Effects of continuous conjugated estrogen and micronized progesterone therapy upon lipoprotein metabolism in postmenopausal women

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Abstract The effects of continuously administering both conjugated equine estrogens (CEE) and micronized progesterone (MP) on the concentration, composition, production and catabolism of very low density (VLDL) and low density lipoproteins (LDL) have not previously been reported. The mechanism of the hormonally induced reductions of plasma LDL cholesterol of S 0–20 (mean 16%, P < 0.005) and LDL apoB (mean 6%, P < 0.025) were investigated by studying the kinetics of VLDL and LDL apolipoprotein (apo) B turnover after injecting autologous 131I-labeled VLDL and 125I-labeled LDL into each of the 6 moderately hypercholesterolemic postmenopausal subjects under control conditions and again in the fourth week of a 7-week course of therapy (0.625 mg/d of CEE + 200 mg/d of MP). The combined hormones significantly lowered plasma LDL apoB by increasing the mean fractional catabolic rate of LDL apoB by 20% (0.32 vs. 0.27 pools/d, P < 0.03). Treatment also induced a significant increase in IDL production (6.3 vs. 3.7 mg/kg/d, P = 0.028). However, this did not result in an increase in LDL production because of an increase in IDL apoB direct catabolism (mean 102%, P < 0.033). VLDL kinetic parameters were unchanged and the concentrations of plasma total triglycerides (TG), VLDL-TG, VLDL-apoB did not rise as often seen with estrogen alone. Plasma HDL-cholesterol rose significantly (P < 0.02). Our major conclusion is that increased fractional catabolism of LDL underlies the LDL-lowering effect of the combined hormones.—Wolfe, B. M., P. H. R. Barrett, L. Laurier, and M. W. Huff. Effects of continuous conjugated estrogen and micronized progesterone therapy upon lipoprotein metabolism in postmenopausal women. J. Lipid Res. 2000. 41: 368–375.

Supplementary key words estrogen • progesterone • VLDL • LDL • cholesterol

Ovarian hormone replacement therapy, defined as treatment with estrogen and progestin (HRT), provides effective relief of vasomotor symptoms (1), prevents osteoporosis (2) and may reduce coronary heart disease (CHD, 3). Although a recent randomized clinical trial in older postmenopausal (PMP) women with advanced CHD including coronary bypass surgery found no overall cardiovascular benefit of HRT (4), observational studies of HRT or replacement of estrogen alone (ERT) have reported reductions in both all-cause and CHD mortality in other PMP women (5, 6).

Elevations of plasma cholesterol (7, 8) and triglycerides (7) have been reported in a high proportion of PMP women and are thought to contribute to the associated increase in CHD (3, 9–11). Estrogen replacement by HRT or ERT has the potential to reduce CHD risk through favorable effects on both lipoproteins and the arterial wall (12). Increases in both plasma triglycerides and HDL cholesterol have been reported in response to ERT in PMP women (13). However, triglyceride responses to HRT involving continuous conjugated estrogen (CEE, 0.625 mg/d) and medroxyprogesterone acetate (MPA, 2.5 mg/d) have been inconsistent. Significant increases in triglycerides have been reported in some, but not all, studies of 12 months or more in duration (14–17), but no significant increase in plasma triglycerides has been reported in shorter studies of 1.7 to 9 months in duration (18–23). MPA has also been reported to significantly reduce the CEE-induced increase in plasma triglycerides (15). Furthermore, estrogen-induced increments in HDL cholesterol have tended to be blunted more by concomitant administration of MPA than by natural ovarian progestin administered as micronized progesterone (MP 14, 24). Because prolonged estrogen therapy that is unopposed by progestin is associated with risk of endometrial neoplasia, there is a need for progestin (14, 25, 26).

Despite clinical usage of MP together with CEE by PMP women (14, 24), there has been no information about the

Abbreviations: CHD, coronary heart disease; CEE, conjugated equine estrogen; MP, micronized progesterone; FCR, fractional catabolic rate; HRT, hormonal replacement therapy; IDL, intermediate density lipoproteins; MPA, medroxyprogesterone acetate; Lp[a], lipoprotein [a].

1 To whom correspondence should be addressed.
The effects of continuously administering CEE and MP (CEE<sub>cont</sub>/MP<sub>cont</sub>) on the concentrations, production, catabolism, and/or composition of the major apolipoprotein (apo) B-containing lipoproteins [very low density (VLDL), intermediate density (IDL) and low density lipoproteins (LDL)] in PMP women. The present study was undertaken to test the hypothesis that HRT with CEE<sub>cont</sub>/MP<sub>cont</sub> would lower LDL-cholesterol by enhancing its fractional catabolism and would blunt the increases in VLDL production.

**SUBJECTS AND METHODS**

**Subjects**

Six participants, who had experienced typical menopausal symptoms and were amenorrheic for at least 6 months (serum follicle stimulating hormone >70 U/L) were recruited from London Health Sciences Centre University Campus out-patient clinics (Table 1). Baseline fasting plasma cholesterol concentrations during Phase I American Heart Association diets prior to entry into the study exceeded 5.2 mmol/L, the cut-point of eligibility for dietary treatment recommended by the National Cholesterol Education Program (27). Two subjects had fasting levels of plasma HDL cholesterol which were below the recommended cut-point of 0.9 mmol/L (27), 2 had plasma triglycerides which were slightly above the 95th percentile (29). One subject had primary hypothyroidism and was treated throughout the control periods. Lipids were measured in these subjects on four occasions at approximately weekly intervals during the control and treatment periods of their studies. Thus, mean values for each subject are the mean of four fasting blood plasma samples obtained over 3 weeks during low fat, low cholesterol diet for each of the control and treatment periods. Significantly different from control, P < 0.01.

**Preparation of labeled lipoproteins and kinetic studies**

The procedures for the lipoprotein turnover studies, including the isolation and preparation of labeled lipoproteins and their reinjection, have previously been described, along with the sampling and fractionation of VLDL (S<sub>35</sub> 0.60–0.40), intermediate density lipoprotein (IDL, S<sub>35</sub> 0.12–0.60), and LDL (S<sub>35</sub> 0.02–0.12) and the isolation of apoB from each lipoprotein fraction by isopropanol precipitation and determination of its specific activity (23, 32–35). The bolus injection of 131<sup>I</sup>-labeled VLDL was immediately followed by the 125<sup>I</sup>-labeled LDL. Isotopic crossover was corrected throughout the studies. Lipids were measured in these lipoprotein fractions as described below.

**TABLE 1. Characteristics of postmenopausal subjects**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Height</th>
<th>Weight</th>
<th>Cholesterol</th>
<th>Triglycerides</th>
<th>HDL-cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>yr</td>
<td>cm</td>
<td>kg</td>
<td>mmol/L</td>
<td>mmol/L</td>
<td>mg/dl</td>
</tr>
<tr>
<td>1</td>
<td>58</td>
<td>164</td>
<td>50</td>
<td>6.70</td>
<td>1.65</td>
<td>0.98</td>
</tr>
<tr>
<td>2</td>
<td>79</td>
<td>152</td>
<td>62</td>
<td>6.10</td>
<td>1.27</td>
<td>1.28</td>
</tr>
<tr>
<td>3</td>
<td>72</td>
<td>151</td>
<td>50</td>
<td>7.22</td>
<td>1.27</td>
<td>1.22</td>
</tr>
<tr>
<td>4</td>
<td>46</td>
<td>158</td>
<td>59</td>
<td>6.59</td>
<td>5.83</td>
<td>0.58</td>
</tr>
<tr>
<td>5</td>
<td>69</td>
<td>161</td>
<td>62</td>
<td>6.21</td>
<td>2.33</td>
<td>1.03</td>
</tr>
<tr>
<td>6</td>
<td>57</td>
<td>159</td>
<td>71</td>
<td>6.30</td>
<td>1.57</td>
<td>1.30</td>
</tr>
<tr>
<td>Mean</td>
<td>64</td>
<td>158</td>
<td>59</td>
<td>6.57</td>
<td>2.32</td>
<td>1.07</td>
</tr>
<tr>
<td>± SE</td>
<td>5</td>
<td>2</td>
<td>3</td>
<td>0.19</td>
<td>0.72</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Values for each subject are the mean of four fasting blood plasma samples obtained over 3 weeks during low fat, low cholesterol diet for each of the control and treatment periods.

Significantly different from control, P < 0.01.

Significantly different from control, P < 0.025.
Kinetic analysis

A multicompartamental model was used to describe VLDL, IDL, and LDL-apoB tracer data. In multicompartamental modeling, each compartment or pool represents a group of kinetically homogeneous particles. In this study the SAAM II program (SAAM Inst., Seattle, WA) was used to fit the model to the observed tracer data. ApoB metabolic parameters are subsequently derived from the model parameters giving the best fit. All tracer data was fit simultaneously using the compartmental model (Fig. 1).

Compartments 1 through 5 are used to describe the kinetics of apoB in the VLDL fraction. It was assumed that all VLDL apoB enters plasma via compartment 1. Compartments 1 through 4 represent a delipidation chain or cascade as originally described by Phair et al. (36). It is assumed that the residence time of particles in each compartment of the chain is equal. In addition, the fraction of each compartment in the cascade converted to the slowly turning over VLDL compartment, compartment 5, is the same. VLDL particles in compartment 4 can be converted to IDL or can be removed directly from plasma. The IDL section of the model includes compartments 6 and 7, a rapidly and slowly turning over pool of IDL particles, respectively. Particles in compartment 6 can be converted to the slow IDL compartment, to LDL, or can be removed directly from plasma. Because labeled IDL was not injected, it was assumed that all IDL was derived from VLDL. The LDL section of the model is characterized by a plasma compartment, compartment 8, and an extravascular exchange compartment, compartment 9. This model assumes that LDL apoB is kinetically homogeneous; however, the isolation of LDL subfractions and/or the collection of urine radioactivity after the injection of labeled LDL, may provide evidence of kinetic heterogeneity within the LDL fraction. Examination of the raw data revealed that significant input of "cold" or unlabeled LDL heterogeneity within the LDL fraction. This model assumes that LDL apoB in the VLDL fraction. It was assumed that all VLDL apoB is kinetically homogeneous; however, the isolation of LDL subfractions and/or the collection of urine radioactivity after the injection of labeled LDL, may provide evidence of kinetic heterogeneity within the LDL fraction. Examination of the raw data revealed that significant input of "cold" or unlabeled LDL must occur, as seen by observing the relationship between the data revealed that significant input of "cold" or unlabeled LDL heterogeneity within the LDL fraction. Examination of the raw data revealed that significant input of "cold" or unlabeled LDL content of VLDL of Sf 0–20 (4.13 ± 0.21 vs. 4.86 ± 0.21 mmol/L, P < 0.005), whereas HDL cholesterol rose by 30 ± 9% (P < 0.025, Table 1). The hormones induced a 29 ± 3% decrease in the mean value for the ratio of total cholesterol to HDL cholesterol versus control (4.6 ± 0.5 vs. 6.6 ± 1.0, P < 0.02) and a 35 ± 2% decrease in the value of the ratio of LDL (Sf 0–20) cholesterol to HDL cholesterol (3.1 ± 0.3 vs. 4.9 ± 0.7, P < 0.01).

Concentrations of lipoprotein lipids and apoB

During weeks 3 to 6 of the administration of CEE<sub>cont</sub>/MP<sub>cont</sub>, there were significant decreases of 8 ± 2% in fasting plasma total cholesterol (6.05 ± 0.27 vs. 6.57 ± 0.19 mmol/L, P < 0.01, Table 1) and 16 ± 2% in the concentration of cholesterol of VLDL of Sf 0–20 (4.13 ± 0.21 vs. 4.86 ± 0.21 mmol/L, P < 0.005), whereas HDL cholesterol rose by 30 ± 9% (P < 0.025, Table 1). The hormones induced a 29 ± 3% decrease in the mean value for the ratio of total cholesterol to HDL cholesterol versus control (4.6 ± 0.5 vs. 6.6 ± 1.0, P < 0.02) and a 35 ± 2% decrease in the value of the ratio of LDL (Sf 0–20) cholesterol to HDL cholesterol (3.1 ± 0.3 vs. 4.9 ± 0.7, P < 0.01).

Data obtained at time of the turnover studies comparing control versus treatment indicated that CEE<sub>cont</sub>/MP<sub>cont</sub> reduced the mean plasma concentration of cholesterol transported in LDL of Sf 0–12 by 13 ± 3% (3.70 ± 0.24 vs. 4.27 ± 0.19 mmol/L, P < 0.005) and increased mean HDL cholesterol by 30 ± 8% (1.32 ± 0.10 vs. 1.01 ± 0.09 mmol/L, P < 0.01, Table 2). Simultaneously, the mean values tended to decline during treatment for each of plasma total triglycerides (1.72 ± 0.36 vs. 2.20 ± 0.34 mmol/L), VLDL triglycerides (0.75 ± 0.17 vs. 0.96 ± 0.20 mmol/L, Table 2), IDL triglycerides (0.52 ± 0.16 vs. 0.69 ± 0.10 mmol/L), and LDL triglycerides (0.28 ± 0.02 vs. 0.36 ± 0.056 mmol/L), however, the changes were not statistically significant.
The changes in the mean values for concentrations of HDL triglycerides (0.17 ± 0.02 vs. 0.19 ± 0.02 mmol/L) and Lp[a] (17 ± 8 vs. 20 ± 10 mg/dl) also failed to be statistically significant. The mean value for concentration of apoB in plasma LDL was significantly lower during hormonal treatment versus control (82 ± 4 vs. 87 ± 3 mg/dl, P < 0.05); however, there was no significant change in mean VLDL apoB concentration (51 ± 0.9 vs. 4.3 ± 0.5 mg/dl, P > 0.2).

The mean value for the ratio of cholesterol to apoB in plasma VLDL (Sf 60–400) was 27 ± 4% lower during hormonal treatment versus control (1.5 ± 0.6 vs. 2.1 ± 0.9, P < 0.02 by Wilcoxon signed rank test), but there was no change in the ratio of triglycerides to apoB (16 ± 6 vs. 17 ± 5, P > 0.5). The mean value for the ratio of cholesterol to apoB in plasma IDL (Sf 12–60) was significantly lower (by 17 ± 2%) during CEE_cont/Mp_cont versus control (1.8 ± 0.1 vs. 2.2 ± 0.2, P < 0.005), but there was no change in the ratio of triglycerides to apoB in IDL (4.0 ± 0.6 vs. 4.1 ± 0.5, respectively, P > 0.5). There was no significant change in the mean value for the ratio of cholesterol to apoB of plasma LDL (Sf 0–12) during CEE_cont/Mp_cont versus control (1.4 ± 0.1 vs. 1.5 ± 0.1, respectively, P > 0.1), nor in the ratio of triglyceride to apoB (0.29 ± 0.02 vs. 0.27 ± 0.03, P > 0.3).

Consistent with a steady-state, there were no systematic changes during the turnover studies in plasma concentrations of the most readily quantified indices, namely total protein content of LDL (mean coefficient of variation of 10.7%, corresponding to a mean SD of 9.2 mg/dl, n = 6 paired studies) and total protein content of VLDL (mean coefficient of variation of 23%, corresponding to a mean SD of 1.7 mg/dl, n = 6 paired studies).

**TABLE 2. Effects of CEE_cont/MP_cont on levels of cholesterol and triglycerides of plasma lipoproteins on first day of turnover studies**

<table>
<thead>
<tr>
<th></th>
<th>Whole Plasma</th>
<th>VLDL</th>
<th>IDL</th>
<th>LDL</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cholesterol</td>
<td>Triglycerides</td>
<td>Cholesterol</td>
<td>Triglycerides</td>
<td>Cholesterol</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>6.31</td>
<td>1.85</td>
<td>0.18</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>6.52</td>
<td>2.44</td>
<td>0.22</td>
<td>1.09</td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>5.30</td>
<td>1.17</td>
<td>0.08</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>4.81</td>
<td>1.27</td>
<td>0.17</td>
<td>0.60</td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>6.90</td>
<td>2.06</td>
<td>0.23</td>
<td>0.72</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>6.52</td>
<td>0.78</td>
<td>0.09</td>
<td>0.28</td>
</tr>
<tr>
<td>4</td>
<td>Control</td>
<td>6.80</td>
<td>3.42</td>
<td>0.52</td>
<td>1.72</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>6.10</td>
<td>3.13</td>
<td>0.34</td>
<td>1.41</td>
</tr>
<tr>
<td>5</td>
<td>Control</td>
<td>6.21</td>
<td>2.93</td>
<td>0.34</td>
<td>1.42</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>5.79</td>
<td>1.27</td>
<td>0.15</td>
<td>0.51</td>
</tr>
<tr>
<td>6</td>
<td>Control</td>
<td>5.71</td>
<td>1.74</td>
<td>0.10</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>5.19</td>
<td>1.45</td>
<td>0.08</td>
<td>0.59</td>
</tr>
</tbody>
</table>

Mean ± SE:

|          | Control      | 6.21 ± 0.25 | 2.20 ± 0.34 | 0.24 ± 0.07 | 0.96 ± 0.20 | 0.70 ± 0.12 | 4.27 ± 0.19 | 1.01 ± 0.09 |
|          | Treated      | 5.82 ± 0.29c | 1.72 ± 0.36 | 0.18 ± 0.04 | 0.75 ± 0.17 | 0.63 ± 0.17 | 3.70 ± 0.24c | 1.32 ± 0.10c |

Values for each subject were based upon fasting blood plasma samples obtained immediately prior to injection of autologous labeled 131I-labeled VLDL (Sf 60–400) and 125I-labeled LDL (Sf 0–12) at the beginning of each turnover study.

Significantly different from control, P < 0.01.
Significantly different from control, P < 0.005.
Significantly different from control, P < 0.001.
nificantly increased conversion of VLDL to IDL during hormone administration partly explains the significant increase in IDL apoB production (6.3 ± 0.5 vs. 3.7 ± 0.8 mg/kg/d, \( P = 0.028 \), Table 4). The increased IDL production would more than offset the trend towards an increase in IDL FCR (1.55 ± 0.29 vs. 1.00 ± 0.11 pools/d, \( P = 0.086 \)), such that there was no significant change in IDL apoB pool size (294 ± 59 vs. 229 ± 42 mg, \( P = 0.4 \)).

The kinetic parameters of LDL turnover are summarized in Table 5. The FCR of LDL apoB increased significantly, by an average of 20 ± 7%, during hormonal administration versus control (0.320 ± 0.021 vs. 0.269 ± 0.016 pools/d, \( P = 0.03 \)). This resulted in a significant 6 ± 2% decrease in LDL apoB pool size (2162 ± 173 vs. 2300 ± 149 mg, \( P < 0.05 \)). Although production of LDL apoB tended to rise, with increases in 5 of the 6 subjects, the change was not significant (\( P = 0.10 \)). LDL direct synthesis was not significantly altered by the combined hormones (8.3 ± 1.5 vs. 7.8 ± 0.9 mg/kg/d, \( P = 0.58 \)) and direct LDL production as a percent of total LDL production was unchanged by hormonal treatment (67 ± 7 vs. 73 ± 4%, \( P = 0.35 \)).

Figure 3 illustrates the effect of CEE\textsubscript{cont}/MP\textsubscript{cont} on the decrease of the \( ^{125} \)I-labeled LDL apoB specific activity from the plasma for a representative subject. Data in Fig. 3 are presented as percent of peak specific activity because absolute values for peak specific activity were different between the treatment and control. The steeper slope during treatment indicates that the fractional catabolic rate of LDL is more rapid during hormonal treatment than control.

### Table 3. Kinetic parameters of human VLDL apoB turnover in CEE\textsubscript{cont}/MP\textsubscript{cont}-treated postmenopausal subjects

<table>
<thead>
<tr>
<th>Subject, Period</th>
<th>VLDL Pool Size</th>
<th>Fractional Catabolic Rate</th>
<th>VLDL Total Production</th>
<th>Conversion of VLDL to IDL</th>
<th>Conversion of VLDL to LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg pools/d</td>
<td>mg/kg/d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Control</td>
<td>140</td>
<td>4.8</td>
<td>13.5</td>
<td>24</td>
<td>20</td>
</tr>
<tr>
<td>Treated</td>
<td>214</td>
<td>3.6</td>
<td>15.3</td>
<td>47</td>
<td>15</td>
</tr>
<tr>
<td>2 Control</td>
<td>92</td>
<td>5.5</td>
<td>8.2</td>
<td>41</td>
<td>35</td>
</tr>
<tr>
<td>Treated</td>
<td>138</td>
<td>7.5</td>
<td>17.3</td>
<td>43</td>
<td>30</td>
</tr>
<tr>
<td>3 Control</td>
<td>92</td>
<td>4.8</td>
<td>8.9</td>
<td>36</td>
<td>35</td>
</tr>
<tr>
<td>Treated</td>
<td>68</td>
<td>6.5</td>
<td>8.8</td>
<td>57</td>
<td>49</td>
</tr>
<tr>
<td>4 Control</td>
<td>101</td>
<td>2.5</td>
<td>4.3</td>
<td>98</td>
<td>61</td>
</tr>
<tr>
<td>Treated</td>
<td>101</td>
<td>2.0</td>
<td>3.4</td>
<td>93</td>
<td>56</td>
</tr>
<tr>
<td>5 Control</td>
<td>142</td>
<td>6.4</td>
<td>14.7</td>
<td>40</td>
<td>22</td>
</tr>
<tr>
<td>Treated</td>
<td>140</td>
<td>6.0</td>
<td>13.4</td>
<td>61</td>
<td>26</td>
</tr>
<tr>
<td>6 Control</td>
<td>112</td>
<td>4.3</td>
<td>6.9</td>
<td>32</td>
<td>23</td>
</tr>
<tr>
<td>Treated</td>
<td>134</td>
<td>5.4</td>
<td>10.2</td>
<td>69</td>
<td>38</td>
</tr>
</tbody>
</table>

Mean ± SE

| Control         | 113 ± 9        | 4.7 ± 0.5                  | 9.4 ± 1.6              | 45 ± 11                  | 33 ± 6                   |
| Treated         | 133 ± 20       | 5.2 ± 0.8                  | 11.4 ± 2.1             | 62 ± 7\(^{a}\)           | 36 ± 6                   |

\(^{a}\) Significantly different from control, \( P < 0.05 \).

### Table 4. Kinetic parameters of human IDL apoB turnover in CEE\textsubscript{cont}/MP\textsubscript{cont}-treated postmenopausal subjects

<table>
<thead>
<tr>
<th>Subject, Period</th>
<th>IDL Pool Size</th>
<th>Fractional Catabolic Rate</th>
<th>IDL Total Production</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg pools/d</td>
<td>mg/kg/d</td>
<td></td>
</tr>
<tr>
<td>1 Control</td>
<td>173</td>
<td>0.93</td>
<td>5.8</td>
</tr>
<tr>
<td>Treated</td>
<td>256</td>
<td>1.39</td>
<td>8.2</td>
</tr>
<tr>
<td>2 Control</td>
<td>145</td>
<td>1.47</td>
<td>3.4</td>
</tr>
<tr>
<td>Treated</td>
<td>199</td>
<td>2.21</td>
<td>7.4</td>
</tr>
<tr>
<td>3 Control</td>
<td>198</td>
<td>0.80</td>
<td>3.2</td>
</tr>
<tr>
<td>Treated</td>
<td>112</td>
<td>2.24</td>
<td>5.0</td>
</tr>
<tr>
<td>4 Control</td>
<td>335</td>
<td>0.74</td>
<td>4.2</td>
</tr>
<tr>
<td>Treated</td>
<td>520</td>
<td>0.36</td>
<td>3.2</td>
</tr>
<tr>
<td>5 Control</td>
<td>173</td>
<td>0.95</td>
<td>3.2</td>
</tr>
<tr>
<td>Treated</td>
<td>256</td>
<td>1.79</td>
<td>7.1</td>
</tr>
<tr>
<td>6 Control</td>
<td>142</td>
<td>1.12</td>
<td>2.2</td>
</tr>
<tr>
<td>Treated</td>
<td>393</td>
<td>1.28</td>
<td>7.1</td>
</tr>
</tbody>
</table>

Mean ± SE

| Control         | 229 ± 42       | 1.00 ± 0.11               | 3.7 ± 0.5             |
| Treated         | 294 ± 59       | 1.55 ± 0.29               | 6.3 ± 0.8\(^{a}\)    |

\(^{a}\) Significantly different from control, \( P = 0.028 \).
DISCUSSION

The present studies are the first to determine the effects of the continuously administered CEE and MP on the production, catabolism, and composition of VLDL, IDL, and LDL. The combined hormones reduced plasma concentrations of LDL-cholesterol and LDL apoB significantly by significantly increasing the FCR of LDL (Table 5). An increase in the FCR of LDL-apoB has also been observed during continuous administration of CEE + MPA (23). However, cyclical estradiol + continuous norgestrel reduce LDL pool size by inhibiting LDL production (32). Whereas a previous study involving the daily administration of 2 mg of estradiol alone to nine PMP women (23) found a significant 21% increase in LDL apoB (13) production, the present study found only a trend towards a small increase (mean 10%, Table 5) suggesting the possibility that MP and/or other progestins could modulate the effects of estrogens on LDL kinetics. Pharmacological doses of ethinyl estradiol in the rabbit have been shown to increase LDL receptor expression (43) and the increase in LDL fractional catabolism with the present hormones could be explained by estrogen-enhancement of LDL receptor activity.

Parameters of LDL metabolism in the present study were determined by multicompartmental analysis of radioiodinated LDL, which remains a reference method for determining LDL-FCR (13). The mean control value for LDL-FCR in the present PMP women of 0.269 pools/d was in the same range as that obtained using similar technology in other groups of estrogen-deficient PMP women (23, 32, 44, 45). Comparisons between the present findings involving exogenous labeling versus studies involving endogenous labeling of LDL should take into account the different experimental methods and different subject

<table>
<thead>
<tr>
<th>Subject, Period</th>
<th>LDL Pool Size</th>
<th>Fractional Catabolic Rate</th>
<th>LDL Total Production</th>
<th>LDL Direct Production</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg pools/d</td>
<td>mg/kg/d</td>
<td>mg/kg/d</td>
<td>%</td>
</tr>
<tr>
<td><strong>1</strong> Control</td>
<td>2093</td>
<td>0.224</td>
<td>10.0</td>
<td>68</td>
</tr>
<tr>
<td>Treated</td>
<td>1913</td>
<td>0.313</td>
<td>11.8</td>
<td>71</td>
</tr>
<tr>
<td><strong>2</strong> Control</td>
<td>2037</td>
<td>0.234</td>
<td>7.7</td>
<td>63</td>
</tr>
<tr>
<td>Treated</td>
<td>1701</td>
<td>0.313</td>
<td>8.9</td>
<td>41</td>
</tr>
<tr>
<td><strong>3</strong> Control</td>
<td>2070</td>
<td>0.261</td>
<td>10.8</td>
<td>71</td>
</tr>
<tr>
<td>Treated</td>
<td>1935</td>
<td>0.232</td>
<td>9.0</td>
<td>51</td>
</tr>
<tr>
<td><strong>4</strong> Control</td>
<td>2336</td>
<td>0.335</td>
<td>13.3</td>
<td>80</td>
</tr>
<tr>
<td>Treated</td>
<td>2257</td>
<td>0.391</td>
<td>15.0</td>
<td>87</td>
</tr>
<tr>
<td><strong>5</strong> Control</td>
<td>2260</td>
<td>0.274</td>
<td>9.4</td>
<td>71</td>
</tr>
<tr>
<td>Treated</td>
<td>2260</td>
<td>0.324</td>
<td>12.0</td>
<td>81</td>
</tr>
<tr>
<td><strong>6</strong> Control</td>
<td>3003</td>
<td>0.284</td>
<td>12.0</td>
<td>87</td>
</tr>
<tr>
<td>Treated</td>
<td>2907</td>
<td>0.349</td>
<td>14.3</td>
<td>73</td>
</tr>
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</table>

Mean ± SE

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL Pool Size</td>
<td>2300 ± 149</td>
<td>2162 ± 173</td>
</tr>
<tr>
<td>Fractional Catabolism Rate</td>
<td>0.269 ± 0.016</td>
<td>0.520 ± 0.021</td>
</tr>
<tr>
<td>LDL Total Production</td>
<td>10.8 ± 0.8</td>
<td>11.8 ± 1.0</td>
</tr>
<tr>
<td>LDL Direct Production</td>
<td>7.8 ± 0.9</td>
<td>8.3 ± 1.5</td>
</tr>
</tbody>
</table>

* Significantly different from control, P < 0.05.

* Significantly different from control, P = 0.03.

**Fig. 3.** Specific radioactivity of LDL apoB expressed as percent of the peak specific activity after intravenous injection of autologous 125I-labeled LDL for subject no 1.
populations. Values for LDL-FCR derived from endoge-

nous labeling studies can be overestimated if the exper-

imental protocol is too short, resulting in insufficient in-

formation on the LDL tracer data to support the existence

of an LDL exchange pool. Similarly, overestimation results

if the studies rely upon LDL tracer data derived from

jected VLDL. Thus, a somewhat higher mean value for

LDL-FCR (i.e., 0.36, range 0.21–0.60) has been obtained

during endogenous labeling of LDL apoB in healthy

young men who had mean levels of LDL cholesterol

which were 13% lower than the present PMP women (46).

Conversely, estrogen replacement increases values for

LDL-FCR in PMP women, tending to raise them into the

same range as those of healthy men with normal to bor-

derline high plasma cholesterol (47, 48). One of the poten-

tial drawbacks of the exogenous approach is that VLDL iso-

lated for labeling and re-injection may under-represent

portion of the VLDL population that is converted to LDL

via the delipidation pathway. In contrast, endogenous la-

beling may allow the experimentalist to see the kinetics of

all VLDL particles, leading to the conclusion that a larger

proportion of LDL is derived from the VLDL fraction that

is seen with exogenous labeling. However, the mean control

value for direct production of LDL in the present PMP

woman (8.3 mg/dl) is similar to that previously re-

ported in other estrogen-deficient PMP women (23, 44).

The 16% reduction in the fasting plasma concentration of

LDL-cholesterol (Sf 0–20) with the CEE cont/MP cont accords

with reductions of 13–15% observed during cyclical ad-

ministration of MP together with continuous or cyclical

CEE (14, 24).

The present findings indicate that CEE cont/MP cont sig-

nificantly increases IDL production (Table 4) and that

this, in turn, is largely explained by increased conversion

of VLDL to IDL (Table 3). However, the increase in pro-

duction of IDL did not result in an increase in LDL pro-

duction because of the increase in direct removal of

IDL, which is consistent with up-regulation of LDL recep-

tors by the CEE. Treatment also significantly altered the

composition of IDL (Sf 12–60), as reflected in 17% lower

value for the ratio of cholesterol to apoB in plasma IDL

(Sf 12–60) and this explains why LDL cholesterol concen-

tration was unchanged despite the tendency for IDL apoB

pool size to increase (Tables 2 and 4).

There have been no previous reports of the effects on

HDL-cholesterol of continuously administering both CEE

and MP. However, the relatively high proportional in-

crease in HDL-cholesterol with the present HRT could be

explained, at least in part, by the observation that subjects

with low HDL experience relatively larger than average in-

creases in HDL-cholesterol during ovarian hormone re-

placement (49). Furthermore, it has been reported that ad-

ministration of continuous CEE and cyclical MP tended to

increase HDL more than CEE alone (24). However, oth-

er studies found that MP, like MPA, blunts estrogen-

induced increases in HDL-cholesterol, but the reductions

in increment are smaller than with MPA (14, 50).

CEE alone increases production of VLDL TG (51) and

estradiol administered alone increases production of

VLDL apoB (13). However, when CEE was combined with

MP in the present study (Table 4) or combined with the C-

19 progestin MPA (23), no significant increase in VLDL

production or TG concentration was observed. This sug-

gests that these progestins blunt the triglyceride-elevating

effect of CEE, consistent with a previous report that the C-

19 progestin norethindrone acetate inhibits hepatic tri-

glyceride secretion in a swine model (50). As mentioned

above, numerous short-term and some long-term studies

are consistent with the view that co-administration of MPA

with CEE blunts the expected estrogen-induced incre-

ments in plasma TG (15–20).

In summary, the CEE cont/MP cont lowered LDL choles-

terol and apoB by increasing the fractional catabolism of

LDL of Sf 0–12 and thereby improved the plasma lipopro-

tein vascular risk profile (52, 53).

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