CLINICAL STUDY

Effects of tibolone and cyclic hormone replacement therapy on glucose metabolism in non-diabetic obese postmenopausal women: a randomized study

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Abstract

Objective and methods: To study the effects of hormone replacement therapy on glucose metabolism, 31 obese (body mass index $\geq 27$ kg/m$^2$) postmenopausal women were randomized to treatment with tibolone (2.5 mg once daily; TIB; $n = 16$) or to oestradiol valerate (2 mg daily)-dydrogesterone (20 mg daily for 2 weeks every 3 months; ED; $n = 15$) for 12 months. Oral (OGTTs) and intravenous glucose tolerance tests (IVGTTs) and a euglycaemic hyperinsulinaemic clamp were performed before and at 6 and 12 months of treatment.

Results: TIB decreased the rates of whole body glucose uptake (WBGU) at 6 ($P = 0.04$) and 12 months ($P = 0.001$), but it did not have a significant effect on glucose tolerance. In OGTTs, serum insulin and C-peptide concentrations 2 h after the oral glucose load were increased ($P < 0.001$ and $P = 0.05$ respectively) at 12 months of treatment with TIB, but no changes in the areas under the curve (AUC) of insulin or C-peptide were observed. Furthermore, TIB did not have a significant effect on insulin secretion, the metabolic clearance rate (MCR) of insulin or hepatic insulin extraction. Treatment with ED did not modify the rates of WBGU, but it increased the MCR of insulin ($P = 0.017$) and hepatic insulin extraction ($P < 0.001$) and tended to decrease the insulin AUC ($P = 0.07$). Moreover, glucose tolerance slightly deteriorated during this treatment ($P = 0.02$). Although early phase insulin secretion evaluated by the serum C-peptide response at 30 min in the OGTT increased ($P = 0.046$), the first-phase insulin response during the IVGTT decreased ($P = 0.05$) during ED treatment.

Conclusions: Despite the impairment in peripheral insulin sensitivity, TIB treatment had a neutral effect on glucose tolerance, possibly due to a compensatory decrease in endogenous glucose production. The increased demand on insulin induced by ED, due to both a stimulatory effect on pancreatic $\beta$ cells and increased insulin metabolism, may explain the slightly detrimental effect on glucose tolerance with this treatment.

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Introduction

Menopause is associated with a progressive increase in insulin resistance and serum insulin concentrations (1, 2). Insulin resistance and factors associated with it, however, are major risk factors for the development of cardiovascular disease and type 2 diabetes mellitus (DM), especially in obese women (1, 3). In previous non-randomized studies, the use of oral oestrogen replacement therapy has been associated with a decreased incidence of coronary heart disease (CHD) in healthy postmenopausal women (4–7). Several factors, such as improved insulin sensitivity and reduction of hyperinsulinaemia, have been suggested to have cardioprotective effects (8–10). However, recent blinded, randomized trials have suggested that hormone replacement therapy (HRT) may even increase the risk of coronary events in previously healthy postmenopausal women (11) and in women with documented CHD prior to randomization (12).

Combined HRT regimens may, however, differ substantially in their effects on insulin and glucose metabolism. Oral treatment with medroxyprogesterone acetate and norgestrel in combination with conjugated equine oestrogens or oestradiol in postmenopausal women has been shown to worsen glucose metabolism (13–15). On the other hand, the increase in insulin resistance seen with the administration of C21 progestogens and 19-nortestosterone derivatives is not observed with dydrogesterone, which has been reported to be neutral as regards glucose metabolism (16, 17).

Tibolone (TIB) is a synthetic steroid compound with oestrogenic and, to a lesser extent, progestogenic and androgenic properties (18). While it relieves climacteric
symptoms (19, 20) and prevents postmenopausal osteoporosis (21, 22), no endometrial proliferation occurs and amenorrhea is produced (23–25). Because of its androgenic properties, TIB is effective in improving well-being and libido, but it has been shown to have an unfavourable effect on the serum lipid profile (26, 27). However, only a few studies have been focused on its effects on insulin sensitivity and glucose metabolism (28–30).

The aim of this study was to evaluate and compare the effects of TIB and oestradiol–dydrogesterone (ED) on glucose metabolism, assessed by oral (OGTTs) and intravenous glucose tolerance tests (IVGTTs) and a euglycaemic clamp technique.

**Subjects and methods**

**Subjects**

Seventy postmenopausal women seeking help for climacteric symptoms were contacted through an advertisement in a local newspaper and 46 obese women (body mass index (BMI) ≥ 27 kg/m²) were screened (by L C M-P). Eight subjects were excluded and 38 were randomized (via computer-generated assignment) to the TIB (2.5 mg once daily, NV Organon, Oss, The Netherlands) or the ED (oestradiol valerate) (2 mg daily, Estrofem, Novo Nordisk, Espoo, Finland) and dydrogesterone (20 mg daily for 2 weeks every 3 months, Terolut, Solway Pharmaceuticals, Weest, The Netherlands) group for 12 months (L C M-P). Because of the different bleeding schedules of the HRTs, neither patients nor investigators could be blinded to treatment. Five women were found to have type 2 DM at entry and they were excluded from the study. Two women (ED group) stopped, one because of nausea and the other was lost to follow-up (Fig. 1).

All women included in the study were 45–65 years of age, had been in natural menopause for at least 6 months, and their serum follicle-stimulating hormone levels were higher than 30 IU/l. The women on HRT underwent a washout period of at least 3 months prior to the study. Alcohol users and those taking drugs known to affect glucose metabolism during the 3 months preceding the study were excluded. Before and at the end of the study, a gynaecological examination was performed, including general and gynaecological history, palpation of the breasts and...
mammography (if not performed in the last year), a
Papanicolaou smear, vaginal ultrasonography and an
endometrial biopsy.

All women underwent an OGTT, an IVGTT and a
euglycaemic hyperinsulinaemic clamp before and at 6
and 12 months of treatment. To ensure a minimal pro-
gestogen effect on glucose metabolism, the subjects in
the ED group were evaluated at least 7 days after the
last dydrogesterone pill. None of the subjects had a
known personal history of glucose or lipid alterations.

The study was approved by the Ethics Committee of
the University of Oulu, Finland, and informed written
consent was obtained from each subject.

**OGTT**

After an overnight fast of 10–12 h, all subjects under-
went an OGTT (a load of 75 g glucose in 300 ml water).
Venous blood samples for blood glucose, serum insulin
and serum C-peptide assays were drawn at 0, 30, 60
and 120 min. The glycaemic response to the OGTT
was defined according to the American Diabetes Asso-
ciation 1997 criteria (31).

**IVGTT**

The first-phase insulin response was determined by
means of an IVGTT. At 0800 h, after a 12-h overnight
fast, an intravenous catheter was placed in an ante-
cubital vein for infusion of glucose. Another cannula
for blood sampling was inserted into a wrist vein of
the other hand, surrounded by a heated box (40°C).
After baseline blood collection and measurement of
gas exchange (see Calorimetry, below), a bolus of glu-
cose (300 mg/kg in a 50% solution) was given
(within 30 s) via the antecubital vein to increase the
blood glucose level acutely. Samples for the measure-
ment of blood glucose and serum insulin were drawn at −5, 0, 2, 4, 6, 8 and 10 min from the wrist vein.

**Euglycaemic hyperinsulinaemic clamp**

The euglycaemic hyperinsulinaemic clamp technique
was used for assessment of peripheral insulin sensitivity
(32). After the IVGTT, a priming dose of insulin infu-
sion (Actrapid, 100 IU/ml; Novo Nordisk, Genstofe,
Denmark) was administered during the initial 10 min
to raise serum insulin acutely to the desired level,
where it was maintained by continuous insulin infusion
of 80 mU/m² body surface area per min. Blood glucose
was clamped at 5 mmol/l for the next 180 min by
adjusting the rate of 20% glucose infusion according
to blood glucose measurements performed every
5 min using a photometric assay (HemoCue AB, Ängel-
holm, Sweden). The M-value (expressing the rate of
whole body glucose uptake (WBGU): μmol/kg per
min) was calculated as the mean value for each
20-min interval during the last 60 min of the clamp.

The higher the M-value, the better is peripheral insulin
sensitivity. The coefficient of variation for blood glucose
was <4% in all clamps in this study. As it has been
previously shown that, in non-diabetic hyperandro-
genic subjects, endogenous glucose production is negli-
gible at this insulin infusion rate, the amount of glucose
infused may be considered equivalent to WBGU (33).
Blood samples for assays of serum insulin and free fatty acids (FFAs) were drawn at 0, 120, 140, 160
and 180 min.

**Calorimetry**

Indirect calorimetry was performed with a computer-
ized flow-through canopy gas analyzer system (DELTA-
TRAC; TM Datex, Helsinki, Finland) in connection with
the euglycaemic clamp, as previously described (34).
This device has a precision of 2.5% for O₂ consumption
and 1.0% for CO₂ production. On the day of the exper-
iment, gas exchange (O₂ consumption and CO₂ pro-
duction) was measured for 30 min after a 12-h fast
before and during the last 30 min of the clamp. The
values obtained during the first 10 min of both time-
periods were discarded, and the mean values for the
remaining 20 min of data were used for calculation.
Protein, glucose and lipid oxidation were calculated
according to Ferrannini (35). Protein oxidation was
 calculated on the basis of the urinary non-protein
nitrogen excretion rate (35). The fraction of carbo-
hydrate non-oxidation during the euglycaemic clamp
was estimated by subtracting the carbohydrate oxida-
tion rate (determined by indirect calorimetry) from
the glucose infusion rate (determined by the euglyca-
emic clamp).

**Assays and calculations**

The concentrations of C-peptide (Diagnostic Products
Corporation, Los Angeles, CA, USA) and insulin (Phar-
macia Diagnostics, Uppsala, Sweden) were analysed by
radioimmunoassays, following the manufacturer’s
instructions. Levels of blood glucose and FFAs were
determined by standard methods. The intra- and inter-
assay coefficients of variation were 5.3 and 7.2% for
C-peptide respectively and 5.3 and 7.6% for insulin
respectively.

The insulin, glucose and incremental C-peptide areas
under the curve (AUC) were calculated by the trapezoi-
dal method. The fasting serum C-peptide/fasting serum
insulin molar ratio was calculated as an index of
hepatic insulin extraction in the fasting state (36).
The metabolic clearance rate (MCR) of insulin was
 calculated from the formula: MCR = (infusion rate of
exogenous insulin during the euglycaemic clamp)/
(mean plasma insulin concentration during the last
60 min of the euglycaemic clamp), expressed as
ml/min per kg. The homeostasis model assessment
(HOMA) index for the estimation of insulin sensitivity
used the formula described by Matthews et al. (37): (fasting insulin × fasting glucose)/22.5.

**Statistical analysis**

Where there were normally distributed variables, analysis of variance for repeated measures was used to compare the clinical, metabolic and hormonal parameter changes within the ED and TIB groups during the treatment, either without or with logarithmic transformation. The Wilcoxon unpaired test was used for variables with persisting skewed distribution after log transformation.

Differences between the two treatments were summarized and compared for all the parameters of the study, as changes from baseline, according to Altman (38).

For comparison between the TIB and ED groups before and at 6 and 12 months of treatment, Student’s two-tailed t-test was used for normally distributed variables, either without or with log transformation. The Mann–Whitney U test was used for variables with persisting skewed distribution after log transformation.

Determinants of blood glucose AUC in the OGTT were evaluated by multiple regression analysis. The factors included in model 1 were the incremental changes in insulin AUC in the IVGTT, WBGU, MCR of insulin, hepatic insulin extraction in the fasting state and the C-peptide level at 30 min in the OGTT, and in model 2 the factors were insulin level at 30 min in the OGTT, WBGU, MCR of insulin, hepatic insulin extraction in the fasting state and the C-peptide level at 30 min in the OGTT.

**Results**

All parameters analysed at 6 and 12 months of treatment were comparable. Therefore, the results at 6 months are shown only when they are of special interest and reinforce the observation.

**Clinical and biochemical characteristics**

The groups were comparable with respect to age, BMI, waist-to-hip ratio (WHR), fasting blood glucose, fasting insulin and fasting C-peptide (Table 1). BMI did not change during either treatment, but the WHR tended to decrease in the ED group compared with the TIB group \( (P = 0.06) \). Fasting blood glucose levels decreased in both groups (TIB group: \( P = 0.02 \); ED group: \( P = 0.01 \)). Fasting insulin concentrations decreased remarkably during ED treatment \( (P = 0.001) \) and this decrease was more pronounced compared with that in the TIB group \( (P = 0.04) \), in whom fasting insulin concentrations did not change significantly. Although no significant changes in fasting C-peptide concentrations were observed in either study group, the ED group showed a greater increase in these concentrations compared with the TIB group \( (P = 0.002) \) (Table 1).

**Glucose tolerance**

At the baseline examination, ten women had normal glucose tolerance (NGT), one woman had IFG and five women had IGT in the TIB group. After 12 months of treatment with TIB, three of the ten women with NGT had converted to IGT, whereas the rest retained NGT. The woman with impaired fasting glycaemia (IFG) had converted to impaired glucose tolerance (IGT). One of the five women with IGT developed DM, whereas the rest of them converted to NGT. In the ED group, nine women had NGT; five women had IFG and one woman had IGT at baseline. After 12 months of treatment with ED, three of the nine women with NGT had developed IGT and the rest retained NGT. One of the women with IFG had converted to IGT and the other four had converted to NGT, as did the woman with IGT in this group.

Glucose tolerance when expressed as blood glucose AUC during the OGTT did not change significantly

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**Table 1** Clinical and biochemical characteristics of the study subjects before and during treatment. The results are given as means±S.E. and the reference ranges are shown in brackets.

<table>
<thead>
<tr>
<th></th>
<th>TIB group</th>
<th>ED group</th>
<th>Effect of TIB vs ED (P)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Before (n = 16)</td>
<td>6 months (n = 16)</td>
<td>12 months (n = 16)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>55.1±1.2</td>
<td>32.9±0.9</td>
<td>32.5±0.9</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>(20–25 kg/m²)</td>
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</tr>
<tr>
<td>WHR (&lt;0.85)</td>
<td>0.88±0.01</td>
<td>0.89±0.01</td>
<td>0.88±0.01</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol/l) (3.3–5.6 mmol/l)</td>
<td>5.4±0.1</td>
<td>5.3±0.1</td>
<td>5.1±0.1</td>
</tr>
<tr>
<td>Fasting insulin (pmol/l) (30–120 pmol/l)</td>
<td>88.1±9.5</td>
<td>88.9±6.6</td>
<td>75.8±7.6</td>
</tr>
<tr>
<td>Fasting C-peptide (pmol/l) (200–1000 pmol/l)</td>
<td>560±0.06</td>
<td>670±0.06</td>
<td>590±0.09</td>
</tr>
</tbody>
</table>

\*P < 0.05, \^P < 0.01 and \$P < 0.001 compared with the level before treatment; NS, not significant.
during TIB treatment, the glucose AUC did, however, increase in the ED group \( (P = 0.02) \), but this change did not significantly differ from that in the TIB group (Table 2 and Fig. 2). Blood glucose levels at 60 min in the OGTT in the TIB group \( (P = 0.02) \) and at 60 min and 120 min in the ED group \( (P = 0.002 \) and \( P = 0.04 \) respectively) increased during these treatments (Fig. 2).

### Insulin secretion and peripheral insulin response

**OGTT** The early phase insulin secretory response, evaluated by C-peptide concentration at 30 min in the OGTT, did not change during TIB treatment, whereas it was increased in the ED group at 12 months \( (P = 0.003) \) (Table 2). The simultaneous serum insulin response at 30 min did not change significantly in either group (Table 2). The insulin secretory response, when expressed as incremental C-peptide AUC, did not increase significantly during either treatment (Table 2 and Fig. 2). Moreover, the peripheral insulin response, when expressed as insulin AUC, did not change during TIB treatment, but tended to decrease in the ED group \( (P = 0.07) \) (Table 2 and Fig. 2b). Furthermore, insulin concentrations at 60 min \( (P = 0.023) \) and 120 min \( (P < 0.001) \) were decreased during ED treatment, whereas in the TIB group both insulin and C-peptide concentrations were increased at 120 min \( (P < 0.001 \) and \( P = 0.005 \) at 12 months respectively; Fig. 2).

**IVGTT** Serum insulin concentrations after the intravenous glucose load were comparable between the study groups at the baseline examination (Fig. 3), insulin concentrations at 2 and 4 min were increased during TIB treatment \( (P = 0.004 \) and \( P = 0.019 \) respectively) (Fig. 3a). In the ED group, insulin concentrations were decreased at 8 and 10 min at 12 months of treatment (Fig. 3b). Furthermore, the first-phase insulin response when expressed as insulin AUC in the IVGTT was decreased during ED treatment \( (P = 0.050) \) (Fig. 3b).

### Insulin metabolism

The MCR of insulin did not change significantly during TIB treatment (baseline: 10.1 ± 0.4 ml/min per kg; 12 months: 10.7 ± 0.5 ml/min per kg; \( P = 0.12) \), but it increased significantly during ED treatment (baseline: 10.2 ± 0.5 ml/min per kg; 12 months: 11.7 ± 0.9 ml/min per kg; \( P = 0.017) \). Furthermore, hepatic insulin extraction in the fasting state was increased significantly in the ED group at 12 months (baseline: 5.6 ± 0.7 pmol per l; 12 months: 10.7 ± 0.8 pmol per l; \( P < 0.001 \)), and also as compared with that in the TIB group \( (P < 0.001) \), but it did not change significantly in the TIB group.

### Insulin sensitivity, lipid and glucose oxidation and serum FFA levels

Treatment with TIB resulted in reduction in the rate of WBGU measured during the hyperinsulinaemic euglycaemic clamp (baseline: 38.1 ± 2.1 pmol/kg per min; 6 months: 33.0 ± 1.8 pmol/kg per min; \( P = 0.04 \); 12 months: 31.1 ± 1.3 pmol/kg per min; \( P < 0.001) \) (Fig. 3a). Treatment with ED, on the other hand, did not have any effect on the rate of WBGU (baseline: 40.8 ± 1.5 pmol/kg per min; 12 months: 40.0 ± 1.5 pmol/kg per min; not significant (NS)) (Fig. 3b). The lower rates of WBGU during TIB treatment were mainly accounted for by lower glucose non-oxidation (baseline: 26.2 ± 1.8 pmol/kg per min; 12 months: 19.4 ± 1.3 pmol/kg per min; \( P = 0.003) \) (Fig. 3a). Glucose non-oxidation did not change in the ED group (baseline: 29.3 ± 1.2 pmol/kg per min; 12 months: 28.1 ± 1.0 pmol/kg per min; NS).

### Table 2 Glucose tolerance, insulin secretory response and peripheral insulin response during the OGTT before and during treatment.

The results are given as means ± S.E.

<table>
<thead>
<tr>
<th></th>
<th>TIB group</th>
<th>ED group</th>
<th>Effect of TIB vs ED (P)</th>
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<tbody>
<tr>
<td></td>
<td>Before ( (n = 16) )</td>
<td>6 months ( (n = 16) )</td>
<td>12 months ( (n = 16) )</td>
</tr>
<tr>
<td>Glucose tolerance</td>
<td></td>
<td></td>
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<tr>
<td>Glucose AUC (mmol/l per h)</td>
<td>14.6 ± 0.8</td>
<td>15.8 ± 0.8</td>
<td>15.6 ± 0.6</td>
</tr>
<tr>
<td>Insulin secretory response</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>C-peptide at 30 min (pmol/l)</td>
<td>1450 ± 217</td>
<td>1600 ± 172</td>
<td>1338 ± 216</td>
</tr>
<tr>
<td>Incremental C-peptide AUC (pmol/l per h)</td>
<td>572 ± 167</td>
<td>881 ± 258</td>
<td>561 ± 166</td>
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<tr>
<td>Peripheral insulin response</td>
<td></td>
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</tr>
<tr>
<td>Insulin at 30 min (pmol/l)</td>
<td>96.5 ± 13.4</td>
<td>99.2 ± 14.2</td>
<td>85.7 ± 13.1</td>
</tr>
<tr>
<td>Insulin AUC (pmol/l per h)</td>
<td>165 ± 22</td>
<td>200 ± 22</td>
<td>171 ± 18</td>
</tr>
</tbody>
</table>

*\( P < 0.05 \) and b \( P < 0.01 \) compared with the level before treatment; NS, not significant.
Figure 2 Serum glucose, insulin and C-peptide concentrations during OGTTs before (□) and at 12 months (●) of (a) TIB and (b) ED treatment. *P < 0.05, †P < 0.01 and ‡P < 0.001 compared with the level before treatment. AUC gluc, change in AUGgluc at 12 months of treatment compared with value before treatment.

Figure 3 Serum insulin concentrations during IVGTTs before (□) and at 12 months (●) of (a) TIB and (b) ED treatment, and WBGU (including glucose oxidation (solid bars) and non-oxidation (open bars)) during the clamp before and at 12 months of TIB and ED treatment. *P < 0.05, †P < 0.01 and ‡P < 0.001 compared with the level before treatment. AUC ins, insulin AUC.
Glucose oxidation rates during the clamp were not affected during either treatment (Table 3).

Glucose oxidation rates in the fasting state remained unchanged during TIB treatment, but they were increased after 12 months of ED treatment ($P = 0.026$), also when compared with the TIB group ($P = 0.04$) (Table 3). Correspondingly, lipid oxidation in the fasting state tended to be decreased at 12 months in the ED group ($P = 0.096$), also when compared with the TIB group ($P = 0.050$), in which it did not change (Table 3). Lipid oxidation during the hyperinsulinaemic clamp did not change significantly, although it tended to be decreased in the ED group at 12 months when compared with that in the TIB group. Serum FFA levels remained unchanged during both treatments (Table 3).

The HOMA index decreased significantly at 1 year of treatment in both groups from $3.53 \pm 0.41$ before treatment to $2.87 \pm 0.30$ ($P = 0.05$) in the TIB group and from $3.39 \pm 0.69$ to $1.72 \pm 0.13$ ($P < 0.001$) in the ED group.

Multiple linear regression analyses

In stepwise multiple linear regression analysis, incremental insulin AUC in the IVGTT was the only significant determinant of glucose tolerance after 12 months of treatment with ED, explaining 63% of the variation of the glycaemic response in the OGTT (model 1).

Discussion

The present study is the first to show that TIB treatment leads to reduction in peripheral insulin sensitivity in non-diabetic obese postmenopausal women. Despite that, it did not significantly affect glucose tolerance, as evaluated by OGTTs, and it did not modify either insulin secretion or insulin metabolism at 12 months of treatment. Treatment with ED, despite having no effect on peripheral insulin sensitivity, led to a slight deterioration of glucose tolerance. Although it resulted in increased insulin secretion, as evaluated by the C-peptide response at 30 min during OGTTs, the subsequent peripheral insulin response decreased, most likely due to increased elimination of insulin.

The women on TIB experienced a significant decrease in peripheral insulin sensitivity at 6 and 12 months of treatment. The effects of TIB on insulin sensitivity have been previously specifically investigated in only one study in a similar group of women (28). In that non-randomized prospective study, treatment with TIB for 3 months led to an improvement in insulin sensitivity, as estimated by the minimal model method (28). This method gives an overall estimate of insulin action in glucose metabolism in insulin-dependent tissues (i.e. the muscle and the liver) at physiological and dynamic insulin levels, where the hepatic and the peripheral components of insulin action cannot be distinguished. In the present study, insulin action was measured at supraphysiological plasma insulin levels capable of fully suppressing hepatic glucose output (33) and thus giving a direct measure of peripheral insulin sensitivity. Interestingly, despite the impairment of peripheral insulin sensitivity, treatment with TIB resulted in the decline of fasting blood glucose levels and unchanged fasting insulin concentrations. This finding strongly suggests that TIB has beneficial effects on insulin action in the liver (i.e. inhibition of hepatic glucose output). This may also explain the opposite results of these two studies. Furthermore, in the present study, insulin sensitivity, estimated by the HOMA index (37), improved during the TIB treatment.

### Table 3 Glucose and lipid oxidation and serum FFA concentrations in the fasting state and during the euglycaemic clamp before and during treatment. The results are given as means±S.E. and the reference ranges of FFAs is shown in brackets.

<table>
<thead>
<tr>
<th></th>
<th>TIB group</th>
<th></th>
<th>ED group</th>
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<th>Effect of TIB vs ED ($P$)</th>
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<tbody>
<tr>
<td></td>
<td>Before</td>
<td>6 months</td>
<td>12 months</td>
<td>Before</td>
<td>6 months</td>
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<td></td>
<td>($n = 16$)</td>
<td>($n = 16$)</td>
<td>($n = 16$)</td>
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<tr>
<td><strong>Fasting</strong></td>
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<tr>
<td>Glucose oxidation</td>
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</tr>
<tr>
<td>($\mu$mol/kg per min)</td>
<td>6.2±0.6</td>
<td>5.0±0.8</td>
<td>6.0±0.5</td>
<td>4.8±0.5</td>
<td>4.5±0.5</td>
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<tr>
<td>Lipid oxidation</td>
<td>0.84±0.05</td>
<td>0.93±0.06</td>
<td>0.82±0.05</td>
<td>0.82±0.03</td>
<td>0.85±0.05</td>
</tr>
<tr>
<td>Serum FFAs (mmol/l)</td>
<td>0.60±0.07</td>
<td>0.91±0.19</td>
<td>0.56±0.05</td>
<td>0.64±0.04</td>
<td>0.58±0.06</td>
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<tr>
<td>(0.08–0.70 mmol/l)</td>
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<td><strong>Euglycaemic clamp</strong></td>
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<tr>
<td>Glucose oxidation</td>
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<tr>
<td>($\mu$mol/kg per min)</td>
<td>11.9±0.7</td>
<td>12.6±0.9</td>
<td>11.7±0.7</td>
<td>11.5±0.7</td>
<td>11.6±0.8</td>
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<tr>
<td>Lipid oxidation</td>
<td>0.46±0.04</td>
<td>0.41±0.05</td>
<td>0.48±0.04</td>
<td>0.45±0.05</td>
<td>0.43±0.04</td>
</tr>
<tr>
<td>Serum FFAs (mmol/l)</td>
<td>0.08±0.02</td>
<td>0.09±0.04</td>
<td>0.15±0.05</td>
<td>0.06±0.02</td>
<td>0.05±0.006</td>
</tr>
</tbody>
</table>

* $P < 0.05$ compared with the level before treatment. NS, not significant.
The mechanisms responsible for the impairment in peripheral insulin sensitivity in this study remain unclear. The androgenic effects of TIB may play an important role, since hyperandrogenism has been shown to induce insulin resistance (39), most likely by increasing the number of less-insulin-sensitive type IIb skeletal muscle fibres (40). Furthermore, increased numbers of these muscle fibres have been shown to be inversely associated with non-oxidative glucose uptake (41). In line with this, in our study, the impairment in insulin sensitivity during TIB treatment was mainly accounted for by lowered rates of glucose non-oxidation.

Although in the present study TIB treatment resulted in an approximately 20% reduction in peripheral insulin sensitivity, it did not have any significant effect on glucose tolerance at 1 year of treatment. This finding is in line with recent data (42), but in contrast with the results of a previous study in which a slight deterioration of glucose tolerance in non-diabetic women treated with TIB was reported (29). In the present study, the reduction in insulin sensitivity was at least partly compensated for by increased insulin secretion at 2 h after an oral glucose load but, on the other hand, TIB treatment did not result in hyperinsulinaemia as judged by the insulin AUC during the OGTT. Since TIB had neutral effects on insulin secretory capacity and insulin metabolism, this finding could be explained by other compensatory mechanisms, possibly by improvement of insulin action in the liver (i.e. decreased hepatic glucose output during the fasting state and after the oral glucose load).

Many investigators have studied the effects of oestrogen substitution alone or in combination with progestogens on insulin sensitivity in postmenopausal women, with contradictory results (10, 15, 43). Our study showed that ED treatment did not have any effect on peripheral insulin sensitivity. In only one study has detailed evaluation of insulin sensitivity when combining cyclic dydrogesterone with oestradiol been reported. In that study, 6 months of ED treatment ameliorated insulin sensitivity in hyperinsulinaemic postmenopausal women, measured by the hyperinsulinaemic euglycaemic clamp, but showed neutral effects in normoinsulininaemic postmenopausal women (44). In our study, the women treated with this combination, albeit obese, were not as hyperinsulinaemic as the women in the study by Cucinelli et al. (44) and showed no change in insulin sensitivity during 12 months of treatment. Thus, on the basis of these two studies, ED treatment has neutral or even favourable effects on insulin sensitivity in obese postmenopausal women.

Despite having no significant effect on WBGU, treatment with ED resulted in a slight improvement of the fasting glucose oxidation rates. According to the hypothesis proposed by Randle et al. (45), FFAs compete with glucose for oxidation in skeletal muscle, and a decrease in lipid oxidation rates can improve glucose disposal (oxidation). Accordingly, in the present study, ED treatment induced a slight reduction in fasting lipid oxidation rates, and it also resulted in a decreased WHR. This effect may be explained by increased insulin binding in hepatocytes (46), which leads to suppression of lipid oxidation rates in the liver, a major part of fatty acid metabolism. This, in turn, would then lead to compensatory stimulation of lipogenic pathways, i.e. triglyceride production (47) and increase glucose oxidation rates and decrease glucose output from the liver. In line with this, oestrogen treatment has been shown to inhibit lipid oxidation and increase glucose oxidation (48). However, other mechanisms mediated through oestrogen-sensitive hepatic proteins and/or oestrogen receptors on the adipocytes (49) may also be possible.

In the present study, ED treatment led to improved insulin secretion, as evaluated by C-peptide concentration at 30 min after the oral glucose load. Furthermore, both the MCR of insulin and hepatic insulin extraction increased during this treatment, leading to a reduction in fasting- and OGTT-induced serum insulin concentrations. In line with these findings, it has been shown that both oestrogen and progesterone increase insulin secretion from the pancreatic β cells (50). Moreover, in a recent study in non-diabetic postmenopausal women, it was demonstrated that there was an exaggerated OGTT-induced plasma C-peptide response in relation to a normal plasma insulin response after 6 months of ED treatment (43), and in another study it was reported that there were increased fasting plasma C-peptide concentrations but lowered fasting plasma insulin concentrations in non-diabetic postmenopausal women after 12 months of treatment with ED (51). These studies and our results strongly suggest that this treatment leads not only to increased insulin secretion but also to increased insulin elimination, most likely as a result of improved hepatic insulin extraction. Moreover, oestradiol and progesterone treatments have been shown to increase insulin binding and degradation in rat hepatocytes (45). Whether potentiated insulin secretion and hepatic insulin extraction reflect distinct effects of ED on β cells and hepatocytes, or whether they represent compensatory mechanisms remains unclear.

In the present study, 12 months of treatment with ED was unexpectedly associated with a slight deterioration of glucose tolerance. The results of many previous studies have suggested that oestradiol treatment could improve glucose tolerance in postmenopausal women (4, 19), and that the addition of dydrogesterone does not oppose this beneficial effect of oestradiol (16, 42, 51). Since obese postmenopausal women are at risk of type 2 DM (1 – 3), it is likely that the increase in blood glucose levels during the OGTT in our study was due to natural impairment of glucose tolerance with age rather than being an effect of ED treatment per se. The worsening of glucose tolerance in individuals at high
risk of type 2 DM is often associated with progressive deterioration of first- and early phase insulin secretory capacity (52). Although early phase insulin secretion estimated by the plasma C-peptide response increased during ED treatment in the present study, the resultant peripheral insulin response was still low in relation to the glycaemic response during the OGGT. Furthermore, the first-phase insulin response in the IVGTT decreased during ED treatment, and in linear regression analyses it explained 63% of the variation in the glycaemic response. Therefore, it cannot be ruled out that the long-term increased demand on insulin induced by ED, due to both a stimulatory effect on pancreatic β cells and increased insulin metabolism, may result in some degree of exhaustion of pancreatic β cells in obese postmenopausal women, leading to impairment of glucose tolerance.

Conclusions
In conclusion, despite the impairment in peripheral insulin sensitivity, TIB treatment seems to have a neutral effect on glucose tolerance in non-diabetic obese postmenopausal women, possibly partly due to an improvement of insulin sensitivity in the liver. The slightly detrimental effect of ED treatment on glucose tolerance might be explained by the increased demand on insulin induced by ED (due to both a stimulatory effect on pancreatic β cells and increased insulin metabolism) or, most likely, to the natural impairment of glucose tolerance with age. Therefore, the present results suggest that TIB is a neutral alternative in HRT as regards glucose tolerance in non-diabetic obese postmenopausal women. However, larger placebo-controlled follow-up studies are needed to confirm these findings.

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