Obesity and metabolic syndrome are important factors in the increased prevalence of cardiovascular disease. One of the hallmarks of obesity in men is a reduction in serum testosterone levels. Biswas et al. reported recently that 45% and 61% of men with type 2 diabetes mellitus had reductions in total and free serum testosterone levels, respectively. The mechanisms responsible for the reduction in androgens with obesity have not been elucidated. It is possible that increases in aromatase activity in the adipose tissue could cause conversion of testosterone to estradiol and, thus, reduce testosterone levels. In support of this contention, Tamer et al. reported recently that bariatric surgery, and subsequent weight loss, increased serum testosterone levels in obese men.

Many investigators have heralded the reduction in androgens in obese men as being a major causative factor for increased cardiovascular disease, with consequences including endothelial dysfunction, erectile dysfunction, and hypertension. As a consequence, if androgen levels are decreased, obese men are often treated with androgen supplements. Although studies in older men show that testosterone supplements improve libido, protect against osteoporosis, and improve overall feelings of well being, no long-term studies have carefully documented the cardiovascular consequences of chronic androgen treatment in men with metabolic syndrome. One concern is that serum testosterone levels are not always well controlled in androgen-treated individuals compared with age-matched men, and studies have not been performed to determine what “adequate” testosterone levels should be targeted, because it is not clear whether chronically increasing androgens in obese men will protect them against cardiovascular disease.

In nonobese male rats that are prone to hypertension, androgens promote increases in blood pressure, because castration often reduces their blood pressure. How androgens increase blood pressure in nonobese males has not been entirely elucidated. The fact that androgens contribute to increased blood pressure in male rats calls into question whether androgen supplements in obese men will further increase blood pressure and promote cardiovascular disease or will actually protect against further development of cardiovascular disease and reduce or attenuate the onset of hypertension.

Just as in obese men, male obese Zucker rats (OZRs) have a reduction in serum testosterone levels as compared with male lean Zucker rats (LZRs). The present study tests the
hypothesis that chronic testosterone supplements in OZRs will promote, rather than protect against, cardiovascular disease and hypertension.

Methods

All of the protocols complied with the Guidelines for the Care and Use of Laboratory Animals by the National Institutes of Health and were reviewed and approved by the institutional animal care and use committee of the University of Mississippi Medical Center.

Rat Model

OZRs and their lean counterparts (LZRs) were obtained at 12 weeks of age from the vendor (Harlan, Indianapolis, IN) and allowed to age to 22 weeks of age. We have preliminary data that blood pressures in male OZRs and LZRs were not different between 12 and 20 weeks of age. Thus, at 22 weeks of age, rats were divided into 2 groups, those implanted with testosterone pellets (LZR: n=13; OZR: n=13) and controls (LZR: n=12; OZR: n=12). Rats were implanted subcutaneously on the back of the neck with testosterone-filled or empty silastic tubes (length, filled with testosterone propionate; Sigma Chemicals, St Louis, MO) that were changed every 3 weeks for 10 weeks. Throughout the testosterone treatment period, food and water intake were measured daily for 1 week per month. Data are presented only for the end of the study. All of the studies were performed in rats, aged 32 weeks, as described below, after 10 weeks of testosterone treatment.

Testosterone Supplements and Plasma Testosterone Levels

Silastic implants (10 mm, ID: 0.062 in; OD: 0.125 in; Dow Chemical Co, Midland, MI) were packed with testosterone decanoate and sealed with silastic glue. Pellets were soaked overnight in 70% ethanol before implantation. Pellets were changed every 3 weeks throughout the experiment. Testosterone levels were measured using a commercially available radioimmunoassay kit (Coat-A-Count testosterone kit; Diagnostic Products Corporation, Los Angeles, CA), as we have described previously.

Measurement of Blood Pressure

At 29 to 30 weeks of age, radiotelemeters were implanted as we have described previously. After a 2-week recovery period, blood pressure was measured for 5 days and recorded.

Urinary Protein Excretion

Rats (n=6–7 per group) were placed in plastic metabolic cages, and urine was collected for 24 hours. Urinary protein excretion was measured using the Bradford method using a commercially available reagent (BioRad, Richmond, CA).

Glomerular Sclerosis

Kidney sections from rats were examined by a pathologist who was not aware of the identity of the groups. Kidneys were embedded in paraffin and cut into 5-μm sections. The sections were stained with periodic acid-Schiff reagent. Three-hundred glomeruli from each kidney were examined, and each was graded for injury as follows: (1) <25% of the glomeruli damaged; (2) 25% to 50% damaged; (3) 50% to 75% damaged; (4) >75% damaged; and (5) global sclerosis. The data from all of the rats in a group were averaged and expressed as a percentage of glomeruli from each kidney exhibiting the 5 levels of injury.

Measurement of Metabolic Parameters

Plasma leptin, insulin, and cholesterol were measured by commercially available kits (insulin, leptin: Linco Research, St Charles, MO; cholesterol: Wako Pure Chemical Industries, Ltd, Richmond, VA). Oral glucose tolerance test was performed after 18 hours of fasting on all rats. Glucose (D-1-[14C]glucose in water; Sigma; 2 g/kg of body weight; total volume: 500 μL) was given by oral gavage, and then blood glucose levels were measured from a drop of tail blood using a glucometer (Accu-check Advantage; Roche) at 0, 30, 60, 90, and 120 minutes afterward and plotted. Data are presented as area under the curves for all of the groups. Visceral fat (as perirenal fat) was measured in all of the groups at the time of euthanization and factored for body weight.

Statistical Analyses

All of the data are expressed as mean±SEM. Statistical differences were determined by ANOVA, performed with SigmaStat software package version 3.1 (Systat Software Inc, San Jose, CA). Differences were considered statistically significant at \( P<0.05 \).

Results

Plasma Testosterone Levels

At 32 weeks of age, plasma testosterone levels were 70% lower in control OZRs compared with LZRs, and testosterone pellets increased plasma testosterone by 10- to 12-fold in both groups (Figure 1).

Body Weights and Kidney Weights

At 22 weeks of age, body weights were higher in OZRs than in LZRs (LZR: 472.6±765.5 g versus OZR: 765.5±17 g; Table). BWs, KWs, and KW/BW for LZR and OZR Controls And Those Treated With Testosterone Supplements

<table>
<thead>
<tr>
<th>Rats</th>
<th>BW, g</th>
<th>KW, g</th>
<th>KW/BW×10⁻³</th>
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<tr>
<td>LC (n=6)</td>
<td>522±14</td>
<td>3.33±0.15</td>
<td>6.31±0.25</td>
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<tr>
<td>LT (n=6)</td>
<td>474±14</td>
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<tr>
<td>OT (n=7)</td>
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<td>7.12±0.18</td>
</tr>
<tr>
<td>( P )</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

LZR indicates lean Zucker rat; OZR, obese Zucker rat; LC, LZR control; LT, LZR+testosterone treatment; OC, OZR control; OT, OZR+testosterone treatment; BW, body weight; KW, kidney weight; KW/BW, kidney weight/buffer weight ratio; NS, not significant.
Kidney weights were higher in control OZRs than LZRs and were slightly increased with testosterone treatment in both groups (Table). However, kidney weight:body weight ratios were significantly higher in LZR controls than OZR controls, and testosterone treatment increased kidney weight:body weight ratios by 21% in testosterone-treated OZRs (Table).

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Blood Pressure and Renal Injury
At 32 weeks of age, mean arterial pressure, as measured for 5 days by radiotelemetry, was significantly higher in OZRs than LZRs (Figure 2). Testosterone treatment had no effect on mean arterial pressure in LZR controls but was different compared with control LZR controls, and body weight was reduced by 21% in testosterone-treated OZRs (Table). Kidney weights were higher in control OZRs than LZRs and were slightly increased with testosterone treatment in both groups. However, kidney weight:body weight ratios were significantly higher in LZR controls than OZR controls, and testosterone treatment increased kidney weight:body weight ratios by 20% and 40% in LZR and OZR controls, respectively.

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Metabolic Parameters
Perirenal fat was significantly greater in control OZRs than LZRs (Figure 5A). Testosterone treatment reduced perirenal fat weight by 40% in LZR controls and by 25% in OZR controls. Visceral adiposity, measured as the mass of perirenal fat tissue factored for body weight, was also significantly higher in control OZRs than LZRs (Figure 5B), but testosterone treatment had no effect in either group. As expected, plasma leptin was considerably higher in control OZRs than LZRs, and testosterone treatment reduced plasma leptin by 30% in OZRs but not LZRs (Figure 6A). Plasma insulin was significantly higher in control OZRs than LZRs (Figure 6B). Testosterone treatment had no effect on insulin levels in LZR controls, but reduced insulin in OZRs by 80%, similar to levels in testosterone-treated LZR controls. Fasted blood glucose was higher in both control OZRs compared with LZR controls (control LZRs: 70±4 versus control OZRs: 123±8 mg/dL; P<0.001) and testosterone-treated OZRs compared with LZR controls (testosterone-treated LZRs: 62±4 versus testosterone-treated OZRs: 91±10; P<0.001). Testosterone treatment significantly reduced fasting glucose in OZRs compared with control OZRs (P<0.001) but not LZR controls (LZR controls versus testosterone-treated LZR controls). Insulin resistance, measured as the area under the curve for oral glucose tolerance test, was significantly greater in control OZRs than LZRs (Figure 6C). Testosterone treatment had no effect in LZR controls but reduced insulin resistance in OZRs by 40%. Plasma cholesterol was 2-fold higher in control OZRs than LZRs and was reduced significantly by testosterone treatment in OZRs but not LZR controls (Figure 6D).

Discussion
Obesity in men is associated with reductions in plasma testosterone levels. In the present study we found similar reductions in plasma testosterone in a model of obesity and metabolic syndrome, the OZR. In the present study we gave testosterone supplements to OZRs that exhibited 80% reductions in testosterone levels compared with their lean counterparts and increased plasma testosterone by 10-fold (>4-fold higher than in untreated LZR controls). We found that testosterone supplements in OZRs significantly reduced their body weight but had no effect on visceral adiposity when factored for body weight. We also found that testosterone supplements improved characteristics of the metabolic syndrome in OZRs, such as reducing plasma insulin, cholesterol, and leptin and improving oral glucose tolerance test. However, despite the positive metabolic syndrome effects, testosterone supplements significantly increased blood pressure in OZRs.

Whether androgen supplements increase blood pressure in men is not clear. There have been no trials in which androgen supplements have been given for long periods of time and
blood pressure followed throughout. There have been studies in female-to-male transsexuals who have been given testosterone supplements, and the data suggest that there is an increase in blood pressure with testosterone supplements. Basaria et al reported that testosterone supplements in aging men caused a significant increase in adverse cardiac events compared with the placebo group. Blood pressure was not reported in this study.

Many studies in rodents have shown that androgens promote hypertension and renal disease, including age-related renal disease. For example, aged male Sprague-Dawley rats exhibit accelerated renal aging and injury compared with females, and castration of males attenuates the injury, whereas ovariectomy of females does not worsen the renal injury. These data suggest that androgens promote renal injury. Young adult male spontaneously hypertensive rats exhibit higher blood pressure than do females, and castration of males attenuates the hypertension, whereas ovariectomy of females has no effect on blood pressure. These data suggest that androgens promote renal injury. We also found that blood pressure is higher in male Dahl salt-sensitive rats compared with females regardless of a high- or low-salt diet. High-salt diet does reduce serum testosterone levels in male DS rats (L.L. Yanes and J.F. Reckelhoff, unpublished data, 2009). Thus based on these data, it is possible that the naturally occurring reduction in androgens in OZRs with progression of obesity and metabolic syndrome may be protective against the development of further cardiovascular disease.

Men with low levels of androgens are given androgen supplements despite the presence of obesity and metabolic syndrome, both harbingers of future cardiovascular disease. Based on our data, testosterone supplements did improve insulin resistance, cholesterol, and leptin levels, despite the fact that the OZR is a leptin receptor–deficient rat. These changes may have been attributed to the effect of testosterone to reduce body weight in the OZR. Wu et al also reported improvement in metabolic syndrome characteristics in young obese men given testosterone supplements. In the present study, absolute levels of perirenal fat were also reduced; however, the level of visceral adiposity was similar despite testosterone treatment because of concomitant loss of fat with reductions in body weight. However, there was no increase in glomerular injury in OZRs with testosterone as determined by morphology. There was an increase in proteinuria with testosterone treatment in OZRs, but that was likely attributed to the increases in blood pressure.

In LZRs, raising plasma testosterone by 10-fold had no effect on their blood pressure but increased urinary protein and albumin excretion and also caused glomerular injury, albeit minimal. These data suggest that, with continued use of supraphysiological levels of androgens, testosterone would eventually increase blood pressure in LZRs because of the progressive renal damage. Individuals with chronic kidney disease have reductions in testosterone levels. Future studies are necessary to determine whether long-term testosterone supplements in individuals with chronic kidney disease would exacerbate their renal injury and lead to further nephron functional loss.
Why testosterone improved the characteristics of the metabolic syndrome but significantly increased blood pressure is not clear. Quan et al27 reported that testosterone supplements in normotensive rats increased proximal sodium reabsorption, which would cause an increase in blood pressure. Obesity is associated with an increase in tubular reabsorption of sodium. Indeed, we found a significant increase in the blood pressure with testosterone treatment only in the obese rats in this study. Alternatively, testosterone supplements may increase vasoconstrictors such as angiotensin II and endothelin. Testosterone supplementation has been shown to increase plasma endothelin levels in female-to-male transsexuals.28 Renin and angiotensinogen expressions are both upregulated in kidneys of male rats compared with their castrated counterparts.29,30 Schwartzman and colleagues31,32 have shown the androgens upregulate the synthesis of cytochrome P450 4A ω-hydroxylases in the vasculature of the kidney that would increase synthesis of prohypertensive 20-HETE. Another possibility is that the reduction in body weight and adipose tissue with androgen supplementation in OZRs could have reduced the amount of estrogens produced by the adipose tissue in the males. Estrogens are known to cause vasodilation via their effect on NO synthesis,33 and, thus, a reduction in adipose-produced estrogens could have contributed to the increase in blood pressure in the OZRs treated with testosterone. Unfortunately, we did not measure plasma estrogen in this study. Thus, future studies will be necessary to determine whether any of these mechanisms contribute to the hypertension in testosterone-treated OZRs.

Perspectives
In this study, chronic supplementation of male OZRs with androgens reduced insulin resistance and dyslipidemia and improved glucose mobilization but significantly increased blood pressure. These data suggest that androgen supplements may have similar beneficial effects on metabolic syndrome in obese men but that it is incumbent on physicians to monitor their blood pressure closely when prescribing androgen supplements. Furthermore, in addition to hypertension, high doses of androgens in men could promote prostate cancer.34

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Disclosures
None.

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