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Effects of short-term transdermal hormone replacement therapy on glycaemic control, lipid metabolism, C-reactive protein and proteinuria in postmenopausal women with type 2 diabetes or hypertension

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BACKGROUND: The study was carried out to evaluate the effects of short-term transdermal hormone replacement therapy (HRT) on glycaemic control, lipid metabolism, C-reactive protein (CRP) and proteinuria in high-risk postmenopausal women. METHODS: A total of 20 well-controlled type 2 diabetic, hypertensive and 21 well-controlled glucose-tolerant, hypertensive postmenopausal women were prospectively enrolled. After 12 weeks of transdermal HRT, the changes in serum lipid sub-fractions, fasting glucose, fructosamine, glycated haemoglobin (HbA_{1c}), CRP, creatinine, 24 h urine protein levels, creatinine clearance and blood pressure were evaluated. RESULTS: After 12 weeks of treatment, serum total-cholesterol and low-density cholesterols (LDL-cholesterol) appeared slightly reduced and serum triglyceride slightly elevated, although non-significantly so in both groups. The increase in HDL-cholesterol ($P < 0.05$) and reduction in very low density (VLDL)-cholesterol ($P < 0.05$) levels were significant in hypertensive patients. Elevation in the Apolipoprotein A1 ($P < 0.05$) and reduction in the Apolipoprotein B ($P < 0.05$) levels were statistically significant in all patients. HRT was associated with significant decreases in serum fasting glucose ($P < 0.05$) and fructosamine ($P < 0.05$) levels in diabetic patients. Serum HbA_{1c}, CRP, creatinine, 24 h urine protein levels, creatinine clearance and systolic and diastolic blood pressure did not change significantly in either group. CONCLUSIONS: There were no detrimental effects of transdermal HRT on lipid profile, glucose metabolism, CRP and urine protein levels in our well-controlled diabetic or hypertensive patients. A decision regarding HRT use should be taken on a case-by-case basis.

Key words: CRP/diabetes/HRT/lipid-glucose metabolism/proteinuria

Introduction

Type 2 diabetes and hypertension are major risk factors for cardiovascular diseases (CVD), which are associated with dyslipidaemia (Niskanen *et al*., 1998; Kaukua *et al*., 2001; Camisasca *et al*., 2002; Jacobsen *et al*., 2002). Diabetic women have a 2–5-fold higher risk for cardiovascular disease than age-matched non-diabetic women (de Stefano *et al*., 1993; Wilson, 1998). A recent study reported that 17% of postmenopausal diabetic women used HRT, compared with 39% of non-diabetics (Keating *et al*., 1999).

There is a lack of overall agreement about whether postmenopausal women can use HRT for the purpose of cardiovascular protection. Although study subjects were relatively old, the results of the Heart and Estrogen/progestin Replacement Study (HERS) (Hulley *et al*., 1998) and the Womens Health Initiative (WHI) Writing Group, (2002) studies suggested that HRT had no advantages for prevention of coronary heart disease (CHD). There was an increase in cardiovascular events in the first year, but, interestingly, fewer events were seen after 2 years of treatment in the hormone group in the HERS. On the other hand, it has been reported that HRT improves lipid–lipoprotein profiles (Hulley *et al*., 1998; Herrington *et al*., 2000; Shlipak *et al*., 2000; Erenus *et al*., 2001; Sendag *et al*., 2002; Ranta *et al*., 2002) and endothelial and vascular smooth muscle function, and has potentially beneficial effects on vascular relaxation in peripheral and coronary vasculatures (Lieberman *et al*., 1994; Reis *et al*., 1994; Perera *et al*., 2002). Noticeably, concerns remain about the use of HRT for cardiovascular protection, which is more intricate than was initially believed. Although there were some recent reports on the use of HRT in diabetic or hypertensive postmenopausal women (Cornu *et al*., 2000; Cacciatore *et al*.,

2001; Darko *et al*., 2001; Perera *et al.,* 2001, 2002), the effects of short-term transdermal HRT on glycaemic control, lipid metabolism, C-reactive protein and urine protein in these high-risk groups have not been studied adequately.

This study was planned to evaluate the effects of 12 weeks of transdermal HRT on mean serum lipid sub-fractions, fasting glucose, fructosamine, glycated haemoglobin (HbA_{1c}), C-reactive protein (CRP), creatinine, 24 h urine protein levels, creatinine clearance and blood pressure.

Materials and methods

We prospectively studied 41 subjects, 20 postmenopausal women with type 2 diabetes and hypertension (DM-HRT group) and 21 postmenopausal women who were glucose-tolerant but hypertensive (HT-HRT group). Diabetes was diagnosed using the criteria of the World Health Organization (World Health Organization, 1980) at least 2 years before entering the study. In order to maintain their glucose levels in an acceptable range, women with type 2 diabetes were on dietary management alone (two patients) or taking oral anti-diabetic drugs that consisted of metformin and sulfonylureas (18 patients). Each diabetic patient received a diabetic diet with <30% of total calories as fat, ⩽10% saturated fat, ⩽10% mono-unsaturated fat and ⩽10% polyunsaturated fat. The women were instructed not to change their diet. None of them were taking insulin. All women took a calcium channel blocker (amlodipine 5–10 mg/day) as an anti-hypertensive drug. Amlodipine, which has no significant effect on lipid metabolism, was prescribed at least 3 months before the study. None of the women were taking anti-lipidaemic, corticosteroid or anti-convulsant therapy. The anti-diabetic and anti-hypertensive medications were left unchanged during the study.

Menopause was confirmed by the absence of menstruation for at least 12 months and by high serum levels of FSH (>30 mIU/ml) and low serum levels of estradiol (E_2) (<20pg/ml). The subjects had not received HRT previously. Gynaecological examination, comprising endometrial biopsy and mammogram, were normal in all subjects.

All study subjects received 12 weeks of transdermal continuous 17β-estradiol (0.05 mg/day) with transdermal sequential norethisterone acetate (NETA) 0.25 mg/day (Estracombi TTS, Novartis, Switzerland). This work was approved by the local medical ethics committee and all participants gave informed consent before the onset of study.

All metabolic and physical examinations were performed at the onset of the study and then again after 12 weeks of receiving HRT. Blood pressure was measured with a mercury sphygmomanometer during two clinical evaluations by an average of three measurements taken in the sitting position before the morning dose of the anti-hypertensive therapy. On the fourth day of the third hormonal cycle (in the estrogen only phase) blood samples were taken after a 10 h fast and serum total cholesterol (total cholesterol), triglyceride (TG), high density cholesterol (HDL-cholesterol), VLDL-cholesterol, apolipoprotein A1 (Apo-A1), apolipoprotein B (Apo-B), serum creatinine (sCr), urine creatinine (uCr), 24 h urine protein, fructosamine, HbA_{1c} and glucose levels were determined in an Hitachi 911 automated analyser by using commercial kits supplied from Roche Diagnostics. Low-density cholesterol (LDL cholesterol) levels were calculated by using Friedewald's formula. Serum CRP was turbidimetrically determined by clinical chemistry system (SPACE, Schiapparelli Biosystems, Netherlands), which gives a quantitative result. For biochemical measurements, the intra-assay coefficients of variation (CV) values ranged between 0.8– 2.1%, and the between-run CV values ranged between 1.2–2.7%.

Statistical analysis

Statistical analysis was carried out with Wilcoxon Two Related-Samples and Mann–Whitney tests. The data are expressed as means ± SEM. Statistical significance was set at $P < 0.05$ and the categorical variables were expressed as percentages. Data were analysed using SPSS (Statistical Package for the Social Science, version 10.0) for Windows 98 (Microsoft Corp.).

Results

All of the women who were enrolled into the investigation completed the study. The mean age of the subjects was 50.9 ± 1.23 and 52.6 ± 0.7 years, and their mean body mass index (BMI) was 31.27 ± 0.23 and 30.95 ± 0.19 kg/m^2 in the DM-HRT and HT-HRT groups respectively. Two subjects (10%) in the DM-HRT group and one subject (4.7%) in the HT-HRT group complained about abnormal vaginal bleeding such as metrorrhagia and four patients (19%) reported breast tenderness in the HT-HRT group. Other adverse effects were not seen.

HRT was associated with statistically significant decreases in serum mean fasting glucose and fructosamine levels, and with a non-significant reduction in serum mean HbA_{1c} level in the DM-HRT group. Significant changes were seen in mean serum VLDL and HDL-cholesterol levels in the HT-HRT group. The increase in mean serum Apo-A1 level and the reduction in mean serum Apo-B level were statistically significant in both treatment groups. The changes in mean serum lipid sub-fractions, fasting glucose, fructosamine and HbA_{1c} levels are given in Table I.

Serum CRP, creatinine, 24 h urine protein levels, creatinine clearance and systolic and diastolic blood pressure did not change significantly during 12 weeks of transdermal HRT in both groups. The baseline values and the effects of HRT on C-reactive protein (CRP), creatinine, 24 h urine protein levels, creatinine clearance and blood pressure are summarized in Table II.

Discussion

Diabetic patients have a high risk of cardiovascular disease and dyslipidaemia (Niskanen *et al*., 1998; Kaukua *et al*., 2001; Jacobsen *et al*., 2002). A number of studies have analysed the effects of HRT on glucose and lipid metabolism or endothelial function and markers in diabetic patients (Andersson *et al.,* 1997, 1999; Cornu *et al*., 2000; Aguilar-Salinas *et al*., 2001; Darko *et al*., 2001; Ferrara *et al*., 2001; Friday *et al*., 2001; Manning *et al*., 2001; Perera *et al.,* 2001, 2002). Some previous studies in type 2 diabetics suggested that HRT improved glucose homeostasis (Brussaard *et al*., 1997a,b; Samaras *et al*., 1999; Aguilar-Salinas *et al*., 2001; Ferrara *et al*., 2001; Friday *et al*., 2001). Estrogen regulates insulin-induced glucose transport through glucose transporters translocation in rat skeletal muscle (Rincon *et al*., 1996). Contrary to these data it was recently demonstrated that glucose utilization was lower in postmenopausal women taking HRT in a human-study (Ryan *et al*., 2002),

In this study, transdermal HRT did not have any worsening effect on glucose metabolism in diabetic patients, moreover the

Table I. Effects of HRT on fasting glucose, fructosamine, glycated haemoglobin (HDA_{2C}) fasting lipid fractions and apolipoproteins (Apo)

Fasting variables		Diabetic + HRT (n = 20)			Hypertensive + HRT (n = 21)		
		Mean values	Change %	P	Mean values	Change %	P
Fasting glucose mg/dl	Baseline	159.80 ± 13.74	–16.9	0.01 [a]	97.14 ± 2.6	–0.8	NS
	3 months	132.85 ± 9.2			96.38 ± 2.7		
HbA1c %	Baseline	5.07 ± 0.4	–6.9	NS	3.73 ± 0.13	–2.4	NS
	3 months	4.72 ± 0.22			3.64 ± 0.11		
Fructosamine mol/ml	Baseline	314.35 ± 16.31	–10.7	0.002 [a]	249.8 ± 10.3	–3.6	NS
	3 months	252.50 ± 6.3			240.66 ± 7.1		
Apo A1 mg/dl	Baseline	10524 ± 4.6	+21.9	0.001 [a]	112.57 ± 3.1	+16.9	0.001 [a]
	3 months	128.63 ± 3.23			131.01 ± 3.8		
Apo B mg/dl	Baseline	110.88 ± 4.7	–16.3	0.001 [a]	117.05 ± 4.14	–15.3	0.001 [a]
	3 months	92.20 ± 5.1			99.69 ± 4.3		
Total chol. mg/dl	Baseline	225.55 ± 8.73	–9.5	NS (0.067)	219.47 ± 8.7	–2.6	NS
	3 months	201.80 ± 8.3			214 ± 9.4		
LDL-chol. mg/dl	Baseline	122 ± 6.53	–4.9	NS	134.09 ± 6.6	–4.4	NS
	3 months	116.05 ± 7.15			128.66 ± 9.4		
VLDL-chol mg/dl	Baseline	49.25 ± 7.14	–14.2	NS	38.14 ± 3.0	–28.9	0.006 [a]
	3 months	41.75 ± 4.84			27.61 ± 5.2		
HDL-chol. mg/dl	Baseline	46.20 ± 2.7	+7.9	NS (0.091)	47.95 ± 2.9	+14.8	0.017 [a]
	3 months	50.5 ± 2.2			54.76 ± 3.5		
Triglyceride mg/dl	Baseline	186.45 ± 22.7	+9.4	NS	168.76 ± 34.7	+5.9	NS
	3 months	205.3 ± 23.9			178.57 ± 20.2		

[a]$P < 0.05$ statistically significant; P for the change between baseline and 3 months, ± SEM.

Table II. Effects of HRT on urine protein excretion and serum CRP, fibrinogen levels

Variable		Diabetic + HRT (n=20)		Hypertensive + HRT (n = 21)	
		Mean values	P	Mean values	P
Urine protein mg/day	Baseline	143.7 ± 32.9	0.64	109 ± 19.9	0.17
	3 months	145.25 ± 25.6		138.95 ± 16.2	
CRP mg/l	Baseline	9.4 ± 1.17	1	9.57 ± 1.7	0.86
	3 months	12.55 ± 3.3		9.8 ± 1.7	
Serum creatinine mg/dl	Baseline	0.83 ± 0.016	0.25	0.85 ± 0.018	0.82
	3 months	0.85 ± 0.016		0.86 ± 0.017	
Creatinine clearance ml/min	Baseline	85.8 ± 4.3	0.16	84.62 ± 4.9	0.61
	3 months	96.7 ± 3.6		85.38 ± 4.15	
SBP mmHg	Baseline	142.68 ± 2.95	0.91	143.8 ± 2.3	0.33
	3 months	142.51 ± 2.4		145 ± 2.24	
DBP mmHg	Baseline	82.68 ± 2.07	0.56	83.1 ± 1.8	0.20
	3 months	82.07 ± 2.3		84.76 ± 1.4	

[a]$P < 0.05$ statistically significant; P for the change between baseline and 3 months ± SEM.
SBP = systolic blood pressure, DBP = diastolic blood pressure.

mean serum fasting glucose and fructosamine levels decreased significantly. These outcomes were consistent with some previous studies, which used the transdermal route for HRT (Andersson *et al*., 1999; Duncan *et al*., 1999). On the other hand the patients enrolled in this study had well-controlled diabetic regulation. Glucose homeostasis and route of HRT administration may contribute to the effect of estrogen on glucose metabolism. In addition to these data, Cornu *et al*. (2000) showed that there was no significant difference between the transdermal and oral routes for glucose homeostasis criteria. In our hypertensive normo-glycaemic postmenopausal women, transdermal HRT did not have any significant effect on glucose metabolism. These results were consistent with data from the Postmenopausal Estrogens/progestins Intervention (PEPI) trial (The Writing Group, 1995), which suggested that the greatest benefit concerning glucose metabolism was achieved in patients who were hyperinsulinaemic at the beginning of HRT.

The lipid profile in type 2 diabetic patients usually comprises higher triglycerides and LDL-cholesterol, and lower HDL-cholesterol (Lehto *et al*., 1997). Most of the studies that deal with the effect of HRT on lipid metabolism have indicated that there is an improvement in lipid profile (Hulley *et al*., 1998; Samaras *et al*., 1999; Herrington *et al*., 2000; Manwaring *et al*., 2000; Chen *et al*., 2001; Erenus *et al*., 2001; Shlipak *et al*., 2001; Sendag *et al*., 2002). Effects of HRT on lipid metabolism due to routes of administration are different. Although a reduction in serum total cholesterol, LDL cholesterol or triglyceride levels was seen in the transdermal route (Perrera *et al*., 2001; Ranta *et al*., 2002; Sendag *et al*., 2002), it was suggested that the oral route had a more favourable effect on lipid profile than the transdermal route, except triglycerides (Darko *et al*., 2001; Vehkavaara *et al*., 2001; Sendag *et al*., 2002).

The current study demonstrated that transdermal HRT caused a significant increase in Apo-A1 levels and a significant reduction in Apo-B levels and did not show any noteworthy

harmful effect on lipid sub-fractions in all study subjects. For instance, serum HDL-cholesterol levels increased by 14.8 and 7.9% in hypertensive normo-glycaemic ($P < 0.05$) and diabetic patients (P = NS) respectively, and also there were no significant changes in total cholesterol, LDL cholesterol and triglyceride levels in either treatment groups. These outcomes might have been dependent on using short-term (3 months) HRT and the transdermal route.

We kept antihypertensive medication constant during the study period and HRT did not have any worsening effect on mean blood pressure in high-risk postmenopausal women. It was shown that HRT did not have an adverse effect on blood pressure control in postmenopausal women (Szekacs *et al*., 2000a). The neutral effects of estrogen on blood pressure might have been achieved by the transdermal route (Affinito *et al*., 2001) or an improvement in parameters of macro-vascular function such as endothelium-mediated vasodilatation by an increase in the biological actions of nitric oxide and a decrease in the action of angiotensin-converting enzyme (ACE) activity (Bush *et al*., 1998; Gerhard *et al*., 1998; Konukoglu *et al*., 2000; Cefalu, 2001; Nogawa *et al*., 2001).

This study showed that 3 months of transdermal HRT in high-risk postmenopausal women did not have any adverse effect on proteinuria and serum creatinine levels. Szekacs *et al*. (2000b) demonstrated that HRT improved proteinuria in the diabetic patient. The ability of estrogen to reduce oxidative stress may be beneficial to ameliorate nephropathy.

Elevated serum C-reactive protein was reported as a risk factor for future cardiovascular events (Ridker *et al*., 1998). CRP represents the increased proinflammatory effect and increases plaque vulnerability and propensity to thrombosis (Cushman *et al*., 1999a,b). Our subjects in this study comprised high-risk postmenopausal women and there was not an increase in CRP level. It was reported that HRT increased the risk of new cardiovascular events occurring in the first year and the risk reduction occurred in subsequent years (Hulley *et al*., 1998). Consistent with this is the observation that serum CRP rapidly becomes elevated in healthy HRT users (Ridker *et al*., 1999; van Baal *et al*., 1999), although, in our study groups, using short-term transdermal HRT, we were unable to confirm this conclusion; an outcome consistent with the results of Vehkavaara *et al*. (2001) and Prelevic *et al*. (2002).

There were many questions about the risk and benefits of estrogen/progestin use in postmenopausal women. The outcomes of the WHI study (Writing Group for the Women's Health Initiative Investigators, 2002), which is a randomized placebo-controlled trial, were derived from the use of a daily combined regimen of conjugated equine estrogen (CEE) and medroxyprogesterone acetate (MPA) in postmenopausal women with an intact uterus. Although no difference was seen in death rates between the study groups, a small but significantly increased risk of non-fatal cardiac events was found in the postmenopausal women using combined HRT, which in combination with an increased risk of breast cancer led inevitably to a discontinuation of the study. This outcome may not be valid for the transdermal route of HRT administration, and also the fact that study subjects were relatively older in this study than in the other studies might have affected this result.

There were no detrimental effects of transdermal HRT on lipid profile, glucose metabolism, CRP and urine protein levels in our well-controlled, relatively younger diabetic or hypertensive patients in short-term interval. Additional research will be necessary to determine the effects of long-term transdermal HRT in high-risk postmenopausal women.

We conclude that decisions about HRT should be taken on a case-by-case basis, and in doing so it is important to include the potential personal benefits and risk profile, especially in high-risk postmenopausal women.

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