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Effects of Postmenopausal Hormone Replacement Therapy on Insulin Resistance

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Postmenopausal hormone replacement therapy (HRT) protects women from the risk of cardiovascular system disease, osteoporosis, and dementia. There are conflicting reports about the effects of HRT on insulin resistance. The purpose of this study was to investigate the effects of HRT on insulin resistance with the hyperinsulinemic euglycemic clamp technique, the most sensitive technique measuring insulin resistance. Conjugated estrogen (0.625 mg/d) and medroxyprogesterone acetate (5 mg/d) were given to 15 postmenopausal women with insulin resistance. After 3 mo of HRT, the *M* value (total glucose consumption) increased 28% ($p < 0.001$), low-density lipoprotein (LDL) cholesterol decreased 12.9% ($p < 0.044$), high-density lipoprotein (HDL) cholesterol increased 17% ($p < 0.009$), total cholesterol decreased 9.1% ($p < 0.016$), and serum insulin decreased 33% ($p < 0.022$) compared to baseline values before HRT was started. No significant changes in glucose, C-peptide, and triglyceride levels were observed. Whereas there were no differences regarding glucose, total cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride levels between the insulin-resistant ($n = 15$) and non-insulin-resistant women ($n = 24$) ($p > 0.05$), there were significant differences in *M* value, insulin, and C-peptide levels between these groups ($p < 0.05$). We believe that HRT with this combination may protect postmenopausal women from coronary artery disease (CAD) through its beneficial effects on insulin resistance, hyperinsulinemia, and lipid levels, which are considered to be important factors in CAD pathogenesis.

Key Words: Insulin resistance; hormone replacement; postmenopausal women.

Introduction

The risks of coronary artery disease (CAD), hypertension, stroke, vasomotor disorders, osteoporosis, and osteoporosis-related bone fractures increase after menopause owing to lack of estrogen. Even though the relationship between the loss of ovarian function and the CAD has been known for many years, the importance of postmenopausal hormone replacement therapy (HRT) has recently been appreciated *(1,2)*. It is believed that HRT decreases the risk of CAD *(3–5)* by changing the lipid and lipoproteins *(5–8)*, by limiting lipid peroxidation *(9)*, by protecting endothelium and myocardium from reperfusion injury with antioxidant action *(10)*, and by increasing endothelium-related vasodilatation in arteriosclerotic coronary vessels *(11)*.

Insulin resistance and hyperinsulinemia are clinically important since the effects of insulin have been shown in the formation of atherosclerotic plaques *(12)*, and, hence, increased risks of hypertension and arteriosclerosis have been attributed to insulin resistance in postmenopausal women. In postmenopausal women, fasting glucose and insulin levels decrease with HRT *(13)*. It has been postulated that insulin resistance could decrease with HRT. Different studies resulted in contradictory conclusions; estrogen and progesterone were shown to decrease insulin sensitivity in one study *(14)* but were reported not to affect insulin sensitivity in others *(15,16)*.

For this reason, we aimed to study the effects of HRT on insulin resistance by the hyperinsulinemic euglycemic clamp technique, which is accepted as the most reliable and the most sensitive test for insulin resistance *(17)*.

Results

Patient age was between 48 and 59 yr, with an average mean of 51.7 ± 7.4 yr. The results of measurements before and after HRT are shown in Table 1.

At the end of the third month of HRT, with respect to the first determined values, *M* value increased an average of 28% ($p < 0.001$), LDL cholesterol decreased an average of 12.9% ($p < 0.044$), in HDL cholesterol increased an average of 17% ($p < 0.009$), total cholesterol decreased an average of 9.1% ($p < 0.016$), and serum insulin decreased an average of 33% ($p < 0.022$). No significant differences were found in basal glucose, C-peptide, and triglyceride values. There were no differences regarding age, glucose, total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides between the insulin-resistant ($n = 15$) and non-insulin-resistant women ($n = 23$) ($p > 0.05$). There were significant differences in *M*

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Table 1
Serum Glucose, Insulin, C-Peptide, and Lipid Levels in Insulin-Resistant Patients (before and after postmenopausal HRT), Control Group (basal and last values), and Patient Group (insulin resistant and non–insulin resistant) [a]

	Insulin-resistant patients (n = 15)			Control group (n = 26)			Patient group (n = 38)		
	Before HRT	After HRT	p	Basal value	Last value	p	Insulin-resistant group (n = 15)	Non-insulin-resistant group (n = 23)	p
Total glucose consumption (mg/[kg·min])	3.3 ± 0.6	4.54 ± 0.9	0.001	4.3 ± 0.6	4.54 ± 0.9	0.234	3.3 ± 0.6	5.74 ± 0.3	0.001
Glucose (mg/dL)	86.4 ± 8.1	88.4 ± 7.5	0.442	88.4 ± 8.1	87.4 ± 7.5	0.487	86.4 ± 8.1	84.4 ± 7.6	0.642
Insulin(μU/dL)	39 ± 11	26.6 ± 10.1	0.022	36.5 ± 11.3	36.6 ± 10.1	0.221	39 ± 11	34.6 ± 9.1	0.012
C-peptide (ng/mL)	3.0 ± 1.1	3.57 ± 0.6	0.167	3.7 ± 1.1	3.57 ± 0.6	0.188	3.0 ± 1.1	2.57 ± 0.3	0.03
Total cholesterol (mg/dL)	230 ± 43	209 ± 27	0.016	227.5 ± 47.6	224.3 ± 27.5	0.654	230 ± 43	220.6 ± 24.8	0.556
LDL cholesterol (mg/dL)	188 ± 6.4	161 ± 8.2	0.044	167.7 ± 8.4	161.2 ± 8.2	0.244	188 ± 6.4	185.7 ± 6.2	0.441
HDL cholesterol (mg/dL)	41.8 ± 6.1	48.4 ± 3.9	0.009	44.8 ± 6.1	45.4 ± 3.9	0.512	41.8 ± 6.1	41.4 ± 2.5	0.239
Triglycerides (mg/dL)	152 ± 65	132 ± 37.9	0.551	144.6 ± 65.8	142.3 ± 37.9	0.351	152 ± 65	148.6 ± 27.1	0.511

[a]Results are given as mean ± SD.

values and insulin and C-peptide levels between these groups ($p < 0.05$).

When the first determined values of the control group were compared with the last determined values after 3 mo, no significant differences were found in laboratory values and M values between the first and last values ($p > 0.05$) (Table 1).

Discussion

Sex hormones, together with glucose and insulin metabolism, play interrelated roles in pathogenesis of CAD. Impaired glucose tolerance, hyperinsulinemia, and insulin resistance increase the risk of CAD *(18)*.

Insulin resistance increases gradually in postmenopausal women. In the present study, we found insulin resistance in 15 of 39 (38%) postmenopausal women when we applied the hyperinsulinemic euglycemic technique. A similar result was obtained in 1994 by Lindheim et al. *(14)*, who applied an insulin tolerance test or iv glucose tolerance test and reported insulin resistance in 44–60% of seemingly healthy postmenopausal women.

After 3 mo of HRT with estrogen and medroxyprogesterone acetate (MPA), we observed that insulin resistance improved 28% and insulin levels decreased 33% compared to baseline values before HRT. Estrogen improves glucose tolerance by increasing receptor binding of insulin *(19)*. The effect of estrogen on insulin sensitivity has been shown to be bimodal. It has been reported that a standard 0.625-mg dose of conjugate oral estrogen increased insulin sensitivity by 25%, but that a 1.25-mg dose of conjugate estrogen decreased insulin sensitivity by 24.7% *(20)*.

In a prospective double-blind study on postmenopausal women, beneficial changes occurred in plasma lipid levels with HRT *(11)*. In that study, significant decreases in fasting glucose and insulin levels were also reported *(11)*. Whereas estrogen decreases LDL, it increases HDL. In our study, after administering 0.625 mg/d of conjugated estrogen and 5 mg/d of MPA, we found a decrease of 12.9% in LDL level, a 9.1% decrease in total cholesterol level, and an increase of 17% in HDL level. It has been determined that 17β-estradiol decreases plasma HDL clearance by 82% *(21)*. Again, in a study similar to ours, 0.625 mg of conjugated estrogen orally was administered for 3 mo, and in the last 14 d, 10 mg of MPA orally was added to the regimen. At the end of the treatment, a 3.5% decrease in total cholesterol, and an 8.7% decrease in LDL was seen. In addition, 6.5% increase in HDL, 9% increase in apolipoprotein A-1, and 16% increase in triglyceride levels were also reported *(3)*. A potential untoward effect of estrogen treatment is a 10–20% increase in triglyceride levels. In our study, we could not report significant changes in fasting glucose and triglyceride levels, probably owing to the number of patients studied (type II error).

Except for an idiosyncratic increase in blood pressure in rare cases, HRT either does not change or minimally changes blood pressure *(15,22,23)*. In a study by Kawecka-Jaszcz et al., it was found that combined percutaneous HRT may reduce the lipid-dependent cardiovascular risk in postmenopausal women with arterial hypertension. In another study, a period of 3 mo of HRT with a progestin in postmenopausal women improved insulin sensitivity and lowered LDL cholesterol levels *(25)*.

In our study, we did not observe any significant change in the patients' blood pressure measurements. There were no side effects during HRT. Weight and body mass index (BMI) of patients were not different at the end of 3 mo. The only problem was bleeding in women with late menopause.

We observed beneficial effects of a combination of estrogen and progesterone as HRT on insulin resistance, hyperinsulinemia, and lipid levels in postmenopausal women. We

believe that this combination of HRT may protect postmenopausal women from CAD through its effects on insulin resistance, hyperinsulinemia, and lipid levels, which are considered to be important factors in CAD pathogenesis.

Materials and Methods

Subjects

After approval by an institutional ethical committee, postmenopausal patients, scheduled for HRT, were informed about menopause, HRT, and insulin resistance and consented to the study. Patients with diabetes mellitus, impaired glucose tolerance, systemic disease, and BMI more than 30 kg/m^2 and previous HRT were excluded. Postmenopausal women were identified using standard criteria including an absence of menstruation for at least 6 mo and elevated levels of follicle-stimulating hormone (above 40 mIU/mL) and low levels of estradiol (<30 pg/mL). The control group consisted of 26 postmenopausal volunteers. The patient group and control group were not statistically different from each other regarding age, total glucose consumption, glucose, insulin, C-peptide, total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides. None of the control subjects were given any drug during the 3-mo period. After 3 mo, control subjects were reevaluated by physical examination, laboratory values, and insulin resistance. The patients (postmenopau-sal) group (n = 38) was divided into two groups: insulin-resistant group (n = 15) and non-insulin-resistant group (n = 23).

Hyperinsulinemic Euglycemic Clamp Technique

The hyperinsulinemic euglycemic clamp technique was applied on 38 postmenopausal women. Fifteen of them, who had insulin resistance, were included in the study. Insulin resistance was evaluated by this method as defined by De Fronzo *(17)*. Blood glucose determination was performed using a B-glucometer (B-Glucose; Hemocue AB, Sweden) both before and during the test, with 10-min intervals. Insulin and 20% dextrose were infused by infusion pumps (Abbott-Show, Life Care Pump, Model 4). Regular insulin was prepared with a concentration of 300 mIU/mL in isotonic saline solution. To prevent the attachment of insulin to the rubber surface of the equipment, 4 mL of patient serum was added to the solution. The test was performed at 9.00 AM following 12 h of fasting. Room temperature was 20°C and the patients were in a supine position until the end of the evaluation. To withdraw venous blood, a 20F-polyethylene catheter was placed in the left antecubital vein. To perform arterialization of venous blood, the right arm was placed in a mechanism in which the temperature was kept constant at 60°C. Before any infusion, the right arm was heated for 30 min, lasting 150 min until the end of the test. According to the table in De Fronzo *(17)*, insulin infusion was started at a rate of 127.6 mIU/m^2 and decreased to 40 mIU/m^2 in first the 10 min. Then the infusion was continued at this final concentration (40 mIU/m^2) for 110 min. To prevent hypoglycemia and antagonistic hormonal response to insulin, 4 min after the beginning of insulin infusion, glucose infusion was started at a concentration of 2 mg/(kg·min). Ten minutes later the glucose infusion rate was increased to 2.5 mg/(kg·min). The glucose infusion rate was adjusted to maintain basal glucose values in the range of ±10% of baseline. Glucose values (which were measured every 10 min for 120 min) were classified in five interval groups, by omitting the values at 10 and 120 min. The values were converted to milligrams/(kilograms·minute) and glucose consumption rate was calculated for each 20-min period. The calculations were named M_1–M_5. The arithmetic means of these M values resulted in the real M indicating total glucose consumption. M values <4 mg/(kg·min) were accepted as insulin resistance *(17)*.

Assay of Blood Samples

Blood samples for insulin and C-peptide measurements were centrifuged at 4000 rpm and preserved at –20°C for measurements. An autoanalyzer (Technicon; RA 1000) for routine biochemical analysis was used. The serum basal insulin value was determined by the coated-tube method (DPC), and C-peptide levels by radioimmunassay method (DSL). Serum insulin values <30 μU/mL and basal C-peptide value 3.5 ng/mL were accepted as normal.

Additional Tests

Hormonal and biochemical tests; breast, pelvic, and abdominal ultrasound; gynecologic examination; cervical smear; and mammography tests were requested. For HRT, 0.625 mg/d of conjugated estrogen and 5 mg/d of MPA were administered. After 3 mo, patients were reevaluated by physical examination, laboratory values, and insulin resistance.

Statistical Analyses

SPSS for Windows 6.0 software was used for statistical analyses. Wilcoxon test was used to compare the measurements before and after HRT. A value of $p < 0.05$ was accepted as statistically significant.

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