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Short-term oestrogen replacement therapy improves insulin resistance, lipids and fibrinolysis in postmenopausal women with NIDDM

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Summary Oestrogen replacement therapy is associated with a decreased risk of cardiovascular disease in postmenopausal women. Patients with non-insulin-dependent diabetes mellitus (NIDDM) have an increased cardiovascular risk. However, oestrogen replacement therapy is only reluctantly prescribed for patients with NIDDM. In a double blind randomized placebo controlled trial we assessed the effect of oral 17 β-estradiol during 6 weeks in 40 postmenopausal women with NIDDM. Glycated haemoglobin (HbA_{1c}), insulin sensitivity, suppressibility of hepatic glucose production, lipoprotein profile and parameters of fibrinolysis were determined. The oestrogen treated group demonstrated a significant decrease of HbA_{1c} and in the normotriglyceridaemic group a significantly increased suppression of hepatic glucose production by insulin. Whole body glucose uptake and concentrations of non-esterified fatty acids did not change. LDL-cholesterol- and apolipoprotein B levels decreased, and HDL-cholesterol, its subfraction HDL_2-cholesterol and apolipotrotein A1 increased. The plasma triglyceride level remained similar in both groups. Both the concentration of plasminogen activator inhibitor-1 antigen and its active subfraction decreased. Tissue type plasminogen activator activity increased significantly only in the normotriglyceridaemic group. Oestrogen replacement therapy improves insulin sensitivity in liver, glycaemic control, lipoprotein profile and fibrinolysis in postmenopausal women with NIDDM. For a definite answer as to whether oestrogens can be more liberally used in NIDDM patients, long term studies including the effect of progestogens are necessary. [Diabetologia (1997) 40: 843–849]

Keywords Oestrogen therapy, non-insulin-dependent diabetes mellitus, glucose regulation, insulin sensitivity, hepatic glucose production, lipoprotein profiles, coagulation factors, fibrinolysis.

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Abbreviations: Apo A1 and apo B, Apolipoprotein A1 and B; ERT, oestrogen replacement therapy; FSH, follicle stimulating hormone; HDL-chol, high density cholesterol; HGP_1 and HGP_2, hepatic glucose production basal (first step) and second step; HGP_{suppr}, suppression of HGP from the first to the second step; LDL-chol, low-density cholesterol; LH, luteinising hormone; NEFA, non-esterified fatty acids; $NEFA_{suppr}$, percentage suppression of NEFA from the first to the second step; t-PA-ag, tissue type plasminogen activator antigen; PAI-1, plasminogen activator inhibitor; VLDL-TG, very low density lipoprotein triglycerides; $WBGU_2$, whole body glucose uptake in the second step of the clamp; WHR, waist hip ratio.

Overt non-insulin-dependent diabetes mellitus (NIDDM) is the end of a continuum in metabolic insulin resistance with decreasing compensation by insulin production from the beta cell [1]. Risk factors for ischaemic heart disease, the most importance cause of death and disability in elderly patients with diabetes, like carbohydrate intolerance or manifest diabetes, visceral obesity, hypertriglyceridaemia [2], low HDL-cholesterol content in plasma [3] and hypertension [2] are often clustered in a more or less complete profile of interdependent metabolic abnormalities, called the insulin resistance syndrome [4]. The balance between coagulation and fibrinolysis is intimately linked with this profile [5–7]. In patients with NIDDM the cardiovascular risk is increased 2

to 5 times when compared with non-diabetic persons [8]. When dyslipidaemia is also present the relative risk may further increase to 15 times [9].

Before the menopause cardiovascular events are less frequent in women than in men. This suggests a protective role for oestrogens. This apparent protection disappears in diabetes [10]. Oestrogen replacement therapy (ERT) in non-diabetic women after the menopause was found to be associated with lower fasting glucose [11] and with lower insulin concentrations [11, 12]. Godsland et al. [13] found no significant difference between users of ERT and non-users. They estimated insulin sensitivity by mathematical modelling of intravenous glucose tolerance tests. Lindheim et al. [14] using insulin tolerance tests found only a difference for low dose conjugated oestrogens. In experimental animal studies lowering of blood glucose induced by insulin was enhanced oestrogen therapy [15, 16]. Recently the Postmenopausal Estrogen/Progestin Interventions (PEPI)-trial [17], a placebo controlled double blind study in non-diabetic women, showed a significant decrease of fasting glucose during oral treatment with conjugated equine oestrogens with no change in insulin levels.

ERT was associated with a 50% reduction of the relative risk in cardiovascular morbidity and mortality in observational studies in non-diabetic women [18]. Selection bias may have caused overestimation of the apparent protective effect of ERT [19]. However, both the observation in animal studies that oestrogens stimulate reverse cholesterol transport with decreased subintimal cholesterol ester accumulation [20] and the direct vasorelaxing effect of oestrogens seen in humans [21] strongly suggest a beneficial effect. Moreover, during ERT the lipoprotein profile [22] and parameters of fibrinolysis [23] changed favourably.

In diabetic postmenopausal women ERT is only reluctantly advised [24]. The adverse effects of oral contraceptives on glucose tolerance in patients with NIDDM [25] may have contributed to the hesitation.

We studied the short term effects of ERT on glycaemic control and on insulin resistance using the euglycaemic hyperinsulinaemic clamp in relation with their effects on lipoprotein profile and fibrinolysis in patients with NIDDM with normal or elevated plasma triglyceride level (cut-off 2.0 mmol/l).

Subjects and methods

Patients. We recruited 40 postmenopausal women with NIDDM from outpatient clinics of the University Hospital Leiden or by advertisements and articles in newspapers. We considered NIDDM to be present if diabetes had been controlled sufficiently with diet and/or oral hypoglycaemic agents for more than 1 year or when detectable plasma C-peptide concentrations after glucagon stimulation were found. Women were considered to be postmenopausal when they had no menstrual cycle for more than 1 year. This was confirmed biochemically by a concentration of follicle stimulating hormone (FSH) in plasma over 20 U/l, higher concentrations of FSH than luteinising hormone (LH) and a concentration of oestradiol under 50 pmol/l. Exclusion criteria were: manifest coronary heart disease; liver or renal impairment; endocrine abnormalities other than NIDDM; hereditary diseases of lipid metabolism, plasma triglyceride levels over 10 mmol/l; oestrogen sensitive tumours or a history of thromboembolic diseases. Also excluded were patients who used metformin, diuretics, lipid lowering drugs, corticosteroids, anticonvulsant therapy or postmenopausal hormonal replacement therapy within the previous 3 months. The study was approved by the committee of medical ethics of the University Hospital Leiden and all women gave informed consent before their entry to the study.

Study protocol. We conducted a 6-week double blind placebo controlled trial of 2 mg micronized 17-β oestradiol (Novo-Nordisk, Zoeterwoude, The Netherlands) in 40 postmenopausal women with NIDDM, who received once daily either ERT or placebo orally. The patients were randomized into two groups of 20 subjects. Randomization was performed by a pharmacist, who was the only person knowing the code. FSH and LH were measured after closure of the trial in order not to break the code indirectly. We postulated that the effect of ERT would be similar in the normotriglyceridaemic group (NTG, TG $\leq$ 2 mmol/l; $n = 27$) and the hypertriglyceridaemic group (HTG, TG > 2 mmol/l; $n = 13$). In the NTG group 14 patients received oestrogen and 13 patients placebo. In the HTG group 6 patients were treated with oestrogen and 7 with placebo. The patients were asked to keep a food diary. Before and after 6 weeks of treatment parameters of insulin resistance, lipoprotein metabolism and fibrinolysis were assessed. After the study all patients were treated with 5 mg medroxyprogesterone acetate orally except those who had undergone a hysterectomy in the past.

Two patients from the oestrogen-group reported vaginal spotting, for which they were referred to the gynaecological department. They did not withdraw from the study.

Metabolic investigations. All patients were admitted to the metabolic ward the day before the clamp study. To achieve euglycaemia regular insulin was infused intravenously during the overnight fasting period. At 08.00 hours a cannula was inserted in the other forearm for blood sampling. The arm was placed in a thermoregulated plexiglass box (50–70 °C) to obtain arterialized blood. Before the start of the clamp blood samples were drawn for the assessment of fibrinolytic parameters and lipoprotein profile.

A sequential euglycaemic (5.4 mmol/l) hyperinsulinaemic three-step clamp was performed in order to measure whole body glucose uptake (WBGU) at various insulin perfusion rates [6]. To assess the hepatic glucose production rate (HGP) we administered tritiated glucose (3-^{3}H-glucose, specific activity 499.5 GBq/mmol, NET-331C; Dupont, Boston, Mass., USA). as a tracer by fixed infusion in a rate of 10 μCi/h for 6 h, preceded by a bolus of 15 μCi. During the clamp study plasma glucose was determined every 5 min (Glucose analyser; Beckman Instruments, Palo Alto, Calif., USA). The duration of each step of the clamp was 2 h. During the last 30 min of each step (the equilibrium phase) four blood samples were drawn to estimate the insulin concentration and the specific activity of glucose. The concentration of non-esterified fatty acid (NEFA) was determined in the last blood sample of each step. A dose response curve was obtained by infusion of regular insulin in the following order: 1) basal step: as much insulin as needed to maintain euglycaemia; 2) second step: 1.25 mU $\cdot$

$kg^{-1} \cdot min^{-1}$, preceded by a bolus of 12.5 $mU \cdot kg^{-1}$; and 3) third step: 10 $mU \cdot kg^{-1} \cdot min^{-1}$, preceded by a bolus of 100 $mU \cdot kg^{-1}$. During the last two steps 20% glucose was infused to maintain euglycaemia. Forty to sixty mmol potassium chloride was added to 1000 ml glucose 20% solution to prevent hypokalaemia.

In the first step of the clamp WBGU was equal to HGP. In the next two steps WBGU was calculated according to the isotope dilution technique, being the quotient between the infusion rate of tritiated glucose and the specific activity of glucose in plasma. The specific activity is the quotient between 3-^{3}H glucose in counts per minute and μmol glucose in the same aliquots. The specific activity of tritiated glucose was assessed after deproteinizing plasma with $ZnSO_4$ and $Ba(OH)_2$.

The HGP in the second and third step of the clamp was calculated by subtracting the mean glucose infusion rate during the last 30 min from the calculated rate as mentioned above. In the third step HGP was zero. HGP suppression (HGP_{suppr}) was calculated as (HGP_1-HGP_2)/HGP_1 (where HGP_1 denotes HGP in step 1 and HGP_2 denotes HGP in step 2 of the three step clamp). The mean glucose infusion rate during the steady state of second and the third step was considered to be the WBGU if the mean glucose infusion rate exceeded the quotient between the infusion rate of tritiated glucose and the specific activity of glucose. Otherwise the WBGU was assumed to be equal to the above mentioned quotient.

Percentage NEFA suppression ($NEFA_{suppr}$) has been calculated as $100 \times (NEFA_1\text{-}NEFA_2)/(NEFA_1\text{-}NEFA_3)$, $NEFA_1$, $NEFA_2$ and $NEFA_3$ being the NEFA concentrations of the steady-state phase of the first, second and third step of the clamp respectively.

Analytical procedures. NEFA were determined using the NEFAC test (Wako Chemicals GmbH, Neuss, Germany) and immunoreactive insulin and C-peptide concentration in plasma with a radioimmunoassay method (Medgenix, Brussels respectively, Biolab, Brussels, Belgium). Glycated haemoglobin (HbA_{1c}) was measured by HPLC (Bio-Rad, Richmond, Calif., USA). Lipoprotein profile was assessed by density gradient ultracentrifugation [26]. Plasma triglyceride concentration was determined with GPO-PAP triglyceride reagent (Boehringer Mannheim, Mannheim, FRG). Plasma glucagon concentration was measured by a specific pancreas glucagon radioimmunoassay (Daiichi, Tokyo, Japan). Apo A1 and Apo B were assessed by rate immunonephelometry using an automated Beckman array analyser (Beckman Instruments). Plasminogen activator inhibitor type 1 antigen (PAI-1-ag) was determined by an ELISA method and active PAI-1-ag by an immunoassay specific for the determination of active PAI-1 (Organon Teknika, Turnhout, Belgium).

Tissue type plasminogen activator antigen (t-PA-ag) was measured by immunoassay (Imulyse; Biopool, Umea, Sweden) [27] and activity (t-PA-act.) by a bioimmunoassay (Coatest BIA t-PA, Chromogenix, Mölndal, Sweden) [28]. The PAI-t-PA complex was measured using an immunoassay (Thrombonostika; Organon Teknika, Boxtel, The Netherlands). Blood samples for assessment of fibrinolysis were collected in Stabilyte tubes to prevent binding of t-PA to PAI-1 [29].

Statistical analysis. The adequacy of the randomization was assessed by comparing the two treatment groups at baseline using the Mann-Whitney U-Test. The same test was used to assess treatment group differences (tgd) in changes from baseline. Correlation analysis was performed with the Spearman rank correlation test. We used the SOLO 4.0 statistical program (J. Hintze; BMDP Statistical Software, Los Angeles, Calif., USA).

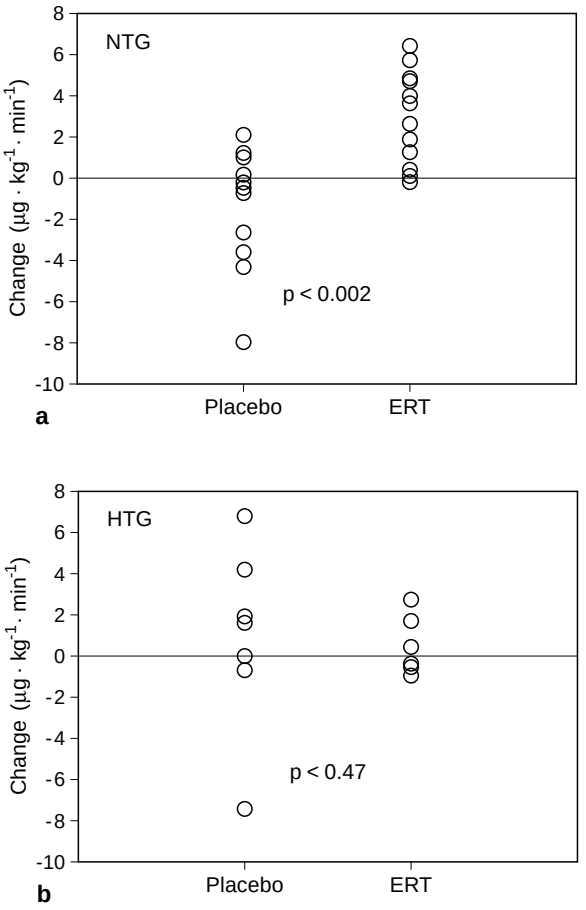

Fig. 1 a, b. Comparison of the effect of 6 weeks oestrogen treatment compared to placebo on hepatic glucose production suppression in an euglycaemic hyperinsulinaemic clamp in NIDDM patients with fasting normotriglyceridaemia (≤ 2.0 mmol/l; NTG) or hypertriglyceridaemia (> 2.0 mmol/l; HTG)

Results

The baseline values and the effects of treatment on insulin resistance, lipids, lipoprotein profile and fibrinolysis are summarized in Tables 1, 2 and 3 respectively. The two study groups did not significantly differ regarding age, BMI, WHR, duration of diabetes, HbA_{1c}, parameters of insulin resistance, parameters of lipoprotein profile and fibrinolysis.

Hypertension was found in 25% of the patients (i.e. systolic pressure > 170 and diastolic pressure > 90 mm Hg), 33% hypertriglyceridaemia (i.e. triglyceride concentration > 2 mmol/l) and 80% visceral obesity (i.e. WHR > 0.90). All patients had a creatinine clearance over 50 ml/min and microalbuminuria was less than 20 μg/min.

None of the patients dropped out of the study. Compliance was checked by measurement of plasma oestradiol levels at the second visit. These levels ranged from 106 to 670 pmol/l in the drug treated group and they were very low or not detectable in the placebo group. Blinding was maintained throughout the

Table 1. Demographics and parameters of insulin sensitivity at baseline and the change during therapy

		Baseline values (a) After therapy (b)		$p_{baseline}$	p_{change}
		Placebo ($n = 20$)	17 β-oestradiol ($n = 20$)		
Age (years)	(a)	60.7 ± 5.2	60.4 ± 5.9	1.00	–
NIDDM (years)	(a)	8.9 ± 8.9	15 ± 13.3	0.12	–
Smoking *(n)*	(a)	3	4	–	–
BMI (kg/m^2)	(a)	28.4 ± 4.7	28.6 ± 6.1	0.99	0.04
	(b)	28.5 ± 4.6	29.0 ± 6.2		
WHR	(a)	0.96 ± 0.07	0.96 ± 0.07	0.80	0.73
	(b)	0.97 ± 0.06	0.98 ± 0.07		
HbA_{1c} (%)	(a)	8.1 ± 1.6	8.7 ± 1.5	0.24	0.03
	(b)	7.7 ± 1.4	8.1 ± 1.3		
C-peptide (nmol/l)	(a)	0.54 ± 0.38	0.38 ± 0.39	0.11	0.26
	(b)	0.52 ± 0.43	0.24 ± 0.22		
HGP_{suppr} ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	(a)	10.9 ± 3.3	9.4 ± 3.0	0.12	0.04
	(b)	10.4 ± 2.3	11.2 ± 3.5		
$WBGU_2$ ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	(a)	21.8 ± 9.2	20.7 ± 15.2	0.19	0.87
	(b)	23.5 ± 10.8	22.1 ± 14.1		
$NEFA_{suppr}$ (%)	(a)	78.6 ± 30.5	82.8 ± 16.9	0.88	0.43
	(b)	84.7 ± 8.1	78.9 ± 22.2		
Glucagon (ng/l)	(a)	166 ± 61.3	156 ± 62.9	0.47	0.18
	(b)	154 ± 60.9	151 ± 56.6		

Values are mean ± SD. The p-values indicate the probability that the mean baseline values ($p_{baseline}$) or the change in the two treatment groups (p_{change}) are identical

study. No significant group-difference was found in treatment related complaints, but in the end all patients guessed the right allocation of treatment group. The total energy intake and dietary composition in terms of protein, fat and carbohydrate content did not differ in the two treatment groups.

Insulin resistance (Table 1). The body mass index (BMI) increased by 1.5% during oestrogen treatment and by 0.7% during placebo (treatment group difference (tgd) $p = 0.04$). No difference in change of WHR was found between the two treatment groups.

The decrease in HbA_{1c} in the oestrogen treated group from 8.7 to 8.1% (mean change – 0.66 ± 0.67) was more than the decrease from 8.1 to 7.7% (mean change – 0.34 ± 0.45) in the placebo group (tgd $p < 0.03$). HGP_1 was not affected by ERT. However the change in HGP_{suppr} (1.77 ± 2.91 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) was significantly greater in the oestrogen treated group than after placebo (0.51 ± 3.61 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) (tgd $p < 0.04$) (Table 1). This was most pronounced in the NTG group (tgd $p < 0.002$, Fig. 1 a). In the HTG group no significant difference in treatment effect was found (tgd $p = 0.47$, Fig. 1 b). The HGP_{suppr} was significantly greater in the NTG than in the HTG group. The plasma level of glucagon, of C-peptide, the WBGU and the percentage of NEFA suppression changed in a comparable way in both groups, with no differences between the NTG and HTG groups. Before ERT, HbA_{1c} was not correlated with HGP or HGP_{suppr}. This was also true for the change in HbA_{1c} during ERT and the change in HGP and HGP_{suppr}.

Lipids, lipoproteins and apolipoproteins (Table 2). Plasma triglyceride levels did not change significantly in either of the two treatment groups, not even in the HTG patients. The same was true for VLDL-cholesterol. Plasma cholesterol concentration changed only slightly, while the LDL-cholesterol levels in the ERT group decreased by 14% compared to an increase of 2% in the control group (tgd $p = 0.0001$). Concomitantly, the plasma apo B levels decreased by 10% compared to an increase by 2% respectively (tgd $p = 0.001$). HDL-cholesterol increased by 22% compared to 3% (tgd $p < 0.0002$). This change in HDL-cholesterol level was mainly due to an increase in the less dense subfraction HDL_2 raising it by 49% compared to 5% (tgd $p < 0.0001$). No significant change was found in HDL_3-cholesterol concentration. Apo A1 was raised by 16% compared to 3% (tgd $p = 0.0001$). No effect in the mean density of the LPL peak or on the oxidisability of LDL [30] was seen.

Fibrinolysis (Table 3). Both total PAI-1 antigen and active PAI-1 antigen concentration decreased significantly during ERT compared with the placebo group (both tgd $p < 0.05$). The change in t-PA activity, t-PA antigen and in the PAI-t-PA complex concentration did not differ between treatment groups.

Table 2. Parameters of lipoprotein profile and the apolipoproteins at baseline and the effect of oestradiol or placebo on concentrations of plasma lipids and (apo)lipoproteins

		Baseline values (a) After therapy (b)		$p_{baseline}$	p_{change}
		Placebo ($n = 20$)	Oestradiol ($n = 20$)		
Plasma cholesterol (mmol/l)	(a)	5.28 ± 0.66	5.25 ± 0.78	0.82	0.02
	(b)	5.32 ± 0.70	4.97 ± 0.70		
LDL-cholesterol (mmol/l)	(a)	3.36 ± 0.68	3.30 ± 0.74	0.68	0.0001
	(b)	3.42 ± 0.69	2.82 ± 0.67		
HDL-cholesterol (mmol/l)	(a)	1.20 ± 0.30	1.20 ± 0.47	0.88	0.0002
	(b)	1.24 ± 0.32	1.47 ± 0.56		
HDL_2-cholesterol (mmol/l)	(a)	0.36 ± 0.19	0.41 ± 0.29	0.89	0.0007
	(b)	0.38 ± 0.20	0.61 ± 0.39		
HDL_3-cholesterol (mmol/l)	(a)	0.84 ± 0.14	0.79 ± 0.21	0.47	0.14
	(b)	0.86 ± 0.17	0.85 ± 0.20		
VLDL-cholesterol (mmol/l)	(a)	0.64 ± 0.35	0.69 ± 0.43	0.86	0.61
	(b)	0.60 ± 0.42	0.62 ± 0.48		
Plasma triglyceride (mmol/l)	(a)	1.53 ± 0.83	1.74 ± 0.95	0.48	0.65
	(b)	1.61 ± 1.09	1.79 ± 1.09		
VLDL-triglyceride (mmol/l)	(a)	1.06 ± 0.63	1.09 ± 0.83	0.78	0.85
	(b)	1.07 ± 0.77	1.09 ± 0.84		
Apo A1 (g/l)	(a)	1.44 ± 0.18	1.39 ± 0.28	0.68	0.0001
	(b)	1.48 ± 0.18	1.61 ± 0.30		
Apo B (g/l)	(a)	1.27 ± 0.28	1.26 ± 0.36	0.89	0.0004
	(b)	1.30 ± 0.32	1.13 ± 0.29		

Values are mean ± SD. The p-values indicate the probability that the mean baseline values ($p_{baseline}$) or the change in the two treatment groups (p_{change}) are identical

Table 3. Parameters of fibrinolysis and the effect of oestradiol or placebo on parameters of fibrinolysis

		Baseline values (a) After therapy (b)		$p_{baseline}$	p_{change}
		Placebo ($n = 20$)	Oestradiol ($n = 20$)		
Active PAI-1 (ng/ml)	(a)	22.9 ± 25.3	17.5 ± 14.1	0.67	0.01
	(b)	25.5 ± 23.5	8.9 ± 5.9		
PAI-1 antigen (ng/ml)	(a)	129 ± 124	100 ± 63	0.83	0.03
	(b)	131 ± 118	72 ± 44		
tPA activity (IU/ml)	(a)	0.23 ± 0.14	0.26 ± 0.16	0.53	0.07
	(b)	0.21 ± 0.13	0.29 ± 0.15		
tPA antigen (ng/ml)	(a)	6.7 ± 2.7	6.7 ± 3.3	0.88	0.17
	(b)	6.7 ± 3.1	5.6 ± 2.7		
PAI-tPA complex (ng/ml)	(a)	12.0 ± 5.7	11.4 ± 6.5	0.68	0.40
	(b)	11.9 ± 5.4	10.3 ± 5.3		

Values are mean ± SD. The p-values indicate the probability that the mean baseline values ($p_{baseline}$) or the change in the two treatment groups (p_{change}) are identical

Discussion

The results of this study demonstrate for the first time that during short term oral ERT the control of NIDDM improves, as indicated by a decrease in HbA_{1c}. Our study indicates that among the three main sites of insulin resistance, the liver, skeletal muscle and adipose tissue [1], the liver (increased HGP suppression) is the major site of improvement of diabetic control during short term ERT. Inhibition of glucagon induced glycogenolysis, as has been shown in ovariectomized mice [16] or an inhibition of alpha-cell function, causing a fall of glucagon secretion [15] may be involved. We were not aware of the plasma glucagon levels in the portal blood in our patients, but the peripheral glucagon levels and their changes were similar in the two treatment groups.

We measured basal HGP after normalizing blood glucose values during the night preceeding the clamp to obtain comparable measurements. HGP is influenced by the blood glucose level, which was sometimes over 10 mmol/l on the evening preceding the study.

We found that the HGP suppression by insulin was not affected by ERT in HTG patients, while the suppression was strongly enhanced in NTG patients (tgd $p < 0.002$). It may be assumed that the increase of triglyceride synthesis during ERT [31] decreases the availability of NEFA for beta-oxidation. Beta-oxidation provides "fuel" for gluconeogenesis, but is rapidly saturated. It may be that in HTG patients due to the increased levels of NEFA, beta-oxidation is already saturated by high substrate availability so that eventual changes in substrate availability are not reflected in changed gluconeogenesis whereas in NTG patients the lower NEFA concentrations allow changes to become visible. An effect of ERT on intracellular availability of NEFA in mitochondria is not known.

Another source of fatty acids for the liver are core remnants of triglyceride rich lipoproteins and HDL_2 particles after they have been attacked by hepatic lipase. Oral administration of oestrogens inhibits hepatic lipase activity [32]. Thus, a reduced NEFA influx from this source may also have contributed to the improved HGP_{suppr} especially in NTG patients. Although the BMI was significantly increased in the ERT group as compared to the placebo group the WHR did not change. The WHR is a measure of abdominal obesity. An increased NEFA release from abdominal fat is important in the insulin resistance syndrome [2]. This is supported by our baseline data, i.e. WHR ($r_s = -0.49$, $p < 0.002$) and BMI ($r_s = -0.45$ $p < 0.005$) were negatively correlated with HGP_{suppr}.

Insulin resistance can also be caused by reduced blood flow in skeletal muscle and reduced capillary density [1, 33, 34]. However, we could not demonstrate an increase in WBGU during ERT. The effect of oestrogens on the re-esterification of NEFA with glycerol phosphate in adipose tissue, a site of insulin resistance [1], is unknown.

The change in plasma lipids and lipoproteins from baseline is in accordance with earlier reports on non-diabetic postmenopausal women [22]. The decrease of cholesterol in plasma is a reflection of the lowering of LDL-cholesterol. This was paralleled by a decrease in apo B concentration and may be explained by an increased LDL-cholesterol and apo B catabolism in liver, probably due to an increased number of apo LDL recetpors during ERT [31]. The effects on HDL-cholesterol were mainly due to an increase of its less dense, variable part, the HDL_2 subfraction. This may be explained by suppression of hepatic lipase activity by ERT [31]. Moreover, the increase in HDL was accompanied by a significant increase of apo A1 concentration probably by increased apo AI synthesis in the liver [35]. Although oestrogens enhance triglyceride synthesis in the liver, producing large "fluffy-puffy" VLDL particles [22], we did not find an increase in plasma VLDL triglyceride, or in VLDL-cholesterol concentration.

A high PAI-1 concentration and activity are associated with insulin resistance [5–7]. The lowering fits in the pattern of other changes observed during ERT, but the mechanism is not clear. Kroon et al. [23] demonstrated that ERT can induce a decrease in the plasma concentration of PAI-1 in non-diabetic postmenopausal women. During treatment with low-dose oral contraceptives a 50% decrease in PAI-1 has been observed [36]. We found a decrease in both active and total PAI-1 levels in postmenopausal women with NIDDM, suggesting an improvement of fibrinolysis.

Oestrogen replacement therapy is increasingly prescribed in postmenopausal women for various reasons. We studied the risk factors influenced by unopposed oestrogens. However, in practice combined therapy with progestogens is prescribed to minimize oestrogen-induced risk of endometrial carcinoma in women with an intact uterus. The effect of progestogens remains to be established. In diabetic women ERT is more reluctantly used [24] because of the presumed negative effects of oestrogens on carbohydrate and lipid metabolism [25]. A long term study on the effects of hormone replacement therapy among postmenopausal women with NIDDM should be performed before a more liberal policy is advisable.

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References

1. DeFronzo RA, Bonadonna RC, Ferrannini E (1992) Pathogenesis of NIDDM, a balanced overview. Diabetes Care 15: 318–368
2. Kaplan NM (1989) The Deadly Quartet, Upper-body obesity, glucose intolerance, hypertriglyceridemia and hypertension. Arch Intern Med 149: 1514–1520
3. Bierman EL (1992) Atherogenesis in diabetes. Arterioscler Thromb 12: 647–656
4. Reaven GM (1991) Resistance to insulin-stimulated glucose uptake and hyperinsulinemia: role in non-insulin dependent diabetes, high blood pressure, dyslipidaemia HTG and coronary heart disease. Diab Metab 17: 78–86
5. Juhan-Vague I, Thompson SG, Jespersen J (1993) Involvement of the hemostatic system in the insulin resistance syndrome, a study of 1500 patients with angina pectoris. Arterioscl Thromb 13: 1865–1873
6. Potter van Loon BJ, Kluft C, Radder JK, Blankenstein MA, Meinders AE (1993) The cardiovascular risk factor

plasminogen activator inhibitor type 1 is related to insulin resistance. Metabolism 42: 945–949
7. Brussaard HE, Gevers Leuven JA, Krans HMJ, Meijer P, Buytenhek R, Kluft C (1994) Non-esterified fatty acids are related with hypofibrinolysis in type 2 diabetes mellitus. Fibrinolysis 8: 25–27
8. DeStefano F, Ford EA, Newman J et al. (1993) Risk factors for coronary heart disease mortality among persons with diabetes. Ann Epidemiol 3: 27–34
9. Assmann G, Schulte H (1989) Diabetes mellitus and hypertension in the elderly: concomitant hyperlipidemia and coronary heart disease risk. Am J Cardiol 63: 33H–37H
10. Barrett-Connor E (1994) Heart disease in women. Fert Ster 62: 127S–132S
11. Nabulsi AA, Folsom AR, White A et al. (1993) For the atherosclerosis risk in communities study investigators. Association of hormone replacement therapy with various cardiovascular risk factors in postmenopausal women. N Engl J Med 328: 1069–1075
12. Ferrara A, Barrett-Connor E, Wingard DL, Edelstein SL (1995) Sex differences in insulin levels in older adults and the effect of body size, oestrogen replacement therapy, and glucose tolerance status. The Rancho Bernardo Study. Diabetes Care 18: 220–225
13. Godsland IF, Gangar K, Walton C et al. (1993) Insulin resistance, secretion and elimination in postmenopausal women receiving oral or transdermal hormone replacement therapy. Metabolism 42: 846–853
14. Lindheim SR, Presser SC, Ditkoff EC, Vijod MA, Stanczyk FZ, Lobo RA (1993) A possible bimodel effect of oestrogen on insulin sensitivity in postmenopausal women and the attenuating effect of added progestin. Fertil Steril 60: 664–667
15. Mandour T, Kissebah AH, Wynn V (1977) Mechanisms of oestrogen and progesterone effects on lipid and carbohydrate metabolism: alteration in the insulin : glucagon molar ratio and hepatic enzyme activity. Eur J Clin Invest 7: 181–187
16. Ahmed-Sorour H, Baily CJ (1980) Role of ovarian hormones in the long-term control of glucose homeostasis. Interaction wiht insulin, glucagon and epinephrine. Hormone Res 13: 396–403
17. The writing group for the PEPI trial (1995) Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women. JAMA 273: 199–208
18. Stampfer MJ, Colditz GA, Willett WC et al. (1991) Postmenopausal oestrogen therapy and cardiovascular disease. Ten year follow-up from the nurses' health study. N Engl J Med 325: 756–762
19. Vandenbroucke JP (1995) How much of the cardioprotective effect of postmenopausal estrogens is real? Epidemiology 6: 207–208
20. Adams MR, Kaplan JR, Manuck SB, Koritnik DR, Parks JS, Wolfe MS (1990) Inhibition of coronary artery atherosclerosis by 17 β-oestradiol in ovariectomized monkeys. Lack of an effect of adding progesterone. Arteriosclerosis 10: 1051–1057
21. Collins P, Rosano GMC, Sarrel PM et al. (1995) 17 β-Estradiol attenuates acetylcholine-induced coronary artery constriction in women but not in men with coronary artery disease. Circulation 92: 24–30
22. Gevers Leuven JA (1994) Sex steroids and lipoprotein metabolism. Pharmac Ther 64: 99–126
23. Kroon UB, Silfverstolpe G, Tengborn L (1994) The effects of transdermal oestradiol and oral conjugated oestrogens on haemostasis variables. Thromb Haem 71: 420–423
24. Hemminki E, Sihvo S (1993) A review of postmenopausal hormone therapy recommendations: a potential for selection bias. Obstet Gynecol 82: 1021–1028
25. Godsland IL, Crook D, Simpson R et al. (1990) The effects of different formulations of oral contraceptive agents on lipid and carbohydrate metabolism. N Engl J Med 323: 1375–1381
26. Gevers Leuven JA, Mooren MV van der, Buytenhek R (1995) The effect of medrogestone on lipids and lipoproteins in postmenopausal women using conjugated oestrogens: an open randomized comparative study. Fertil Steril 64: 525–531
27. Ranby M, Bergsdorf N, Nilsson T, Mellbring G, Winblad B, Bucht G (1986) Age dependence of tissue plasminogen activator concentrations in plasma as studied by an improved enzyme linked immuno-sorbent. Clin Chem 32: 2160–2165
28. Meijer P, Boon R, Jie AFH, Rosen S, Kluft C (1992) Bioimmunoassay for tissue-type plasminogen activator (t-PA) in human plasma: evaluation of blood sampling and handling procedures and comparison with other t-PA activity methods. Fibrinolysis 6: 97–99
29. Ranby M, Sundell IB, Nilsson ThJJ (1989) Blood collection in strong acidic citrate anticoagulants used in a study of dietary influence and basal tPA activity. Thromb Haemost 62: 617–622
30. Brussaard HE, Gevers Leuven AJ, Kluft C, Krans HMJ, Duyvenvoorde W van, Huytenhek R, Laarse A van der, Princen HMG (1997) Effect of 17 β-estradiol on plasma lipids and LDL oxidation in postmenopausal women with type II diabetes mellitus. Arterioscler Throm Vasc Biol 17: 324–330
31. Walsh BW, Schiff I, Rosner B, Greenberg L, Ravnikar V, Sacks FM (1991) Effects of postmenopausal oestrogen replacement on the concentrations and metabolism of plasma lipoproteins. N Engl J Med 325: 1196–1204
32. Westerveld HT, Kock-Herman LAW, Rijn JM van, Erkelens DW, Bruin TWA de (1995) 17-beta estradiol improves postprandial lipid metabolism in postmenopausal women. J Clin Endocrin Metab 80: 249–253
33. Krotkiewski M, Seidell JC, Bjorntorp P (1990) Glucose tolerance and hyperinsulinaemia in obese women: role of adipose tissue distribution, muscle fibre characteristics and androgens. J Intern Med 228: 385–392
34. DelPrato S, Bonadonna RC, Bonora E et al. (1993) Characterization of cellular defects of insulin action in type 2 (non-insulin-dependent) diabetes mellitus. J Clin Invest 91: 484–494
35. Walsh BW, Li H, Sacks FM (1994) Effects of postmenopausal hormone replacement with oral and transdermal oestrogen on high density lipoprotein metabolism. J Lip Res 35: 2083–2093
36. Gevers Leuven JA, Kluft C, Dersjant-Roorda MC, Harthoorn-Lasthuizen EJ, Boer R de, Helmerhorst FM (1995) Effects of low-dose ethinylestradiol oral contraceptives differing in progestogenic compound on coagulation and fibrinolytic risk variables for venous and arterial thromboembolic diseases. In: Glas-Greenwalt P (ed.) Fibrinolysis in disease: molecular and hemovascular aspects of fibrinolysis. CRC Press, Boca-Raton, pp 226–234