# Effect of Menopausal Status on Lipolysis: Comparison of Plasma Glycerol Levels in Middle-Aged, Premenopausal and Early, Postmenopausal Women

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**To determine whether menopausal status affects systemic lipolysis, we measured plasma glycerol concentrations following an overnight fast and during euglycemic hyperinsulinemic conditions (40 mU · $m^{-2}$ · $min^{-1}$) in 43 middle-aged, premenopausal women (mean ± SE; 47 ± 0.4 years) and 26 early, postmenopausal (51 ± 0.8 years) women. In addition, body composition was measured by dual-energy x-ray absorptiometry and abdominal fat distribution by computed tomography (CT). Postmenopausal women had greater amounts of whole body (fat mass, 22.8 ± 1.4 *v* 17.4 ± 1.2 kg; percent fat, 34.7 ± 1.2 *v* 29.1 ± 1.4; both $P < .01$) and intra-abdominal fat (89.0 ± 6.5 *v* 55.9 ± 4.4 $cm^2$; $P < .01$) compared with premenopausal women. Despite greater adiposity, plasma glycerol concentrations were similar between pre- and postmenopausal women following an overnight fast (142.7 ± 9.7 *v* 136.1 ± 6.4 μmol/L) and at 30 minutes (112.7 ± 5.5 *v* 108.4 ± 4.5 μmol/L ) and 120 minutes (92.7 ± 4.5 *v* 97.5 ± 5.9 μmol/L ) into the euglycemic hyperinsulinemic clamp. Plasma glycerol levels remained similar after statistical adjustment for fat mass, percent fat, and intra-abdominal fat. Moreover, no differences in plasma glycerol were observed in pre- and postmenopausal women matched (± 5%) for fat mass (n = 22/group) or intra-abdominal fat (n = 15/group). In premenopausal women, plasma glycerol levels at 30 and 120 minutes of hyperinsulinemia were positively related to adiposity measures (range, $r = .314$ to $r = .493$; $P < .05$ to $P < .01$), although no relationships were found in postmenopausal women. Our results suggest no effect of menopausal status on plasma glycerol levels under postabsorptive or hyperinsulinemic conditions.**



FEMALE SEX HORMONES have been implicated in the regulation of adipose tissue metabolism in humans. In vitro studies suggest that the availability of estrogen and/or progesterone may regulate lipid uptake and release in isolated adipocytes.[1,2] The role of these hormones in controlling adipose tissue metabolism in vivo, however, is less clear. Jensen et al[3] recently showed that estrogen-deficiency in postmenopausal women was associated with increased systemic lipolysis, as measured by free fatty acid kinetics, compared with the estrogen-replaced state. These findings suggest that ovarian hormone availability may partially regulate systemic lipolysis. To our knowledge, however, no study has examined the effect of menopausal status, per se, on systemic lipolysis. Moreover, we know of no study that has investigated the effect of menopausal status on the sensitivity of lipolysis to insulin, an important hormonal regulator of adipose tissue metabolism.[4,5] Thus, our goal in the present study was to assess the effect of menopausal status on plasma glycerol concentrations, a proxy measure of systemic lipolysis. To accomplish this objective, we measured plasma glycerol concentrations after an overnight fast and under euglycemic hyperinsulinemic conditions in middle-aged, premenopausal and early, postmenopausal women. By studying middle-aged, premenopausal and early, postmenopausal women, we sought to minimize the confounding effect of age on adipose tissue metabolism[6] and other hormonal and physiologic systems that may regulate lipolysis. According to the results of Jensen et al[3] and the effects of ovarian hormones on insulin sensitivity,[7,8] we hypothesized that lipolysis would be increased in postmenopausal women under both postabsorptive and hyperinsulinemic conditions.

## SUBJECTS AND METHODS

### *Subjects*

Volunteers were recruited for 2 on-going studies from Burlington, VT and surrounding areas through advertisements in local newspapers. Premenopausal volunteers were recruited to participate in the Vermont Longitudinal Study of the Menopause, a 5-year study examining changes in energy expenditure, body composition, abdominal fat distribution, and metabolic function in women as they traverse the menopause. Data from the first year evaluation are presented. Postmenopausal women were recruited to participate in a randomized, double-blind study examining the effect of hormone replacement therapy on glucose homeostasis and abdominal fat distribution. Baseline, pretreatment data from this study are presented. Data from these cohorts have been used previously to examine the effect of menopausal status on body composition and insulin sensitivity.[9,10] The nature, purpose, and possible risks of each study were explained to each subject before she gave written consent to participate. The experimental protocols were approved by the Committee on Human Research at the University of Vermont.

The inclusion criteria for premenopausal women were: (1) between 40 and 52 years of age; (2) premenopausal, as defined by the occurrence of 2 menses in the 3 months preceding testing, no increase in cycle irregularity in the 12 months preceding testing, and a follicle-stimulating hormone level less than 30 IU/L; (3) nonsmoking; (4) normal electrocardiogram at rest and during an exercise test; (5) weight stability (± 2 kg) during the 6 months prior to testing; and (6) body mass index ≤30 $kg/m^2$. Premenopausal 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

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could affect metabolic function; (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-hour glucose level of greater than 7.77 mmol/L following a 75-g oral glucose load.

The inclusion criteria for early, postmenopausal women were: (1) early, postmenopausal status, as defined by absence of menses for at least 6 months, but not greater than 5 years and a follicle-stimulating hormone level greater than 30 IU/L and (2) body mass index $\leq$30 kg/m$^2$. The exclusion criteria for postmenopausal women were identical to premenopausal women except that glucose intolerance was defined as a fasting glucose level greater than 6.22 mmol/L.

### *Experimental Protocol*

Following an outpatient screening visit, eligible volunteers were studied during inpatient visits to the General Clinical Research Center (GCRC). For 3 days prior to inpatients visits, all subjects consumed a standardized, weight-maintenance diet provided by the Metabolic Kitchen of the GCRC (60% carbohydrate, 25% fat, 15% protein). Premenopausal women underwent 2 inpatients visits. The first inpatient visit occurred during the follicular phase of the menstrual cycle and the second during the luteal phase. Computed tomography (CT) scans were performed the evening of admission of the first inpatient visit and dual energy x-ray absorptiometry the following morning. The second inpatient visit occurred 10 days following the first visit. Plasma glycerol levels were measured following an overnight fast directly proceeding hyperinsulinemia (-10 minutes) and during euglycemic hyperinsulinemic conditions (30 and 120 minutes). Postmenopausal women underwent 1 inpatient visit. CT scans were performed the evening of admission. After an overnight fast, glycerol levels were measured prior to starting the euglycemic hyperinsulinemic clamp (-10 minutes) and 30 and 120 minutes during the clamp. Dual energy x-ray absorptiometry measurements were performed directly proceeding the clamp.

### *Plasma Glycerol Measurements*

Blood glycerol was measured according to the 1-step method developed by Boobis and Maughan.[11] Briefly, plasma samples (20 $\mu$L) were treated with 0.25 mol/L perchloric acid. After centrifugation (1,200*g*, 5 minutes, 4°C), 50 $\mu$L supernatant was used for glycerol measurement. The assay was based on the reduction of the cofactor $\beta$-nicotinamide adenine dinucleotide (NAD) during the conversion of glycerol to dihydroxyacetone by glycerol dehydrogenase (Roche Molecular Biochemicals, Indianapolis, IN). The assay buffer contained 0.2 mol/L hydrazine, 1 mmol/L EDTA, 0.1 mmol/L 2-amino-2-methylpropan-1-ol, $\beta$-NAD, glycerol dehydrogenase, and 50 $\mu$L glycerol standard or plasma extract and was incubated for 2 hours at room temperature. The reaction was stopped with 1 mL sodium carbonate buffer (20 mmol/L, pH 10). Fluorescence was measured at 350/458 nm. An equal number of plasma samples from pre- and postmenopausal women were measured in each assay. Plasma samples from baseline and insulin-stimulated conditions for each patient were measured within the same assay. The coefficients of variation for duplicate measurements averaged 2.7% for all the samples. The interassay coefficient of variation was 6.6%.

### *Hyperinsulinemic Euglycemic Clamp*

A 2-hour hyperinsulinemic euglycemic clamp was performed according to the method of DeFronzo et al.[12] Briefly, insulin was infused (40 mU $\cdot$ m$^{-2}$ $\cdot$ min$^{-1}$) to approximate postprandial insulin levels. Euglycemia was maintained by a variable rate infusion of 20% dextrose. Plasma glucose level was monitored every 5 minutes and the dextrose infusion rate adjusted to maintain euglycemia. The average glucose infusion rate (mg/min) from 90 to 120 minutes was calculated as a measure of insulin sensitivity (ie, insulin-stimulated glucose disposal).

### *CT*

Intra-abdominal and abdominal subcutaneous adipose tissue areas were measured by CT with a GE High Speed Advantage CT scanner (General Electric Medical Systems, Milwaukee, WI), as described.[13] Subjects were examined in the supine position with both arms stretched above the head. The scan was performed at the L4-L5 vertebrae level using a scout image of the body to establish the precise scanning position. Intra-abdominal adipose tissue area was quantified by delineating the intra-abdominal cavity at the internal most aspect of the abdominal and oblique muscle walls and the posterior aspect of the vertebral body. Adipose tissue was highlighted and computed using an attenuation range from -190 to -30 Hounsfield units. The subcutaneous adipose tissue area was quantified by highlighting adipose tissue located between the skin and the external most aspect of the abdominal muscle wall.

### *Body Composition*

Fat mass, fat-free mass, and bone mineral mass were measured by dual energy x-ray absorptiometry using a Lunar DPX-L densitometer (Lunar Co, Madison, WI). All scans were analyzed using the Lunar Version 1.3y DPX-L extended-analysis program for body composition.

### *Hormone Measurements*

Serum insulin was determined by a double antibody radioimmunoassay (Diagnostic Products Co, Los Angeles, CA). The intra-and interassay coefficients of variation for insulin were 4% and 10%, respectively.

### *Statistics*

Differences between variables were determined by unpaired, Student's *t* test. Analysis of covariance was used to examine differences between groups after statistically removing the effect of fat mass, percent fat, and intra-abdominal fat. In addition to statistical adjustment, plasma glycerol levels were compared between subsamples of pre- and postmenopausal women matched for either fat mass (n= 22/group; premenopausal, 22.6 $\pm$ 1.5 *v* postmenopausal, 22.3 $\pm$ 1.5 kg; $P$ = .90) or intra-abdominal fat (n = 15/group; premenopausal, 79.7 $\pm$ 8.6 *v* postmenopausal, 79.3 $\pm$ 8.7 cm$^2$; $P$ = .98). In both cases, each pair of pre- and postmenopausal women were matched to within $\pm$ 5% of the matching variable. Relationships between variables were determined by Pearson product-moment correlation coefficients. Because glycerol levels at baseline and 30 minutes of hyperinsulinemia in premenopausal women were not normally distributed (Shapiro Wilks test; $P < .02$ for both), relationships between these variables and body composition measurements were assessed by Spearman rank correlation coefficients. All data are expressed as mean $\pm$ SE.

## RESULTS

Physical characteristics of pre- and postmenopausal women are shown in Table 1. Body mass index was greater ($P < .05$) in postmenopausal women compared with premenopausal women (24.1 $\pm$ 0.6 *v* 22.5 $\pm$ 0.4 kg/m$^2$; range, pre: 18.3 to 29.5 kg/m$^2$; post, 17.8 to 29.5 kg/m$^2$; data not shown in Table 1). Premenopausal women were younger than postmenopausal women ($P < .01$). Fat mass was 31% greater ($P < .01$), percent body fat 19% greater, and intra-abdominal fat 59% greater in postmenopausal compared with premenopausal women. No differences were found in body mass, fat-free mass, or subcutaneous abdominal fat.

**Table 1. Physical Characteristics of Pre- and Postmenopausal Women**

| Variable | Premenopausal | Postmenopausal |
|---|---|---|
| No. | 43 | 26 |
| Age (yr) | 46.5 ± 0.4 | 50.8 ± 0.8* |
| Height (cm) | 164.6 ± 0.8 | 162.4 ± 1.1 |
| Body mass (kg) | 61.5 ± 1.3 | 65.3 ± 1.9 |
| Fat mass (kg) | 17.4 ± 1.2 | 22.8 ± 1.4* |
| Fat-free mass (kg) | 40.5 ± 0.6 | 39.1 ± 0.7 |
| Percent fat (%) | 29.1 ± 1.4 | 34.7 ± 1.2* |
| Intra-abdominal fat ($cm^2$) | 55.9 ± 4.4 | 89.0 ± 6.5* |
| Subcutaneous abdominal fat ($cm^2$) | 224.4 ± 17.1 | 270.4 ± 17.3 |

*$P < .01$.

Insulin-stimulated glucose disposal was not different between pre- and postmenopausal women if expressed on an absolute basis (435.7 ± 20.0 *v* 452.3 ± 23.7 mg/min) or per unit of fat-free mass (10.73 ± 0.45 *v* 11.72 ± 0.68 mg · kg $FFM^{-1}$ · $min^{-1}$). Insulin levels were similar between pre- and postmenopausal women following 30 minutes (574.6 ± 27.2 *v* 575.9 ± 42.8 pmol/L) and 120 minutes (582.11 ± 24.5 *v* 562.8 ± 48.8 pmol/L) of euglycemic hyperinsulinemia.

Figure 1 shows differences in plasma glycerol levels following an overnight fast and during euglycemic hyperinsulinemia. No differences were found in plasma glycerol concentrations at baseline (142.7 ± 9.7 *v* 136.1 ± 6.4 μmol/L) and at 30 minutes (112.7 ± 5.5 *v* 108.4 ± 4.5 μmol/L) and 120 minutes (92.7 ± 4.5 *v* 97.5 ± 5.9 μmol/L) of hyperinsulinemia. Statistical adjustment for fat mass, percent fat, intra-abdominal fat, or a combination of all 3 covariates did not affect differences in plasma glycerol levels at any time point (data not shown). Moreover, no differences were found in plasma glycerol levels in subgroups of pre- and postmenopausal women matched (± 5%) for fat mass (n = 22/group; basal glycerol, 140.5 ± 8.4 *v* 140.8 ± 6.9 μmol/L; 30 minutes, 122.1 ± 6.1 *v* 112.3 ± 4.6 μmol/L; 120 minutes, 105.5 ± 6.2 *v* 102.3 ± 6.3 μmol/L); or intra-abdominal fat (n = 15/group; basal glycerol, 136.1 ± 8.6 *v* 128.0 ± 8.0 μmol/L; 30 minutes, 110.1 ± 7.3 *v* 101.6 ± 5.0 μmol/L; 120 minutes, 100.7 ± 6.8 *v* 89.8 ± 6.8 μmol/L). No difference in the relative suppression of plasma glycerol levels by insulin was observed at either 30 minutes (pre, -14 ± 5 *v* post, -19% ± 2%) or 120 minutes (pre, -28 ± 4 *v* post, -29% ± 2%).

Correlation coefficients for the relationship of plasma glycerol levels to adiposity measures and insulin sensitivity in pre- and postmenopausal women are shown in Table 2. Plasma glycerol levels at 30 and 120 minutes were positively related to fat mass, percent fat, intra-abdominal fat, and subcutaneous abdominal fat in premenopausal women (range, $r = .314$ to $r = .493$; $P < .05$ to $P < .01$). After statistical control for percent body fat, correlations of glycerol levels to intra-abdominal (30 minutes, $r = -.063$; 120 minutes, $r = .075$) and subcutaneous abdominal fat (30 minutes, 0.064; 120 minutes, $r = .071$) were no longer significant. In contrast, no relationships were observed between plasma glycerol concentrations and adiposity at baseline or under hyperinsulinemic conditions in postmenopausal women.

## DISCUSSION

Our goal was to examine the effect of menopausal status on whole-body lipolysis. To accomplish this objective, we measured plasma glycerol levels in middle-aged, premenopausal and early, postmenopausal women following an overnight fast and under hyperinsulinemic conditions. We found no difference in plasma glycerol levels between pre- and postmenopausal women under postabsorptive or hyperinsulinemic conditions. These results suggest that menopausal status, per se, does not influence whole-body lipolysis.

No menopause-related differences in plasma glycerol levels were noted under postabsorptive or hyperinsulinemic conditions. In addition, no effect of menopause on plasma glycerol levels was observed when data were statistically adjusted for body composition and fat distribution measures or when pre- and postmenopausal women were matched (± 5%) for total (kilograms) or intra-abdominal ($cm^2$) fat. Our results differ from previous studies that have shown increased lipolysis in postmenopausal women in the estrogen-deficient compared with the estrogen-replaced state.[3] The reasons for divergent results between studies may reside in the different study designs used. Jensen et al[3] examined the acute (8 weeks) effect of estradiol availability on lipolysis in postmenopausal women using constant-release, transdermal estradiol replacement. This system of hormone replacement does not mimic normal ovarian function in the type of hormones provided or the cyclicity of their release. In contrast, our study examined the effect of menopausal status on lipolysis. Our results were influenced not only by ovarian hormone availability, but also changes in other physiologic and hormonal systems that occur with the menopause transition. Thus, our findings are not directly comparable to those of Jensen et al.[3]

Perhaps more surprising was the lack of differences in glycerol levels in postmenopausal women despite their greater amounts of total and intra-abdominal fat. Several studies have shown that increased total body[14,15] and abdominal[15-17] fat is associated with increased lipolysis and resistance to suppression by insulin. Thus, we would have expected that increased adiposity in postmenopausal women would contribute to increased lipolysis. This expectation, however, is based on the

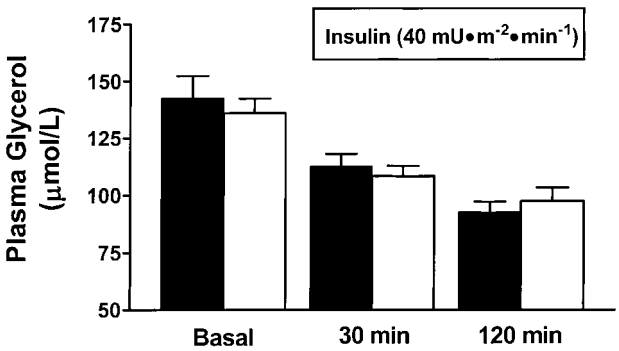


**Fig 1. Differences in plasma glycerol between pre- and postmenopausal women under postabsorptive (basal) and hyperinsulinemic conditions. Closed bars represent premenopausal women and open bars represent postmenopausal women. Values are mean ± SE.**

**Table 2. Correlation Coefficients for the Relationship of Plasma Glycerol Levels at Baseline (0 min) and During Hyperinsulinemia (30 and 120 min) With Adiposity and Insulin Sensitivity in Pre- and Postmenopausal Women**

| Variable | Premenopausal | | | Postmenopausal | | |
|---|---|---|---|---|---|---|
| | 0 Minutes | 30 Minutes | 120 Minutes | 0 Minute | 30 Minutes | 120 Minutes |
| Fat mass | .133 | .348* | .493† | −.085 | −.100 | −.075 |
| Percent fat | .155 | .314* | .475† | −.104 | −.067 | −.096 |
| Intra-abdominal fat | −.016 | .154 | .390* | −.024 | .144 | −.003 |
| Subcutaneous abdominal fat | .162 | .349* | .440† | .055 | .107 | .057 |
| Insulin-stimulated glucose disposal | .153 | .082 | −.155 | −.098 | −.066 | −.064 |

NOTE. All values are Pearson correlation coefficients except for values for 0- and 30-minute glycerol concentrations in premenopausal women which are Spearman rank coefficients. Insulin-stimulated glucose disposal values are expressed as mg · kg $FFM^{-1}$ · $min^{-1}$.

*$P < .05$.

†$P < .01$.

assumption that lipolysis is positively related to total and abdominal fat. Although several studies have shown that increased total and abdominal adiposity is associated with greater basal lipolysis and resistance to the suppressive effects of insulin,[14-17] these studies were conducted in groups of lean and obese subjects. Whether modest differences in total and abdominal adiposity are associated with increased lipolysis in a nonobese population has not been clearly established. In our total cohort (n = 69), we found no association between adiposity indices and basal lipolysis and found only weak positive correlations between adiposity indices and plasma glycerol levels during hyperinsulinemic conditions (30 and 120 minutes; range, $r = .237$ to $r = .313$; $P < .05$ to $P < .01$), suggesting a limited role for the amount of total or abdominal fat in determining plasma glycerol levels in nonobese women. A more likely explanation for the absence of differences in glycerol levels between pre- and postmenopausal women is the similarity in insulin sensitivity. That is, increased total and central adiposity may need to be accompanied by insulin resistance for alterations in systemic lipolysis to manifest.

The relationship of plasma glycerol levels to adiposity indices differed in pre- and postmenopausal women. Total and abdominal adiposity measures were correlated to plasma glycerol levels at 30 and 120 minutes of hyperinsulinemia in premenopausal, but not postmenopausal women. The relationships of intra-abdominal and subcutaneous abdominal fat to plasma glycerol levels were no longer significant after statistical control for total body fat, suggesting that these associations were a function of the overall mass of adipose tissue and not abdominal adiposity, per se. A positive relationship between adiposity and plasma glycerol levels during hyperinsulinemia would be consistent with the observation that sensitivity to the antilipolytic effects of insulin is reduced with increasing total and regional adiposity in premenopausal women.[15-17] The reason for menopause-related differences in correlation coefficients is not readily apparent. To our knowledge, no study has considered the effects of female sex steroids on insulin-induced suppression of lipolysis in vitro or in vivo. Estradiol and progesterone receptors are present in fat cells.[18,19] Moreover, estradiol and progesterone have been shown to alter tissue insulin sensitivity.[7,8] Thus, it is plausible to speculate that alterations in ovarian hormone levels with the menopause transition may influence the relationship of insulin-induced suppression of lipolysis to adiposity. The physiologic significance of menopause-related differences in these relationships, however, is unclear.

Several caveats to our results should be noted. First, plasma glycerol levels may not accurately reflect whole-body lipolysis. In a metabolic steady state, the amount of plasma glycerol is a function of the appearance of glycerol into the blood, primarily from lipolysis, and the removal from blood. Under postabsorptive and fasting conditions, plasma glycerol levels are highly correlated to the appearance rate of glycerol into the plasma as measured by isotope dilution.[20-22] Moreover, recent findings show that plasma glycerol levels closely reflect changes in abdominal and femoral adipose tissue cell lipolysis in response to hyperinsulinemia in premenopausal women.[17] Thus, we believe that plasma glycerol level is an acceptable proxy measure of whole-body lipolysis under postabsorptive and hyperinsulinemic conditions. Second, we cannot discount the possibility that differences in other hormonal or physiologic systems contributed to the absence of menopause-related differences. Finally, the cross-sectional nature of this study does not preclude the possibility that genetic variation and other factors contributing to individual variability in lipolysis confounded our results.

In summary, our findings suggest that menopausal status does not influence postabsorptive lipolysis or the suppression of lipolysis by insulin.

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