Effects of testosterone and estradiol on cutaneous vasodilation during local warming in older men

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Submitted 16 August 2007; accepted in final form 19 September 2007.

Sokolnicki LA, Khosla S, Charkoudian N. Effects of testosterone and estradiol on cutaneous vasodilation during local warming in older men. Am J Physiol Endocrinol Metab 293:E1426–E1429, 2007.—Microvascular vasodilation in humans can become impaired with age, leading to cardiovascular diseases ranging from mild to life-threatening. Reproductive hormones may confer some protection on the vascular system in women; however, it is unclear whether the same is true in men. Our goal was to evaluate the impact of four hormonal conditions (testosterone only, estradiol only, testosterone and estradiol, no testosterone and no estradiol) on microvascular vasodilator responsiveness in the skin of older men. We hypothesized that in older healthy men estradiol promotes cutaneous microvascular dilation during local warming of the skin and that testosterone inhibits this dilation. We measured skin blood flow using laser Doppler flowmetry during 35 min of cutaneous local warming to 42°C in 52 healthy men (average age 67 ± 1 yr). Subjects were randomized to one of the four hormonal conditions and were studied before and after hormone treatments. The endothelium-dependent vasodilator response to local warming was not different among groups either before or after hormone treatment. For example, with testosterone-only treatment this vasodilator response was 220 ± 13 AU, and with estrogen only the response averaged 246 ± 12 AU (P > 0.05). We conclude that, within the doses employed in the present study, testosterone and estradiol did not consistently alter cutaneous vasodilator responsiveness in healthy older men.

reproductive hormones; skin blood flow; aging; microvasculature

A major health concern associated with the growing elderly population in the United States is the increased risk for cardiovascular diseases with advancing age. The ability of the microvasculature to dilate has significant implications for protection against these diseases, including hypertension, stroke, and myocardial infarction. Microvascular endothelial function in health and disease has been evaluated by measurement of the cutaneous vasodilator response to local warming (6, 9, 11, 12). During local warming, cutaneous vasodilation occurs via two independent pathways: an initial rapid peak caused by local sensory nerves, followed by a slow rise in skin blood flow (SKBF) that plateaus after ~30 min of heating. The plateau phase of vasodilation is mediated in large part by local nitric oxide (NO) release (6, 9).

Previously, Charkoudian et al. (3) demonstrated that cutaneous vasodilation to local warming was augmented by exogenous reproductive hormones (oral contraceptives) in young women. The authors hypothesized that this was primarily an effect of estrogen to augment the NO-dependent vasodilator response. In men, testosterone may inhibit NO-dependent vasodilation (5). The effects of estrogen on vascular control in women have been well documented; however, vascular influences of reproductive hormones remain poorly understood in men. Additionally, although aging affects vasodilation, potential hormonal influences have not been studied in older men. In the present study, our goal was to utilize a known NO-dependent vasodilator stimulus (local warming of the skin) to evaluate potential influences of estradiol and testosterone on microvascular vasodilation in older healthy men.

Recently, Sanyal et al. (10) studied the influences of reproductive hormones on bone metabolism by randomizing healthy, older men to one of four groups: no testosterone and no estradiol, estradiol only, testosterone only, and combined testosterone and estradiol. We took advantage of this protocol to evaluate the impact of these four hormonal conditions on vasodilator responsiveness in the skin. We tested the hypothesis that in older healthy males estradiol augments cutaneous microvascular dilation during local warming of the skin and that testosterone inhibits this dilation.

METHODS

Subjects. The present study was a substudy of a larger study of 59 elderly men (10); of these, 52 men ranging in age from 50 to 70 yr consented to participate in this study. Subjects were asked to complete a health history questionnaire and a complete blood screen analysis prior to the study. Blood screening samples included a complete blood count, serum calcium, phosphorus, albumin, alkaline phosphatase, creatinine, thyroid-stimulating hormone, prostate-specific antigen, and 25-hydroxyvitamin D level. All blood values fell within the normal range for all subjects. No subject had a history of cardiovascular or bone disease. Subjects were randomly assigned to one of four treatment groups: Group A (−T, −E), Group B (−T, +E), Group C (+T, −E), and Group D (+T, +E). All study visits were performed in the Mayo Clinic Clinical Research Unit (CRU). Informed consent was obtained before any procedures began. The protocol for this experiment was approved by the Institutional Review Board at the Mayo Clinic in Rochester, MN.

Protocol. As described in detail elsewhere (10), to standardize hormone levels for the start of the study, all subjects were given an intramuscular injection of leuprolide, a GnRH agonist, to suppress endogenous testosterone and estrogen production. Simultaneously, an aromatase inhibitor, letrozole, was administered to block the conversion of androgens to estrogens. For the initial 3-wk baseline period, physiological circulating levels of testosterone and estradiol were maintained by using a testosterone gel (AndroGel; Solvay Pharmaceuticals, Marietta, GA; 5 g/day, delivering 50 mg/day of testosterone.

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transdermally) and an estrogen patch (Vivelle 0.0375 mg/day; Novartis, East Hanover, NJ). These doses were established from previous work from our group (4).

Three weeks later, subjects returned to the CRU for a baseline SkBF measurement and assessment of the cutaneous vasodilator response to local warming. SkBF was measured as cutaneous red blood cell flux using Periflux laser-Doppler flowmetry (LDF) as previously described (11, 13). One LDF probe (Periflux System 5000; Perimed, Stockholm, Sweden) was attached adhesively to the ventral side of the left forearm in a specialized holder that measures and controls temperature over an area of 12 cm².

Prior to local warming, baseline data were recorded for 7–10 min while local temperature was held constant at 32°C. Local temperature was then increased to 42°C at a rate of 1°C/s, and held at 42°C for 35 min, as previously described (11, 13). Subjects were asked whether they felt any pain or discomfort during the local warming; no subject reported feeling any pain or discomfort.

After the baseline visit (week 3), subjects were randomized into one of four treatment groups. Group A discontinued the testosterone gel and estrogen patch; Group B discontinued the testosterone gel and continued the estrogen patch; Group C continued the testosterone gel but discontinued the estrogen patch; and Group D continued both the testosterone gel and the estrogen patch. All subjects continued treatment with letrozole and received a second injection of leuprolide to ensure the inhibition of endogenous testicular function during the remainder of the protocol. Subjects reported to the CRU 21 days after group randomization for a final visit. Identical procedures from the baseline study visit were followed for the posttreatment local warming visit.

Data analysis. All data are expressed as means ± SE. SkBF data are expressed in arbitrary units (AU) measured by the Perimed LDF unit. Resting SkBF was calculated as the average of the final 2 min of the period before local warming. SkBF responses to local warming were analyzed as follows. The initial peak was assessed as a 60-s average of the highest point during the initial rapid vasodilator response (first ~3–5 min). The plateau response was calculated as the average of the last 2 min during local warming. The baseline and initial peak phases are then expressed as a percentage of the maximal SkBF value. This latter value is referred to as %plateau. To evaluate the influence of reproductive hormone status on these variables, we compared initial peak, plateau, and %plateau between baseline and intervention conditions in each of the four groups. This was done using ANOVA with repeated measures on time and independent measures on group. In addition, since the study involved a 2 × 2 factorial design, we also used a two-factor ANOVA model to compare the changes in the variables in the +E (Groups B and D) vs. the −E (Groups A and C) and the +T (Groups C and D) vs. the −T (Groups A and B) groups, as described previously (4, 10). We calculated that this approach gave us >90% power to detect a minimum 10% difference in SkBF responses as a function of hormonal treatments. Statistical significance was accepted for P < 0.05.

RESULTS

Subject characteristics, as measured during the screening visit, are listed in Table 1. All treatment groups were of comparable height, weight, heart rate, mean arterial pressure, and BMI. Subjects’ ages among groups were similar except that Group C was slightly older than Group B, (P < 0.05). Table 1 also includes blood hormone levels of testosterone and estradiol measured in each subject 3 wk after the initiation of the treatment phase. Consistent with the study design, testosterone was significantly elevated in the +T groups (C and D) compared with the −T groups (A and B). In the +T groups, levels were consistent with normal male circulating testosterone levels, whereas the −T groups had testosterone levels similar to those seen in castrated men. Estradiol levels were significantly elevated in the +E groups, consistent with levels seen in normal elderly men (4).

Average baseline LDF values (%plateau), for all four groups are shown in Fig. 1 for the pre- and posttreatment time points. There were no significant differences among any of the groups, nor were there any differences within groups between the pre- and posttreatment study visits (P > 0.05). Additionally, the two-factor ANOVA did not identify significant effects of estrogen or testosterone independently (P > 0.05 for both comparisons).

Initial peak values were similar among the four hormonal conditions (Group A pretreatment: 75 ± 3, posttreatment: 78 ± 4%plateau; Group B pretreatment: 78 ± 4, posttreatment: 81 ± 3%plateau; Group C pretreatment: 83 ± 4, posttreatment: 78 ± 3%plateau; Group D pretreatment: 77 ± 3, posttreatment: 81 ± 3%plateau; P > 0.05 for all comparisons). As with baseline LDF, two-factor ANOVA did not identify specific effects of E or T (P > 0.05 for both comparisons). Figure 2 illustrates the changes (Δ) in the initial peak response from pre-

Table 1. Baseline subject characteristics and circulating sex hormone levels for the 4 groups

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age, yr</th>
<th>Height, cm</th>
<th>Weight, kg</th>
<th>HR, beats/min</th>
<th>MAP, mmHg</th>
<th>BMI, kg/m²</th>
<th>T, ng/dl</th>
<th>E, pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>12</td>
<td>65 ± 3</td>
<td>176 ± 1</td>
<td>86 ± 4</td>
<td>67 ± 3</td>
<td>98 ± 3</td>
<td>28 ± 1</td>
<td>30 ± 5</td>
<td>&lt;5 ± 0</td>
</tr>
<tr>
<td>B</td>
<td>13</td>
<td>65 ± 2</td>
<td>176 ± 3</td>
<td>86 ± 4</td>
<td>62 ± 3</td>
<td>99 ± 4</td>
<td>28 ± 1</td>
<td>28 ± 4</td>
<td>39 ± 8</td>
</tr>
<tr>
<td>C</td>
<td>14</td>
<td>70 ± 2</td>
<td>178 ± 2</td>
<td>88 ± 2</td>
<td>68 ± 2</td>
<td>103 ± 3</td>
<td>28 ± 1</td>
<td>314 ± 36</td>
<td>&lt;5 ± 0</td>
</tr>
<tr>
<td>D</td>
<td>13</td>
<td>66 ± 3</td>
<td>179 ± 2</td>
<td>91 ± 4</td>
<td>63 ± 3</td>
<td>98 ± 4</td>
<td>28 ± 1</td>
<td>293 ± 53</td>
<td>23 ± 3</td>
</tr>
</tbody>
</table>

Data are means ± SE; n, no. of subjects; HR, heart rate; MAP, mean arterial pressure; BMI, body mass index; T, testosterone; E, estradiol.
to posttreatment. These Δs were not significantly different from zero ($P > 0.05$).

The plateau responses to local warming are shown in Fig. 3. There were no significant differences seen during the plateau phase among the four groups ($P > 0.05$). Responses before and after treatment within groups were also similar. As above, there were no specific influences of estrogen or testosterone by two-factor ANOVA ($P > 0.05$ for both comparisons).

**DISCUSSION**

The major new findings of the present study were that there were no consistent influences of physiological levels of testosterone and estradiol on cutaneous microvascular vasodilation during local warming of the skin in older healthy men. This was somewhat surprising considering previous evidence that vasodilator responsiveness in the skin is augmented by estrogen in both young and older women (2, 3). However, several factors could contribute to these differences between studies. First, aging per se causes a decrease in cutaneous vasodilator responsiveness (7, 8); so our older male subjects likely had several factors influencing their vasodilation in addition to the hormone levels. Second, the studies in women that have shown influences on vasodilation have involved much higher levels of estrogens. For example, women using combination oral contraceptive pills had augmented cutaneous vasodilator responses during the high-hormone phase compared with the low-hormone phase (placebo pills) (3).

There is some evidence that testosterone may affect endothelium-dependent vasodilation, but its specific influences remain unclear. With regard to conduit vessels, it was found that males with end-stage kidney disease and who were androgen deficient had decreased flow-mediated and nitrate-mediated dilation of the brachial artery (5). In contrast to those studies, 6 mo of testosterone treatment was shown to decrease microvascular endothelium-dependent vasodilation in hypogonadal men (1). In our present study, testosterone did not appear to have a consistent effect. It is likely that the specific circulations studied, as well as the initial condition of the research subjects (healthy individuals vs. patients) contributed to the variability in the conclusions of studies to date. Another important consideration regarding potential hormonal influences is that older or more frail individuals, in whom the microvasculature is likely further compromised at baseline, might exhibit different responsiveness to estrogen and testosterone compared with that seen in our present group of healthy men.

Local warming is used as a paradigm to study local microvascular vasodilation because of the previously identified mechanisms involved in the response (6, 9). During local warming, there is an initial rapid rise in flow (initial peak) mediated via the local sensory nerves 3–5 min after local temperature is increased to 42°C. Following this peak, SkBF begins to plateau after 25–30 min of warming during a slower response that is primarily NO dependent (6, 9). Therefore, using the local warming protocol, we were able to study a specific population and try to identify the mechanisms in the response that may be impaired by testosterone and estrogen.

A caveat to the interpretation that cutaneous vasodilation was unaffected by hormone levels in the present study relates to the use of laser Doppler technology to measure SkBF. LDF measurements are semiquantitative and can be affected by local microvascular density. Although it is unlikely that local vascularity was altered during the protocol, heterogeneity of microvascular density at LDF sites, both within and among subjects, may have increased intersite variability to the extent that we were not able to observe a significant difference between groups. Additionally, since we were not able to measure arterial pressure during these experiments, we were unable to calculate cutaneous vascular conductance values to account for blood pressure variations among subjects.

Although LDF has its limitations, laser Doppler measurements of SkBF provide continuous beat-to-beat information specific to cutaneous microvascular perfusion in a noninvasive manner. Therefore, in combination with the known mechanisms of vasodilation during local warming of the skin, LDF measurements provide a robust approach to evaluate mechanisms of microvascular dilation and their modification by physiological perturbations such as changes in circulating levels of reproductive hormones.
Other considerations regarding the design of our study relate to endogenous testosterone levels and potential effects of leuprolide and/or letrozole on endothelial function. In this context, our study was designed to control for variability among individuals in endogenous hormone levels by studying all men under fixed exogenous administration of testosterone and estradiol as the baseline condition. Furthermore, we have previously shown that the doses of testosterone used in the present study result in circulating levels that are similar to pre-leuprolide levels (4). Finally, we are not aware of influences of leuprolide or letrozole on endothelial or microvascular function; however, both pre- and post-hormone studies were conducted in the presence of these agents, thereby controlling for any potential influences they may have had.

In summary, we found in the present study that estradiol and testosterone did not have consistent effects on cutaneous microvascular dilation during local warming of the skin in healthy older men. In contrast, previous studies in women have suggested that estrogen augments cutaneous vasodilator responsiveness (2, 3). Potential influences of testosterone on endothelium-dependent vasodilation are less clear (1, 5). This is the first study that has looked at the microvascular effects of estrogen and testosterone in healthy older men. Because of the complexity of studying microvascular dilation in older men, there is a need for further research on the potential interactions of reproductive hormones and aging in this group.

ACKNOWLEDGMENTS
We are grateful to the subjects for their participation in these studies, and to James Peterson for assistance with statistical analysis.

GRANTS
This work was supported by National Institutes of Health Grants P01 AG-004875 and UL1 RR-024150.

REFERENCES