Role of ovarian hormones in the regulation of protein metabolism in women: effects of menopausal status and hormone replacement therapy

Michael J. Toth, Cynthia K. Sites, and Dwight E. Matthews

Departments of Medicine and Obstetrics and Gynecology, University of Vermont, Burlington, Vermont

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Toth, Michael J., Cynthia K. Sites, and Dwight E. Matthews. Role of ovarian hormones in the regulation of protein metabolism in women: effects of menopausal status and hormone replacement therapy. Am J Physiol Endocrinol Metab 291: E639–E646, 2006.—The aim of this study was to determine the role of ovarian hormones in the regulation of protein metabolism in women. Whole body protein turnover, whole body protein breakdown, and whole body protein synthesis were measured in 18 premenopausal and 18 postmenopausal women. Oral treatment with conjugated estrogens (0.625 mg) and medroxyprogesterone (2.5 mg) for 1 yr did not alter whole body protein turnover. However, to address the hypothesis that ovarian hormones regulate protein metabolism, we examined the effect of menopausal status and hormone replacement therapy (HRT) on protein turnover. Whole body protein turnover was measured under postabsorptive conditions using [13C]bicarbonate (99% 13C) from Cambridge Isotope Laboratories (Andover, MA). Chemical and isotopic purities were determined by gas chromatography-mass spectrometry (GC-MS). Hormone replacement therapy (HRT) (0.625 mg conjugated estrogens + 2.5 mg medroxyprogesterone acetate, n = 9) or placebo (n = 9). No differences in whole body protein breakdown, oxidation, or synthesis were found between premenopausal and postmenopausal women. Protein metabolism remained similar between groups after statistical adjustment for differences in adiposity and when subgroups of women matched for percent body fat were compared. In postmenopausal women, no effect of HRT was found on whole body protein breakdown, synthesis, or oxidation. In contrast, our results support a stimulatory effect of HRT on albumin fractional synthesis rate, although this did not translate into alterations in circulating albumin concentrations. In conclusion, our results suggest no detrimental effect of ovarian hormone deficiency coincident with the postmenopausal state, and no salutary effect of hormone replacement with HRT, on rates of whole body protein turnover, although oral HRT regimens may increase the synthesis rates of albumin.

Menopausal and postmenopausal women. Protein metabolism remained similar between groups after statistical adjustment for differences in adiposity and when subgroups of women matched for percent body fat were compared. In postmenopausal women, no effect of HRT was found on whole body protein breakdown, synthesis, or oxidation. In contrast, our results support a stimulatory effect of HRT on albumin fractional synthesis rate, although this did not translate into alterations in circulating albumin concentrations. In conclusion, our results suggest no detrimental effect of ovarian hormone deficiency coincident with the postmenopausal state, and no salutary effect of hormone replacement with HRT, on rates of whole body protein turnover, although oral HRT regimens may increase the synthesis rates of albumin.

FAT-FREE TISSUE MASS declines with age (6, 9). In women, this decline appears to be accelerated around the time of menopause (3, 9, 32, 37). Because diminished ovarian hormone production is the hallmark of menopause, it is plausible to hypothesize a role for ovarian hormones in the maintenance of fat-free mass (FFM). In further support of this notion, several studies have suggested that pharmacological replacement of ovarian hormones in postmenopausal women has anabolic effects (17, 30) or diminishes the age-related loss of FFM (7), although these findings are not unanimous (4, 13, 14, 21). At present, our understanding of the role that ovarian hormones play in the regulation of FFM is limited.
ment (0.625 mg of conjugated equine estrogens + 0.25 mg medroxyprogesterone acetate; Prempro) and placebo tablets were obtained from Wyeth-Ayerst (Philadelphia, PA).

**Experimental subjects.** Data from two separate cohorts were used to address our hypotheses.

Healthy, middle-aged premenopausal women from Burlington, VT, and surrounding areas were recruited to participate in a 5-yr longitudinal study where the effect of the menopause transition on substrate and energy metabolism was examined. Details regarding this population have been described previously (35). A subset of women from this cohort (n = 15) were invited to participate in studies where the effect of menopausal status on protein metabolism was examined. These measurements were conducted between years 2 and 3 of the 5-yr longitudinal study (1999 and 2000). The inclusion criteria for these women were as follows: 1) between 40 and 52 yr of age; 2) premenopausal, as defined by the occurrence of two menses in the 3 mo preceding testing; no increase in cycle irregularity in the 12 mo preceding testing, and a follicle-stimulating hormone level <30 IU/l; 3) nonsmoking; 4) normal electrocardiogram at rest and during an exercise stress test; 5) body mass index ≤30 kg/m². The premenopausal status of these women was confirmed by menstrual cycle monitoring throughout the first 2 yr of the longitudinal study. Women were excluded if they 1) were or planned on becoming pregnant; 2) had a history or present diagnosis of diabetes, heart disease, hypertension, or other chronic disease; 3) were taking HRT, hormone-based contraceptives, chronic steroid therapy, neuroleptics, or other medication that could affect estrogenic state, energy expenditure, insulin sensitivity, or protein metabolism; 4) had a history of alcohol or drug abuse; or 5) were glucose intolerant, defined as having a fasting glucose level of 6.22 mmol/l or higher or a 2-h glucose level of >7.77 mmol/l following a 75-g oral glucose load. The nature, purpose, and possible risks of the study were explained to each subject before she gave written consent to participate. The experimental protocol was approved by the Committee on Human Research at the University of Vermont.

Healthy, middle-aged postmenopausal women (n = 18) were recruited from Burlington, VT, and surrounding areas to participate in a study where the effect of HRT on protein metabolism was examined. Testing on these women was conducted between 1999 and 2001. The inclusion criteria were as follows: 1) between 45 and 60 yr of age; 2) postmenopausal, as defined by no menses within the 6 mo preceding testing, and a follicle-stimulating hormone level >30 IU/l; 3) nonsmoking; and 4) body mass index ≤30 kg/m². Women were excluded if they 1) had a history or present diagnosis of diabetes, heart disease, hypertension, or other chronic disease; 2) were taking HRT or any other medication that could affect estrogenic state or protein metabolism; 3) had a history of alcohol or drug abuse; or 4) had a fasting glucose level of greater than 7.77 mmol/l following a 75-g oral glucose load. The nature, purpose, and possible risks of the study were explained to each subject before she gave written consent to participate. The experimental protocol was approved by the Committee on Human Research at the University of Vermont.

Experimental protocol. Both pre- and postmenopausal women underwent an outpatient screening visit, at which time medical history, physical examination, blood work, and other tests were performed. Volunteers that met the eligibility criteria listed above were invited to participate in each respective study. Premenopausal women were admitted to the General Clinical Research Center (GCRC) for an overnight visit during the luteal phase of their menstrual cycle. For 5 days prior to admission, subjects were instructed to consume a meat-free (but not dairy-free) diet by the research dietician. Twenty-four-hour urine collections were performed on the 2 days prior to admission. Subjects were asked to refrain from strenuous activity for 2 days prior to protein metabolism measurements. Volunteers were admitted to the GCRC on the evening prior to each infusion study. They were fasted that evening and through completion of the infusion study the following day.

At ~0600 the subject was awakened, and a similar experimental protocol as described above for premenopausal women was followed, with a few modifications. In addition to the leucine tracer, a primed (2.6 μmol/kg FFM), continuous (3.1 μmol·kg·FFM⁻¹·h⁻¹) infusion of [ring-²H₅]phenylalanine was started at time 0 h and continued for 8 h. Blood and breath samples were drawn at 3.5 h and at half-hour intervals between 5 and 8 h for measurement of tracer enrichments in plasma amino acids and in albumin. Plasma was isolated and stored at −70°C until analysis. Oxygen consumption and carbon dioxide production rates were determined using the ventilated hood technique (DeltaTrac, Yorba Linda, CA).

Postmenopausal women were randomized, using a stratified block approach, to receive oral, combination HRT (0.625 mg of conjugated equine estrogens and 2.5 mg of medroxyprogesterone acetate) or placebo daily for 2 mo. Groups were stratified for age and body mass index. Patients and all personnel performing metabolic testing were blinded to treatment status. Periodic telephone calls were made to each subject, and pill counts were performed at the completion of the 2-mo treatment regimen to define compliance. Of the 19 women who were originally enrolled in the study, 18 completed the study. There was a problem obtaining blood samples from the sampling catheter during the second infusion of one patient, and her data were excluded.

Protein metabolism measurements were performed on two occasions: immediately prior to beginning HRT or placebo therapy and after 2 mo of treatment. Directly preceding each infusion study, volunteers were instructed to consume a meat-free (but not dairy-free) diet for 5 day by the research dietician. Twenty-four-hour urine collections were performed on the 2 days prior to admission. Subjects were asked to refrain from strenuous activity for 2 days prior to protein metabolism measurements. Volunteers were admitted to the GCRC on the evening prior to each infusion study. They were fasted that evening and through completion of the infusion study the following day.

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**Body composition.** Body mass was measured on a metabolic scale (Scale-Tronix, Wheaton, IL). Fat mass, FFM, and bone mineral mass were each measured by dual-energy X-ray absorptiometry (DEXA) using Lunar DPX-L densitometer (Lunar, Madison, WI). Bone mass data are not reported. Appendicular FFM was measured using skeletal landmarks, as described previously (15), and served as an estimate of appendicular skeletal muscle mass (39). In addition, urinary creatinine excretion was measured over a 2-day period to estimate muscle mass. Urine volume was measured to the nearest milliliter and an aliquot stored at −20°C until analysis. Creatine concentration was measured using a colorimetric assay (Sigma Diagnostics, St. Louis, MO). Skeletal muscle mass was predicted from creatinine excretion as 21.8 × creatinine (g/day) (38). Creatinine excretion was not measured in one premenopausal woman.

**Analytical methods.** Plasma leucine enrichment and concentration were measured by negative chemical ionization GC-MS and α-ketoisocaprate (KIC) enrichment and concentration by electron impact ionization GC-MS, as described previously (24, 33). Enrichments were calculated from ion current ratios, as described previously (12). The ¹³C enrichment of expired CO₂ in the breath samples was...
measured by isotope ratio mass spectrometry (VG SIRA II; Midwich, Cheshire, UK).

The rate of albumin synthesis was determined from measurement of incorporation of [1,2-13C2]leucine and [ring-2H5]phenylalanine into plasma albumin. Albumin was isolated from plasma using the method of Korner and Debro (19) and was hydrolyzed in 6 M HCl for 24 h at 110°C. Amino acids were isolated by ion exchange chromatography and derivatized to the N-(O,S)-ethoxycarbonyl (ECF) derivatives (16) for the measurement of the leucine 13C enrichment and to N-heptafluorobutyryl-N-propyl (HFBP) ester derivatives (24) for measurement of [ring-2H5]phenylalanine enrichments. ECF-leucine enrichments were measured by gas chromatography-combustion isotope ratio mass spectrometry (Finnegan Delta Plus, Bremen, Germany). ECF-leucine enrichment measurements were not completed on the baseline sample for one volunteer in the HRT group because of technical problems. The [ring-2H5]phenylalanine enrichment in albumin was measured from the HFBP derivatives by positive chemical ionization GC-MS (model 5973A; Agilent Technologies, Palo Alto, CA) with selected ion monitoring of the minor isotope ions [mass-to-charge ratio (m/z) = 406 and 409] of the MH+ ion (m/z = 408), following the method of Patterson et al. (28). The enrichment measurements were not completed on the posttreatment sample for one volunteer in the placebo group because of technical problems.

Plasma albumin concentrations were measured using the bromocresol green method (8).

Calculations. Leucine rate of appearance (μmol·kg·FFM·h⁻¹), an index of whole body protein breakdown, was calculated as described previously (33), with plasma KIC enrichment as a proxy of intracellular leucine enrichment. The rate of leucine oxidation was calculated as described previously (34). Nonoxidative disposal of leucine, a proxy of protein synthesis, was calculated as leucine rate of appearance minus leucine oxidation.

The fractional synthesis rate of albumin was calculated as FSR = m_{ab}/E_p, where m_{ab} is the rate of increase in enrichment of either [1,2-13C2]leucine or [ring-2H5]phenylalanine in albumin per unit time, and E_p is the enrichment of the precursor pool. The rate of increase of tracer enrichment in albumin (m_{ab}) was determined from the linear regression of the [1,2-13C2]leucine and [ring-2H5]phenylalanine enrichments vs. time. A recent study (2) has shown that plasma KIC enrichment is a more reliable indicator of the precursor pool for protein synthesis in the liver than plasma leucine enrichment. Because there are few data regarding the most appropriate index of hepatic precursor pool enrichment, we chose to calculate albumin fractional synthesis rate using plasma leucine and KIC enrichment for E_p for the [1,2-13C2]leucine tracer. Plasma [ring-2H5]phenylalanine enrichment was used for E_p for the [ring-2H5]phenylalanine tracer. The absolute synthesis rate of albumin was calculated from the fractional synthesis rate, the plasma albumin concentration, and an estimate of plasma volume assuming 45 ml of plasma volume per kilogram of FFM.

### Table 1. Physical characteristics of pre- and postmenopausal women

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>Body mass index, kg/m²</th>
<th>Height, cm</th>
<th>Weight, kg</th>
<th>Fat mass, kg</th>
<th>Body fat, %</th>
<th>Fat-free mass, kg</th>
<th>Appendicular fat-free mass, kg</th>
<th>Skeletal muscle mass, kg</th>
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</thead>
<tbody>
<tr>
<td>Premenopausal</td>
<td>49.2±0.8</td>
<td>22.8±0.6</td>
<td>164.2±1.3</td>
<td>61.6±1.8</td>
<td>17.2±1.5</td>
<td>28.3±1.2</td>
<td>40.0±0.9</td>
<td>24.1±0.9</td>
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<tr>
<td>Postmenopausal</td>
<td>53.1±0.7</td>
<td>24.5±0.5</td>
<td>162.0±1.6</td>
<td>64.2±1.6</td>
<td>21.3±1.1*</td>
<td>32.9±1.3*</td>
<td>39.7±0.9</td>
<td>20.5±0.6*</td>
</tr>
</tbody>
</table>

Data are means ± SE.; *P < 0.05; †P < 0.01. Note that n = 14 for skeletal muscle mass in the premenopausal group.

### RESULTS

Physical characteristics of pre- and postmenopausal women are shown in Table 1. Postmenopausal women were older (P < 0.01) and had higher body mass indexes (P < 0.05) than premenopausal women. The higher body mass index was due to greater adiposity (P < 0.05). Skeletal muscle mass derived from creatinine excretion was lower in post- than in premenopausal women (P < 0.01). No differences were found in height, weight, FFM, or appendicular FFM (FFM measurements by DEXA).

The effect of menopausal status on protein metabolism is shown in Fig. 1. No differences were found in the rate of leucine appearance [premenopausal (Pre): 143.8 ± 4.2 μmol·kg·FFM⁻¹·h⁻¹ vs. postmenopausal (Post): 146.9 ± 2.7 μmol·kg·FFM⁻¹·h⁻¹, P = 0.525], nonoxidative leucine disposal (Pre: 112.3 ± 3.4 μmol·kg·FFM⁻¹·h⁻¹ vs. Post: 116.4 ± 2.5 μmol·kg·FFM⁻¹·h⁻¹, P = 0.325), or leucine oxidation (Pre: 31.4 ± 1.6 μmol·kg·FFM⁻¹·h⁻¹ vs. Post: 30.4 ± 0.9 μmol·kg·FFM⁻¹·h⁻¹, P = 0.569). No differences in circulating leucine (Pre: 101.0 ± 4.1 μM vs. Post: 106.1 ± 3.6 μM, P = 0.355) or KIC (Pre: 36.4 ± 1.6 μM vs. Post: 41.1 ± 2.1 μM, P = 0.102) concentrations were noted.

Because of differences in body fat between the groups (Table 1), and the fact that we (36) have shown adiposity to be a determinant of protein metabolism in women, we compared protein metabolism between groups after statistically adjusting for body fat. A significant difference in adiposity, we compared protein metabolism within a subgroup of pre- and postmenopausal women (n = 10/group) matched (±10%) for percentage of body fat (Pre: 31.0 ± 2.4% vs. Post: 31.6 ± 2.1%, P = 0.859). No differences in leucine rate of appearance (P = 0.140), nonoxidative leucine disposal (P = 0.391), or leucine oxidation (P = 0.112) were evident.

![Fig. 1. Effect of menopausal status on postabsorptive leucine metabolism. Ra, leucine rate of appearance; NOLD, nonoxidative leucine disposal; Ox, leucine oxidation. Data are means ± SE.](http://ajpendo.physiology.org/ by http://ajpendo.physiology.org/ by http://ajpendo.physiology.org/)
Table 2. Effect of menopausal status on protein metabolism after statistical control for body fat percentage and in subgroups of women matched for body fat percentage

<table>
<thead>
<tr>
<th></th>
<th>Premenopausal</th>
<th>Postmenopausal</th>
</tr>
</thead>
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<tr>
<td>n</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>Ra</td>
<td>146.0±3.6</td>
<td>144.4±3.2</td>
</tr>
<tr>
<td>NOLD</td>
<td>114.7±3.1</td>
<td>114.1±2.1</td>
</tr>
<tr>
<td>Ox</td>
<td>31.3±1.5</td>
<td>30.0±1.3</td>
</tr>
</tbody>
</table>

Subgroups matched for body fat percentage

<table>
<thead>
<tr>
<th>n</th>
<th>10</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ra</td>
<td>148.4±4.8</td>
<td>140.0±1.9</td>
</tr>
<tr>
<td>NOLD</td>
<td>115.4±4.3</td>
<td>111.1±2.3</td>
</tr>
<tr>
<td>Ox</td>
<td>33.0±1.6</td>
<td>29.3±1.4</td>
</tr>
</tbody>
</table>

Data are means ± SE and in μmol·kg⁻¹·h⁻¹. Ra, leucine rate of appearance; NOLD, nonoxidative leucine disposal; Ox, leucine oxidation. Analysis of covariance was used to adjust protein metabolism for body fat percentage. Pre- and postmenopausal women were matched for body fat percentage to within ±10% (premenopausal: 31.0 ± 2.4% vs. postmenopausal: 31.6 ± 2.1%, P = 0.859).

The physical characteristics of postmenopausal women randomized to HRT and placebo at baseline and following 2 mo of treatment are shown in Table 3. The randomization procedure produced treatment groups that were matched for both age (HRT: 53.1 ± 0.5 yr vs. Placebo: 53.1 ± 1.4 yr) and body mass index (HRT: 24.8 ± 0.5 kg/m² vs. Placebo: 24.2 ± 0.9 kg/m²). In addition, no differences were found between treatment groups at baseline for any body size or composition variable (range of P values: 0.165–0.742). Similarly, there were no group by time interaction effects (range of P values: 0.161–0.968) for any body size or composition variable.

The effect of 2 mo of HRT or placebo administration on postabsorptive, whole body leucine turnover is shown in Fig. 2, A and B, respectively. None of differences in leucine metabolism were found at baseline between women randomized to HRT or placebo (range of P values: 0.508–0.816). No group-by-time interaction effects were found for the rate of leucine appearance (HRT: 145.8 ± 4.8 to 147.4 ± 4.9 μmol·kg⁻¹·FFM⁻¹·h⁻¹ vs. placebo: 147.9 ± 2.7 to 145.4 ± 4.1 μmol·kg⁻¹·FFM⁻¹·h⁻¹, P = 0.392), nonoxidative leucine disposal (HRT: 115.6 ± 4.4 to 119.2 ± 4.3 μmol·kg⁻¹·FFM⁻¹·h⁻¹ vs. placebo: 117.2 ± 2.3 to 115.5 ± 3.6 μmol·kg⁻¹·FFM⁻¹·h⁻¹, P = 0.195), or leucine oxidation (HRT: 30.2 ± 1.3 to 28.2 ± 1.0 μmol·kg⁻¹·FFM⁻¹·h⁻¹ vs. placebo: 30.1 ± 1.5 to 29.8 ± 1.0 μmol·kg⁻¹·FFM⁻¹·h⁻¹, P = 0.585). Similar to results where the leucine tracer was used, no group by time interaction effect was found for phenylalanine rate of appearance (HRT: 44.1 ± 1.7 to 44.7 ± 1.7 μmol·kg⁻¹·FFM⁻¹·h⁻¹ vs. placebo: 46.8 ± 2.4 vs. 45.3 ± 2.2 μmol·kg⁻¹·FFM⁻¹·h⁻¹, P = 0.825; data not shown).

Plasma leucine, KIC, and phenylalanine enrichment data are shown in Fig. 3. No group-by-time interaction effects were observed for the average enrichment values calculated from these data (leucine: P = 0.403; KIC: P = 0.327; phenylalanine: P = 0.17). These average enrichment values were used for calculation of albumin fractional synthesis rates together with the rate of increase in enrichment of either [¹³C₅]leucine or [³H₅]phenylalanine in albumin per unit time, which are shown in Fig. 4.

Table 4 shows data for fractional and absolute synthesis rates of albumin using the [¹³C₅]leucine and [³H₅]phenylalanine tracers. For the [¹³C₅]leucine tracer, both plasma leucine and KIC were used as surrogates of precursor pool enrichment in the calculation of synthesis rates. No differences were found in the fractional synthesis rates of albumin at baseline between women randomized to HRT and placebo (range of P values: 0.508–0.831). Albumin fractional synthesis rate measured with the [³H₅]phenylalanine tracer increased in the HRT group compared with placebo (P < 0.01). In contrast, no group-by-time interaction effect was found for albumin synthesis rates when calculated using the [¹³C₅]leucine tracer with leucine (P = 0.231) or KIC (P = 0.166) as the measure of precursor pool enrichment. Analysis of changes within each group showed that albumin fractional synthesis rates increased in the HRT group with the use of the [³H₅]phenylalanine tracer (P < 0.05) and with the [¹³C₅]leucine tracer, using plasma leucine (P < 0.05) or KIC (P < 0.05) as a precursor. In contrast, albumin fractional synthesis rates did not change in the placebo group when the [¹³C₅]leucine tracer was used (P = 0.699 and P = 0.902 for leucine and KIC as precursors, respectively), although a trend toward a decrease was noted with the [³H₅]phenylalanine tracer (P = 0.06). Similar group-by-time interaction effects were found when absolute albumin synthesis rates (g/day) were calculated; HRT increased absolute albumin synthesis rates when calculated from the [³H₅]phenylalanine...
DISCUSSION

Several studies suggest that menopause accelerates the normal age-related loss of fat-free tissue (3, 9, 32, 37) and that HRT in postmenopausal women may have anabolic effects (7, 17, 30). In an effort to explore the role of ovarian hormones in the regulation of FFM, the present study examined the effect of menopausal status and combined estrogen-progestin HRT on protein turnover. Our results show that neither ovarian hormone deficiency associated with the postmenopausal state nor HRT in postmenopausal women alters whole body protein metabolism. In contrast, our findings suggest that HRT may increase plasma albumin fractional synthesis rates in postmenopausal women.

Contrary to our hypothesis, we found no differences in protein metabolism between pre- and postmenopausal women. Although postmenopausal women were older than their premenopausal counterparts (+4 yr), it is unlikely that such a small age difference would affect our comparisons, given the slow age-related change in whole body protein turnover (29). Despite similar inclusion criteria for body mass index, however, our comparisons were complicated by the fact that postmenopausal women had greater amounts of body fat (Table 1). Previous studies from our laboratory (36) and others (18, 40) have shown that increasing adiposity is associated with higher protein turnover rates. Thus a lower rate of protein metabolism in postmenopausal women could be masked by increased protein turnover secondary to greater body fat. In an attempt to account for differences in body fat, groups were compared after statistical control for adiposity and in subgroups of women matched for percentage of body fat. In both cases, protein metabolism remained statistically similar between pre- and postmenopausal women. We did note that, when women were matched for percentage of body fat, postmenopausal women began to show a tendency toward lower (4–11%) rates of protein turnover, although these differences did not reach significance. Nonetheless, our findings suggest that menopausal status, and by extrapolation ovarian hormone deficiency, does not alter whole body protein metabolism.

Our observation of differences in muscle mass between pre- and postmenopausal women warrants brief comment. Muscle mass estimated from urinary creatinine excretion was lower in postmenopausal than in premenopausal women. These findings disagree with muscle mass estimates from DEXA (i.e., appendicular FFM), which did not differ between groups. We believe that this disagreement reflects an effect of ovarian hormones on creatinine excretion rather than an effect of menopausal status on muscle mass per se. For instance, creatinine-based estimates of muscle mass were highly correlated to those derived from...
DEXA in premenopausal women ($r = 0.813$, $P < 0.001$), whereas no relationship was found in postmenopausal women ($r = 0.340$, $P = 0.167$). In support of our conclusion, Hassager and Christiansen (14) found a similar disagreement between urinary creatinine excretion and DEXA data in postmenopausal women receiving HRT. Collectively, these results raise questions about the validity of creatinine excretion to estimate muscle mass in women that vary in ovarian hormone status.

In postmenopausal women, oral estrogen/progestin HRT did not alter whole body protein metabolism. Our findings agree with recent data from Smolders et al. (31), who showed that a 3 mo of oral estradiol or estradiol plus dydrogesterone did not alter protein synthesis or breakdown in postmenopausal women, as measured by $[\text{methyl}-^2\text{H}_3,1^{-13}\text{C}]$methionine. Measurement of protein turnover using isotope labeled methionine, however, employs assumptions about the intracellular dilution of the tracer that might not be valid (23). This could affect the ability to detect changes in protein synthesis and breakdown in response to HRT if the hormones affect intracellular methionine metabolism (26, 27). For this reason, we believe that our study is the first to examine rigorously the effects of HRT on whole protein metabolism in postmenopausal women.

Whole body protein metabolism reflects the average protein turnover rates of all organs and tissue systems. Thus alterations in protein turnover in one organ or tissue system could be obscured by reciprocal changes in another. In an attempt to assess organ-specific effects of HRT, we also measured plasma albumin synthesis rate. We chose to measure the synthesis rate of an hepatic protein because we reasoned that, given the high first-pass concentrations of the hormones caused by the oral route of administration, turnover of hepatic proteins would show a more robust response to HRT compared with other organs. Albumin was chosen because it is the major secretory protein produced by the liver, comprising ~15% of total liver protein synthesis (5). Results from the $[^2\text{H}]{\text{H}}$phenylalanine tracer showed that HRT increased albumin fractional synthetic rates, whereas data from the $[^13\text{C}]$leucine tracer, calculated using either plasma leucine or KIC as a precursor pool, showed a nonsignificant increase in albumin fractional synthesis rate in the HRT group of ~15%. Similarly, HRT increased the absolute rates of albumin synthesis when calculated from the $[^2\text{H}]$phenylalanine tracer, but not when calculated from the $[^13\text{C}]$leucine tracer. The reason why an HRT effect was observed with the phenylalanine tracer only is not readily apparent but deserves further discussion.

To our knowledge, only one other study (10) has utilized both leucine ($[^1-13\text{C}]$) and phenylalanine ($[^15\text{N}]$) tracers to measure hepatic protein synthesis in humans. Using measurements obtained from cohorts of young, middle-aged, and elderly men and women, Fu and Nair (10) examined the effect of age on the synthesis rate of albumin and fibrinogen. With respect to albumin fractional synthesis rates, leucine and phenylalanine tracers similarly showed no effect of age, regardless of the surrogate used to estimate precursor pool enrichment. The tracers differed, however, in their ability to detect gender differences. Use of either the leucine or the phenylalanine tracer revealed a sex effect on albumin synthesis rate when their respective plasma enrichments were used as surrogates of precursor pool enrichment. In contrast, no sex difference was noted with the $[^13\text{C}]$leucine tracer when plasma KIC enrichment was used. Additionally, the pattern of age-related differences in fibrinogen synthesis rate varied according to the tracer used, with a drop in synthesis rate between young and middle-aged groups being discriminated by the leucine tracer, but not by the phenylalanine tracer. As in the present study, the reasons for divergent results among tracers are not apparent. These findings demonstrate, however, that variation in the ability of leucine and phenylalanine tracers to detect effects on synthesis of hepatic proteins is not unique to our study.

On balance, the effect of HRT on albumin protein synthesis was similar among tracers, although this effect was significant only for the $[^2\text{H}]$phenylalanine tracer. Thus we favor the interpretation that oral HRT increases hepatic albumin synthesis. Importantly, an effect of HRT on albumin metabolism is not unprecedented. Smolders et al. (31) showed that 3 mo of oral HRT reduced circulating plasma albumin levels in postmenopausal women. Thus the increase in albumin protein synthesis could be a compensatory response to falling plasma albumin levels. Although we observed a slight drop (5%) in plasma albumin in the HRT group, this effect did not reach significance. The implication of a stimulatory effect of HRT on albumin synthesis is not clear, and we urge caution in extrapolating findings from albumin protein synthesis to other hepatic proteins or hepatic protein synthesis in general (5). At a minimum, we believe that our results suggest that the role of ovarian hormones in the regulation of protein metabolism may

### Table 4. Effect of 2 mo of HRT or placebo on fractional and absolute synthesis rates of albumin and plasma albumin concentration

<table>
<thead>
<tr>
<th></th>
<th>HRT</th>
<th>Placebo</th>
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<tbody>
<tr>
<td></td>
<td>Baseline 2 mo</td>
<td>Baseline 2 mo</td>
</tr>
<tr>
<td><strong>Fractional synthesis rate (%/day) calculated using</strong></td>
<td></td>
<td></td>
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<tr>
<td>$[^2\text{H}]$phenylalanine</td>
<td>5.60±0.19</td>
<td>6.72±0.45</td>
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<tr>
<td>$[^13\text{C}]$leucine</td>
<td>6.21±0.36</td>
<td>7.08±0.28</td>
</tr>
<tr>
<td>$[^13\text{C}]$KIC</td>
<td>7.82±0.46</td>
<td>9.00±0.28</td>
</tr>
<tr>
<td><strong>Absolute synthesis rate (g/day) calculated using</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$[^2\text{H}]$phenylalanine</td>
<td>4.00±0.22</td>
<td>4.57±0.29</td>
</tr>
<tr>
<td>$[^13\text{C}]$leucine</td>
<td>4.55±0.32</td>
<td>4.96±0.29</td>
</tr>
<tr>
<td>$[^13\text{C}]$KIC</td>
<td>5.74±0.41</td>
<td>6.31±0.37</td>
</tr>
<tr>
<td>Plasma albumin, g/dl</td>
<td>3.97±0.11</td>
<td>3.78±0.09</td>
</tr>
</tbody>
</table>

Data are means ± SE. KIC, ketoisocaproate; *$P < 0.01$ for group by time interaction effect. Note that $n = 8$ for placebo group for $[^2\text{H}]$phenylalanine tracer and $n = 8$ for the HRT group for $[^13\text{C}]$leucine and $[^13\text{C}]$KIC data.
be complex, with effects that are organ, tissue, or protein specific.

In conclusion, we found no evidence for an effect of menopausal status or HRT on whole body protein turnover. Thus our results do not support the hypothesis that variation in ovarian hormone levels alters whole body protein metabolism. Taken together with previous studies (31), however, our findings do support an effect of oral HRT on hepatic albumin metabolism. The relevance of this effect on albumin metabolism is not clear but suggests that the regulation of protein metabolism by ovarian hormones may be complex, with effects that are unique to the organ, tissue, or protein studied.

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REFERENCES


