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Hormone Therapy is Associated with Preserved Smooth Muscle Structure and Dilation in the Arterial Vasculature of the Leg in Older Women

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Abstract

Long-term hormone therapy (HT) is associated with reduced intima-medial thickness (IMT), an established risk factor for atherosclerotic disease, in the femoral artery of healthy older women relative to age-matched non-hormone users. However, the influence of continuous, long-term HT on the relation between age, IMT, and smooth muscle dilation has not been investigated in the popliteal artery, an artery prone to stiffening and calcification. In the present study, popliteal artery IMT and smooth muscle dilation (the increase in diameter to sublingual nitroglycerin, NTG) were assessed with Doppler ultrasound in young (Y: $n=16$; age 23 ± 1 [mean $\pm$ S.E.M]), older non-HT (O non-HT: $n=14$; age 69 ± 1), and older HT (O HT: $n=8$; age 67 ± 1) healthy women. The ~ 0.5 mm increase in resting diameter observed in older non-HT women relative to young women was absent in older HT women, as was the age-related increase in IMT (Y: 0.52 ± 0.02 mm; O non-HT: 0.63 ± 0.02 mm; O HT: 0.56 ± 0.02 mm; $p < 0.05$ for age and hormone comparisons). NTG dilation (percent change above rest) was similarly attenuated in older non-HT women (Y: 8.6 ± 1 %; O non-HT: 3.0 ± 0.7 %; O HT: 7.4 ± 1.7 %; $p < 0.05$ for age and hormone comparisons), and NTG dilation was inversely related to IMT ($p < 0.01$). Collectively, these results suggest that long-term, continuous HT may alleviate the detrimental effects of aging on both structural changes and smooth muscle dilation of the popliteal artery in healthy women.

Keywords

Estrogen; intima-medial thickness; age; popliteal artery

INTRODUCTION

Aging is associated with increased arterial wall intima-medial thickness (IMT) in lower extremity arteries [1,2], which, in turn, is a risk factor for coronary and peripheral arterial disease (PAD) [3,4]. Moreover, up to 25% of women aged 55–74 years may be diagnosed with PAD [5], and the incidence of adverse cardiovascular events in women increases markedly following menopause [6]. Thus, understanding the risk factors and interventions that modify vascular disease is particularly important in older women.

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To this end, it has been shown that healthy postmenopausal women who chronically use hormone therapy (HT) have lower femoral artery IMT [2] than healthy age-matched non-hormone users. The influence of HT on lower extremity IMT in healthy older women has not been well-characterized in the popliteal artery [7], which is the second most-common site of aneurysm behind the aorta [8] and appears particularly susceptible to calcium accumulation [9], arterial tortuosity [10] and reduced distensibility [11] with age. As such, the popliteal artery may be a particularly sensitive and relevant site in which to investigate the influence of age and hormone therapy on vascular risk factors, particularly given that vascular changes attributable to disease and hormone therapy differ between upper extremity/central arteries and lower extremity arteries [7,12,13]. In addition, to the best of our knowledge, the relation between increased lower extremity IMT and a functional marker of smooth muscle dilation—response to a nitric oxide (NO) donor eliciting endothelium-independent dilation—has not been systematically documented with respect to age or hormone therapy in any peripheral artery. We previously observed attenuated dilation to nitroglycerin in the popliteal artery of older, non-hormone-using women compared to young women [14,15]; since increases in IMT in healthy adults are attributed to growth of smooth muscle cells within the medial layer [16, 17], it is important to understand whether the relation between hormone therapy and lower extremity IMT is accompanied by measurable benefits to smooth muscle function.

Accordingly, we sought to examine the influence of age and HT on both popliteal artery IMT and popliteal dilation to nitroglycerin (an NO donor) in women. Since some of the discrepant health findings associated with HT have been attributed to the age at which HT was initiated post-menopause (i.e. immediate vs delayed-onset replacement; [18-20], we included only women who had a continuous history of HT since menopause in the study population to control for this potentially confounding factor. Given the established benefits of HT on femoral IMT and our previous investigations concerning the influence of age on popliteal artery dilation Parker [14], we hypothesized that HT would mitigate the influence of age such that popliteal artery IMT would be smaller and smooth muscle dilation to an NO donor preserved in older women using hormone therapy compared to age-matched non-hormone users.

METHODS

Subject Characteristics

Sixteen young (20–30 yr), fourteen older non-hormone users (62–74 yr), and 8 older hormone users (62–73 yr) women completed the study. Data regarding endothelium-independent dilation in young vs. older non-hormone users has been published previously [14]. All subjects were non-obese ($BMI \leq 30$), nonsmokers, had clinically normal blood chemistry (i.e. hemoglobin concentrations ranged from $11.6\text{--}14.8\text{ g}\cdot\text{dL}^{-1}$, total cholesterol $\leq 240\text{ mg/dl}$, LDL cholesterol $\leq 150\text{ mg/dl}$, and resting supine ankle-brachial index ratings (ABI between 0.90 and 1.30; VP2000, Colin Medical). All subjects were normotensive (resting brachial blood pressure $\leq 140/90\text{ mmHg}$) and were neither extremely sedentary nor extremely fit (i.e. had cycle ergometer $VO_{2\text{peak}}$ values between 20 and 80% of age-predicted norms [21]). Subjects were free of overt chronic diseases as evaluated by medical history questionnaire, a physical examination and resting ECG. Additionally, no subjects were taking medications having significant hemodynamic effects, including oral contraceptives (young subjects). All subjects gave their written, informed consent to participate. This study was approved by the Office for Research Protections and the Institutional Review Board at The Pennsylvania State University, and procedures were followed in accordance with the Declaration of Helsinki of the World Medical Association.

Subjects also completed a physical activity questionnaire to assess routine physical activity (Yale Physical Activity Questionnaire [22] for older and Baecke Questionnaire of Habitual Physical Activity [23] for young). None of the subjects participated in moderate to high

intensity aerobic exercise $> 3 \text{ d}\cdot\text{wk}^{-1}$ or regular lower body resistance training $> 2 \text{ d}\cdot\text{wk}^{-1}$ during the past 12 months. To objectively quantify aerobic fitness status, all of the subjects performed a continuous incremental leg cycle ergometer test (Lode) to maximal exertion to determine peak oxygen uptake ($\text{VO}_{2\text{peak}}$), as described in detail previously [24].

Ankle-brachial index (ABI) and regional (femoral-ankle and carotid-femoral) pulse wave velocity (PWV) were measured after 10 minutes quiet supine rest using the VP2000 vascular profiling machine (Colin Medical) as previously described [25].

Total and regional body composition were estimated using dual-energy X-ray absorptiometry (DXA; model QDR 4500W, Hologic, Waltham, MA) with subjects in the supine position as described previously [26]. Region of interest software (version 9.80 D, Hologic) was used to determine bone-free lean mass and percent fat for the calf (from the proximal tibia to the distal tibia or fibula) of each subject's left limb. Subject characteristics are presented in Table 1.

Assessment of Hormone Replacement Status

Older women were classified as non-hormone-users (non-HT) if they were not currently taking any form of hormone replacement and had not taken any form of hormone replacement for at least 12 months prior to the study. Older women were classified as hormone users (HT) if they had a continuous history of hormone therapy (either estrogen + progesterone or estrogen alone) since menopause or hysterectomy (continuous time on HT was 18 ± 3 yrs). Types and doses of hormone-replacement taken by subjects are presented in Table 2.

Study Protocol

On study day, subjects were asked to refrain from caffeine, aspirin, ibuprofen, or herbal supplements for at least 12 hours prior to testing. Young subjects were studied in days 1–7 of their menstrual cycle to minimize the influence of cyclical changes in female hormones. On a single study visit, Doppler ultrasound was used to assess left leg popliteal diameters at rest and during nitroglycerin administration. The room was thermoneutral with temperature consistently maintained at $23.5 \pm 0.1^\circ\text{C}$. Blood pressure was measured at heart level in the dominant arm with an automatic blood pressure monitor (Omron HEM-705CP; Vernon Hills, IL) and heart rate was continuously monitored using a 3-lead electrocardiogram.

Study Protocol—The artery was imaged immediately proximal to the bifurcation (usually at or slightly above the popliteal fossa) using a 6–11MHz multifrequency linear array probe attached to the Acuson 128XP duplex ultrasound imaging system (Siemens, New York, NY, USA); once a satisfactory image using optimal B-mode imaging was obtained, the placement of the probe was marked on the leg to ensure that the site of measurement did not change during the trial. Ultrasound parameters were not changed during the study. After the subject had rested in the prone position for 10 min, resting popliteal artery diameters and IMT were measured for 1 minute. 0.4 mg sublingual nitroglycerin (NTG), a nitric oxide donor eliciting maximal dilation representative of smooth muscle function, was then administered. Diameters were measured continuously for 10 minutes following administration of NTG.

Measurements and Calculations

Measurement of popliteal artery diameter—Diameter measurements were sampled at end-diastole (ECG-gating was used to select images that were triggered by the R-wave of the cardiac cycle) using Brachial Imager software (Medical Imaging Applications; Iowa City, IA). Post-test analysis of diameters was performed using edge-detection software (Brachial Analyzer Software, Medical Imaging Applications; Iowa City, IA); briefly, the technician (always the same and blind to any subject information) selected a region of interest along the arterial wall and the edge of the wall was detected by pixel density and represented by a line

of best fit. Each sequence of images was reviewed by the technician and adjusted to ensure that diameter measurements were always calculated from the intima-lumen interface at both the distal and proximal vessel wall. Diameters were calculated as a 10 second average of images taken over the 1 min baseline (rest) and at each minute following NTG administration. Once the peak diameter was identified for each individual, NTG dilation was calculated as the percent change in dilation relative to baseline ((peak diameter-resting diameter)/resting diameter *100).

Popliteal artery IMT—Brachial Imager edge-detection software was again used to determine popliteal IMT; the software coupled with the ultrasound provided a minimum detectable difference of 0.06 mm [27]. IMT was defined as the distance from the leading edge of the lumen-intima interface to the leading edge of the media-adventitia interface [28]. A different technician, always the same and blind to all subject data as well as the results from the NTG analysis, took 10 measurements of IMT for each subject. The mean value of these 10 measurements was reported. Wall thickness was normalized for lumen size and was expressed as IMT/lumen diameter ratio.

Reproducibility of techniques—To assess day-to-day reproducibility of popliteal measurements and analysis, resting diameter and IMT measurements were collected on seven subjects on different days. The between-day coefficient of variation (standard deviation of each subject's measurements / average of each subject's measurements *100) for resting popliteal and IMT measurements was $2.5 \pm 0.5\%$ and $3.0 \pm 0.6\%$, respectively.

Power calculations—Since we hypothesized that vascular outcomes in the HT group would resemble those in young subjects, power calculations were derived from expected group differences between either older, non-HT women vs. young women or older, non-HT women vs older, HT women. Based on data concerning femoral IMT and HT in older women [29], we predicted that a group difference of 0.10 mm and a standard deviation of 0.05 mm would necessitate a sample size of 6 in each of the older groups with a power of 0.90. Based on previous data regarding popliteal NTG dilation and age in women [15], we predicted that an age-group difference of 5.6% and a standard deviation of 2.7% would necessitate a sample size of 6 in each of the older groups with a power of 0.90.

Statistical Analysis—Statistical analyses were performed using Minitab (Minitab, State College, PA) and SPSS (SPSS 13.0, Chicago, IL) software. All data are reported as mean $\pm$ S.E.M. and significance was set at $p < 0.05$. A one-way ANOVA and Tukey post-hoc analysis were used to compare differences between groups. In addition, simple linear regression was used to assess whether there was a significant relationship between dilation to nitroglycerin or pulse wave velocity and IMT.

RESULTS

Subject Characteristics (Table 1)

Older non-HT and HT subjects were well matched for baseline characteristics; with the exception of 17- β estradiol, the older women did not exhibit significant differences between groups in any baseline characteristics. Compared to young women, older women exhibited higher systolic and diastolic pressure, total cholesterol, HDL cholesterol (HT women only) and LDL cholesterol (non-HT women only), as well as lower VO_{2peak} .

Influence of age and HT on IMT (Figures 1 and 2)

Popliteal IMT was significantly greater in older non-HT but not HT subjects compared to young subjects (Figure 1); normalizing IMT to lumen diameter abolished age and HT group differences (Figure 2).

Influence of age on sublingual nitroglycerin responses (Figure 3)

Smooth muscle dilation to an NO-donor was reduced in older non-HT subjects but not older HT subjects comparable to young subjects (Figure 3).

Relation between femoral-ankle pulse wave velocity (faPWV) and both IMT and popliteal diameter (Figure 4)

There was a significant positive association between PWV and both IMT and popliteal diameter (Figure 4); the regression equations were $IMT (mm) = 0.310 + 0.000258 PWV (cm/s)$; $r^2 = 0.16$; $p = 0.01$, and $Diameter (mm) = 3.80 + 0.00149 PWV (cm/s)$; $r^2 = 0.13$; $p = 0.02$.

Relation between IMT and dilatory response to NTG (Figure 5)

There was a significant inverse association between IMT and dilation to NTG (Figure 5); the regression equation was $NTG Dilation (\%) = 18.4 - 21.7 IMT (mm)$; $r^2 = 0.23$; $p < 0.01$.

DISCUSSION

The purpose of the present investigation was to examine the influence of age and long-term, chronic HT on an established risk factor for atherosclerotic vascular disease (increased IMT) as well as a functional indicator of smooth muscle dilatory capacity, the response to sublingual nitroglycerin, in the popliteal artery of healthy women. The popliteal artery appears particularly susceptible to detrimental aging-related structural changes such that it more closely resembles a central elastic rather than muscular artery [11]. Our HT study population consisted of older women on continuous hormone supplementation since menopause, as the potential effects of HT on the vasculature may be confounded by a delayed onset of therapy with respect to menopause [18,19]. The major finding was older women on HT exhibited a similar IMT (Figure 1) and smooth muscle dilation (Figure 3) relative to young women, whereas older non-HT women displayed increased IMT and blunted dilation relative to the aforementioned groups.

As noted in the introduction, Moreau et al. observed that hormone therapy exerted a positive influence on preventing the age-associated increase in femoral artery IMT among healthy women [2]. Previous investigations in the popliteal artery are limited to a subset analysis conducted in the Atherosclerosis Risk in Communities (ARIC) study, in which authors noted only a nonsignificant decrease in popliteal IMT among postmenopausal women taking hormone therapy [7]. Given that the ARIC study population included women with hypertension, coronary heart disease, and diabetes, the present study is the first such investigation in healthy postmenopausal women taking hormone therapy continuously since menopause.

The mechanisms underlying the relatively preserved IMT in HT women compared to non-HT women could be explained by the differences in resting lumen diameter. Women taking HT exhibited baseline popliteal diameters similar to young women whereas older non-HT women displayed an average diameter increase of approximately 0.5 mm (Table 1). Normalizing IMT to lumen diameter abolished age and hormone differences between all three subject groups (Figure 2), similar to findings in the femoral artery [2]. This suggests that the beneficial mechanisms of HT may be more directly related to the forces that underlie age-associated arterial enlargement rather than IMT per se. Age-related arterial remodeling may be caused by the number of elastic fibers decreasing with age [30,31], heightened resting blood pressure

increasing the distending force acting on the arterial wall [32], or atherosclerotic-mediated plaque deposition evoking generalized vascular dilation [33]. As increased IMT may then be a compensatory response to enlarging lumen diameters in order to diminish circumferential wall stress [17], the mechanisms through which hormone therapy influence lumen diameter and the resultant IMT may occur through the preservation of elastic fibers and reduced stiffening as well as mitigation of atherosclerotic processes [34]. In the present study, femoral-to-ankle pulse wave velocity, which provides an approximation of the stiffness of the lower extremity vasculature, was marginally lower in older HT women compared to older non-HT women (O non-HT: 1117 ± 28 cm/s; O HT: 1026 ± 36 cm/s; $p = 0.06$) and was significantly related to both popliteal lumen diameter and IMT in the entire study sample (Figure 4). Other beneficial actions of HT may include a reduction of vascular inflammatory factors such as cell adhesion molecules and matrix metalloproteinases, as well as inhibition of vascular endothelial growth factor, proliferation and/or migration of vascular smooth muscle cells [35-37].

An additional finding in the present study was that a functional marker of smooth muscle cell dilation—the dilatory response to nitroglycerin—was also preserved in older women on HT compared to older non-HT women. It is thought that smooth muscle cell hypertrophy underlies increased IMT [16,17]; these structural alterations in smooth muscle then limit vascular dilatory capacity [38] as well. It is likely, then, that any structural benefits of HT underlying lumen diameter and IMT in the older HT women in the current study would translate into preserved dilation to an NO donor (i.e. an indirect effect of HT on dilation). In addition, there may be indirect effects of HT on popliteal artery dilation as well, including modulation of smooth muscle cell contraction, as shown in monkeys treated with hormone therapy [39,40], and potentiation of smooth muscle cell relaxation to NO [41]. The observed significant relationship between IMT and dilation (Figure 5), suggests that the mechanisms through which HT influences IMT in older women may also underlie the preservation of smooth muscle cell dilation. However, IMT explained only 23% of the variability in dilation to nitroglycerin, indicative of additional factors important to smooth muscle dilation that may be influenced independently by HT as well.

It is worth noting that while our current findings as well as those of Moreau et al. present evidence for a positive effect of long-term HT on lower extremity IMT, studies of a similar nature investigating the relation between HT and carotid artery IMT have not always yielded positive results [2,42]. Interestingly, in the present study, although we did not measure carotid IMT, carotid-femoral PWV, representative of central vascular stiffness, was significantly higher in HT women vs non-HT women (non-HT women: 1267 ± 80 cm/s; HT women: 1692 ± 208 cm/s; $p < 0.01$). This observation may provide insight into the differing effects of HT on the vasculature and why clinical trials do not uniformly support a positive influence of HT on mitigating systemic vascular disease [43].

Limitations

In the current study, our criteria of continuous HT since menopause elicited a tradeoff: while removing a potentially confounding factor regarding timing/initiation of HT [18,19], this experimental design limited the HT group to a small sample of women in whom we were not able to delineate between the different forms of therapy and measured vascular outcomes. Hormone therapy may produce differing effects on the vasculature depending on the mode of delivery (i.e. transdermal vs oral; [44]) and type of hormone administration (i.e. estrogen only vs estrogen + progesterone; [45]). There were no overt trends regarding the latter in the current study (i.e. comparing the HT women with respect to estrogen only therapy vs estrogen + progesterone therapy yielded no differences). While our conclusions are thus limited to a small sample of women taking general hormone therapy irrespective of mode or type, we believe

that limiting the sample of HT women to those continuously taking HT since menopause strengthened the study design and our subsequent interpretations.

Given the cross-sectional nature of the study, an alternative explanation of the data is that the physiological and personal characteristics of the subjects in the hormone-replaced group differed from those in the non hormone-replaced group [46]. However, older subjects were well matched for fitness (performance on a $\text{VO}_{2\text{peak}}$ test) and physical activity (both groups of subjects reported performing approximately 2200–2500 minutes of physical activity/week) as well as resting blood pressure, % body fat, and BMI. In addition, LDL cholesterol, the oxidation of which influences vascular function [47], was only different between young and older non-HT women. Although it is impossible to exclude the influence of other personal factors such as diet and education, it is unlikely that these characteristics would have exerted a significant impact on IMT and dilation but not the above-mentioned physiological characteristics. Thus, we do not believe the cross-sectional design prohibits making conclusions about chronic HT use and aging in the present study.

Conclusions

The current results provide evidence that chronic hormone therapy may alleviate aging-related changes in the lower extremity arterial vasculature that are closely associated with morbidity and atherosclerotic disease in women.

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Table 1

Baseline Subject Characteristics

	Young (n=16)	Older, non-HT (n=14)	Older, HT (n=8)
Age	23 ± 1	69 ± 1 [*]	67 ± 1 [*]
BMI (kg/m ²)	23.6 ± 0.2	24.7 ± 0.6	24.8 ± 1.5
Body Fat (%)	29.7 ± 1.5	32.7 ± 1.4	32.0 ± 2.4
VO _{2peak} (mL·min ⁻¹ ·kg ⁻¹)	32.8 ± 2.9	20.3 ± 1.2 [*]	19.6 ± 1.5 [*]
Triglycerides (mg/dL)	83.5 ± 6.3	93.6 ± 13.2	98.9 ± 7.6
Total Cholesterol (mg/dL)	164.2 ± 8.1	203.7 ± 5.7 [*]	207 ± 5.6 [*]
HDL Cholesterol (mg/dL)	56.4 ± 2.5	69.5 ± 15.6	73.4 ± 5.2 [*]
LDL Cholesterol (mg/dL)	90.8 ± 6.9	115.6 ± 4.2 [*]	113.8 ± 8.0
Systolic Pressure (mmHg)	109 ± 3	129 ± 4 [*]	134 ± 7 [*]
Diastolic Pressure (mmHg)	65 ± 1	72 ± 2 [*]	73 ± 2 [*]
Popliteal Diameter (mm)	5.1 ± 0.1	5.6 ± 0.2 [*]	5.1 ± 0.1 [†]

Data are expressed as group averages ± S.E.M.

* represents significant (p < 0.05) difference from young subjects.

[†] represents significant (p < 0.05) difference between non-HT and HT subjects.

Table 2

Types and doses of HT taken by subjects

HT (Generic Name)	Dose	# Subjects
Micronized progesterone w/ Estradiol transdermal biweekly patch	200 mg 0.05 mg	1
Micronized progesterone w/ Conjugated estrogens	100 mg 0.45 mg	1
Conjugated estrogens/ medroxyprogesterone	0.3–0.45/1.5 mg	3
Conjugated estrogens	0.45–0.625 mg	3