Abstract—Current hormone replacement therapy may not optimize cardiovascular health in women with premature ovarian failure. We compared the effects of physiological and standard sex steroid replacement regimens on cardiovascular health in these women. In an open-label, randomized, controlled crossover trial, 34 women with premature ovarian failure were randomly assigned to 4-week cycles of physiological (transdermal estradiol and vaginal progesterone) and standard (oral ethinylestradiol and norethisterone) therapy for 12 months. Cardiovascular health was assessed by 24-hour ambulatory blood pressure, arterial stiffness, and renal and humoral factors. Eighteen women (19 to 39 years of age) completed the 28-month protocol. Both regimens caused similar suppression of luteinizing hormone and follicle-stimulating hormone and provided symptom relief. In comparison with the standard regimen, physiological sex steroid replacement caused lower mean 24-hour systolic and diastolic blood pressures throughout the 12-month treatment period (ANOVA; \( P \leq 0.0001 \) for both): systolic blood pressure was 7.3 mm Hg (95% CI: 2.5 to 12.0 mm Hg) and diastolic was 7.4 mm Hg (95% CI: 3.9 to 11.0 mm Hg) lower at 12 months. Although there were no differences in arterial stiffness, physiological sex steroid replacement reduced plasma angiotensin II (ANOVA; \( P = 0.007 \)) and serum creatinine (ANOVA; \( P = 0.015 \)) concentrations without altering plasma aldosterone concentrations. In comparison with a standard regimen, physiological sex steroid replacement in women with premature ovarian failure results in lower blood pressure, better renal function, and less activation of the renin-angiotensin system. These findings have major implications for the future cardiovascular health of young women who require long-term sex steroid replacement therapy. (Hypertension. 2009;53:805-811.)

Key Words: \[ \text{ovarian failure} \quad \text{premature} \quad \text{hormone replacement therapy} \quad \text{renin-angiotensin system} \quad \text{ambulatory blood pressure monitoring} \]

Premature ovarian failure, the onset of the menopause before the age of 40 years, is a relatively common problem, affecting 1% of women. Sufferers are exposed to prolonged estrogen deficiency and have an increased risk of premature death, mainly from cardiovascular disease. In an attempt to reduce this risk, sex steroid replacement is usually offered in the convenient form of the combined oral contraceptive pill or hormone replacement therapy (HRT) designed for postmenopausal women. These standard regimens often use nonphysiological synthetic hormones and do not restore normal serum sex steroid hormone concentrations.

The use of the oral contraceptive pill in healthy young women and HRT in postmenopausal women has been linked to increases in blood pressure and the associated outcomes of stroke and subarachnoid hemorrhage. Although hormone replacement will frequently be prescribed until the age of normal menopause, there has been no formal assessment of these therapies in younger women with premature ovarian failure. In these women, the combined cardiovascular risk associated with the prolonged use of sex steroid regimens may be higher than reported in other groups, with even small differences in safety and efficacy having major implications for morbidity and mortality in later life.

Compared with standard regimens, physiological sex steroid replacement therapy improves uterine characteristics in women with premature ovarian failure. However, the consequences for cardiovascular health are unknown. We hypothesized that use of a physiological transdermal estrogen-based regimen may lead to lower systemic blood pressures throughout the 12-month treatment period (ANOVA; \( P \leq 0.0001 \) for both): systolic blood pressure was 7.3 mm Hg (95% CI: 2.5 to 12.0 mm Hg) and diastolic was 7.4 mm Hg (95% CI: 3.9 to 11.0 mm Hg) lower at 12 months. Although there were no differences in arterial stiffness, physiological sex steroid replacement reduced plasma angiotensin II (ANOVA; \( P = 0.007 \)) and serum creatinine (ANOVA; \( P = 0.015 \)) concentrations without altering plasma aldosterone concentrations. In comparison with a standard regimen, physiological sex steroid replacement in women with premature ovarian failure results in lower blood pressure, better renal function, and less activation of the renin-angiotensin system. These findings have major implications for the future cardiovascular health of young women who require long-term sex steroid replacement therapy. (Hypertension. 2009;53:805-811.)

Key Words: \[ \text{ovarian failure} \quad \text{premature} \quad \text{hormone replacement therapy} \quad \text{renin-angiotensin system} \quad \text{ambulatory blood pressure monitoring} \]
pressure and less activation of the renin-angiotensin system than synthetic oral estrogen–based therapies. The aim of this study was to compare the effects of a physiological sex steroid replacement therapy with a standard regimen on measures of cardiovascular health in young women with premature ovarian failure.

Methods

Subjects
Forty-two women with premature ovarian failure attributed to chemotherapy or radiotherapy, idiopathic or surgical treatment, or Turner syndrome were recruited between February 2002 and September 2004. The trial ran from February 2002 to November 2006 and was performed with the approval of the local research ethics committee, given clinical trial authorization by the Medicines and Healthcare products Regulatory Agency (United Kingdom), in accordance with the Declaration of Helsinki, and with the written informed consent of all of the participants.

Study Design
This was an open-label, randomized, controlled crossover trial. At entry, all of the patients were receiving a standard nonphysiological hormone replacement regimen. After a 2-month washout period of no therapy, patients were randomly assigned to receive either the standard regimen (Loestrin 30, Galen Ltd; ethinylestradiol 30.0 μg and norethisterone 1.5 mg daily for weeks 1 to 3, followed by 7 “pill-free” days) or physiological sex steroid replacement (Estraderm TTS patches, Novartis Pharmaceuticals UK Ltd; transdermal estradiol 100 μg daily for week 1 and 150 μg for weeks 2 to 4; and Cyclogen vaginal pessaries, Actavis UK Ltd; progesterone 200 mg twice daily in weeks 3 to 4). Some women used oral progesterone (Duphaston, Solvay Healthcare Ltd; dydrogesterone 10 mg twice daily) in preference to vaginal pessaries. After the first 12-month period, a further 2-month washout was completed before receiving the alternative treatment. Assessments were made at the start of the first washout period, at the end of each washout period, and at 3, 6, and 12 months of each treatment period.

Outcome Measures
Study procedure did not inform investigators of the current study treatment, although this was an open-labeled study. Calculation of all of the cardiovascular and renal and humoral measures was performed by investigators blind to treatment allocation.

Blood Pressure and Heart Rate
At each visit, blood pressure was measured at the brachial artery using a validated semi-automated oscillometric sphygmomanometer (Omron 705IT, Omron), and then patients were fitted with a validated automated ambulatory blood pressure monitor (Del Mar Reynolds) to record 24-hour blood pressure and heart rate. Data were processed using Spacelabs proprietary software (Spacelabs Healthcare).

Arterial Stiffness
Subjects were asked to abstain from food for 4 hours and caffeinated drinks for ≥8 hours before their visit. After resting supine for 30 minutes, peripheral arterial pressure waveforms were recorded at the radial artery and the ipsilateral carotid artery using a high-fidelity micromanometer (Colin Corporation) and the SphygmoCor system (AtCor Medical). Waveforms were gated to the R-wave of the ECG to allow for calculation of carotid-radial pulse wave velocity, and central arterial blood pressure was calculated using the SphygmoCor software.

Biochemical Analyses
Blood samples were obtained at each visit, collected into EDTA and serum gel, and kept on ice until centrifuged at 2000g for 30 minutes. Plasma and serum samples were immediately frozen at −80°C until subsequent analysis. Plasma angiotensin II (Ang II) concentrations were determined by radioimmunoassay (Peninsula Laboratories Europe Ltd) after extraction using Bond Elut columns (Varian), as described previously. Plasma renin activity (PRA) was measured under standard conditions through the generation of angiotensin I, as determined by radioimmunoassay. Plasma aldosterone concentrations were determined by radioimmunoassay (Bachem UK Ltd). Serum urea nitrogen, creatinine, and electrolyte concentrations were determined by the clinical biochemistry reference laboratory (Royal Infirmary) using an Olympus AU2700 automated analyzer (all of the reagents supplied by Olympus).

Serum luteinizing hormone and follicle-stimulating hormone concentrations were determined by fluoroimmunoassay (PerkinElmer Life and Analytic Sciences). Serum 17β-estradiol concentrations were measured by a specific (not sensitive to estrone or ethinylestradiol) radioimmunoassay (Adalis Italia SpA) and serum progesterone concentrations by established in-house radioimmunoassay, as described previously.

Statistical Analysis
Equal 1:1 randomization was performed separately for each etiology in balanced blocks of 10 by opaque multipart assignment “envelopes” produced at the Medical Statistics Unit (University of Edinburgh). Subjects were enrolled and assigned to their group by nursing staff at the clinical research facility.

Baseline values were defined as the last measurement of the preceding 2-month washout period and change calculated by subtracting this baseline from subsequent measurements. Analysis of each treatment was pooled regardless of treatment order, and comparisons were made using 2-way ANOVA with repeated measures, including time and treatment as variables. Bonferroni posttests were performed to assess treatment differences at individual time points. Estradiol concentrations were skewed and, therefore, log transformed before analysis. Subjects with missing data points were excluded from analysis. Statistical significance was taken as a 2-sided P<0.05. All of the analysis was performed using GraphPad Prism (version 4 for Macintosh, GraphPad Software). All of the data are expressed as means (95% CIs) unless otherwise stated.

Results
Given the intensive nature of the protocol, 18 patients (52%) completed the study (Figure 1). Women were young (19 to 39 years of age), and their premature ovarian failure was principally idiopathic or surgically induced, or attributed to Turner syndrome (Table). Both treatments were generally well tolerated, although some women reported adverse reactions to the patch adhesive when taking physiological treatment (see online data supplement, available at http://hyper.ahajournals.org) and were withdrawn from the study. No women withdrew because of unacceptable hormonal symptoms during their treatment (Figure S1). In general, more women withdrew during the first treatment phase (14 vs 1), suggesting a lack of tolerance of the research protocol rather than the intervention. The suppression of plasma luteinizing hormone and follicle-stimulating hormone concentrations for both treatments was similar (P>0.05 for both), suggesting equivalent sex steroid replacement (Figure 1).

Blood Pressure and Arterial Stiffness
Although none of the women were clinically hypertensive, in comparison with the standard regimen, physiological sex-
steroid therapy was associated with a lower blood pressure ($P<0.0001$ for both systolic and diastolic blood pressures; Figure 2) and differed from the standard regimen at 3 ($P<0.05$), 6 ($P<0.05$), and 12 months ($P<0.01$). The same was seen in derived central arterial blood pressures ($P<0.0001$ for both systolic and diastolic pressures; data not shown) and clinic blood pressures, although the magnitude of effect was greater (Figure 2). At 12 months, there was an overall 7.3 mm Hg (95% CI: 2.5 to 12.0 mm Hg) 24-hour mean systolic and 7.4 mm Hg (95% CI: 3.9 to 11.0 mm Hg) 24-hour mean diastolic blood pressure benefit with physiological sex-steroid therapy. There were no differences in carotid-radial pulse wave velocity ($P=0.411$) or 24-hour mean heart rate ($P=0.511$) through the study period (data not shown).

**Renal and Humoral Factors**

Compared with the standard regimen, physiological sex-steroid therapy reduced plasma Ang II ($P=0.007$) and serum creatinine concentrations ($P=0.015$), without affecting plasma aldosterone concentrations (Figure 3). There was an apparent reduction in PRA, but this did not reach statistical significance ($P=0.180$). Body mass index ($P=0.146$), serum urea nitrogen ($P=0.063$), sodium ($P=0.402$), and potassium ($P=0.895$) concentrations did not change during the study (data not shown).

**Discussion**

In comparison with a 12-month standard regimen, physiological sex steroid replacement therapy resulted in lower blood pressure, better renal function, and less activation of the renin-angiotensin system in women with premature ovarian failure. These differences are likely to have major consequences for the future long-term cardiovascular risk of these women.

Major randomized, controlled trials of primary prevention using HRT in older postmenopausal women have failed to demonstrate cardiovascular benefits and have even suggested harm. Indeed, postmenopausal women maintained on HRT have an increased risk of stroke. These surprising findings have generated much debate.

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**Table. Baseline Characteristics of Study Participants at Study Entry by Treatment Order**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Physiological/Standard</th>
<th>Standard/Physiological</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (range), y</td>
<td>26 (20 to 34)</td>
<td>24 (19 to 39)</td>
</tr>
<tr>
<td>Height, median (range), cm</td>
<td>158 (149 to 163)</td>
<td>162 (148 to 175)</td>
</tr>
<tr>
<td>Weight, median (range), kg</td>
<td>55 (46 to 105)</td>
<td>70 (51 to 102)</td>
</tr>
<tr>
<td>BMI, median (range), kg/m²</td>
<td>24 (19 to 40)</td>
<td>27 (21 to 37)</td>
</tr>
<tr>
<td>24-h mean systolic blood pressure, mm Hg</td>
<td>114 (109 to 119)</td>
<td>114 (107 to 121)</td>
</tr>
<tr>
<td>24-h mean diastolic blood pressure, mm Hg</td>
<td>70 (68 to 71)</td>
<td>72 (67 to 77)</td>
</tr>
<tr>
<td>Serum urea nitrogen, mg/dL</td>
<td>13.2 (10.1 to 16.5)</td>
<td>12.9 (10.4 to 15.1)</td>
</tr>
<tr>
<td>Serum creatinine concentration, mg/dL</td>
<td>0.78 (0.70 to 0.88)</td>
<td>0.94 (0.83 to 1.04)</td>
</tr>
<tr>
<td>Plasma renin activity, ng/mL per h</td>
<td>1.9 (1.3 to 2.5)</td>
<td>2.4 (0.8 to 4.0)</td>
</tr>
<tr>
<td>Plasma Ang II concentration, pg/mL</td>
<td>9.6 (6.4 to 12.8)</td>
<td>11.8 (4.2 to 19.5)</td>
</tr>
</tbody>
</table>

Data for 24-hour blood pressure, n = 17; renal and humoral markers, n = 13. Data are expressed as mean (95% CIs) unless otherwise stated. BMI indicates body mass index.
Several factors have been highlighted as obscuring a potentially beneficial effect of the hormonal intervention, including the age of study participants and the preparations of HRT administered (these studies used a variety of nonhuman and conjugated estrogens, with and without progestins). Many patients were elderly (>70 years of age), and posthoc analyses restricted to younger patients (50 to 59 years of age) have suggested apparent benefit. This latter observation awaits prospective confirmation in the ongoing Early Versus Late Intervention Trial With Estradiol and the Kronos Early Estrogen Protection Study due to report in 2010.

Young women with untreated premature ovarian failure have an increased risk of osteoporosis, cardiovascular

![Figure 2. Changes in mean 24-hour ambulatory systolic (A) and diastolic (C) and clinic systolic (B) and diastolic (D) blood pressures with physiological sex steroid (■) and standard (□) regimens (mean±SEM). P<0.0001 for treatment effect for all 4 measurements (2-way ANOVA with repeated measures; N=17 for all).](http://hyper.ahajournals.org/)

![Figure 3. Changes in (A) serum creatinine (P=0.015), (B) PRA (P=0.180), (C) plasma Ang II (P=0.007), and (D) plasma aldosterone (P=0.784) concentrations after treatment with physiological sex steroid (■) and standard (□) regimens (mean±SEM; N=13 for all; 2-way ANOVA with repeated measures).](http://hyper.ahajournals.org/)
disease, and cognitive impairment. They are frequently prescribed hormone replacement until the age of normal menopause in accordance with national guidelines. The median age of natural menopause is reported to range between 49 and 52 years, and, thus, these young women with early loss of ovarian function may require 4 decades of sex hormone replacement.

The oral contraceptive pill is known to increase blood pressure in women with normal ovarian function and is linked to adverse outcomes associated with blood pressure, including ischemic stroke and subarachnoid hemorrhage. It is important to recognize that such hormonal intervention is deliberately targeted at producing supraphysiological concentrations of sex steroids to inhibit ovulation and produce the desired contraceptive effect. Moreover, oral contraceptive therapy may be intermittent and often interrupted for planned pregnancies. The needs and consequences for women with premature ovarian failure are very different from fertile women requiring effective contraception. Our data would suggest that, whereas the administration of hormonal therapies based on contraceptive regimens provides adequate symptom relief, longer-term continuous use may be associated with potentially harmful cardiovascular effects.

There have been no previous prospective studies assessing the use of hormone replacement regimens on cardiovascular outcomes in young women with premature ovarian failure. Although our study uses different hormone regimens from those used in the large HRT trials, we have presented the first data comparing 2 sex steroid replacement regimens and the potential consequences for cardiovascular health. In this group of young women, we have demonstrated substantial effects on blood pressure that are more marked than those reported previously in postmenopausal women. If these data are substantiated in a larger long-term study, there would be major implications for cardiovascular health.

We did not demonstrate any difference in pulse wave velocity between the treatment regimens, suggesting that the hypertensive effect is not mediated through an increase in arterial stiffness. We suggest that the competing influences of effective estrogen replacement (known to improve vascular endothelial function and reduce arterial stiffness) and increased blood pressure (robustly associated with reduced arterial compliance) on vascular tone cancel out any potential changes in arterial compliance resulting in unchanged pulse wave velocity through the study. However, we suggest that our findings may be explained by an effect on the kidney and the renin-angiotensin system. A direct hypertensive effect would be anticipated to cause reflex suppression of the renin-angiotensin system with downregulation of PRA and Ang II concentrations. In contrast, we report no suppression in PRA and an increase in plasma Ang II concentrations, suggesting a causal relationship with the activation of the renin-angiotensin system.

The profile and consequences of estrogens are highly dependent on their route of administration. Parenteral administration by estrogen patches leads to a lower peak plasma concentration, lower hepatic exposure, and a more stable steady-state profile. In contrast, oral estrogen administration leads to wide plasma variations with high concentrations demonstrable within an hour of ingestion. Moreover, to achieve adequate systemic effects, oral estrogen therapies are given at high doses because of extensive first-pass hepatic metabolism. Consequently, after oral administration, the liver is exposed to particularly high portal venous concentrations of estrogens, and this may underlie the stimulation of the renin-angiotensin system and elevation of blood pressure. In both preclinical and clinical models, oral estradiol administration upregulates hepatic angiotensinogen mRNA expression and increases plasma angiotensinogen concentrations. Angiotensinogen is the substrate for renin, and plasma concentrations have been linked to clinical hypertension. Upregulation of angiotensinogen production without suppression of PRA provides a clear explanation for the elevation in plasma Ang II concentrations. This, in turn, might account for the undoubted blood pressure changes seen in our study. Thus, we suggest that oral therapy causes high hepatic exposure to estrogens leading to excessive angiotensinogen production, activation of the renin-angiotensin system, and systemic hypertension: an effect avoided by transdermal estrogen preparations.

It is interesting to note the increase in serum creatinine in this study. This may be a consequence of alterations in renal blood flow secondary to renin-angiotensin system activation. Alternatively, this may be because of changes in renal blood flow attributable directly to the effect of estrogen. Finally, it may be attributable to the sustained changes in blood pressure. It is conceivable that hemodilution may have caused an apparent change in serum creatinine, although this variable was not measured in this study, and this may need to be addressed in future studies. Assuming that the change in creatinine is a real phenomenon, whatever the underlying mechanism, this does represent a clinically important change in renal function.

An important weakness of our study is the heterogeneous etiology of the women with premature ovarian failure. We predominantly included patients with idiopathic or surgically induced premature ovarian failure who are typical of the majority of women presenting with this condition. However, we also included patients with Turner syndrome and survivors of cancer who underwent treatment either premenarche or postmenarche. Patients with Turner syndrome have an increased incidence of cardiovascular abnormalities, including aortic coarctation and hypertension. Patients who are survivors of radiotherapy and chemotherapy have had a toxic insult to the whole body that may influence the cardiovascular system. Thus, the response to the intervention may vary according to etiology of ovarian failure. In our study, we observed no interaction of disease etiology with treatment effect, although our subgroup sizes are too small to draw any firm conclusions. However, we used a crossover design to enable each patient to act as his or her own control, as well as to maximize the power to detect differences in treatment regimens.
The other significant weakness of our study is the high drop out rate, with only 18 of 34 randomly assigned subjects (52%) completing the whole study, although more women withdrew during the first treatment phase, suggesting an intolerance of the research protocol rather than the intervention. Of the dropouts, only 4 women clearly withdrew because of intolerance of the treatment, and this was entirely attributed to adverse reactions to the patch adhesives. Improved adhesives may help to reduce these intolerances and make the treatment more acceptable.

Perspectives
Although none of the women in this study were clinically hypertensive, in comparison with a 12-month standard regimen, physiological sex steroid replacement therapy resulted in lower blood pressure, better renal function, and less activation of the renin-angiotensin system in women with premature ovarian failure. These important differences reinforce the view that the type and profile of sex steroid replacement are critical and underscore the importance of the appropriate selection of sex steroid regimens in the prevention and avoidance of cardiovascular disease. If substantiated in a larger long-term study, these findings will have major implications for the future cardiovascular health of young women who require long-term sex steroid replacement therapy.

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Disclosures
None.

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Cardiovascular effects of physiologic and standard sex steroid replacement regimens in premature ovarian failure

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Figure S1. Study consort flow chart.