig. 2B). Relative to PT, there was a small decrease in plasma estradiol in LT and a significant increase ($P = 0.023$) in HT.

Exercise oxygen consumption and heart rate. As expected, given that the absolute work intensity was matched among conditions, oxygen consumption expressed in absolute terms, or as a percentage of $V_O2\text{max}$, and heart rate were very similar in PT, HT, and LT (Table 2). These results suggest that the relative exercise intensity did not vary across the three conditions. In addition, subjects’ rating of perceived exertion was almost identical across conditions (data not shown).

Blood concentrations of substrates. Blood glucose (Fig. 3A) concentrations declined slightly (~10%) over time during exercise. Blood levels of lactate rose from resting values at the onset of exercise and plateaued around 1.5 mM for the final hour of exercise (Fig. 3B). Free fatty acid concentrations rose progressively throughout exercise (Fig. 3C). There were no significant differences among conditions in any of the circulating compounds, either at rest or during exercise.

Gas exchange. At rest, there were no significant differences in resting RER (PT = 0.84 ± 0.01, LT = 0.83 ± 0.01, HT = 0.81 ± 0.02, $P = 0.231$). RERs rose during exercise and remained fairly steady around 0.90 during the last 45 min of exercise (Fig. 4). A modest depression in the HT condition (~0.02 unit) was not significantly different from either of the other two conditions, either at rest or during exercise. Based on RERs, rates of carbohydrate (PT = 2.64 ± 0.48, LT = 2.36 ± 0.29, HT = 2.18 ± 0.35 mg·kg⁻¹·min⁻¹, $P = 0.144$) and lipid oxidation (PT = 1.44 ± 0.09, LT = 1.35 ± 0.10, HT = 1.54 ± 0.13 mg·kg⁻¹·min⁻¹, $P = 0.321$) at rest were calculated and were not different among conditions. Steady-state exercise values (averaged over the last 45 min of exercise) are shown in Table 3. Small mean differences in carbohydrate oxidation (9% lower in HT compared with PT) between conditions were not statistically significant. Conversely, the mean rate of lipid oxidation was slightly elevated in the HT condition (16% higher than PT), but there was no statistically significant difference.

Glucose kinetics. Isotopic enrichment of plasma glucose was ~1.4% at rest and was maintained at approximately the same
value (1.3–1.5%) during the last 45 min of exercise in all three conditions (data not shown). Resting glucose Ra was not different among conditions (PT/H11005 4.09/H11006 0.43, LT/H11005 3.78/H11006 0.46, HT/H11005 4.37/H11006 0.51 mg/kg•min, P=0.303). During steady-state exercise, glucose Ra for PT, LT, and HT were very similar, with no significant differences among conditions (Table 3). Because blood glucose concentrations declined slightly during exercise, glucose Rd were marginally higher than the Ra but followed the same pattern across conditions (Table 3). Expressed as the difference between total carbohydrate oxidation and glucose Rd, EMGU was almost identical in the HT vs. LT condition. The EMGU in both conditions was 9–10% lower than PT, but this minor difference was not statistically significant.

Contribution to total energy expenditure. The proportion of total energy expenditure attributable to lipid oxidation, blood glucose use, and EMGU is shown in Fig. 5. There are slight variations in the exact mixture of substrates oxidized across conditions, but, overall, the patterns are markedly similar.

Relationship between hormone concentrations and substrate use. Correlations between concentrations of circulating testosterone and carbohydrate use, fat use, glucose kinetics, or estimated glycogen use were all very weak (r² < 0.20) and nonsignificant. The same pattern of correlations was observed between substrate use and circulating concentrations of estrogen or the testosterone-to-estrogen ratio.

DISCUSSION

The main finding in this study was that suppression and replacement of testosterone to create distinctly different concentrations of circulating testosterone had very little impact on the balance between blood glucose, glycogen, and fat utilization during exercise in young men. Subtle differences in the HT condition hinted at a nudge toward greater use of fat and sparing of carbohydrate, both at rest and during exercise, but the changes were not statistically significant and unlikely to be physiologically relevant.

Due to the conflicting results obtained from short-term studies, our data are consistent with some, but certainly not all, published results. In two studies in humans, lipoprotein lipase activity increased, and there was a reduction in visceral and subcutaneous fat in response to testosterone supplementation, suggesting a rise in fat utilization (31, 37). In vitro studies by Xu and associates (42) showed that testosterone treatment increased lipolysis in rats. Also, in rats, testosterone supplementation reduced exercise glycogen use and increased resting muscle glycogen content (40) and reversed the elevated rates of glycogenolysis induced by castration (30). In contrast, catecholamine-stimulated lipolysis was not increased in isolated adipocytes when hypophysectomized rats were given testosterone (43). Testosterone treatment actually lowered the rate of catecholamine-stimulated lipolysis (but only in subcutaneous fat cells) in human preadipocytes (12) and reduced lipolysis and increased antilipolytic activity in brown adipocytes from rats (28). To add complexity to the story, Giudicelli et al. (16)
reported that testosterone may upregulate both lipolytic and antilipolytic responses in rat and hamster visceral fat. Rebuffe-Scrive et al. (31) reported that testosterone increased basal, but not catecholamine-stimulated, lipolysis in human visceral adipose tissue, whereas, in subcutaneous fat, catecholamine-stimulated lipolysis actually went down in the presence of testosterone. It is unclear why the responses to short-term changes in circulating testosterone concentrations are so variable. It is probable that the difficulty of controlling all of the potential confounding variables (e.g., diet, physical activity, circadian rhythm, other stressors) obscures any impact of acutely changing the testosterone environment. In a previous study, using a similar method to effect acute changes in circulating estrogen and progesterone concentrations, however, we did see marked metabolic effects in women (11). Taken together, the implication from these two studies is that the metabolic effects of short-term variations in circulating testosterone, if any, are subtle.

The present findings are not consistent with data from long-term human studies, which generally show that chronic (weeks to months) exposure to testosterone increases lean mass and reduces fat mass in men (1, 38). It is very likely that this discrepancy is related to differences in the length of exposure. Over the long term (months), subtle changes in nutrient storage and/or utilization could alter adipocyte mass while being undetectable when viewed at a “snapshot” point in time. Chronic exposures to elevated levels of testosterone can also alter receptor sensitivity, e.g., the upregulation of receptor sensitiv-

### Table 3. Summary of metabolic data during steady-state exercise

<table>
<thead>
<tr>
<th>Condition</th>
<th>PT</th>
<th>LT</th>
<th>HT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Testosterone, ng/ml</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>4.8±0.29</td>
<td>0.7±0.04</td>
<td>9.7±1.0</td>
</tr>
<tr>
<td><strong>Δ from PT</strong></td>
<td>-4.1</td>
<td>-0.7</td>
<td>-4.9</td>
</tr>
<tr>
<td>95% CI</td>
<td>(-6.2, -1.9)</td>
<td>(-2.8, 7.2)</td>
<td></td>
</tr>
<tr>
<td><strong>Exact P value</strong></td>
<td>0.0001</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td><strong>RER</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.910±0.012</td>
<td>0.914±0.016</td>
<td>0.894±0.007</td>
</tr>
<tr>
<td><strong>Δ from PT</strong></td>
<td>0.004</td>
<td>0.016</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>(-0.039, 0.046)</td>
<td>(-0.059, 0.027)</td>
<td></td>
</tr>
<tr>
<td><strong>Exact P value</strong></td>
<td>0.83</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td><strong>Total CHO use, mg kg⁻¹ min⁻¹</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>27.1±1.7</td>
<td>25.5±2.0</td>
<td>24.9±1.4</td>
</tr>
<tr>
<td><strong>Δ from PT</strong></td>
<td>-1.6</td>
<td>-2.2</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>(-8.3, 5.0)</td>
<td>(-8.8, 4.5)</td>
<td></td>
</tr>
<tr>
<td><strong>Exact P value</strong></td>
<td>0.53</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td><strong>Estimated muscle glycogen use, mg kg⁻¹ min⁻¹</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>18.8±1.6</td>
<td>17.2±1.7</td>
<td>17.1±1.1</td>
</tr>
<tr>
<td><strong>Δ from PT</strong></td>
<td>-1.6</td>
<td>-1.7</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>(-7.0, 3.3)</td>
<td>(-7.2, 3.7)</td>
<td></td>
</tr>
<tr>
<td><strong>Exact P value</strong></td>
<td>0.46</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td><strong>Blood glucose rate of appearance, mg kg⁻¹ min⁻¹</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>8.3±0.7</td>
<td>8.2±0.7</td>
<td>7.8±0.7</td>
</tr>
<tr>
<td><strong>Δ from PT</strong></td>
<td>-0.1</td>
<td>-0.5</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>(-2.5, 2.4)</td>
<td>(-2.8, 2.0)</td>
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<tr>
<td><strong>Exact P value</strong></td>
<td>0.96</td>
<td>0.65</td>
<td></td>
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<tr>
<td><strong>Blood glucose rate of disappearance, mg kg⁻¹ min⁻¹</strong></td>
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<td></td>
</tr>
<tr>
<td>Mean</td>
<td>8.4±0.7</td>
<td>8.4±0.7</td>
<td>8.0±0.7</td>
</tr>
<tr>
<td><strong>Δ from PT</strong></td>
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<td>-0.4</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>(-2.5, 2.4)</td>
<td>(-2.8, 2.0)</td>
<td></td>
</tr>
<tr>
<td><strong>Exact P value</strong></td>
<td>0.96</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td><strong>Total lipid oxidation, mg kg⁻¹ min⁻¹</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>5.1±0.7</td>
<td>5.4±0.6</td>
<td>5.9±0.4</td>
</tr>
<tr>
<td><strong>Δ from PT</strong></td>
<td>0.3</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>(-1.7, 2.3)</td>
<td>(-1.2, 2.8)</td>
<td></td>
</tr>
<tr>
<td><strong>Exact P value</strong></td>
<td>0.69</td>
<td>0.32</td>
<td></td>
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</table>

Values are means ± SE. Statistical comparisons for LT and HT conditions are expressed relative to PT. CI, confidence interval; Δ, group mean difference; RER, respiratory exchange ratio; CHO, carbohydrate.

![Fig. 5. Contribution of plasma glucose, estimated muscle glycogen, and lipid-to-total energy expenditure during steady-state exercise (45–90 min).](image-url)
ity that occurs during puberty (17). As previously mentioned, short-term variations in circulating testosterone have been reported to alter specific metabolic pathways, with increased rates of visceral adipocyte lipolysis as a common, although not universal, result. Although we observed clear and distinct variations in circulating testosterone concentrations on the day of the metabolic measurements, we cannot be certain that the patterns observed were consistently present during all 3 days of the treatments. Perhaps with longer exposures, the individual would adapt via transcription of new protein and/or upregulation of receptors so that the shift toward greater fat use would become more evident at the level of whole body metabolism. In addition, if longer exposure to testosterone increased lean body mass, a rise in total energy expenditure and mitochondrial mass could increase the capacity for fat oxidation and elevate net lipid utilization.

It is conceivable that, although our “suppression/replacement” model clearly altered circulating concentrations of total testosterone, the impact on free testosterone (not bound to sex hormone-binding globulin) was less affected. In general, however, supplementation with exogenous testosterone or compounds that change endogenous testosterone production have roughly equivalent effects on both total and free testosterone (27, 29, 38). It has been reported that an acute bout of exercise differentially affects circulating concentrations of free and bound testosterone (2), but we expect that, if this were indeed the case, all three of our conditions would be equally impacted and the overall pattern of results would not change.

A potential confounding variable that we suspected could interfere with interpretation of our results is the in vivo conversion of testosterone to estrogen via the enzyme aromatase. There is evidence that supplementing with exogenous testosterone or its precursors, dehydroepiandrosterone and androstenedione, elevates blood concentrations of testosterone and increases traffic through this pathway, resulting in elevated blood concentrations of estradiol or estrone (5). Elevated concentrations of estradiol can increase lipolysis, upregulate enzymes related to β-oxidation of fatty acids, and downregulate the use of carbohydrate for energy (9, 11, 14, 25, 33). In the present study, we did see the estradiol concentration in the blood rise significantly in the HT condition. There was no obvious impact on exercise substrate use, however. It is possible that the effect of estradiol to constrain carbohydrate use negated an opposing effect of testosterone, but, based on several pieces of evidence, this scenario seems unlikely. First, the estradiol concentrations noted in the HT condition are well below values reported to cause changes to exercise substrate metabolism (4, 21). Second, there were no relationships between circulating testosterone, estrogen, or the testosterone-to-estrogen ratio and any aspect of substrate use. Third, physiologically relevant changes in the blood concentration of estradiol would be expected to increase plasma free fatty acid levels, and this did not occur in our study. Finally, if testosterone at physiological concentrations does have a major regulatory impact on substrate use, we would expect a difference between the physiological and suppressed conditions, which we did not observe.

To reduce the probability of missing a true effect of varying the testosterone environment, we tried to minimize sources of variability by exerting experimental control over the most likely confounding variables. Subjects included in the study were all lean men with high levels of habitual physical activity and considerable cardiorespiratory fitness. The exercise intensity was chosen to ensure that all subjects would be working below the lactate threshold so that the contribution from nonoxidative metabolism was minimal. The long-duration exercise task was chosen because prior studies have shown that gender differences may not emerge until exercise duration exceeds 60 min (19, 35). For each subject, testing took place at the same time of day for all three trials with a minimum of 7 days between trials. Subjects were provided all of the food consumed as a preexercise meal 12 (morning testing) or 3 h (afternoon testing) before each trial, with a standardized energy content and macronutrient composition. It is conceivable that protein oxidation was altered by testosterone suppression or replacement. Because protein oxidation was not measured or estimated in the present study, a change in the contribution of protein oxidation to total energy expenditure could affect the actual contribution of carbohydrate and lipid in ways that would be missed by indirect calorimetry. We estimate that, given the observed steady-state RER of ~0.89, even a 50% increase or reduction in the contribution of protein oxidation to total energy expenditure would alter the “true” RER by <0.01 units. Most importantly, we used a suppression/replacement model to create three distinct and reproducible testosterone environments rather than relying on natural variations in circulating testosterone or testosterone precursors. The lack of change in group means across conditions was not a result of averaging positive and negative responses; the failure of testosterone suppression or replacement to alter substrate kinetics or oxidation was consistent across subjects.

In response to acute exercise or training, plasma concentrations of growth hormone tend to parallel the pattern of change in testosterone (22). Because growth hormone increases the rate of lipolysis and shifts substrate use toward greater fat utilization, effects of testosterone suppression or supplementation on substrate use may have been mediated by a concomitant change in circulating growth hormone concentrations. It would have been illuminating to assess whether plasma growth hormone concentrations tracked the obvious differences in circulating testosterone in response to suppression or replacement, but plasma growth hormone concentrations were not measured.

Because none of our outcome measures was impacted by altering the circulating testosterone environment, the role of growth hormone as a mediator of those responses is moot.

Based on the results from the present study, it is unlikely that circulating concentrations of testosterone that characterize the typically “male” sex hormone environment explain the greater reliance on carbohydrate in men compared with women working at the same relative exercise intensity. Although acute changes in circulating ovarian hormones, particularly estradiol, do appear to have a physiologically relevant impact on metabolic regulation during exercise in women, changes of similar magnitude in circulating testosterone appear to have far less potent effects in men. Longer term suppression or elevation of testosterone may induce genomic or proteomic changes that are manifested in altered regulation of metabolic pathways.

From the perspective of clinicians prescribing testosterone to patients and the men and women using it, results from the present study suggest that blood glucose use and the balance between carbohydrate and fat oxidation are not impacted by acute changes in circulating testosterone concentrations.
contrast, acute changes in circulating estrogen and progesterone (for example, in response to oral contraceptives or hormone replacement) do appear to cause significant changes in the metabolic regulation of carbohydrate and fat metabolism. Whether there are metabolic effects of longer term manipulation of circulating testosterone concentrations that could impact fat oxidation, fat storage, or blood glucose uptake remains to be seen.

ACKNOWLEDGMENTS
The authors thank the research subjects for exceptional commitment of time and effort. We also acknowledge helpful assistance from Miki Takeda, Christine Pikul, Elizabeth Mitchell, Carrie Sharoff, and Dr. Steve Black. Thank you to Serrono, USA, for donating the GnRH antagonist Cetroxide.

GRANTS
Funding for this study was provided by a grant from the Baystate/UMASS Biomedical Research Program.

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