Effects of Oral and Transdermal 17β-Estradiol With Cyclical Oral Norethindrone Acetate on Insulin Sensitivity, Secretion, and Elimination in Postmenopausal Women

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Few studies have examined the effects of 17β-estradiol on parameters of insulin and glucose metabolism. We studied 42 healthy, untreated postmenopausal women seeking relief from menopausal symptoms. They were randomized to receive either oral 17β-estradiol 2 mg daily combined with sequential oral norethindrone acetate (NETA) 1 mg daily from days 12 to 22, or transdermal 17β-estradiol 0.05 mg daily combined with sequential oral NETA 1 mg daily from days 17 to 28. Intravenous glucose tolerance tests (IVGTTs) were performed at baseline and after 46 weeks (estrogen-alone phase) and 48 weeks (combined phase) of completed therapy. Mathematical modeling analysis of plasma glucose, insulin, and C-peptide concentration profiles provided measures of insulin resistance, secretion, and elimination. Both types of therapy were associated with a decrease in fasting insulin and glucose levels. Insulin sensitivity was increased by oral estradiol during the estrogen-alone phase but was reversed by the addition of NETA. Transdermal estradiol did not affect insulin sensitivity. Hepatic insulin uptake and insulin secretion were increased with both types of treatment. The oral regimen of estradiol therapy was favorable to both insulin elimination and sensitivity. Transdermal estradiol therapy had relatively few effects on insulin metabolism.

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THERE IS INCREASING interest in the role of disturbances in insulin metabolism not only in the pathogenesis of diabetes but also in the development of coronary heart disease (CHD). Insulin may play a role in atherogenesis,[1] and there is epidemiological evidence to suggest that hyperinsulinemia is an independent risk factor for CHD.[2] Moreover, hyperinsulinemia and its associated insulin resistance (reduced insulin sensitivity) are responsible for multiple disturbances in metabolic risk factors for CHD which themselves may augment the risks associated with increased insulin levels and insulin resistance.[3]

In accordance with the postmenopausal increase in the incidence of CHD, menopause is associated with adverse changes in a range of metabolic risk factors,[4,5] including those relating to insulin and glucose metabolism.[6-8] The weight of the evidence suggests that estrogen replacement in postmenopausal women can counter menopause-related declines in insulin secretion and increases in insulin resistance.[9] Therefore, some contribution to the beneficial effects of hormone replacement therapy (HRT) on CHD, and possibly diabetes,[10] could be made by changes in carbohydrate metabolism. However, these beneficial effects can be modified by inclusion of a progestagen in combined HRT therapy.[9] For example, medroxyprogesterone acetate increases insulin resistance, whereas norethindrone acetate (NETA) alone is neutral[9]—although there is some evidence that it may oppose estrogen-related improvements in insulin sensitivity.[11] Furthermore, little is known about the differential effects of oral and nonoral routes of estrogen administration on carbohydrate metabolism.

In the present study, we have compared the effects of orally or transdermally administered 17β-estradiol, both with cyclical oral NETA, during estrogen-alone and combined phases of the treatment cycle. Mathematical modeling analysis of intravenous glucose tolerance test (IVGTT) glucose, insulin, and C-peptide profiles was used for the rigorous evaluation of a number of factors responsible for changes in glucose and insulin metabolism, including insulin sensitivity, secretion, and elimination.

SUBJECTS AND METHODS

Subjects

Sixty-four healthy postmenopausal women were recruited from menopause clinics in London, UK. They were aged less than 65 years (range, 45 to 65), were at least 1 year beyond the menopause, and required relief from menopausal symptoms. All were within 20% of their ideal body weight (as defined by Metropolitan Life tables), were nonsmokers, and consumed less than 10 units of alcohol per week. None were taking medications known to affect lipid metabolism, nor did they receive sex steroids within the previous 3 months or estrogen implants within the previous 6 months. Postmenopausal status was confirmed by measurement of gonadotropin levels (follicle-stimulating hormone > 35 IU/mL). Each participant provided written consent and the study was approved by the local Ethics Committee.

Study Design

Women were randomly allocated to receive either oral therapy consisting of a 28-day cycle of 17β-estradiol and estriol 2 mg and 1 mg daily, respectively, with 10 days of oral NETA 1 mg daily (days 12 to 22) or transdermal 17β-estradiol 0.05 mg daily with 12 days of oral NETA 1 mg daily (days 17 to 28). Patches for transdermal delivery of 17β-estradiol were changed twice weekly. The women were studied before commencement of therapy and after 46 and 48 weeks of

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treatment, during the estrogen-alone and combined phases of the treatment cycle, respectively.

Procedures

Each subject was instructed to eat more than 200 g/d carbohydrate for 3 days prior to testing to minimize dietary-induced differences in the pancreatic insulin response to glucose. They attended the Wynn Department metabolic day ward following a 12-hour overnight fast. Height and weight were measured and a general history was obtained, including details of exercise habits and alcohol consumption and family history of diabetes and heart disease. Blood pressure was recorded after 10 minutes of bed rest with the subject semirecumbent, after which cannulae were inserted into the antecubital veins of each arm; the cannula in the nondominant arm was used for blood sampling. Blood samples for measurement of fasting plasma glucose, insulin, and C-peptide (lithium-heparin anticoagulant) were obtained through this cannula. Additional samples were taken for a second measurement of fasting glucose, insulin, and C-peptide after an interval of 10 minutes. An intravenous glucose injection (0.5 g/kg body weight as 50% dextrose solution) was administered over 3 minutes via the opposite cannula, which was then removed. Samples for measurement of glucose, insulin, and C-peptide and were obtained at 3, 5, 7, 10, 15, 20, 30, 45, 60, 75, 90, 120, 150, and 180 minutes following commencement of the injection.

Laboratory Assays

Plasma glucose was determined within 24 hours on samples stored at 4°C by a glucose oxidase method.[12] Plasma insulin and C-peptide levels were measured in batches on samples stored at −20°C by double-antibody radioimmunoassay with materials supplied by Guildhay (Surrey, UK). Quality control was monitored with commercially available lyophilized sera and by participation in national schemes. The within- and between-batch coefficients of variation were between 2% and 3% for plasma glucose, 4% and 6% for plasma insulin, and 7% and 9% for plasma C-peptide.

Data Analysis

Mean fasting plasma glucose, insulin, and C-peptide concentrations were derived from the 2 basal measurements, and IVGTT incremental glucose, insulin, and C-peptide areas (ie, the area between the fasting level and the IVGTT concentration profile) were calculated by the trapezium rule. Measures of insulin sensitivity, secretion, and elimination were derived by mathematical modeling analysis of the IVGTT glucose, insulin, and C-peptide concentration profiles using programs written in Fortran 77 run on a PDP-11/83 microcomputer (IBM, New York). Insulin sensitivity (S_i) and glucose effectiveness (S_g) were determined using the minimal model of glucose disappearance[13] whereby the prediction of the glucose concentration profile from the observed insulin concentrations allows measurement of these 2 parameters. The relatively high glucose dose (0.5 g/kg) we use provides for a

Table 1. Treatment Group Characteristics (mean ± SD)

Parameter	Oral 17β-Estradiol	Transdermal 17β-Estradiol
No. of subjects completing study	19	23
Age (yr)	56.7 ± 4.5	56.1 ± 4.4
BMI (kg/m²)	24.1 ± 2.3	24.4 ± 2.1
Age at menopause (yr)	46.6 ± 5.5	49.0 ± 4.2
Systolic blood pressure (mm Hg)	112 ± 14.8	117 ± 15.9
Diastolic blood pressure (mm Hg)	69 ± 8.6	72 ± 8.2
Alcohol intake (weekly units)	4 ± 4.6	6 ± 6.3
Time since menopause (yr)	10 ± 6.3	7 ± 5.0

Table 2. Fasting and IVGTT Glucose, Insulin, and C-Peptide Concentrations

Parameter	Oral Therapy (n = 19)			Transdermal Therapy (n = 23)		
	Baseline	E	E + P	Baseline	E	E + P
Fasting plasma concentration						
Glucose (mmol · L⁻¹)	5.16 (0.33)	5.02 (0.35)*	5.02 (0.36)*	5.2 (0.40)	4.95 (0.30)†	4.98 (0.24)†
Insulin (pmol · L⁻¹)§	40.5 (−14.5, +22.5)	36.9 (−16.8, +30.9)	30.8 (−16.8, +37.1)*	37.9 (−15.7, +26.9)	25.8 (−14.1, +31.2)*	33.0 (−11.9, +18.7)
C-peptide (pmol · L⁻¹)§	0.40 (−0.12, +0.17)	0.46 (−0.14, +0.2)	0.46 (−0.12, +0.16)	0.46 (−0.14, +0.19)	0.49 (−0.13, +0.17)	0.51 (−0.15, +0.22)
IVGTT incremental area						
Glucose (mmol · L⁻¹ · min)	493.2 (172.2)	479.3 (161.4)	517.9 (146.7)	433.9 (187.8)	465.9 (175.3)	458.8 (189.4)
Insulin (10⁴ pmol · mL⁻¹ · min)§	2,794 (−4,648, +7,297)	9,750 (−4,429, +81,165)*	13,213 (−5,066, +8,216)‡	11,885 (−4,854, +8,206)	2,618 (−3,808, +5,453)	3,274 (−5,070, +8,204)
C-peptide (pmol · mL⁻¹ · min)§	57.6 (−37.3, +83.4)	82.7 (−36.0, +58.6)	96.6 (−27.6, +37.2)*	70.0 (−27.3, +41.3)	98.1 (−30.5, +42.5)*	94.9 (−36.6, +56.1)*

NOTE. See Table 4 for data information.

Table 3. Insulin Sensitivity (S_I) and Glucose-Dependent Glucose Disposal (S_g)

IVGTT Model-Derived Measures	Oral Therapy (n = 19)			Transdermal Therapy (n = 23)		
	Baseline	E	E + P	Baseline	E	E + P
Insulin sensitivity, S_I ($min^{-1} \cdot \mu U^{-1} \cdot mL$)§	2.64 (−1.62, +2.40)	3.74 (−2.15, +3.05)*	2.72 (−1.58, +2.27)‡	3.64 (−1.6, +2.0)	3.62 (−0.25, +3.8)	3.45 (−1.95, +2.75)
Glucose-dependent glucose disposal, S_g (min^{-1})§	1.85 (−0.8, +1.4)	1.68 (−0.74, +1.32)	1.66 (−0.62, +0.98)	1.83 (−0.9, +1.9)	1.84 (−0.75, +1.30)	1.98 (−0.80, +1.31)

NOTE. See Table 4 for data information.

sufficient endogenous insulin response in nondiabetics without recourse to additional augmentation of pancreatic insulin secretion. This is apparent in the high rate of model identification and good correlation with measures of insulin sensitivity derived from the euglycemic clamp ($r = .92$) that we obtain.[14,15] Insulin delivery characteristics were evaluated using the minimal model of posthepatic insulin delivery,[16] which provides a measure of the fractional insulin elimination rate and the responsiveness of first- and second-phase posthepatic insulin delivery to glucose. A combined model of insulin and C-peptide delivery[17,18] was used to quantify the fractional insulin and C-peptide elimination rate, a measure of the fraction of newly secreted insulin that passes out of the liver, and basal and incremental insulin secretion during the IVGTT in total and during the first and second phases of secretion. The models describing insulin delivery have been evaluated previously in animals and humans.[14,17,19] Some women withdrew from the study soon after commencing therapy; in these cases, no data are available to interpret the effects of therapy on carbohydrate metabolism. Statistical analysis using Systat (Evanston Inc, Illinois) was performed on data for women who completed the study. Before analysis, transformations were applied as appropriate to normalize distributions. Variables that were 4 standard deviations or more from the mean were excluded from the final statistical analysis. Significant changes from baseline were identified within each group by 1-way repeated-measures ANOVA across data for each of the 3 visits. Where significant variation was found, post hoc pairwise testing was used for visits 2 and 3 relative to baseline by forming linear contrasts.

RESULTS

From a total of 64 women who were recruited, 45 completed the trial. The reasons for failure to enter the trial were as follows: impaired glucose tolerance at baseline (n = 3), triglycerides more than 3 mmol/L at baseline (n = 1), hypercholesterolemia at baseline (n = 1), and hypertension at baseline (n = 1). Nine women withdrew from the study within 6 weeks of treatment due to adverse side effects of therapy. These symptoms were nausea (n = 3), abdominal pain (n = 2), headaches (n = 1), and heavy withdrawal bleeding (n = 3). Data from 7 women were excluded from the final analysis due to poor attendance (n = 4) or because blood samples were not obtained during the appropriate time of the treatment cycle (n = 3). Forty-two women were thus included in the final analysis (19 receiving oral and 23 receiving transdermal estrogen).

Group characteristics are shown in Table 1. The age of the women in both treatment groups was not significantly different. Women in the transdermal group had a higher menopausal age, but this did not reach statistical significance. There were no differences in the body mass index (BMI) or blood pressure in the 2 groups at baseline. BMI values in both treatment groups remained the same at the end of the study. Blood pressure (systolic and diastolic) did not change in either group throughout the course of the study. Baseline data for the women who withdrew from the trial because of side effects (n = 9) or who were excluded from the final analysis because of poor attendance or incorrectly timed blood samples (n = 7) were not significantly different versus data for both treatment groups who completed the study.

The fasting concentration and IVGTT incremental area for glucose, insulin, and C-peptide are listed in Table 2. In both treatment groups, there was a significant decrease in fasting glucose ($P < .05$) in both the estrogen-alone and combined phases of therapy. Fasting insulin levels were reduced during the combined phase of therapy in the oral estradiol group ($P < .05$) and during the estrogen-alone phase in the transdermal estradiol group. There were no significant changes in the incremental glucose area in either treatment group. With oral estradiol, there was a decrease in the incremental insulin area during the estrogen-alone phase ($P < .05$) that was no longer apparent during the combined phase. The IVGTT C-peptide incremental area was increased during the combined phase with oral therapy ($P < .05$) and during both phases with transdermal therapy (both $P < .05$).

Results from the modeling analysis of IVGTT glucose and insulin concentration profiles are shown in Table 3. Women who received oral estrogens showed a 42% increase in insulin sensitivity ($P < .05$) during the estrogen-alone phase of therapy. Insulin sensitivity returned to near-baseline values during the combined phase of treatment ($P < .05$). In the group receiving transdermal estradiol, insulin sensitivity was unchanged during both the estrogen-alone and combined phases of therapy. Glucose-dependent glucose disposal (S_g), which represents the rate constant for non–insulin-dependent glucose metabolic pathways, was unchanged with both types of therapy.

Parameters derived from the posthepatic insulin delivery model were unaffected by both types of therapy (Table 4). There was a 35% increase in hepatic insulin uptake in women using oral estrogens, and this was evident during both phases of treatment ($P < .05$). Transdermal estradiol was associated with a 25% increase in hepatic insulin uptake during the estrogen-alone phase only ($P < .05$). Net incremental insulin secretion during the IVGTT in women receiving oral therapy showed no change during the estrogen-alone phase but a 64% increase during the combined phase ($P < .05$). In women using transdermal estradiol, there was a significant increase in the pancreatic insulin secretion response during the combined phase of therapy ($P < .05$).

DISCUSSION

The number of women who failed to complete the study appears high (22 of 64; 34%), but more than half of these

Table 4. Model-Derived Measures of Posthepatic and Pancreatic Insulin Delivery, Hepatic Throughput, and Insulin and C-Peptide Elimination

Parameter	Oral Therapy (n = 19)			Transdermal Therapy (n = 23)		
	Baseline	E	E + P	Baseline	E	E + P
Posthepatic insulin delivery model						
n_i (min^{-1})§	0.12 (−0.06, +0.07)	0.13 (−0.06, +0.07)	0.11 (−0.06, +0.09)	0.12 (−0.05, +0.06)	0.14 (−0.06, +0.07)	0.11 (−0.05, +0.06)‡
Φ_1 ($\mu U \cdot mL^{-1} \cdot min \cdot mg^{-1} \cdot mL$)§	2.81 (−1.25, +2.26)	2.23 (−0.98, +1.77)	2.76 (−1.28, +2.38)‡	3.31 (−1.35, +2.27)	3.24 (−1.67, +3.45)	3.5 (−1.7, +3.27)
Φ_2 ($\mu U \cdot mL^{-1} \cdot min^{-2} \cdot mg^{-1} \cdot mL$)§	6.5 (−3.4, +7.0)	7.27 (−4.4, +11.2)	6.95 (−4.07, +9.87)	6.9 (−4.8, +15.9)	9.03 (−4.6, +9.4)	7 (−4.12, +8.83)
Pancreatic insulin secretion model						
ff§	0.77 (−0.31, +0.52)	0.5 (−0.18, +0.29)*	0.53 (−0.22, +0.4)*	0.67 (−0.19, +0.27)	0.5 (−0.18, +0.28)*	0.58 (−0.18, +0.27)
k_i (min^{-1})§	0.09 (−0.04, +0.07)	0.08 (−0.03, +0.06)	0.09 (−0.04, +0.07)	0.08 (−0.03, +0.05)	0.10 (−0.03, +0.04)	0.09 (−0.04, +0.08)
k_e (min^{-1})§	0.018 (−0.003, +0.004)	0.02 (−0.008, +0.015)	0.017 (−0.005, +0.006)	0.016 (−0.005, +0.007)	0.02 (−0.005, +0.008)	0.016 (−0.006, +0.009)
IS (pmol · mL^{-1} · min)§	1.39 (−0.45, +1.46)	1.52 (−0.52, +1.58)	2.28 (−0.74, +1.8)†‡	1.15 (−0.77, +1.44)	1.67 (−0.9, +1.51)	1.92 (−0.97, +1.62)*
IS_1 (pmol · mL^{-1} · min)	0.65 (0.35)	0.75 (0.37)	0.78 (0.4)	0.86 (0.45)	1.07 (0.4)†	1.01 (0.42)
IS_2 (pmol · mL^{-1} · min)§	0.85 (−0.08, +1.92)	0.87 (−0.1, +2.01)	1.59 (−0.3, +2.32)†‡	0.35 (−0.89, +1.36)	1.17 (−1.1, +1.5)†	1.04 (−1.2, +1.7)*

NOTE. Data are presented as the mean (SD), except for modeling measures, which are expressed as the mean and asymmetrical SD (−SD, +SD). Logarithmic transformation was applied to mean fasting insulin and C-peptide values and incremental insulin and C-peptide areas.

Abbreviations: E, estrogen alone; E + P, combined therapy; f, hepatic throughput index; IS, IS_1, and IS_2, net, phase 1, and phase 2 incremental pancreatic insulin secretion, respectively.

*Within-group change from baseline, $P < .05$.

†Within-group change from baseline, $P < .01$.

‡Within-group change between E and E + P phase, $P < .05$.

§Data transformed before analysis.

women failed the screening part of the study or withdrew due to side effects of the therapy. The discontinuation rate due to adverse effects of therapy (9 of 58) is within the range of previous reports.[20,21]

Menopause is associated with a decline in insulin secretion and is followed by a progressive increase in insulin resistance and hepatic insulin throughput, ie, less insulin is taken up by the liver.[6-8] The expected physiological actions of estrogen replacement would therefore be an increase in insulin sensitivity and secretion and hepatic insulin uptake. Improvements in insulin sensitivity have been reported in association with estrogen replacement both orally and transdermally, although estrogen therapy to induce supraphysiological concentrations is associated with a deterioration of insulin sensitivity.[9] In the present study, there was an improvement in insulin sensitivity with oral therapy, but this was only apparent during the estrogen-alone phase of treatment. This accords with the possibility that NETA opposes the beneficial effect of estrogen replacement on insulin sensitivity.[8] The lack of change in insulin sensitivity with transdermal treatment accords with our previous study in which both 17β-estradiol and NETA were administered transdermally.[11] Possibly, there is some carryover effect of NETA from the previous treatment cycle in combination therapy. Our observation that the oral route of administration of estradiol is sufficient to overcome this effect suggests that the exposure of the liver to higher estrogen concentrations with this route may be involved in the improvement of insulin sensitivity. This accords with the observation by Brussaard et al[22] that oral estradiol therapy in postmenopausal women with non–insulin-dependent diabetes mellitus increases hepatic but not peripheral insulin sensitivity, and focuses attention on the liver as the most likely site of estrogen action in improving insulin sensitivity. In this respect, the reduced basal glucose and insulin concentrations observed by our group and many others may be relevant. These changes result from estrogen-induced reductions in glucagon sensitivity and secretion,[9] which would be expected to reduce opposition to the effects of insulin by glucagon, primarily at the liver.

The effects of postmenopausal estrogen replacement on pancreatic insulin secretion and hepatic insulin handling have not been well studied. The expected actions of estrogen replacement to reverse menopause-induced changes in these parameters were clearly apparent in our study. The improvement in insulin secretion in response to estrogen replacement may be a relatively long-term effect resulting from pancreatic hyperplasia or hypertrophy.[23] Since there were no overall changes in the insulin concentration, it would appear that the observed increases in pancreatic insulin secretion and hepatic insulin uptake compensate for each other so that glucose homeostasis is maintained. Whether there is a mechanistic link between changes in the two processes is not known, although the existing experimental evidence would suggest that the changes in insulin secretion are primary. It is noteworthy that increased insulin uptake by the liver appears to be associated with a favorable metabolic risk factor profile, particularly with regard to high-density lipoprotein metabolism.[24]

There has previously been considerable confusion regarding the effects of estrogens on carbohydrate metabolism. This has largely been engendered by inappropriate extrapolation from

the effects of oral contraceptives. The considerable variability in the study design used to evaluate the effects of estrogens on carbohydrate metabolism is also likely a confounding factor. Nevertheless, the weight of the evidence, both from experimental studies in animals and from studies of the effects of estrogens administered alone to postmenopausal women, indicates that physiological estrogen replacement in postmenopausal women is associated with an improvement in insulin sensitivity.[9] In contrast, the use of higher-dose (eg, 1.25 rather than 0.625 mg conjugated equine estrogens) or more potent estrogens (eg, ethinyl estradiol) is associated with a deterioration of carbohydrate metabolism, probably due to an estrogen-induced increase in corticosteroid levels.[9] The inclusion of a progestagen in combined-estrogen replacement HRT regimens may also modify the effects of estrogens. There are still relatively few studies on the effects of combination HRT on carbohydrate metabolism, but the evidence indicates that the addition of the commonly used progestagens such as medroxyprogesterone acetate and levonorgestrel adversely affects glucose tolerance,[11,25-27] whereas norethindrone-containing combinations[11] are relatively neutral, and with dydrogesterone, the beneficial effects of estradiol may even be preserved to some extent.[28]

The effects of HRT on insulin sensitivity in postmenopausal women demonstrated in this and other studies appear relatively small, particularly when the cyclic or continuous administration of progestagens that oppose the effects of estradiol are considered. Whether such changes may affect long-term glucose homeostasis and the development of diabetes in postmenopausal women is uncertain, although when considering HRT for women with established diabetes, there would seem to be a case for favoring therapies that may diminish insulin resistance. Studies designed to evaluate this possibility are clearly needed. HRT-induced changes in pancreatic insulin secretion and insulin elimination have rarely been considered in previous studies, but such changes may be more important than changes in insulin sensitivity. Menopause per se is not associated with increased insulin resistance, although increasing time since menopause is. However, menopause is associated with a 50% reduction in the pancreatic insulin response to glucose,[29] and there is some evidence that this may be associated with a commensurate increase in the incidence of diabetes.[30] It is also well established in animal models that estrogen administration can prevent the development of diabetes.[31] In the present study, pancreatic insulin secretion increased by about 60% for both treatments. Prospective studies in HRT users show either no change[32] or a reduction[10] in the incidence of diabetes. A reduction in the incidence accords with the experimental data and recommends further research in this field.

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REFERENCES

1. Stout R: Insulin and atheroma: 20-year perspective. Diabetes Care 13:631-654, 1990

2. Despres J-P, Lamarche B, Mauriege P, et al: Hyperinsulinaemia as an independent risk factor for ischaemic heart disease. N Engl J Med 334:952-957, 1996

3. Reaven G, Laws A: Insulin resistance, compensatory hyperinsulinaemia, and coronary heart disease. Diabetologia 37:948-952, 1994

4. Ley C, Lees B, Stevenson J: Sex- and menopause-associated changes in body fat distribution. Am J Clin Nutr 55:950-954, 1992

5. Stevenson J, Crook D, Godsland I: Influence of age and menopause on serum lipids and lipoproteins in healthy women. Atherosclerosis 98:83-90, 1993

6. Proudler A, Felton C, Stevenson J: Ageing and the response of plasma insulin, glucose and C-peptide concentrations to intravenous glucose in postmenopausal women. Clin Sci (Colch) 83:489-494, 1992

7. Godsland I, Walton C, Stevenson J: Impact of menopause on metabolism, in Diamond M, Naftolin F (eds): Metabolism in the Female Life Cycle. Rome, Italy, Ares Serono Symposia, 1993, pp 171-189

8. Walton C, Godsland I, Proudler A, et al: The effects of the menopause on insulin sensitivity, secretion and elimination in non-obese, healthy women. Eur J Clin Invest 23:466-473, 1993

9. Godsland I: The influence of female sex steroids on glucose metabolism and insulin action. J Intern Med 240:1-60, 1996 (suppl 738)

10. Manson J, Rimm E, Colditz G, et al: A prospective study of postmenopausal estrogen therapy and subsequent incidence of non–insulin dependent diabetes mellitus. Am J Epidemiol 2:665-673, 1992

11. Godsland I, Gangar K, Walton C, et al: Insulin resistance, secretion and elimination in postmenopausal women receiving oral or transdermal hormone replacement therapy. Metabolism 42:846-853, 1993

12. Trinder P: Determination of blood glucose using an oxidase-peroxidase system with noncarcinogenic chromogen. J Clin Pathol 22:158-161, 1969

13. Bergman R, Ider Y, Bowden C, et al: Quantitative estimation of insulin sensitivity. Am J Physiol 236:E667-E677, 1979

14. Walton C, Godsland I, Proudler A, et al: Evaluation of four mathematical models of glucose and insulin dynamics with analysis of the effects of age and obesity. Am J Physiol 262:E755-E762, 1992

15. Swan J, Walton C, Godsland I: Assessment of insulin sensitivity in man: A comparison of minimal model and euglycaemic clamp derived measures in health and heart failure. Clin Sci (Colch) 86:317-322, 1994

16. Toffolo G, Bergman R, Finegood D, et al: Quantitative estimation of beta-cell sensitivity to glucose in the intact organism. Diabetes 29:979-990, 1980

17. Volund A, Polonsky K, Bergman R: Calculated pattern of intraportal insulin appearance without independent assessment of C-peptide kinetics. Diabetes 36:1195-1202, 1987

18. Watanabe R, Volund A, Roy S, et al: Prehepatic beta-cell secretion during the intravenous glucose tolerance test in humans: Application of a combined model of insulin and C-peptide kinetics. J Clin Endocrinol Metab 69:790-797, 1989

19. Godsland I, Felton C, Proudler A, et al: Evaluation of two methods for estimating pancreatic insulin secretion by modelling analysis of the intravenous glucose tolerance test. Diabet Med 8:23A, 1991 (suppl 1, abstr)

20. Crook D, Godsland I, Hull J, et al: Hormone replacement therapy with dydrogesterone and 17-beta oestradiol: Effects on serum lipoproteins and glucose tolerance during 24 month follow-up. Br J Obstet Gynaecol 104:298-304, 1997

21. Burch D, Spowart K, Jesinger D, et al: A dose ranging study of the use of cyclical dydrogesterone with continuous 17 beta oestradiol. Br J Obstet Gynaecol 102:243-248, 1995

22. Brussaard H, Gevers-Leuven J, Frolich M, et al: Short-term oestrogen replacement therapy improves insulin resistance, lipids and fibrinolysis in postmenopausal women with NIDDM. Diabetologia 40:843-849, 1997

23. Houssay B, Foglia V, Rodriguez R: Production and prevention of some types of experimental diabetes by oestrogens or corticosteroids. Acta Endocrinol (Copenh) 17:146-164, 1954

24. Godsland I, Crook D, Walton C, et al: Influence of insulin resistance, secretion, and clearance on serum cholesterol, triglycerides, lipoprotein cholesterol, and blood pressure in healthy men. Arterioscler Thromb 12:1030-1035, 1992

25. Writing Group for the PEPI: Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women: The Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial. JAMA 273:199-208, 1995

26. Lindheim S, Presser S, Ditkoff E, et al: A possible bimodal effect of estrogen on insulin sensitivity in postmenopausal women and the attenuating effect of added progestin. Fertil Steril 60:664-667, 1993

27. Goldman J, Ovadia J: The effect of estrogen on intravenous glucose tolerance in women. Am J Obstet Gynecol 103:172-178, 1969

28. Crook D, Godsland I, Hull J, et al: Hormone replacement therapy with dydrogesterone and 17 beta-oestradiol: Effects on serum lipoproteins and glucose tolerance during 24 month follow-up. Br J Obstet Gynaecol 104:298-304, 1997

29. Walton C, Godsland I, Proudler A, et al: The effects of the menopause on carbohydrate metabolism in healthy women. J Endocrinol 129:97A, 1991 (suppl, abstr)

30. Seige K, Hevelke G: The effect of female gonadal function on the manifestation and frequency of diabetes mellitus, in Sixth Symposium of the German Endocrinological Society: Modern Developments in Progestagenic Hormones in Veterinary Medicine. Kiel, Germany, Springer Verlag, 1959, pp 274-279

31. Rodriguez R: Influence of estrogen and androgens on the production and prevention of diabetes, in Leibel B, Wrenshall G (eds): On the Nature and Treatment of Diabetes. New York, NY, Excerpta Medica, 1965, pp 288-307

32. Gabal L, Goodman-Gruen D, Barrett-Connor E: The effect of postmenopausal estrogen therapy on the risk of non–insulin dependent diabetes mellitus. Am J Public Health 87:433-435, 1997