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Regulation of Protein Metabolism in Middle-Aged, Premenopausal Women: Roles of Adiposity and Estradiol*

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ABSTRACT

The age-related loss of fat-free mass (FFM) is accelerated in women during the middle-age years and continues at an increased rate throughout the postmenopausal period. Because protein is the primary structural component of fat-free tissue, changes in FFM are largely due to alterations in protein metabolism. Knowledge of the hormonal and physiological correlates of protein metabolism in middle-aged women, therefore, has important implications for understanding the mechanisms underlying changes in FFM. We measured leucine kinetics (expressed relative to FFM: μmol/kg FFM·h) in 46 middle-aged, premenopausal women (mean ± SD, 47 ± 3 yr) after an overnight fast (*i.e.* basal) and during euglycemic hyperinsulinemia (40 mU/m^2·min) using a 5.5-h infusion of [1-^{13}C]leucine. Additionally, we measured insulin-stimulated glucose disposal by euglycemic hyperinsulinemic clamp, body composition by dual energy x-ray absorptiometry, abdominal fat distribution by computed tomography, and hormone levels by RIA as possible correlates of protein metabolism. Under basal conditions, stepwise regression analysis showed that leucine appearance (*i.e.* protein breakdown) was related to percent body fat and serum estradiol ($r^2 = 40\%$; $P < 0.01$), and leucine oxidation was related to serum estradiol and percent body fat ($r^2 = 26\%$; $P < 0.05$). Under euglycemic hyperinsulinemic conditions, no variables correlated with the percent change in leucine appearance. The percent change in leucine oxidation was related to intraabdominal adipose tissue area and glucose disposal rate ($r^2 = 48\%$; $P < 0.01$). Correlates and r^2 values for nonoxidative leucine disposal (*i.e.* protein synthesis) under basal and euglycemic hyperinsulinemic conditions were similar to those observed for leucine appearance. From these results, we conclude that adiposity and/or serum estradiol may contribute to the regulation of protein metabolism and FFM in middle-aged, premenopausal women. (*J Clin Endocrinol Metab* **85:** 1382–1387, 2000)

FAT-FREE MASS (FFM) declines with age in women (1–3). During the middle-age years, the loss of FFM is accelerated and continues at an increased rate throughout the postmenopausal years (3–6). Loss of FFM, particularly skeletal muscle mass, contributes to reduced functional capacity and the development of disability in older women (7–9). In addition to its importance for physical functioning, FFM is a strong determinant of resting energy expenditure (3, 10), fat oxidation (11), and insulin-stimulated glucose disposal (12). Thus, loss of FFM may have adverse effects on physical and metabolic function in women during the postmenopausal years.

On a chemical level, protein is the primary structural component of fat-free tissue mass (13). Thus, changes in FFM are largely due to alterations in protein metabolism. The protein mass of the body is regulated by the dynamic balance between protein synthesis and protein breakdown. Knowledge of the hormonal and physiological correlates of protein synthesis and breakdown in middle-aged women, therefore, has important implications for understanding the mechanisms underlying changes in FFM. The goal of this study was to examine the hormonal and physiological correlates of protein turnover in middle-aged women. To accomplish this objective, we measured whole body leucine kinetics under postabsorptive and euglycemic hyperinsulinemic conditions in a cohort of middle-aged, premenopausal women. We chose to assess protein metabolism during hyperinsulinemia because of the prominent role of insulin in the regulation of whole body protein metabolism (14, 15). In addition, we measured several hormonal and physiological factors that may modulate protein metabolism under postabsorptive conditions or the response to hyperinsulinemia. Based on our previous studies that showed a sharp decline in FFM during the menopause transition (3, 4), a period characterized by reduced ovarian hormone secretion, we hypothesized that circulating levels of estradiol, progesterone, or both would correlate to leucine turnover measurements.

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Materials and Methods

Materials

L-[1-^{13}C]Leucine (99% ^{13}C) and sodium [^{13}C]bicarbonate (98% ^{13}C) were obtained from Tracer Technologies (Somerville, MA). Chemical and isotopic purities were determined by gas chromatography mass spectrometry (GCMS). Solutions of each isotope were prepared using aseptic technique. Each compound was dissolved in weighted volumes of sterile, pyrogen-free saline and filtered through a 0.22-μm pore size

filter (Millipore Corp., Bedford, MA) before use. An aliquot of the sterile solution was initially verified to be pyrogen free before administration.

Subjects

Middle-aged, premenopausal women (n = 46) were recruited to participate in the Vermont Longitudinal Study of the Menopause, a 5-yr study examining changes in energy expenditure, body composition, abdominal fat distribution, and insulin sensitivity in women as they traverse the menopause. Data from the first year evaluation of these volunteers are presented. Two manuscripts have been previously published using data from this cohort to examine correlates of energy expenditure and substrate oxidation (16) and to examine the relationship among plasma hormones, abdominal fat distribution, and cardiovascular disease risk factors (17).

Participants were recruited from Burlington, VT, and surrounding areas through advertisements in local newspapers. Women included 1) were between 40–52 yr of age; 2) were premenopausal, as defined by the occurrence of two menses in the 3 months preceding testing, no increase in cycle irregularity in the 12 months preceding testing, and a FSH level less than 30 IU/L; 3) were nonsmoking; 4) had a normal electrocardiogram at rest and during an exercise test; 5) were weight stable ($\pm$2 kg) during the 6 months before testing; and 6) had a body mass index of 30 kg/m^2 or less. Women were excluded if they 1) were or planned on becoming pregnant; 2) had a history or current diagnosis of diabetes, heart disease, hypertension, or other chronic disease; 3) were taking hormone replacement therapy, oral contraceptives, chronic steroid therapy, neuroleptics, or other medication that could affect energy expenditure, insulin sensitivity, or protein metabolism; 4) had a history of alcohol or drug abuse; or 5) were glucose intolerant, defined as a fasting glucose level of 6.22 mmol/L or higher or a 2-h glucose level of greater than 7.77 mmol/L after 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

Each prospective volunteer underwent an out-patient screening visit, at which time medical history, physical examination, biochemical laboratory tests, a treadmill test, and an oral glucose tolerance test were performed. Volunteers that met the eligibility criteria after screening and consented to participate were studied during two in-patient visits to the General Clinical Research Center approximately 10 days apart. The first in-patient visit occurred during the follicular phase of each woman's cycle, and the second in-patient visit was during the luteal phase. For 3 days before each admission, all subjects consumed a standardized, weight maintenance diet provided by the Metabolic Kitchen of the General Clinical Research Center (1988 $\pm$ 193 kcal/day; 60% carbohydrate, 25% fat, and 15% protein). Body composition and abdominal fat distribution measurements were performed the evening of admission for the first in-patient visit. All other measurements reported here were made during the second in-patient visit.

On the second in-patient visit, after an overnight fast (12 h), leucine kinetics were measured under basal, postabsorptive conditions and during a euglycemic hyperinsulinemic clamp. At approximately 0600 h, the subject was awakened and allowed to void. Teflon catheters were placed in an antecubital vein (18-gauge) for infusion and retrograde in a dorsal hand vein of the contralateral arm (20-gauge). Catheters were kept patent with a slow infusion of saline (30 mL/h). The hand was placed in a warming box to obtain arterialized venous blood (18). At 0630 h, a primed (4.5 μmol/kg), continuous (4.5 μmol/kg·h) infusion of [1-^{13}C]leucine was started. The bicarbonate pool was also primed (1.6 μmol/kg) with sodium [^{13}C]bicarbonate. A euglycemic-hyperinsulinemic clamp was begun at 210 min according to the method of DeFronzo *et al.* (19). Insulin was infused at 40 mU/m^2·min to approximate postprandial insulin levels. Euglycemia was maintained by a variable rate infusion of 20% dextrose. Plasma glucose levels were monitored every 5 min, and the dextrose infusion rate was adjusted to maintain euglycemia. The average glucose infusion rate (mg/min) from 90–120 min was calculated as a measure of insulin sensitivity and was expressed relative to FFM (mg/kg FFM·min). All infusions were stopped at 330 min, except for the 20% dextrose infusion, which was continued and tapered until no longer required to maintain normal glycemia.

Blood and breath samples were obtained just before the start of infusion for the measurement of baseline enrichments and hormone levels. Blood and breath samples were obtained simultaneously at 165, 180, 195, and 210 min for measurement of baseline leucine kinetics and at 285, 300, 315, and 330 min for measurement of leucine kinetics under euglycemic hyperinsulinemia. Blood samples were placed in heparinized tubes and stored on ice until the plasma was separated by centrifugation (4 C) and frozen at $-$70 C until analysis. Breath samples were placed into 20-ml evacuated tubes. Blood was drawn every 5 min from 210–330 min to monitor plasma glucose levels. Blood samples for measurement of plasma insulin concentration were taken at 210, 225, 240, 255, 270, 280, 300, 315, and 330 min. Oxygen consumption and carbon dioxide production rates were determined at 60 and 165 min for 10 min and at 300 min for 30 min using the ventilated hood technique (DeltaTrac, Yorba Linda, CA).

Analytical methods

Plasma leucine enrichment was measured by negative chemical ionization GCMS and α-ketoisocaproate (KIC) plasma enrichment by electron impact ionization GCMS, as previously described (20). Before measurement by GCMS, amino and keto acids were isolated from plasma and derivatized to the *N*-heptafluorobutyryl, *n*-propyl ester and *t*-butyldimethylsilyl-quinoxalinol derivatives, respectively (20). Injections of the *N*-heptafluorobutyryl, *n*-propyl amino acid derivatives were made into the GCMS (model 5988A, Hewlett-Packard Co., Palo Alto, CA) with selected monitoring of the $[M\text{-}HF]^-$ ion. Ions with mass to charge ratios (m/z) of 349 and 350 were monitored for unlabeled and [1-^{13}C]leucine, respectively. The *t*-butyldimethylsilyl-quinoxalinol derivative of the keto acids was measured by GCMS (model 5971A, Hewlett-Packard Co.), with selected monitoring of the $[M\text{-}57]^+$ ion at m/z 259 and 260 for unlabeled and [1-^{13}C]KIC, respectively. For each measurement, the background corrected enrichment in mole percent excess (mpe) was calculated from peak area ratios, as previously described (21). The enrichment of expired CO_2 (mpe $\times$ 1000) was measured by isotope ratio mass spectrometry (VG Sira II, Middlewich, Cheshire, UK). Plasma glucose concentrations were measured by a glucose analyzer (YSI, Inc., Yellow Springs, OH).

Calculations

The rate of appearance (R_a) of leucine into plasma (μmol/kg FFM·h) was calculated as: $R_a = I(E_i/E_p - 1)$ (Eq I), where I is the infusion rate (μmol/kg FFM·h) of the tracer, and E_i and E_p are the enrichment (mpe) of tracer in the infusate and plasma, respectively. Plasma KIC enrichment, a proxy measure of intracellular leucine enrichment, was used for E_p (*i.e.* reciprocal pool approach) (22). Leucine oxidation (C) was calculated as: $C = F_{13C}(1/E_p - 1/E_i) \times 100$ (Eq II), where F_{13C} is the rate of $^{13}CO_2$ excretion into expired air (μmol ^{13}C/kg FFM·h). F_{13C} was calculated as: $F_{13C} = F_{CO2} \times E_{CO2}/W[(60 \times 44.6)/(100 \times 0.81)]$ (Eq III), where F_{CO2} is the CO_2 production rate (cm^3/min), E_{CO2} is the enrichment of expired CO_2 (mpe $\times$ 1000), and W the subject's FFM (kg). The constants 60 (min/h) and 44.6 μmol/cm^3 convert F_{CO2} to μmol/h, the factor 100 changes mpe to a fraction, and 0.81 accounts for the retention of $^{13}CO_2$ in the bicarbonate pool.

Substrate oxidation patterns will be altered during the euglycemic hyperinsulinemic clamp. Specifically, the use of endogenous fat will be decreased in favor of exogenous glucose (23). Changes in substrate oxidation will alter $^{13}CO_2$ excretion because of the different ^{13}C abundances of endogenous and exogenous substrates (23, 24). To account for changes in $^{13}CO_2$ excretion due to changes in substrate oxidation induced by the euglycemic hyperinsulinemic clamp, we measured $^{13}CO_2$ excretion in a group of six postmenopausal women (51 $\pm$ 6 yr) undergoing an identical clamp protocol as the current study, but with no ^{13}C isotopes administered. Average $^{13}CO_2$ enrichments at 285, 300, 315, and 330 min were used to correct $^{13}CO_2$ enrichments obtained in the present study for contribution from exogenously administered glucose. The correction factors for $^{13}CO_2$ excretion at 285, 300, 315, and 330 min were $-$0.0013, $-$0.0018, $-$0.0023, and $-$0.0027 mpe, respectively.

Nonoxidative leucine disposal (S), an index of leucine incorporation into protein, was calculated as: $S = R_a - C$ (Eq IV).

Body composition

Body mass was measured on a metabolic scale (Scale-Tronix, Inc., Wheaton, IL). Fat mass, FFM, and bone mineral mass were each measured by dual energy x-ray absorptiometry using a Lunar Corp. DPX-L densitometer (Lunar Corp., Madison, WI). All scans were analyzed using the Lunar Corp. version 1.3y DPX-L extended analysis program for body composition. In our laboratory, the coefficient of variation for repeat determinations in seven older women was 1% for total body fat mass and 2% for total body FFM.

Computed tomography

Intraabdominal and abdominal sc adipose tissue areas were measured by computed tomography with a GE High Speed Advantage computed tomography scanner (General Electric Medical Systems, Milwaukee, WI), as previously described (25). The scan was performed between the L4–L5 vertebrae using a scout image of the body to establish the precise scanning position. Intraabdominal adipose tissue area was quantified by delineating the intraabdominal cavity at the internal-most aspect of the abdominal and oblique muscle walls surrounding the cavity and the posterior aspect of the vertebral body. The sc adipose tissue area was quantified by highlighting adipose tissue located between the skin and the external-most aspect of the abdominal muscle wall. The coefficient of variation for duplicate analysis of 10 subject's scans (*i.e.* reproducibility of the analysis of each scan) was less than 1%.

Hormone measurements

Serum insulin was determined with a double antibody RIA (Diagnostic Products, Los Angeles, CA). The intra- and interassay coefficients of variation for insulin were 4% and 10%, respectively. Serum 17β-estradiol, progesterone, testosterone, dehydroepiandrosterone sulfate, and sex hormone-binding globulin concentrations were measured by RIA (Diagostics Systems Laboratories, Inc., Webster, TX). The intra- and interassay coefficients of variations were 7.6% and 8% for 17β-estradiol, 5.6% and 3.3% for progesterone, 7.7% and 10.5% for testosterone, 7.8% and 10% for dehydroepiandrosterone sulfate, and 4% and 2% for sex hormone-binding globulin, respectively. Insulin-like growth factor I was measured by RIA after acid-ethanol extraction followed by cryoprecipitation according to the method of Breier *et al.* (26), as previously described (27). The intra- and interassay coefficients of variation were 4% and 1%, respectively.

TABLE 1. Physical characteristics of 46 middle-aged, premenopausal women

Variable	Mean ± SD	Range
Age (yr)	47 ± 3	40–51
Ht (cm)	165 ± 5	154–178
Body mass (kg)	63 ± 11	47–84
Fat-free mass (kg)	41 ± 4	34–54
Fat mass (kg)	18 ± 9	8–45
% Body fat	30 ± 9	16–50
Intraabdominal fat (cm^2)	61 ± 34	24–185
Subcutaneous fat (cm^2)	240 ± 116	32–573

TABLE 2. Fasting glucose and hormone levels and insulin-stimulated glucose disposal

Variable	Mean ± SD	Range
Fasting glucose (mmol/L)	4.61 ± 0.44	3.44–5.77
Fasting insulin (pmol/L)	6.5 ± 2.0	5–12
Estradiol (pg/mL)	51 ± 6	5–184
Progesterone (ng/mL)	5.1 ± 0.7	0.4–14.7
Dehydroepiandrosterone sulfate (μmol/L)	2.23 ± 0.16	0.76–5.81
Testosterone (nmol/L)	0.97 ± 0.31	0.31–1.77
Sex hormone-binding globulin (nmol/L)	57 ± 4	20–115
Insulin-like growth factor I (μg/L)	157 ± 7	69–278
Insulin-stimulated glucose disposal (mg/kg FFM·min)	10.7 ± 0.45	5.67–19.2

FFM, Fat-free mass.

Statistics

Means and SDs were calculated for all variables. Relationships between variables were determined by Pearson product-moment correlation coefficients. Because intraabdominal fat, estradiol, progesterone, dehydroepiandrosterone sulfate, sex hormone-binding globulin, and insulin-like growth factor I had skewed distributions (by Shapiro-Wilks test, $P < 0.05$ for all), relationships between these variables and leucine kinetic measurements were assessed by Spearman rank correlation coefficients. To examine the effect of hyperinsulinemia on leucine kinetic measurements, we expressed data as a relative change from baseline values. The percent change in each variable was calculated as the basal value minus the euglycemic hyperinsulinemic value divided by the basal value. Stepwise regression analysis was used to determine which hormonal and physiological variables explained variation in basal leucine kinetic measures and the relative change in leucine appearance and oxidation induced by euglycemic hyperinsulinemia. Possible predictor variables were entered into the stepwise model if a physiological basis for explaining variation in protein turnover was supported by prior studies and if a significant bivariate relationship was observed between the measure of protein turnover and the predictor variable. Because some predictor variables had skewed distributions, all dependent and predictor variables were ranked prior to entrance into the stepwise regression model.

Results

Physical characteristics of the subjects are shown in Table 1. This cohort spans a wide range with respect to adiposity and abdominal fat distribution. For example, the coefficients of variation ranged from a low of 30% for percent body fat to a high of 56% for intraabdominal fat.

Fasting glucose and hormone levels and insulin-stimulated glucose uptake are shown in Table 2. Volunteers were characterized by normal fasting insulin and glucose values. Insulin levels averaged 598 ± 158 pmol/L during the last 60 min of the euglycemic hyperinsulinemic clamp (data not shown).

Leucine kinetic data under basal and euglycemic hyperinsulinemic conditions are shown in Fig. 1. Both leucine and KIC were in isotopic steady state in plasma during the last 45 min of each infusion period (data not shown). Enrichment

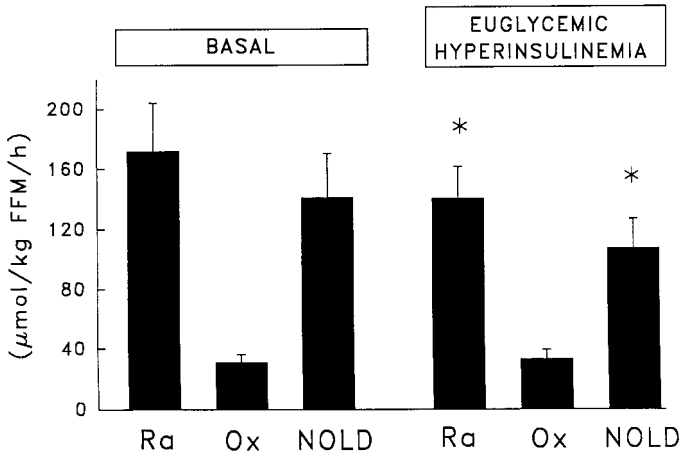

FIG. 1. Leucine kinetic measurements during basal conditions and euglycemic hyperinsulinemia. Ra, Appearance; Ox, oxidation; NOLD, nonoxidative leucine disposal. Values are the mean ± SD. *, $P < 0.05$ less than basal.

TABLE 3. Relationships between leucine kinetic measurements and selected hormonal and physiological variables

Variables	Basal		Euglycemic hyperinsulinemia	
	Leucine Ra	Leucine Ox	% Change in leucine Ra	% Change in leucine Ox
% Body fat	0.48[a]	0.35[b]	0.27	0.40[a]
Subcutaneous fat	0.36[b]	0.39[a]	0.14	0.39[a]
Intraabdominal fat	0.26	0.19	0.27	0.62[a]
Insulin-stimulated glucose disposal	−0.39[a]	−0.35[b]	−0.10	−0.54[a]
Estradiol	0.45[a]	0.42[a]	−0.10	0.28
Progesterone	0.33[b]	0.30	−0.10	0.11
Testosterone	0.03	0.03	0.18	0.03
Sex hormone-binding globulin	−0.15	−0.16	−0.18	−0.04
Dehydroepiandrosterone sulfate	0.25	0.16	0.04	0.04
Insulin-like growth factor I	0.11	0.21	0.13	0.12

Ra, Rate of appearance; Ox, oxidation. Units for variables are similar to those in Tables 1 and 2 and Fig. 1. Correlations for nonoxidative leucine disposal were similar to those for leucine appearance. Correlations for percent fat, sc fat, insulin-stimulated glucose disposal, and testosterone are Pearson product-moment coefficients. All others are Spearman rank coefficients. The percent changes in leucine appearance and leucine oxidation were calculated as the difference between basal and euglycemic hyperinsulinemic values divided by basal values.

[a] $P < 0.01$.

[b] $P < 0.05$.

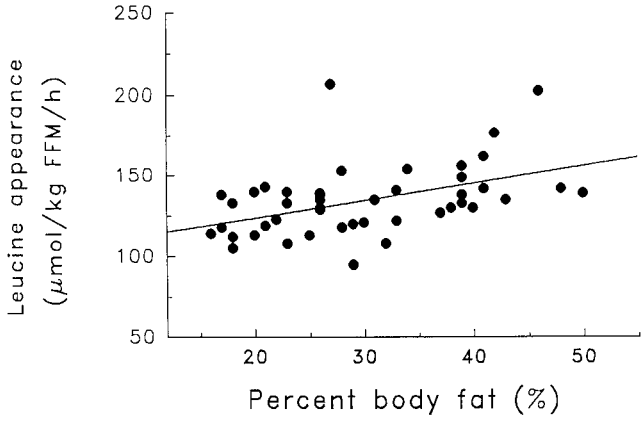

FIG. 2. Relationship of leucine appearance to percent body fat ($r = 0.48$; $P < 0.01$). The regression equation for this relationship is provided in *Results*.

data were averaged for each period and each subject to define mean enrichments for each experimental condition. Euglycemic hyperinsulinemia reduced leucine appearance (basal, 172 ± 32; euglycemic hyperinsulinemia, 141 ± 21 μmol/kg FFM·h; $P < 0.05$) and nonoxidative leucine disposal (basal, 141 ± 29; euglycemic hyperinsulinemic, 108 ± 19 μmol/kg FFM·h; $P < 0.05$), but did not affect leucine oxidation (basal, 31 ± 5; euglycemic hyperinsulinemia, 33 ± 6 μmol/kg FFM·h). The relative change in leucine appearance averaged −18 ± 4%, and leucine oxidation +6 ± 11%.

Correlation coefficients for the relationships between leucine appearance and oxidation measured under basal and euglycemic hyperinsulinemic conditions and various physiological and hormonal predictors are shown in Table 3. Correlations for nonoxidative leucine disposal are not presented because they were similar to correlations shown for leucine appearance. The similarity occurred because nonoxidative disposal is calculated from the difference between leucine appearance and oxidation and because the prominent term is leucine appearance. Measures of adiposity (*i.e.* percent body fat and intraabdominal fat) were the strongest and most consistent correlates of leucine kinetic measurements. In addition, as hypothesized, we observed significant correlations of estrogen and progesterone to leucine kinetic measurements. Correlations between fat mass (kg) and leucine kinetic measurements were similar to those observed for percent body fat and, therefore, are not presented.

Figure 2 shows the relationship of leucine appearance to percent body fat. The regression equation for the relationship of leucine appearance to percent body fat was: leucine appearance (μmol/kg FFM·h) = 0.491 × (percent body fat) + 11.63.

Table 4 shows the results of stepwise regression analysis examining the hormonal and physiological correlates of leucine metabolism. Predictors for nonoxidative leucine disposal and percent variability accounted for by these predictors were similar to those observed for leucine appearance (data not shown). During basal conditions, the leucine appearance rate was predicted by percent body fat ($r^2 = 24\%$; $P < 0.01$) and serum estrogen ($r^2 = 16\%$; $P < 0.01$). Leucine oxidation was predicted by serum estrogen ($r^2 = 17\%$; $P < 0.01$) and percent body fat ($r^2 = 9\%$; $P < 0.05$). Variation in the relative change in leucine oxidation induced by hyperinsulinemia was predicted by intraabdominal fat ($r^2 = 38\%$; $P < 0.01$) and insulin-stimulated glucose disposal ($r^2 = 10\%$; $P < 0.01$). No variable in the present study correlated with the relative change in leucine appearance.

Discussion

The present study sought to identify the hormonal and physiological correlates of protein metabolism in middle-aged, premenopausal women. The major findings are as follows: 1) measures of adiposity were positively related to leucine kinetic measurements; and 2) serum estradiol concentrations were positively related to leucine kinetic measurements. Assuming that leucine kinetic measurements reflect whole body protein turnover, our results suggest that adiposity and/or serum estradiol concentrations may contribute to the regulation of protein metabolism in middle-aged, premenopausal women.

TABLE 4. Stepwise regression analysis examining predictors of leucine kinetics under basal conditions and euglycemic hyperinsulinemia

Dependent variable	Step no.	Predictor variable	r^2	*P* value
Basal				
Leucine appearance	1	% body fat	0.24	<0.01
	2	Estradiol	0.40	<0.01
Leucine oxidation	1	Estradiol	0.17	<0.01
	2	% body fat	0.26	<0.05
Euglycemic hyperinsulinemia				
% Change in leucine oxidation	1	Intraabdominal fat	0.38	<0.01
	2	Insulin-stimulated glucose disposal	0.48	<0.01

The r^2 value represents the cumulative squared correlation coefficient for the stepwise regression model. None of the variables in the present study correlated with the percent change in leucine appearance.

Adiposity and protein metabolism

Percent body fat was the most consistent correlate of basal leucine kinetic measurements. Specifically, an increasing degree of adiposity was associated with greater leucine appearance, oxidation, and nonoxidative disposal. Our findings agree with studies showing increased leucine turnover with increasing body fatness (28–30), but differ from others that find no relation (31–34). The reasons for divergent results among these studies are not clear. Strong support for an effect of adiposity on protein metabolism, however, is provided by Welle and co-workers (35). In this study, leucine kinetics were compared between normal weight women and obese women studied before and after weight loss. The basal leucine appearance rate, expressed relative to lean body mass, was 9% higher in obese women compared to normal weight women. After a subsample of the obese women was reduced in weight to a similar fat mass as controls, however, no difference in leucine appearance was noted. These results suggest that leucine turnover is elevated in women with increased body fat and is reduced by weight loss. Taken together with our results, these findings support a role for adiposity in the regulation of protein turnover in premenopausal women.

Estrogen and protein metabolism

To our knowledge, this is the first study to examine the relationship between serum estradiol concentrations and measures of leucine turnover. Early studies by Calloway and Kurzer (36) showed a biphasic pattern in urinary nitrogen excretion that was related to menstrual cycle phase and suggested that female sex hormones regulate protein metabolism. More recent studies by Lariviere and co-workers (37) showed that leucine turnover was greater during the luteal phase compared to the follicular phase of the menstrual cycle. Because estradiol levels are greater during the luteal phase, these results implied that leucine turnover may be related to estrogen levels, although estradiol concentrations were not measured. As we hypothesized, positive relationships were found in the present study between estradiol levels and basal leucine appearance, oxidation, and nonoxidative disposal. Taken together, these findings support a role for estrogen in the regulation of whole body protein metabolism in premenopausal women.

The physiological relevance of a positive relationship between estrogen levels and leucine turnover and oxidation is not clear. Studies showing that the menopause transition is associated with a loss of FFM (4, 6) and that estrogen replacement therapy slows the age-related loss of FFM in postmenopausal women (38) provide circumstantial evidence that estrogen partially regulates FFM. These studies do not, however, suggest that estrogen has an anabolic effect *per se*, but that estrogen may be required for the maintenance of protein balance and FFM. The positive relationship between estrogen and leucine oxidation may appear paradoxical to this notion. However, it should be pointed out that leucine metabolism was only measured in the postabsorptive state. An increase in postabsorptive leucine oxidation could be compensated for by decreased leucine oxidation during the postprandial period. Although the relationship between estrogen and postprandial leucine oxidation was not measured in the present study, we would expect some compensatory mechanism to be operative, because women were weight stable at the time of evaluation and were not actively losing FFM.

Contrary to our findings, Mauras (39) found no effect of ethinyl estradiol therapy on whole body leucine turnover in hypogonadal, prepubertal girls. Comparison of the results of Mauras (39) to our findings, however, should be made with caution. First, hypogonadal, prepubertal girls may not respond to estrogen in a similar fashion as adult women. Second, we examined the relationship between leucine turnover and endogenous estradiol, whereas Mauras examined the effect of synthetic estrogen administered at different doses and via different routes on leucine turnover. Finally, the lack of an effect of synthetic estrogen on leucine turnover in hypogonadal, prepubertal girls may relate to other factors associated with their clinical syndrome.

Euglycemic hyperinsulinemic condition

The relative change in leucine oxidation induced by euglycemic hyperinsulinemia was related to intraabdominal fat area. Specifically, greater amounts of intraabdominal fat were associated with a greater insulin-induced suppression of leucine oxidation. We hypothesize that the relationship between intraabdominal fat and insulin-induced changes in leucine oxidation may be explained by free fatty acid availability. That is, women with greater amounts of intraabdominal fat would be expected to be resistant to the antilipolytic effects of insulin (40), which, in turn, would contribute to increased plasma free fatty acid concentrations. Greater free fatty acid concentrations would be expected to spare leucine from being oxidized (41). In support of this notion, we found a positive relationship between plasma glycerol concentrations measured during euglycemic hyper-

insulinemia, a proxy measure of lipolysis, and computed tomography-derived measures of abdominal adiposity (Tchernof, A., unpublished results). Thus, the sparing effect of free fatty acids on leucine oxidation may partially explain the relationship between intraabdominal adiposity and changes in leucine oxidation induced by hyperinsulinemia.

None of the variables examined in the present study correlated to insulin-induced suppression of leucine appearance. This may relate to the limited variability in the response of leucine appearance to hyperinsulinemia. For example, the variability (*i.e.* SD) in the relative change in leucine appearance (±4%) was 3-fold lower than that observed in the relative change in leucine oxidation (±11%). The limited variability in the response of leucine appearance to hyperinsulinemia may be explained by the fact that we studied women with normal glucose tolerance and because the insulin infusion achieved plasma insulin levels that would be expected to induce near-maximal suppression of leucine appearance (15).

In conclusion, our results suggest that adiposity and plasma estradiol levels may partially regulate protein metabolism in middle-aged premenopausal women. Because correlations do not signify cause and effect, studies that examine changes in whole body protein metabolism and FFM in women longitudinally as they undergo the menopause transition are needed to determine the physiological importance of our preliminary results.

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