GENERAL GYNECOLOGY

Insulin resistance with hormone replacement therapy: associations with markers of inflammation and adiposity

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OBJECTIVE: This study was undertaken to determine whether insulin resistance associated with combination hormone replacement therapy (HRT) is mediated by changes in serum markers of inflammation or in serum adipocyte hormones.

STUDY DESIGN: Forty-five postmenopausal women, aged 55 ± 7 years, were examined from a randomized, double-blind placebo-controlled trial evaluating the effect of HRT on insulin-stimulated glucose disposal and body composition. Volunteers were randomly assigned to conjugated estrogens 0.625 mg plus medroxyprogesterone acetate 2.5 mg vs placebo for 1 year. At baseline and at 1 year, body composition was assessed by dual photon x-ray absorptiometry scans; body fat distribution was measured by computed tomographic scans at the L4/L5 vertebral disk space; insulin sensitivity was measured by euglycemic hyperinsulinemic clamp; interleukin-6 (IL-6), leptin, and adiponectin were measured by enzyme-linked immunosorbent assay; and c-reactive protein (CRP) was measured by radioimmunoassay.

RESULTS: HRT increased CRP by 121% compared with a 32% increase with placebo ($P = .03$); HRT decreased glucose disposal by 17% compared with no change with placebo ($P = .04$) as reported previously. HRT did not affect body composition, body fat distribution, IL-6, leptin, or adiponectin. The increase in CRP did not correlate with the decrease in glucose disposal in the HRT group ($R = 0.11$, $P = .65$).

CONCLUSION: Treatment with HRT for one year increases CRP, but does not alter IL-6, adiponectin, or leptin. The change in CRP was not, however, related to the decrease in glucose disposal with HRT treatment.

Key words: c-reactive protein, hormone replacement therapy, insulin sensitivity, interleukin-6, leptin

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Insulin sensitivity worsens after menopause,[1-2] which increases the risk of diabetes and cardiovascular disease.[3] Part of this risk may be explained by changes in body fat and body composition, with increased total and central fat in postmenopausal women compared with premenopausal women.[4-6] Hormone replacement therapy (HRT) has been proposed to ameliorate the increased central body fat and insulin resistance in postmenopausal women. Studies evaluating the effect of HRT on glucose disposal in postmenopausal women have led to mixed results depending on the regimen used.[7-14] However, studies evaluating HRT with combined estrogen and continuous progestin demonstrated a decrease in insulin sensitivity without affecting body composition.[7-9] The mechanism behind the effect of HRT on decreasing insulin sensitivity in women has not been explained.

Postmenopausal women also have increased circulating markers of inflammation compared with premenopausal women.[16,17] Specifically, high-sensitivity C-reactive protein (CRP) and interleukin 6 (IL-6) are associated with increased cardiovascular events in these women.[16,18] Because adipose tissue produces IL-6,[19] it is possible that circulating markers of inflammation mediate the effect of fat on insulin sensitivity. We have reported that CRP is associated with increased visceral fat and decreased glucose disposal in postmenopausal women.[17] HRT increases CRP[20-22]; however, it is unknown if the effect of HRT on glucose disposal is associated with circulating markers of inflammation.

Leptin and adiponectin are hormones produced by fat that have been associated with amount of fat, insulin resistance, and the development of diabetes.[23-26] The effect of HRT on leptin is unclear, with some studies showing increases with HRT[27-29] and others finding either no change[30-40] or even a decrease with HRT.[41-44] There is little information available regarding the effect of HRT on adiponectin.[45,46] Furthermore, it is unknown if the effect of HRT on insulin sensitivity is associated with changes in leptin or adiponectin.

As continuous combined HRT decreases insulin sensitivity,[7] we sought to determine what factors may be associated with this decline. The purpose of this study is to determine whether the effect of HRT on insulin sensitivity is associated with changes in circulating inflammatory markers or adipocyte hormones. We hypothesize that HRT in-

creases CRP, IL-6, and leptin, while decreasing adiponectin, thus mediating the change in insulin sensitivity.

MATERIALS AND METHODS
Subjects

Forty-five postmenopausal women, aged 55 ± 7 years (mean ± SD), were recruited as part of a randomized, double blind, placebo-controlled trial of the effect of HRT on insulin-stimulated glucose disposal and body composition. The effect of HRT on body composition and insulin-stimulated glucose disposal in early postmenopausal women has been reported previously by our group,[7] and predictors of worsening insulin sensitivity (including age, years since menopause, lipids, family history of diabetes) has also been reported.[8] To be included in the current study, volunteers were required to have adequate serum for additional studies of leptin, adiponectin, CRP, and IL-6 at baseline and at 1 year; all volunteers who met this criteria were included in the current study.

Subjects were randomly assigned by block design to oral conjugated estrogen 0.625 mg plus medroxyprogesterone acetate 2.5 mg (n = 21) or placebo (n = 24). (Prempro was generously donated by Wyeth Ayerst, Philadelphia, PA.) Of these subjects, 43 were white, 1 was Abenaki Indian, and 1 was Asian. Menopausal status was defined by the absence of menses for at least 6 months with a follicle-stimulating hormone (FSH) greater than 30 mIU/mL. Other inclusion criteria included a body mass index (BMI) less than 30 kg/m^2, fasting glucose less than 112 mg/dL, and waist circumference less than 94 cm. These inclusion criteria were chosen because we were interested in examining the effect of HRT on disease prevention in healthy, nonobese, postmenopausal women. This study was approved by the Institutional Review Board at The University of Vermont, and all women signed statements giving their informed consent.

Insulin-stimulated glucose disposal

We performed euglycemic hyperinsulinemic clamps according to the method of DeFronzo et al[47] with modifications as previously described by our group.[48-50] After 3 days of standardized meals (55% carbohydrate, 30% fat, and 15% protein), volunteers were tested after a 12-hour overnight fast. An intravenous catheter was placed in an antecubital vein for the infusion of insulin and 20% dextrose. A second catheter was placed in the volunteer's contralateral hand and kept in a hot box (60°C) for sampling of arterialized blood samples. A constant infusion of insulin (40 mU/min per m^2) was started at 9:00 AM to approximate postprandial insulin levels. A 20% dextrose solution was also started at 9:00 AM. Plasma glucose levels were measured every 5 minutes during the insulin infusion to titrate the dextrose infusion and maintain fasting glucose levels.

Body composition

Fat mass, percent fat, and lean body mass were measured by dual-photon x-ray absorptiometry (DEXA) with a Lunar DPX-L densitometer (Lunar Corp, Madison, WI) as reported previously.[48,50] Scans were analyzed by using the Lunar version 1.3y DPX-L extended analysis program for body composition. The coefficient of variation for repeated measurements in our laboratory is 1% for fat mass. Waist circumference in the standing position was measured as the smallest distance around the abdomen.

Computed tomography

Intraabdominal fat and subcutaneous abdominal fat were measured at the L4-L5 vertebral disk space at an attenuation range of −190 to −30 Hounsfield units by computed tomography (CT) with a GE High-Speed Advantage scanner (General Electric Medical Systems, Milwaukee, WI).[48,50] Sagittal diameter was measured as the anterior-to-posterior distance in millimeters at this disk level. The within subject variation for repeated analysis of fat measurements in our laboratory is less than1%.

Laboratory analysis

Plasma glucose concentrations were measured by the glucose oxidase method with an automated glucose analyzer (YSI Instruments, Yellow Springs, OH). Serum insulin concentrations were determined with a double-antibody radioimmunoassay (RIA) (Diagnostic Products Corp, Los Angeles, CA: inter- and intraassay coefficients of variation 10% and 4%, respectively). Serum FSH was measured with a chemiluminescent assay (Bayer Diagnostics, Tarrytown, NY). The inter- and intraassay coefficients of variation in the range of 53.1 mIU/mL 2.2% and 0.3%, respectively.

Blood for CRP and other markers was collected in a standardized fashion, centrifuged, separated immediately into small aliquots, and frozen at −80°C until analysis. Samples were run in batch for all assays. We measured serum CRP in a single assay by a colorimetric competitive enzyme-linked immunosorbent assay (ELISA) developed in our laboratory.[51] Biotinylated CRP competes with CRP in the sample for the coated antibody. Detection is performed with the horseradish peroxidase enzyme conjugated in an avidin-biotin complex, followed by the color reagent substrate, orthophenylene diamine. Standardization is performed by using the World Health Organization CRP reference standard. The intraassay coefficient of variation is 5%, and the normal range is 0.18-5.05 mg/L.

Serum IL-6 levels were measured by using a sandwich-type assay by an ultra-sensitive ELISA (R&D Systems, Minneapolis, MN). The lower detection limit is 0.10 pg/mL, and the usual detection range is 0.156-10.0 pg/mL. The intra-assay coefficient of variation is 6%.

Serum leptin levels were measured by using a double-antibody RIA (Linco Research Inc, St. Charles, MO). The lower and upper detection limits are 0.5 ng/mL and 100.0 ng/mL, respectively; and the usual detection range is 0.9-22.2 ng/mL. The inter- and intraassay coefficients of variation are 5% and 4.5%, respectively.

Serum adiponectin levels were measured by using a sandwich-type ELISA (R&D Systems). The lower detection limit is 0.246 ng/mL, and the usual detection range is 3.9-125.0 ng/mL. The inter- and intraassay coefficients of variation are 6.5% and 3.5%, respectively.

TABLE 1
Baseline characteristics and body composition

	Baseline		1 y	
	HRT	Placebo	HRT	Placebo
Age (y)	55.2 ± 1	54.6 ± 2		
Weight (kg)	69.5 ± 3.0	66.7 ± 2.3		
Fasting glucose (mg/dL)	81.1 ± 1.2	81.0 ± 1.3		
Fasting insulin (μU/mL)	9.9 ± 1.0	8.6 ± 0.9		
Glucose disposal (mg/min)*	336.8 ± 29.7	387.9 ± 5.4	277.1 ± 28.2	387.2 ± 24.4
Intraabdominal fat (cm^2)	115.3 ± 10.8	90.0 ± 9.0	117.6 ± 12.1	94.60 ± 8.1
SC abdominal fat (cm^2)	333.9 ± 23.5	288.4 ± 3.2	337.9 ± 24.9	311.9 ± 24.8
Total fat (kg)	24.1 ± 1.9	22.9 ± 1.8	24.6 ± 1.9	23.8 ± 1.8
Percent fat (%)	35.5 ± 1.4	34.2 ± 1.6	36.2 ± 1.6	35.0 ± 1.6

* $P = .04$ for time × treatment in repeated measures analysis of variance.

Statistical analysis

Means and SEs were calculated for all variables. The distribution of variables was examined by using the Shapiro-Wilk test. In both HRT and placebo groups, CRP, IL-6, insulin-stimulated glucose disposal (M), and M/kg fat-free mass had nonnormal distributions ($P < .05$). Log$_{10}$-transformation corrected for the nonnormal distribution. We compared means and SEs of outcome variables by the paired and unpaired Student t test for comparisons within the placebo and HRT group or between groups, respectively. Repeated measures analysis of variance was used to examine treatment effects between groups over time. Pearson correlation coefficients were determined for variables of interest. P values less than or equal to .05 were considered significant. All analyses were conducted with the use of SPSS statistical software (v 12.0, Chicago, IL).

Food intake and physical activity

Methods of monitoring food intake and physical activity in these study volunteers has been reported previously.[7] Briefly, study volunteers recorded food intake for 3 days at each time point. Diaries were analyzed by computer in the General Clinical Research Center by using the Food Intake Analysis System program.[52,53] Leisure time physical activity at each time point was measured by us-ing the Minnesota Leisure Time Physical Activity Questionnaire.[54]

RESULTS

Baseline characteristics and baseline and follow-up body composition data are shown in Table 1. There were no significant differences between HRT and placebo groups with regard to age, weight, fasting glucose, fasting insulin, or glucose disposal. Body composition over the course of the study did not differ significantly between or within the 2 groups at any point. Glucose disposal decreased in the HRT group compared with the placebo group over time. Food intake and leisure time physical activity did not differ within or between groups as reported previously.[7]

The figure shows the effect of HRT on glucose disposal, CRP, IL-6, leptin, and adiponectin. HRT produced a 17.8% decrease in glucose disposal compared with no change with placebo (HRT: 337 ± 30 to 277 ± 28 mg/min vs placebo: 388 ± 25 to 387 ± 24 mg/min, $P = .04$). CRP levels increased by 121% with HRT treatment compared with a 32% increase with placebo (HRT: 1.55 ± 0.30 to 3.42 ± 0.79 μg/mL vs Placebo: 1.13 ± 0.29 to 1.49 ± 0.27, $P = .03$). There was no significant change in leptin levels with HRT compared with placebo. IL-6 and adiponectin declined with HRT and not with placebo, but these differences were not statistically significant.

Pearson correlations between the change in glucose disposal and changes in the various biomarkers are shown in Table 2. The decrease in glucose disposal did not correlate with the increase in CRP in the HRT group (r = 0.11, $P = .65$). There were no correlations between changes in CRP and glucose disposal in the placebo group. There were no significant correlations between the change in glucose disposal and changes in IL-6, leptin, or adiponectin when all patients were analyzed together or when each group was analyzed separately.

COMMENT

We report that HRT increased CRP in postmenopausal women, and that this increase was not correlated with the decrease in glucose disposal observed with HRT in this trial.[7] Furthermore, IL-6, leptin, and adiponectin were not significantly altered with HRT, and change over time of these potential mediators did not explain the decrease in glucose disposal with HRT. To our knowledge, no previous study has examined circulating markers of inflammation or adipocyte hormones as possible mediators of the effect of HRT on glucose disposal.

Because circulating inflammatory markers or adipocyte hormones are not related to the decrease in glucose disposal with HRT, other possible mechanisms should be considered. One possi-

TABLE 2
Relationships between the change in glucose disposal and the change in serum markers

	HRT		Placebo	
	r	*P* value	*r*	*P* value
CRP	0.113	.65	−0.195	.37
IL-6	−0.152	.54	−0.362	.09
Leptin	0.045	.83	−0.257	.37
Adiponectin	−0.172	.59	0.139	.64

ble mechanism is an effect of estrogen and/or progestins on glucose uptake or on glycogen synthesis by skeletal muscle. We have reported that the decrease in insulin sensitivity by HRT in postmenopausal women occurs at the level of skeletal muscle or fat rather than at the level of the liver or kidney.[7] In a study of rats, Kumagai et al[55] reported a decrease in

glucose uptake and glycogen synthesis by combination estrogen plus progesterone. Additional studies from our group have shown that estradiol regulates skeletal muscle glycogen synthase activity.[56] This suggests that HRT reduces insulin sensitivity at a peripheral level, either by a direct mechanism or through other mediators.

The production of CRP occurs primarily in the liver by hepatocytes as part of the acute phase response. IL-6, which originates at sites of inflammation, stimulates the production of CRP. We found that combination HRT increased CRP by 17.8%, but did not change IL-6, suggesting that a generalized proinflammatory effect with HRT is not occurring. Our finding that HRT increases CRP is consistent with previous reports.[20-22,57-59] Our finding that HRT did not affect IL-6 is consistent with another randomized trial,[57] but not with a cross-sectional study that found lower levels of IL-6 with HRT.[58]

It appears that the route of HRT determines whether CRP increases. In both a cross-sectional study[59] and a randomized trial,[57] oral estradiol but not transdermal estradiol is associated with in-

FIGURE
Effect of HRT on glucose disposal, inflammation, and adipocyte hormones
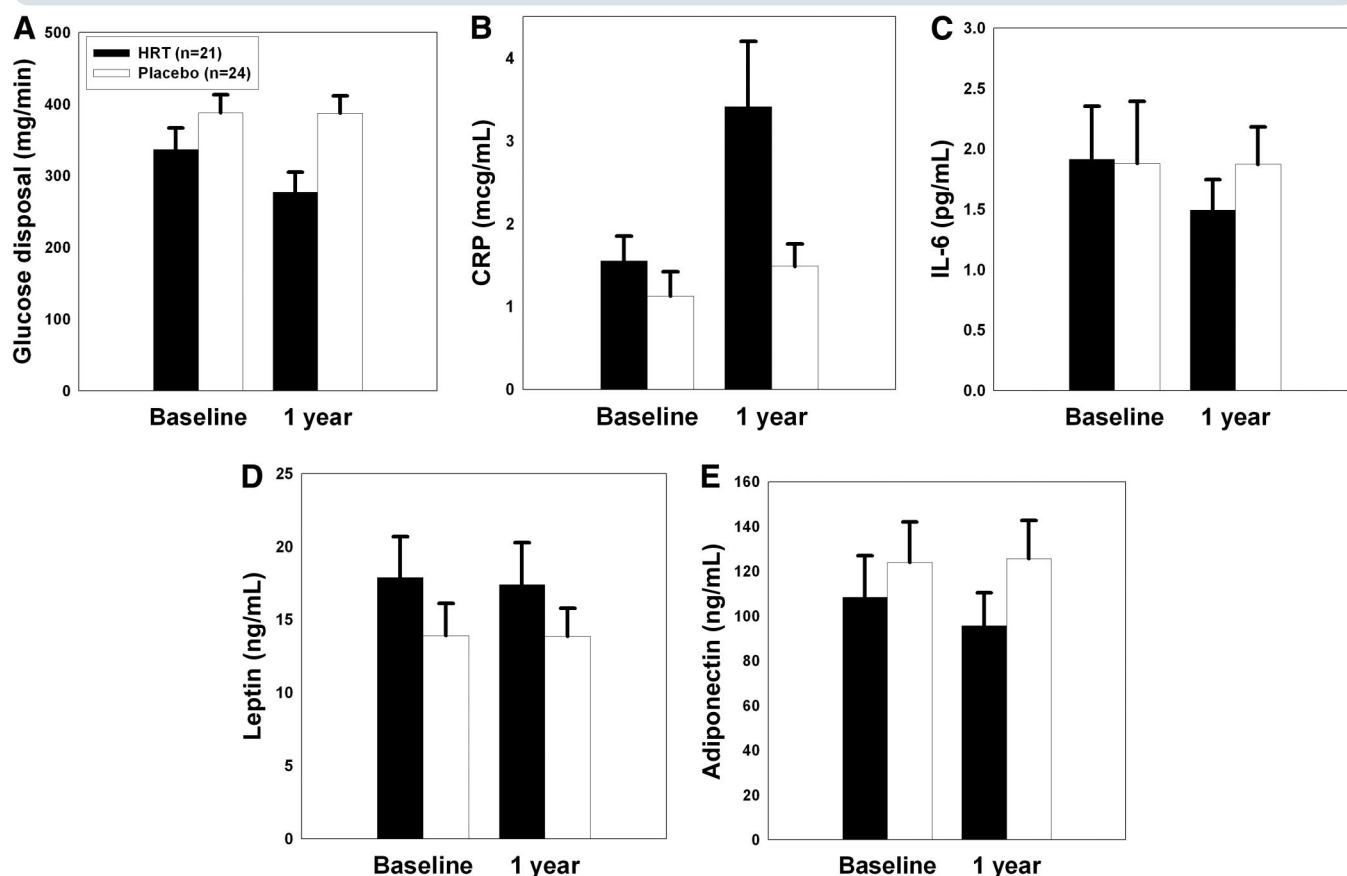
Effect of hormone replacement therapy (HRT) on glucose disposal, CRP, IL-6, leptin, and adiponectin. *P* = .04 for treatment effect of HRT on glucose disposal. *P* = .03 for treatment effect of HRT on CRP by repeated measures analysis of variance.

creased CRP. Because transdermal estradiol reduces the first-pass effect of the hormone in the liver and oral estradiol does not, it is possible that oral estradiol increases CRP production by the liver. Our study is consistent with the findings that the increase in CRP is related to hepatic effects and not because of an inflammatory upregulation.

Despite our report of no changes in total body fat or body fat distribution with HRT based on imaging techniques,[7] it is possible that HRT affects the production of fat hormones that could affect insulin sensitivity (leptin or adiponectin). We report no change in leptin levels with HRT. Our finding is consistent with Hadji et al,[30] who reported in a cross-sectional study that, after controlling for BMI, the use of HRT was not related to leptin level. However, Lavoie et al[29] reported that HRT increased leptin levels in a small short-term trial, despite not increasing BMI, absolute total fat mass, or waist-to-hip ratio. Other studies have shown either a decrease in leptin with HRT or the prevention of increases seen in an untreated group[41-44]; however, these leptin changes could be explained by changes in body composition in the untreated group compared with the HRT group. Taken together, our findings suggest that changes in leptin, body fat, or body fat distribution do not explain the decrease in insulin sensitivity with HRT.

Little information is available regarding the effect of HRT on adiponectin levels. Because adiponectin is also directly related to body composition,[25,26] and this did not change with HRT, we would expect no change in adiponectin with HRT. Our finding of no change in adiponectin levels with HRT is consistent with a 6-month trial[45] and with a cross-sectional study.[46]

Our study has several strengths as well as some limitations. Strengths include the fact that volunteers were recruited for a randomized, double-blind, placebo-controlled trial. Our methods for determining CRP, IL-6, leptin, and adiponectin included sensitive assays. We used the euglycemic hyperinsulinemic clamp to determine glucose disposal, the CT to determine fat distribution, and the DEXA to assess body composition. Limitations include our relatively small sample size. However, CRP and IL-6 have been shown to correlate strongly with markers of insulin sensitivity.[18,60] In our volunteers, CRP, IL-6, and leptin correlated strongly with glucose disposal at baseline ($r = -0.382$, -0.338, -0.561, respectively, all P's $< .01$). To detect this magnitude of correlation between the changes in these respective variables with 80% power and 95% CI, a study would require 19-54 subjects. Furthermore, our study involved mostly thin white women, so it may not be applicable to obese subjects and other racial and ethnic groups.

The health implications of a decrease in insulin sensitivity with ongoing HRT in postmenopausal women are unknown. In older postmenopausal women, HRT has been reported to reduce the incidence of diabetes mellitus by 17% in women without known heart disease[61] and by 35% in women with known heart disease.[62] Whether this reduction in the incidence of diabetes occurs through changes in insulin resistance is unclear because only 8% of the women without heart disease had insulin resistance reported by the homeostasis model of assessment-insulin resistance (HOMA-IR),[60] and too few women with heart disease had fasting glucose, fasting insulin, and HOMA-IR performed.[62] Our data would suggest that if HRT reduces the incidence of diabetes mellitus as reported, it may do so by mechanisms other than by improving insulin sensitivity.

We conclude that the decrease in glucose disposal with oral HRT is not related to the increase in CRP. The mechanism to explain the decrease in insulin sensitivity with HRT does not appear to be a generalized proinflammatory effect, or an alteration in levels of adipocyte hormones. Further studies are needed to examine the effect of HT on glucose and insulin metabolism by skeletal muscle.[15] ∎

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