Hormone replacement therapy has been associated with lower rates of cardiovascular diseases in postmenopausal women.\(^{2,3}\) The underlying metabolic basis for the reduced vascular risk has been explained, in part, by favorable changes in LDL, HDL, and thrombotic markers such as plasminogen activator inhibitor, fibrinogen, and D-dimer.\(^{3-5}\) In contrast, most postmenopausal estrogen formulations increase plasma triglycerides by 24% to 67%.\(^{6,7}\) Elevations in triglycerides are associated with a dose-dependent increase in plasma viscosity\(^8\) that may mitigate the cardiovascular benefit of hormone replacement therapy.

Elevated plasma viscosity is an established predictor of initial and recurrent cardiovascular events and mortality in coronary heart disease patients.\(^{9-11}\) Plasma viscosity is influenced by the concentration of lipoproteins and other plasma proteins, with the major contribution resulting from fibrinogen.\(^2\) Because hormone replacement therapy is accompanied by lipid and fibrinogen changes that may both increase and decrease plasma viscosity, we evaluated whether a 3-month period of daily hormone replacement therapy with either estrogen alone or estrogen and progesterone changed plasma viscosity levels.
Baseline and 3-Month Treatment Changes as a Function of Hormonal Treatment Assignment

<table>
<thead>
<tr>
<th></th>
<th>Estradiol + MPA (n=7)</th>
<th>Estradiol Alone (n=8)</th>
<th>Placebo (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Difference</td>
<td>Difference</td>
<td>Difference</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>3 mo</td>
<td>0</td>
</tr>
<tr>
<td><strong>Plasma viscosity, mPa·s</strong></td>
<td>1.449 ± 0.085</td>
<td>1.392 ± 0.051</td>
<td>1.420 ± 0.039</td>
</tr>
<tr>
<td></td>
<td>−0.057 ± 0.052</td>
<td>−0.055 ± 0.022†</td>
<td>+0.047 ± 0.047</td>
</tr>
<tr>
<td><strong>Fibrinogen, g/L‡</strong></td>
<td>3.37 ± 0.60</td>
<td>3.09 ± 0.52</td>
<td>3.33 ± 0.57</td>
</tr>
<tr>
<td></td>
<td>−2.76 ± 2.02</td>
<td>−4.46 ± 5.33</td>
<td>−0.96 ± 3.25</td>
</tr>
<tr>
<td><strong>Triglycerides, mmol/L</strong></td>
<td>1.49 ± 0.85</td>
<td>0.98 ± 0.23</td>
<td>1.55 ± 0.70</td>
</tr>
<tr>
<td></td>
<td>−0.50 ± 0.81</td>
<td>+0.01 ± 0.91</td>
<td>+0.09 ± 0.56</td>
</tr>
<tr>
<td><strong>Cholesterol, mmol/L</strong></td>
<td><strong>Total</strong></td>
<td>6.58 ± 1.51</td>
<td>5.44 ± 0.92</td>
</tr>
<tr>
<td></td>
<td>−1.14 ± 1.13</td>
<td>−0.86 ± 1.17</td>
<td>−0.48 ± 1.04</td>
</tr>
<tr>
<td></td>
<td><strong>HDL</strong></td>
<td>1.40 ± 0.30</td>
<td>1.28 ± 0.22</td>
</tr>
<tr>
<td></td>
<td>−0.12 ± 0.30</td>
<td>+0.19 ± 0.45</td>
<td>−0.06 ± 0.20</td>
</tr>
<tr>
<td></td>
<td><strong>LDL§</strong></td>
<td>4.61 ± 1.73</td>
<td>3.64 ± 1.28</td>
</tr>
<tr>
<td></td>
<td>−0.97 ± 0.86</td>
<td>−1.10 ± 1.12</td>
<td>−1.03 ± 0.62</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD. Differences are between 3-month and baseline values.

Significant values are as follows: *group×time interaction, P<0.01; †change across time, P<0.01; ‡change across time, regardless of treatment group, P=0.003; §significant change across time, regardless of treatment group, P<0.01.

Estradiol (n=8); and (3) placebo control (n=8). Estradiol (Estrace) and the corresponding placebo were provided by Mead Johnson Laboratories (Princeton, NJ). Medroxyprogesterone acetate (Provera) and the corresponding placebo were provided by The Upjohn Company (Kalamazoo, Mich). One subject experienced vaginal bleeding on combined hormone treatment, and therapy was discontinued at 5 weeks. Follow-up laboratory studies were obtained, and these are included in the analyses. There were 2 subjects who reported adverse reactions but continued therapy. The reported symptoms included breast tenderness, vaginal bleeding, cramps, and swelling in the hands and feet. The 22 subjects who completed the trial were completely adherent with the intervention, as based on pill counts performed at 6 and 12 weeks.

**Blood Handling**

All subjects fasted for a minimum of 12 hours. Blood was drawn from subjects who were seated for a minimum of 5 minutes. A tourniquet was applied lightly and for <1 minute, as described previously. Blood was drawn into vacuum tubes containing a serum separation gel for plasma viscosity and total protein quantification; 15% K$_3$ EDTA for plasma lipid analysis and plasma viscosity measurements; and 0.129 mol/L (3.8%) sodium citrate solution for fibrinogen analysis. Serum and plasma were separated at 4°C for 10 minutes at 3000 rpm. Aliquots of plasma and serum were transferred with disposable plastic pipettes into stoppered plastic tubes. All samples were refrigerated at 4°C until the time of analysis. Plasma and serum aliquots for viscosity determinations were shipped on wet ice to the Lipoprotein and Hemorheology Research Facility, Chicago, Ill. Viscosity measurements were made within 24 hours after venipuncture.

**Laboratory Analyses**

Viscosity measurements were made on plasma by using a coaxial cylinder microviscometer and DIN 412 measuring cup (Mettler-Toledo AG), as described previously. The batch coefficient of variation for plasma viscosity is 2.8%. Plasma concentrations of total cholesterol, HDL cholesterol, and triglycerides were assayed by standard chemical techniques. The LDL cholesterol concentration was estimated by the Friedewald formula. Plasma fibrinogen measurements were made by the Clauss method (Data-Fibrinogen determination kit) and represent an average of 3 individually acquired measurements that were selected to minimize previously recognized methodological variability. The batch coefficient of variation for fibrinogen replicates is 5.8%, and the 3-month coefficient of variation using an average of 3 replicate samples is 8.1%. Because the intrAudit variability in plasma fibrinogen and triglycerides is large, 2 independent blood samples were obtained 1 week apart both at baseline and after 3 months.

**Statistics**

Statistical analyses were performed with SPSS Windows version 6.01. All subject data were analyzed on the basis of intention to treat. Histograms of variables were examined for normality and transformed, if necessary. Data are expressed as means and SDs for continuous variables and as proportions for the categorical variables, such as history of angina and prior history of myocardial infarction. Because 2 blood samples were obtained 1 week apart at baseline and after 3 months, the reported analytes reflect the average of these 2 independent samples at baseline and at 3 months. Pearson correlation tests were used to examine the associations between select variables that included age, body mass index, and waist-to-hip ratio with plasma viscosity. Percent differences in each variable were calculated as follows: (3-month value—baseline value)/baseline value×100. Differences across time and across treatment groups were compared by using a repeated-measures ANOVA followed by paired t tests with an adjusted Bonferroni correction (α of 0.01). Stepwise multivariate linear regression analyses were also performed to evaluate the potential influence of group assignment, age, body mass index, waist-to-hip ratio, and changes in plasma lipids and fibrinogen on plasma viscosity changes.

**Results**

There were no significant differences in age (mean ± SD, 65.1 ± 7.7 years), body mass index (26.2 ± 3.8 kg/m²), waist-to-hip ratio (0.78 ± 0.06), heart rate (72.4 ± 12.2 bpm), systolic (132.3 ± 19.5 mm Hg) or diastolic (80.7 ± 7.8 mm Hg) blood pressure, or previous history of angina or myocardial infarction among the 3 treatment groups. No significant differences in baseline plasma lipids, fibrinogen, or viscosity levels among treatment groups were observed (the Table). Plasma viscosity levels were positively correlated with fibrinogen (r=0.46, P=0.03) but not with age (r=−0.06, P=0.78).
Hormone Replacement Therapy and Plasma Viscosity

Three-month use of either hormone replacement formulation against placebo did not result in significant changes in plasma lipids or fibrinogen. In contrast, changes in plasma viscosity levels were significant ($P<0.01$). The estradiol plus medroxyprogesterone group had a nonsignificant ($P=0.03$) lowering of plasma viscosity from (mean±SD) 1.449±0.085 to 1.392±0.051 mPa·s ($\approx 4\%$), the estradiol-assigned group had a significant ($P<0.01$) reduction in plasma viscosity from 1.420±0.039 to 1.365±0.041 mPa·s ($\approx 4\%$), and the placebo-assigned women experienced a nonsignificant ($P=0.03$) increase in plasma viscosity ($\approx 3\%$) from 1.417±0.082 to 1.464±0.091 mPa·s (the Table). No other significant changes among groups were found. However, significant reductions in fibrinogen ($P=0.003$) and LDL cholesterol ($P<0.01$) levels were observed after 3 months, regardless of treatment assignment.

Three-month differences in plasma viscosity were best explained by hormonal treatment group when body mass index; age; waist-to-hip ratio; systolic and diastolic blood pressures; treatment group; and changes in fibrinogen, LDL cholesterol, HDL cholesterol, total cholesterol, total triglycerides, or the cross product of LDL cholesterol and fibrinogen were available for selection (adjusted $r^2=0.44$, $P=0.0004$). Treatment status and age were selected by backward selection analyses with an adjusted $r^2=0.50$, $P=0.0004$.

Discussion

One well-known consequence of most common hormone replacement formulations is an increase in plasma triglycerides (24% to 67%) that occurs through estrogen-induced stimulation of hepatic VLDL secretion and inhibition of hepatic triglyceride lipase.$^{5,6}$ Elevated triglyceride levels in women are independent predictors of coronary heart disease mortality and angiographic progression of coronary atherosclerosis.$^{20,21}$ Thus, any resulting increase in plasma triglycerides may negate the beneficial influence of hormone replacement therapy in some women, because elevated triglycerides are associated with elevations in plasma viscosity.$^5$

In contrast to potential hypertriglyceridemia-induced hyperviscosity, hormone replacement therapy lowers fibrinogen,$^3$ which is a major determinant of plasma viscosity.$^{12,14}$ The Postmenopausal Estrogen Progestin Interventions trial showed that placebo-treated women experienced significant increases in plasma fibrinogen levels that were blunted by estrogen alone or combined hormone replacement treatment.$^3$ Findings from the Atherosclerosis Risk in Communities study,$^{22}$ and the FINRISK Hemostasis study$^{23}$ showed that women using estrogen alone or combined hormone formulation had lower fibrinogen concentrations than those observed in nonusers.

In this study of postmenopausal women with fasting triglycerides $<3.2$ mmol/L (283 mg/dL), short-term estradiol therapy lowered plasma viscosity by 0.055±0.022 mPa·s ($P<0.01$). Combined hormone replacement therapy lowered plasma viscosity by a similar magnitude (0.057±0.052 mPa·s); however, these changes were not statistically significant at an $\alpha$ of 0.01 ($P=0.03$). This magnitude of plasma viscosity change approximates a 1-SD change in women as determined by our group.$^{14}$ The Caerphilly and Speedwell studies reported that a 2% plasma viscosity difference, or 0.010 mPa·s, in men was associated with a 4% change in coronary heart disease risk.$^{21}$ An age-standardized difference of 0.032 to 0.047 mPa·s discriminated between men with and without incident ischemic heart disease.$^{21}$ In this study, hormone replacement therapies resulted in much larger changes in plasma viscosity, but we are not ascertain whether the magnitude of such changes would occur among women with higher plasma triglyceride levels or whether these changes are maintained with a longer treatment duration.

Estradiol treatment was accompanied by a nonsignificant increase in plasma triglycerides of $+19\%$ that did not offset the favorable changes on plasma viscosity. The lipid changes with estradiol are consistent with 2 other studies that used this estrogen formulation.$^{23}$ In contrast to estradiol treatment alone, women on combined hormone therapy had a nonsignificant reduction in triglycerides of $-21\%$, and women assigned to placebo therapy had a nonsignificant increase in triglycerides of 10%. Plasma fibrinogen levels did not change as a function of treatment assignment, although there was a significant fall in fibrinogen levels with time ($P=0.003$). The reasons for the observed reductions in fibrinogen and LDL cholesterol are not apparent. This outcome would be unlikely in studies of longer duration and may reflect a Hawthorne effect. In addition, the variability in plasma fibrinogen exceeds that of plasma viscosity and may have precluded detection of a group×time interaction for fibrinogen measurement ($P=0.22$).

The literature on hormonal therapy and plasma viscosity is sparse. In 1 report, oral contraceptive users had no significant changes in plasma viscosity, but these conclusions were limited by small sample size.$^{26}$ Another small study of women treated with triphasic oral contraceptives showed an increase in plasma viscosity after 3 to 6 months of treatment.$^{27}$ This difference may be due to the higher concentrations of estrogen and progestins in oral contraceptive preparations compared with those for hormone replacement.

To our knowledge, this is the first report that evaluates the influence of hormone replacement therapy on plasma viscosity in postmenopausal women. Plasma viscosity is a more sensitive indicator of change as a consequence of hormonal replacement therapy than are triglycerides$^{17}$ or fibrinogen,$^{19}$ owing to smaller measurement variability.$^{18,28}$ Our work supports a new mechanism through which estrogen replacement therapy reduces cardiovascular risk in postmenopausal women. This beneficial property was reduced by concomitant progestin administration. Further studies are needed to evaluate the safety of hormone replacement therapy in postmenopausal women with moderate to severe elevations in triglycerides and the relationship between fasting versus postprandial triglyceride levels on plasma viscosity. In conclusion, estrogen replacement therapy lowers plasma viscosity in postmenopausal women with fasting triglycerides $<3.2$ mmol/L.

Acknowledgment

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References


Hormone Replacement Therapy Improves Cardiovascular Risk by Lowering Plasma Viscosity in Postmenopausal Women
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