

Changes in Intra-abdominal Fat in Early Postmenopausal Women: Effects of Hormone Use

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Abstract

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Objective: Intra-abdominal fat (IAF) accumulates with age, is greater among postmenopausal vs. premenopausal women, and is linked to risk for both type 2 diabetes and cardiovascular disease. Whether hormone replacement therapy (HRT) prevents or attenuates changes in IAF and related risk factors is not clear. The objectives of this observational study were to 1) determine whether HRT attenuated the expected age-related increase in IAF and 2) identify the independent effects of HRT and fat distribution on changes in disease risk factors.

Research Methods and Procedures: Subjects were early postmenopausal white women 45 to 55 years of age. Women either used HRT at the time of enrollment ($n = 33$) or did not ($n = 17$). Subjects were evaluated at baseline and 2 years for body composition (DXA), body fat distribution (computed tomography), insulin sensitivity (Si; minimal model), and serum lipids.

Results: IAF increased significantly over 2 years, and this increase was not attenuated by HRT. HRT users had less IAF throughout the study. HRT users showed an increase in Si, whereas non-users showed a decrease. Superficial sub-

cutaneous adipose tissue was significantly and independently related to total cholesterol, whereas IAF was related to high-density lipoprotein cholesterol, triglycerides, and Si. **Discussion:** HRT users had less IAF at baseline and throughout the study. Whether HRT altered the relationship between total body fat and IAF or whether differences between groups existed before the study should be addressed through a randomized, interventional study design. HRT had a significant effect on Si; IAF and superficial subcutaneous adipose tissue were significant determinants of disease risk factors.

Key words: visceral fat, cardiovascular disease, lipids, insulin sensitivity, hormone replacement therapy

Introduction

Among women, the menopause transition is associated with gains in both total and central body fat (1). The increase in central body fat is likely to be in the intra-abdominal or visceral compartment, although few studies have directly assessed the influence of menopause on intra-abdominal fat (IAF).¹ Cross-sectional data, comparing pre- and postmenopausal women, have indicated that IAF is greater among postmenopausal women (2). However, increasing age is consistently associated with IAF accumulation (3,4), making it difficult to discern an independent effect of menopause. Because IAF is uniquely associated with risk for cardiovascular disease, type 2 diabetes, and the metabolic syndrome (5), it is important to identify factors that regulate IAF accrual.

The use of postmenopausal hormone replacement therapy (HRT) has been associated with attenuation of body composition and fat distribution changes associated with meno-

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¹ Nonstandard abbreviations: IAF, intra-abdominal fat; HRT, hormone replacement therapy; SuperSAAT, superficial subcutaneous abdominal adipose tissue; DeepSAAT, deep subcutaneous abdominal adipose tissue; LBM, lean body mass; CV, coefficient of variation; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; Si, insulin sensitivity; AIR_g, acute insulin response to glucose; SEE, standard error of the estimate.

pause, but data are discrepant. Discrepancies among studies are likely caused by differences among protocols, particularly the type of HRT used and the duration of the observation period. In addition, cross-sectional studies may yield different results from longitudinal or interventional studies.

In general, results of interventional or longitudinal studies indicate that women who used estrogen or combined estrogen-progestin therapy either lost body fat or gained less total and/or abdominal fat or body weight over the study period, relative to the placebo group (6,7). However, results varied with regimen; transdermal estrogen users, similar to placebo users, showed a gain in body fat that was primarily in the trunk area (8). Estrogen use did not prevent the loss of lean mass unless combined with an androgenic progestin (6). In cases where women were treated with androgens, lean mass was maintained or gained, but effects on adiposity were variable (8–10).

Few studies have examined the effect of HRT on accumulation of IAF. The only relatively long-term, longitudinal studies have involved Japanese (11) and Swedish (12) women; in both cases, HRT users had less visceral fat than non-users after 1 year of hormone use. These observations confirm cross-sectional data suggesting that HRT use limits IAF deposition (13).

Likewise, few studies have examined associations among changes in individual adipose tissue compartments and changes in disease risk factors in postmenopausal women using or not using HRT. Although IAF is uniquely associated with disease risk, subcutaneous adipose tissue compartments (superficial and deep) also have been associated with risk factors in some populations (14–18). Thus, it is of interest to determine both the influence of HRT on body fat distribution and the association of HRT-mediated changes in fat distribution with changes in disease risk factors.

The objectives of this study were to 1) determine whether HRT attenuated the expected age-related increase in IAF over a 2-year period and 2) determine the independent effects of HRT use and changes in IAF, superficial subcutaneous abdominal adipose tissue (SuperSAAT), deep subcutaneous abdominal adipose tissue (DeepSAAT), and thigh fat on changes in cardiovascular disease risk factors in postmenopausal women.

Research Methods and Procedures

Experimental Subjects

Subjects were 50 postmenopausal women 45 to 55 years of age. Only white women were involved in this study, because ethnicity affects both body fat distribution (19) and insulin sensitivity (17). Only women who experienced a natural menopause, with the time of menopause known to occur at least 6 months before contact, were recruited. Both women using HRT and women not using HRT were recruited. Among hormone users ($n = 33$), subjects predom-

inantly used conjugated equine estrogens, 0.625 mg/d, and medroxyprogesterone acetate, 2.5 mg/d. Four women used unopposed oral estrogen (conjugated equine estrogens), and one used a transdermal estradiol patch in combination with an oral progestin. In cases where use was cyclic, testing was conducted during the combined (estrogen + progestin) portion of the cycle. No HRT use ($n = 17$) was defined as no current use and no use within the past 6 months. Over the 2-year observation period, several women in the HRT group changed their hormone use: five women changed dosage of the same preparation; two women switched to a different formulation of the same hormone (e.g., from Provera to Prometrium); three women altered their hormone use (e.g., from estrogen only to estrogen plus progestin); and two women discontinued HRT. Two women in the control group started using HRT during the course of the study (one used Premarin vaginal cream; the other used Estrace/Prometrium). Results from analyses excluding women who started or stopped oral formulations did not differ in significance or interpretation. Therefore, these subjects were retained in the analyses, and their baseline hormone use classifications were used. None of the women smoked. Data were collected over a 27-hour period during an in-patient visit to the Department of Nutrition Sciences and the General Clinical Research Center at the University of Alabama at Birmingham. The protocol was approved by the Institutional Review Board for Human Use at the University of Alabama at Birmingham, and all subjects signed an informed consent form before testing.

Materials and Methods

Protocol. Testing took place over a 2-year period. At baseline and after 2 years, subjects came to the University of Alabama at Birmingham for an overnight visit with a comprehensive metabolic evaluation (body composition, fat distribution, and insulin sensitivity testing). One year after the baseline visit, subjects reported to the University of Alabama at Birmingham for a brief, outpatient evaluation (fasting blood draw and body composition analysis only). At each comprehensive visit, subjects arrived at the Department of Nutrition Sciences at ~9:00 AM in the fasted condition (12-hour fast). Body composition was determined by DXA. At ~12:00 PM, subjects were escorted to the University of Alabama at Birmingham General Clinical Research Center. Subjects remained at the General Clinical Research Center for ~24 hours, departing at noon the following day. At ~7:00 PM, subjects were escorted to Radiology for computed tomography scanning. While subjects were at the General Clinical Research Center, all food was provided. The evening snack was consumed before 7:00 PM. Subjects fasted until fasting blood draw and intravenous glucose tolerance test the following morning (~7:00 AM).

Body Composition and Fat Distribution. Total and regional body composition [fat mass and lean body mass (LBM)] were measured by DXA using a Lunar DPX-L densitometer (LUNAR Radiation Corp., Madison, WI). Subjects were scanned in light clothing while lying flat on their backs with arms at their sides. DXA scans were performed and analyzed with adult software version 1.5g. IAF, SuperSAAT, DeepSAAT, and thigh fat were analyzed by computed tomography scanning with a HiLight/Advantage Scanner (General Electric, Milwaukee, WI) as previously described (18,20). A scout scan was first performed to locate the L₄-L₅ intervertebral space. Subsequently, a 5-mm scan of this abdominal site was taken. Thigh fat was assessed in a cross-section at midthigh. Scans were later analyzed for cross-sectional area (centimeters squared) of adipose tissue using the density contour program with Hounsfield units for adipose tissue set at -190 to -30. We have shown the test-retest reliability for IAF to be 1.7% (21). Scans were analyzed by an individual blinded to the hormone use status of the study participants.

Assay of Glucose, Insulin, and Lipids. Glucose was measured in 10 μ L of sera using an Ektachem DT II System (Johnson and Johnson Clinical Diagnostics, Rochester, NY). In our laboratory, this analysis has a mean intra-assay coefficient of variation (CV) of 0.61%, and a mean inter-assay CV of 1.45%. Insulin was assayed in duplicate 200- μ L aliquots with Diagnostic Products Corp. (DPC, Los Angeles, CA) "Coat-A-Count" kits. According to the supplier, cross-reactivity of this assay with proinsulin is ~40% at midcurve; C-peptide is not detected. In our laboratory, this assay has a sensitivity of 1.9 μ IU/mL, a mean intra-assay CV of 5%, and a mean interassay CV of 6%. Commercial-quality control sera of low, medium, and high insulin concentration (Lyphochek; Bio-Rad, Anaheim, CA) were included in every assay to monitor variation over time.

Total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglycerides (TGs) were measured with the Ektachem DT II System. With this system, HDL-C is measured after precipitation of low-density lipoprotein cholesterol (LDL-C) and very-low-density lipoprotein cholesterol with dextran sulfate and magnesium chloride. Control sera of low and high substrate concentration are analyzed with each group of samples, and values for these controls must fall within accepted ranges before samples are analyzed. The DT II is calibrated every 6 months with reagents supplied by the manufacturer. LDL-C was estimated using the Friedewald formula (22).

Intravenous Glucose Tolerance Test and Minimal Modeling. At ~7:00 AM, after a 12-hour fast, flexible intravenous catheters were placed in the antecubital spaces of both arms. Three blood samples were drawn over a 40-minute period, and sera were subsequently separated and pooled for analysis of lipids. Three additional blood samples were

taken over a 20-minute period for determination of basal glucose and insulin (the average of the values was used for basal "fasting" concentrations). At time 0, glucose (50% dextrose; 11.4 g/m²) was administered intravenously. Blood samples (2.0 mL) were collected at the following times (minutes) relative to glucose administration at 0 minutes: 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180. Tolbutamide (125 mg/m²) was injected intravenously at 20 minutes. Sera were analyzed for glucose and insulin, and values were entered into the MINMOD computer program (ver. 3.0; Richard N. Bergman) for determination of insulin sensitivity (Si) and the acute insulin response to glucose (AIR_g) (23-25). AIR_g is the integrated incremental area under the curve for insulin during the first 10 minutes of the test.

Physical Activity. Physical activity was assessed at baseline using a modification of the technique published by Sallis et al. (26). Details have been published (27). In brief, the women were interviewed regarding their participation (hour per week) in physical activity over the previous week. Study personnel subsequently categorized activities as light, moderate, hard, or very hard and assigned energy expenditure values to each activity. Measured resting metabolic rate was used to calculate total and activity-related energy expenditure for the week. For the purpose of this study, kilocalories per week of light and moderate physical activity were tested in statistical models for potential contribution to variance in body composition and risk factors.

Statistical Analyses. Body composition, lipid, and insulin variables were log-transformed before analyses to ensure normality of distribution. Student's *t* test was used to compare baseline values between the HRT user and non-user groups. Repeated-measures models (two measurements per subject), with the compound symmetry covariance structure (PROC MIXED, SAS ver. 9.0), were used to examine changes in body composition and body fat distribution and potential relationships with age, HRT, and the HRT \times time interaction. This analysis allows for the examination of the effects of both time and HRT use status on the outcomes of interest while statistically adjusting for confounding factors. The output includes mean values for each group at each time-point that are adjusted for relevant confounding factors (covariates). Total body fat was used as a covariate in the models for IAF, SuperSAAT, DeepSAAT, and thigh fat; weight was used as a covariate in the model for LBM. The relationships between changes in IAF and risk factors were likewise examined using similar repeated-measures models. The selection of body composition and fat distribution variables for use in each final model was determined in preliminary analyses that tested total body fat, IAF, SuperSAAT, Deep SAAT, and thigh fat as potential covariates. Only significant fat terms were included in the final models. The interaction of HRT and time was examined by including an interaction term in preliminary models; this interaction term

Table 1. Baseline descriptive statistics

	Non-users (<i>n</i> = 17)	HRT users (<i>n</i> = 33)	<i>p</i>
Age (years)	51.6 ± 2.8 (46 to 55)	50.3 ± 2.5 (45 to 55)	0.08
Weight (kg)	66.7 ± 9.5 (49.1 to 91)	67.4 ± 9.3 (52.8 to 90.4)	0.78
BMI (kg/m ²)	24.9 ± 3.85 (18.5 to 34.4)	25 ± 3.5 (20.1 to 34.2)	0.93
Age at menopause (years)	48.6 ± 3.4 (42 to 54)	47.6 ± 2.5 (43 to 52)	0.24
Years of hormone use		2.8 ± 2.2 (0.5 to 13)	
Total fat mass (kg)	25.9 ± 7.6 (10.4 to 39.8)	24.7 ± 8.1 (10.7 to 42.5)	0.62
IAF (cm ²)	120.5 ± 52.6 (57 to 224.7)	86.8 ± 32.5 (24.9 to 159.1)	0.02
SuperSAAT (cm ²)	161.9 ± 54.8 (72.9 to 284.6)	144.6 ± 54.3 (57.7 to 255.9)	0.30
DeepSAAT (cm ²)	145.6 ± 52.9 (17.4 to 218.9)	149.8 ± 71.2 (48.7 to 331.7)	0.83
Thigh fat (cm ²)	265.7 ± 76.8 (147 to 455)	272.5 ± 97.5 (93.2 to 501.9)	0.80
Total lean mass (kg)	37.5 ± 3.4 (32.9 to 47.4)	39.4 ± 3.4 (32.6 to 46.0)	0.07
Cholesterol (mg/dL)	182 ± 26 (132 to 220)	181 ± 27 (128 to 235)	0.89
HDL-C (mg/dL)	46 ± 10 (30 to 62)	54 ± 15 (33 to 97)	0.07
LDL-C (mg/dL)	115 ± 30 (60 to 165)	105 ± 24.7 (53 to 148)	0.21
Triglycerides (mg/dL)	107 ± 45 (56 to 181)	108 ± 42 (47 to 226)	0.95
Insulin sensitivity [$\times 10^{-4}$ min ⁻¹ / (uIU/mL)]	6.7 ± 2.7 (2.1 to 10.8)	6.9 ± 3.8 (2 to 20.2)	0.88
Fasting insulin (uIU/mL)	8 ± 3 (4 to 13)	7 ± 3 (3 to 13)	0.18
AIR _g (uIU/mL $\times 10$ minutes)	332 ± 180 (29 to 797)	347 ± 226 (76 to 1070)	0.82
Light physical activity (kcal/wk)	7,740 ± 2,416 (0 to 10,751)	8,612 ± 1,294 (5,298 to 10,711)	0.19
Moderate physical activity (kcal/wk)	2,024 ± 2,074 (0 to 8,415)	2,164 ± 1,388 (328 to 6,718)	0.78

HRT, hormone replacement therapy; IAF, intra-abdominal fat; SuperSAAT, superficial subcutaneous abdominal adipose tissue; DeepSAAT, deep superficial subcutaneous abdominal adipose tissue; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; AIR_g, acute insulin response to glucose. Values are mean ± standard deviation (range).

was not included in the final models if not significant. In all models, the effect of explanatory variables was examined by parameter estimates and *p* values. The significance level was set at *p* < 0.05.

Post Hoc Power Analysis. Because of the limitations on the sample size in this observational study, a power analysis was performed for the IAF outcome using an *n* of 17 per group. A univariate, two-group, repeated-measures ANOVA using the Greenhouse-Geisser correction to nominal degrees of freedom would have 67% power to detect a variance among the group, and marginal means of 321.306 would have 66% power to detect a variance of 38.131 among the means of the two time-points and would have 6% power to detect an interaction between groups and time with a variance of 1.156, assuming that the between-group error term is 59.43, the within-group error term is 20.80, and the measure of sphericity of the covariance matrix, ϵ , is 1.00, when the significance level is 0.050 and the sample size in each of the two groups is 17.

Results

Descriptive information on the study participants at baseline is shown in Table 1. HRT users and non-users did not differ with respect to age or body composition (*p* > 0.05), although HRT users tended to have greater LBM (*p* = 0.063). HRT users had less IAF than non-users (*p* < 0.05). Lipid concentrations did not differ with HRT use, although HDL-C tended to be higher among HRT users (*p* = 0.065). Neither fasting insulin, Si, nor AIR_g differed with HRT use. Reported physical activity fell primarily into the light and moderate categories and did not differ with HRT use.

Results from the repeated-measures analysis for body composition and fat distribution variables are shown in Table 2, along with adjusted means and standard error of the estimate (SEE). For the dependent variable IAF, both HRT and time were statistically significant. IAF increased with time, and HRT users had less IAF across both visits (Figure 1). The HRT \times time interaction was not significant (*p* = 0.321), indicating that the change in IAF with time did not

Table 2. Predicted mean \pm standard error of the estimate and main effects for body composition and fat distribution variables from repeated-measures analysis

	Visit	Non-users	HRT users	<i>p</i> for main effects
IAF (cm ²)	1	103.13 \pm 9.01	82.24 \pm 5.05	Age, <i>p</i> < 0.01
	2	108.89 \pm 9.84	92.40 \pm 5.65	HRT, <i>p</i> < 0.001 Time, <i>p</i> < 0.01 Total fat, <i>p</i> < 0.001 HRT \times time, <i>p</i> = 0.321
SuperSAAT (cm ²)	1,2	148.56 \pm 9.25	137.40 \pm 6.11	Age, <i>p</i> = 0.438 HRT, <i>p</i> = 0.840 Time, <i>p</i> = 0.318 Total fat, <i>p</i> < 0.001 HRT \times time, <i>p</i> = 0.527
DeepSAAT (cm ²)	1,2	125.69 \pm 8.56	137.53 \pm 6.69	Age, <i>p</i> = 0.588 HRT, <i>p</i> = 0.704 Time, <i>p</i> = 0.577 Total fat, <i>p</i> < 0.001 HRT \times time, <i>p</i> = 0.757
Thigh fat (cm ²)	1,2	253.4 \pm 12.6	257.8 \pm 9.0	Age, <i>p</i> = 0.404 HRT, <i>p</i> = 0.953 Time, <i>p</i> = 0.814 Total fat, <i>p</i> < 0.001 HRT \times time, <i>p</i> = 0.538
Total body fat mass (kg)	1,2	24.37 \pm 2.06	23.66 \pm 1.41	Age, <i>p</i> = 0.365 HRT, <i>p</i> = 0.957 Time, <i>p</i> = 0.242 HRT \times time, <i>p</i> = 0.476
LBM (kg)	1,2	37.70 \pm 0.70	39.28 \pm 0.52	Age, <i>p</i> = 0.353 HRT, <i>p</i> = 0.058 Time, <i>p</i> = 0.275 Weight, <i>p</i> < 0.001 HRT \times time, <i>p</i> = 0.287

HRT, hormone replacement therapy; IAF, intra-abdominal fat; SuperSAAT, superficial subcutaneous abdominal adipose tissue; DeepSAAT, deep superficial subcutaneous abdominal adipose tissue; LBM, lean body mass.

Main effects tested were age, HRT, and time; the interaction of HRT and time also was tested. In addition, total body fat was included in the model for IAF, SuperSAAT, DeepSAAT, and weight was included in the model for LBM. All means shown are adjusted for the variables listed in the right column. Where “time” was not significant, the adjusted mean shown is the same for both visits.

differ with hormone use. For the dependent variables SuperSAAT, DeepSAAT, thigh fat, and total body fat, neither HRT nor time was significant. For the dependent variable LBM, HRT was marginally significant (*p* = 0.058); after adjusting for body mass, HRT users had more LBM across both visits. The HRT \times time interaction was not significant in any of the models.

Analogous results for cardiovascular disease risk factors are shown in Table 3, along with adjusted means and SEE.

For the dependent variables total cholesterol, HDL-C, and TGs, neither HRT nor time was significant. SuperSAAT was significantly related to total cholesterol, whereas IAF was significantly related to HDL-C and TGs. In addition, kilocalories per week of light physical activity was inversely related to TG. For the dependent variable Si, HRT, IAF, and the HRT \times time interaction were significant. Si increased with time among HRT users, whereas it decreased with time among non-users (Figure 2).

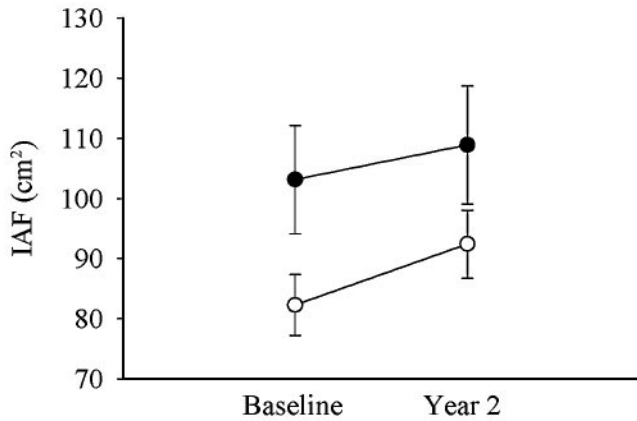


Figure 1: Predicted means \pm SEE for IAF at baseline and 2 years of follow-up in HRT users (○) and non-users (●). Predicted means were derived from repeated-measures analysis and adjusted for age and total body fat. $p < 0.01$ for age, and <0.001 for HRT and total body fat; the HRT \times time interaction was not significant (the two groups did not differ with respect to the change in IAF over time).

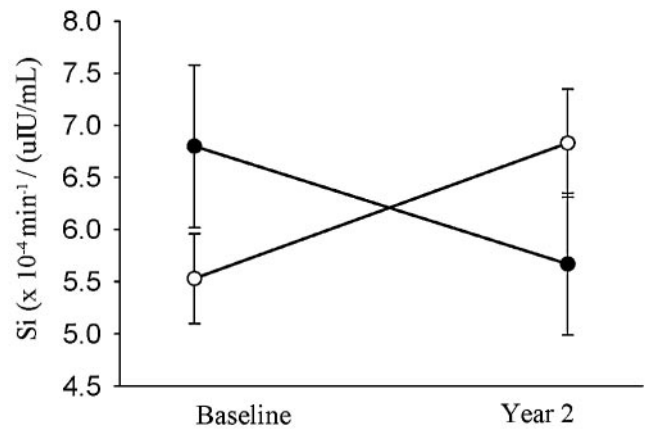


Figure 2: Predicted means \pm SEE for Si at baseline and 2 years of follow-up in HRT users (○) and non-users (●). Predicted means were estimated from a mixed model and adjusted for age and IAF. $p < 0.01$ for HRT; $p < 0.001$ for IAF and the HRT \times time interaction.

Table 3. Predicted mean \pm standard error of the estimate and main effects for cardiovascular disease risk factors from repeated-measures analysis

	Visit	Non-users	HRT users	<i>p</i> for main effects
Total cholesterol (mg/dL)	1,2	178 \pm 7	179 \pm 5	Age, $p = 0.390$ HRT, $p = 0.839$ Time, $p = 0.819$ SuperSAAT, $p < 0.05$
HDL-C (mg/dL)	1,2	47 \pm 3	50 \pm 2	Age, $p = 0.760$ HRT, $p = 0.333$ Time, $p = 0.205$ IAF, $p < 0.01$
TGs (mg/dL)	1,2	89 \pm 10	104 \pm 6	Age, $p = 0.954$ HRT, $p = 0.188$ Time, $p = 0.327$ IAF, $p < 0.05$ PA, $p < 0.01$
Insulin sensitivity [$\times 10^{-4}$ min ⁻¹ /(uIU/mL)]	1	6.80 \pm 0.78	5.53 \pm 0.43	Age, $p = 0.805$
	2	5.67 \pm 0.68	6.83 \pm 0.52	HRT, $p < 0.01$ Time, $p = 0.793$ IAF, $p < 0.001$ HRT \times time, $p < 0.001$

HRT, hormone replacement therapy; SuperSAAT, superficial subcutaneous abdominal adipose tissue; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; IAF, intra-abdominal fat; PA, light physical activity (kilocalories per week); DeepSAAT, deep superficial subcutaneous abdominal adipose tissue.

Main effects tested were age, HRT, time, fat (total fat, IAF, SuperSAAT, DeepSAAT, thigh fat), and physical activity; the interaction of HRT and time was also tested. All means shown are adjusted for the variables listed in the right column. Where “time” was not significant, the adjusted mean shown is the same for both visits.

Discussion

This study is one of the few studies to examine the change in IAF over a multiyear period among postmenopausal HRT users and non-users and to link this change in IAF to outcomes related to disease risk. Results indicate that IAF increased significantly over 2 years in early postmenopausal women, and this increase was not attenuated by use of HRT. Although HRT users had less IAF at baseline and throughout the study, it is not clear whether HRT altered the relationship between total body fat and IAF or whether differences between groups existed before the study. Among early postmenopausal women, both SuperSAAT and IAF were significant determinants of lipid risk factors (SuperSAAT was associated with total cholesterol and IAF with HDL-C and TGs); HRT use did not have an independent effect. However, HRT users showed an increase in Si over the 2-year period, whereas non-users showed a decrease.

Several previous studies have suggested that HRT limits IAF deposition. Looking only at studies that directly measured IAF by computed tomography scan or magnetic resonance imaging, one found that HRT did not affect IAF (28), and several found that HRT users had less IAF than non-users (11–13). However, this study is one of few to report longitudinal data on the accumulation of IAF over time among hormone users and non-users and the only to report these data in a U.S. study population. Among Japanese women who were either untreated ($n = 26$) or treated for 1 year with a combination of conjugated estrogens (0.625 mg/d) and medroxyprogesterone acetate (2.5 mg/d; $n = 35$), IAF increased only among the untreated women (11). Furthermore, subjects with an android fat distribution at baseline showed a decrease in IAF when treated with HRT, whereas those with a gynoid fat distribution at baseline showed no change in IAF in response to HRT. Similarly, Swedish women treated with estradiol ($n = 23$) showed decreases in total fat and IAF after 1 year, relative to those given placebo ($n = 23$) (12). These studies differ from this study in that IAF did not increase among HRT users. However, these earlier studies were of shorter duration; perhaps if followed for a longer time, the Japanese and Swedish HRT users would likewise have shown an increase in IAF. Acute treatment with HRT may have unique effects on IAF accrual that are not maintained during prolonged exposure to HRT.

In this study, at baseline, HRT users had less IAF, independent of total fat. Because of limitations of study design, it is not clear whether this indicates an acute effect of HRT or reflects an inherent difference between groups at baseline in subject characteristics. It is possible that women who have less IAF are more likely to choose to use HRT or that more health-conscious women not only choose to use HRT, but also engage in a healthier lifestyle (e.g., more healthful diet, more physical activity). Although it is possible that

HRT acutely modulates fat distribution, such that less fat is partitioned to the visceral space, an intervention study is needed to confirm this hypothesis. However, it is clear that this difference in fat distribution was maintained over time; although both HRT users and non-users gained IAF over the 2-year study period, the rate of gain did not differ with hormone use, and the relative proportion of visceral to total body fat remained constant in both groups of women.

In addition to IAF, SAAT has been associated with disease risk factors. Total SAAT has been independently associated with insulin sensitivity in children (17), premenopausal women (15), and relatively lean men (14). When SAAT has been subdivided into superficial and deep, DeepSAAT has been independently associated with fasting insulin among men (18). In addition, DeepSAAT and IAF were correlated with insulin sensitivity among men and women combined, whereas SuperSAAT was not (16). No previous study has examined longitudinal changes in SuperSAAT and DeepSAAT in early postmenopausal women, nor has any previous study evaluated the influence of exogenous hormones on accumulation of these depots. Results from this study indicated that HRT use did not influence the accumulation of either subcutaneous fat depot. Likewise, the proportion of fat deposited in the abdominal subcutaneous spaces vs. elsewhere in the body did not differ between hormone users and non-users.

Previous data have suggested that HRT use redistributes adipose tissue from the visceral space to the gluteofemoral area, although no study has directly addressed this possibility by measuring both visceral and peripheral adipose tissue over time. Over a 3-year observation period, women randomized to HRT gained less trunk fat by DXA, and more leg fat by DXA, on a percentage basis, than women randomized to placebo (7). In contrast, this study suggests that HRT may redistribute fat to all non-visceral spaces. At baseline, only IAF differed statistically with hormone use; neither total fat nor the individual subcutaneous compartments differed by group. Likewise, longitudinal results indicated that neither time nor HRT use was significant for total fat or any individual subcutaneous compartment over the test period. A larger sample size may be required to observe the potential positive effect of HRT on non-visceral fat deposition; this study was not powered to detect changes in subcutaneous body fat. However, it is also possible that HRT use results in a relatively subtle and uniform redistribution of fat to the subcutaneous spaces that is difficult to detect statistically.

The mechanism through which HRT alters fat distribution is not known with certainty, but it is likely through estrogen effects on local adipose tissue metabolism. In subcutaneous, but not visceral, adipose tissue, estradiol acts through estrogen receptor- α to increase the number of antilipolytic α -2A adrenergic receptors, an effect that would act to limit lipolysis (29). Estradiol may also affect the activity

of the enzyme lipoprotein lipase, which, in turn, would affect TG uptake by adipose tissue (30). However, the effects of estrogen are dependent on the route of delivery (31), the dose (32), and the endocrine milieu (33). For example, oral, but not transdermal, estrogen has been associated with a depression in fat oxidation and an increase in central and total body fat as assessed by DXA (31), a difference that may be caused by the hepatic first-pass effect of oral estrogen and the resultant greater dose of estrogen achieved at the level of the liver (34). The metabolic effects of estrogen, and the mechanisms through which it acts, seem to be complex and incompletely understood at this time.

It might be hypothesized that lower IAF among HRT users vs. non-users would be reflected in a relatively more favorable lipid profile and higher insulin sensitivity. Results indicated that fat distribution was, in fact, related to both lipids and insulin sensitivity. Total cholesterol was significantly related to SuperSAAT but not IAF. This observation agrees with many other reports indicating that total cholesterol generally does not cluster with other components of the metabolic syndrome (35) and, thus, is not associated with IAF (36). However, as predicted, HDL-C, TG, and Si, components of the metabolic syndrome, were independently related to IAF. HRT use was not significantly related to lipid risk factors independent of body fat, suggesting that any potential beneficial influence of HRT on the lipid profile may be indirect through limitation of IAF deposition. In addition, HRT has a well-documented, undesirable effect on TG; through action on the liver, oral estrogen elevates circulating TG (37). This phenomenon was somewhat apparent in this study, where mean serum TG concentration was higher among HRT users compared with non-users (107 ± 6 vs. 89 ± 10 mg/dL; adjusted mean \pm SEE; $p = 0.188$), after adjusting for the beneficial influence of lower IAF among HRT users.

HRT use also may have affected Si independent of body composition and body fat distribution. HRT users showed an increase in Si over the 2-year study period, whereas non-users showed a decrease. This interactive effect was independent of IAF and, therefore, may have been directly related to hormonal status. This observation agrees with studies showing a positive effect of estrogen on Si. In rats, estrogen treatment improved insulin-stimulated glucose uptake by skeletal muscle (38), and clinically, women show higher Si, as assessed by the euglycemic clamp, than men, after controlling for BMI (39). In the Postmenopausal Estrogen/Progestin Intervention Study, treatment with estrogen, with or without a progestin, lowered the mean fasting insulin concentration by 16.1% (40), suggesting an improvement in Si. In this study, the decrease in Si observed among the non-users may reflect the early postmenopausal status of the subjects at baseline and, perhaps, a cumulative effect of estrogen deprivation on Si with time. However,

because of the observational nature of this study, it also is possible that the divergence in Si displayed by the two groups reflects the "regression toward the mean" phenomenon (41); further study is required to clarify this issue.

This study has several strengths. It is the only study to report 2-year longitudinal observations on body fat distribution in HRT users and non-users and the only longitudinal study of any length in U.S. women. Measures of IAF were obtained using computed tomography scanning and of Si using minimal modeling. Limitations of the study include the observational, rather than interventional, study design, the relatively small number of study subjects, the absence of ethnic groups other than white, and the change in hormone use status of several participants over the course of the study.

In conclusion, results of this study indicate that IAF increased significantly over 2 years in early postmenopausal women, and this increase was not attenuated by use of HRT. However, lower IAF, independent of total fat, among HRT users at baseline (and throughout the study) may suggest that HRT alters the relationship between total body fat and IAF. However, cross-sectional observations regarding the health effects of HRT have not always been observed during intervention studies; thus, these data should be interpreted with caution, and hypotheses generated herein should be verified through a randomized, interventional study design. Among early postmenopausal women, abdominal fat (SuperSAAT and IAF) was a significant determinant of disease risk factors, independent of hormone use.

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