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A randomized trial on the effect of oral combined estradiol and drospirenone on glucose and insulin metabolism in healthy menopausal women with a normal oral glucose tolerance test☆

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ABSTRACT

Background: Menopause is often associated with a central accumulation of body fat. This provokes insulin resistance. The resulting hyperinsulinemia may increase the risk of diabetes, cardiovascular disease and breast cancer. Long-term studies indicate that menopausal hormone therapy (MHT) reduces insulin resistance. To broaden knowledge of the mechanisms behind the influence of MHT on glucose homeostasis we focused on the direct short-term effects of MHT with oral combined estradiol and drospirenone on glucose and insulin metabolism in healthy postmenopausal women.

Methods: This randomized, placebo-controlled study recruited 80 healthy postmenopausal women. Women were randomized to treatment with estradiol 1 mg continuously combined with drospirenone 2 mg or placebo for 6–8 weeks. All participants underwent an oral glucose tolerance test (OGTT) before and after the treatment period. Glucose, insulin, fructosamine and C-peptide levels were measured in serum before and 30, 60, 90, 120 and 150 min after a 75-gram oral glucose challenge.

Results: After intervention, significantly higher glucose levels at 120 min ($p < 0.024$) and 150 min ($p < 0.030$) were observed in the MHT group compared with the placebo group. These glucose levels remained within the normal range.

A significantly lower insulin peak serum level ($p < 0.040$) and a non-significantly smaller area under the curve (AUC) for insulin levels ($p = 0.192$) was observed in the MHT group at the end of the study period relative to baseline. No significant change in the insulin AUC in the placebo group was observed. There were no significant differences in fructosamine, HOMA-IR and C-peptide levels between the MHT group and the placebo group.

Conclusion: This double-blind randomized study (EC/2008/694) indicates that treating healthy, postmenopausal women with 1 mg estradiol continuously combined with 2 mg drospirenone significantly decreases peak insulin levels and increases peak glucose levels during an OGTT compared to placebo. These glucose levels remained within the normal range.

1. Introduction

Menopause is often associated with a central accumulation of body fat [1]. This may provoke insulin resistance, leading to hyperinsulinemia [2]. An increased insulin level is a risk factor for diabetes [3,4] and cardiovascular disease [5]. Moreover, the Women's Health Initiative (WHI) trial revealed that hyperinsulinemia is an independent risk factor for postmenopausal breast cancer [6]. As obese postmenopausal women have higher levels of estrogen, it is believed that estrogens are linked to increased breast cancer risk. However, the association between obesity and estrogen receptor (ER)-negative breast cancer indicates that this connection is partially independent of estrogen in postmenopausal women.

Today, obesity is reaching epidemic proportions worldwide, and has

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a higher incidence in women than in men [7]. In addition, the years surrounding the menopause are associated with decreased physical activity and further weight gain [8]. During the menopause, body fat tends to shift from a gynoid to an android pattern, designated 'abdominal (central) adiposity'. There is strong evidence that MHT may partly prevent this menopause-related change in body composition and the associated metabolic sequelae [9–11]. As reviewed by Mauvais-Jaris et al. [12], several randomized and observational studies report a beneficial influence of menopausal hormone therapy on insulin resistance, fasting glucose, HbA1c and the incidence of diabetes.

To study the influence of MHT on glucose homeostasis, some studies use the euglycemic, hyperinsulinemic clamp while others use serum insulin, glucose and C-peptide concentrations during an IVGTT (intravenous glucose tolerance test) or an OGTT (oral glucose tolerance test) [12]. Differences in body mass index (BMI), age at inclusion, type of MHT (oral versus transdermal), type of estrogen (Equine estrogen versus natural estrogen; low versus high dose), type of progestogen (androgenic progestogen versus natural or non-androgenic progestogen) may all contribute to the differences observed between studies.

Since the intake of oral glucose (during meals or sugared drinks) is natural way of glucose intake, we preferred to use an OGTT in our study. The study of Godsland and coworkers [13] suggest that MHT improves insulin secretion and elimination in postmenopausal women. However, as this study lasted more than 2 years, subtle changes in body composition may have affected the findings.

To expand our knowledge on the influence of MHT on glucose and insulin homeostasis we conducted a double-blind, randomized controlled trial studying the short-term influence of a natural estrogen containing formulation continuously combined with a non-androgenic progestogen, in healthy young menopausal women, with a normal OGTT, not using any other medication.

2. Materials and methods

2.1. Study population

In total 98 women were recruited for this study (Fig. 1). Although all women met the criteria of menopause (1 year in amenorrhea or less than 1 year in amenorrhea with the following criteria: vasomotor symptoms, follicle stimulating hormone (FSH) levels greater than 40 U/L- obtained from the blood results from the referring physician) 6 women were excluded because they still had significant estradiol levels in the serum at study inclusion. Another 12 women were excluded because they were on medication (thyroid medication, lipid-lowering medication, antihypertensive drugs, psychotropic drugs, corticosteroids, pain medication, anti-reflux medication,...) or had fasting glucose levels above 100 mg/dl or had a history of hormone-sensitive tumors.

After exclusion of 18 women, 80 healthy Caucasian postmenopausal women, aged 47–63 years (median age: 54 ± 3 years in MHT and 55 ± 4 years in placebo group) and with an intact uterus, were included in the study. All OGTT tests were performed at the menopause center of the gynecology clinic in the Ghent University Hospital.

A routine gynecologic check-up was performed at recruitment, including a transvaginal ultrasound scan.

The study was approved by the medical ethics committee of the University Hospital of Ghent (EC/2008/694) and all patients signed an informed consent form before entering the study. This clinical trail was registered (eurdaCT number: 2008/003/661/19).

At the time of data analysis, 5 women were excluded as they were not completely menopausal at their first or second visit, 4 women were excluded because they had an OGTT value > 200 mg/dL after 120 min and were considered diabetic, and one woman had a local corticoid injection in her shoulder during the study period so study medication was stopped and the second OGTT was not performed. After these exclusions, the MHT group consisted of 37 women and the placebo group consisted of 33 women. There were no significant differences in baseline characteristics between the placebo group and the MHT group (Table 1).

2.2. Study protocol

This was a 6- to 8-week double-blind randomized controlled trial between 2010 and 2014. The women were randomized in 2 groups. The randomization was performed by a hospital pharmacist, who was the only person who knew the code. One group received orally administered daily doses of 1 mg estradiol and 2 mg drospirenone (the study medication, Angeliq®, was provided by Bayer-Schering (Belgium)). The study medication was repackaged in the hospital pharmacy in order to be indistinguishable from the placebo. The other group was administered an oral placebo. Both groups had to take one tablet a day.

All women had 2 visits, one before treatment and one after 6–8 weeks of treatment. The minimum duration of study medication intake was 6 weeks. Some women took the study medication for up to 8 weeks for practical reasons. Most women were still working at the time of inclusion for the study. Hence, intake of study medication was extended for one or maximum two weeks to fit in with work commitments. If week 6 coincided with a vacation, again further intake of study medication – placebo or active medication- was allowed for a short period. There was no difference in duration of intake of study medication

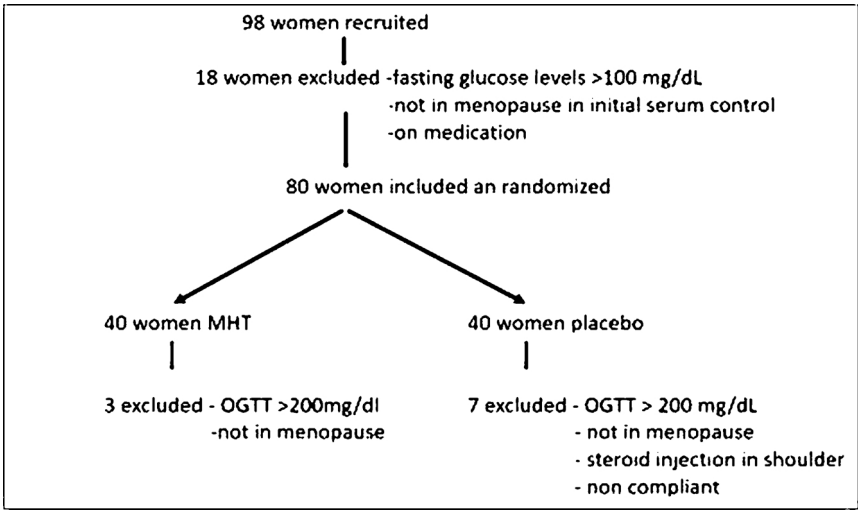

Fig. 1. flow chart of study inclusion.

Table 1
Baseline characteristics of the MHT and placebo groups.

	MHT (n = 37) Mean ± SD Median (Q1 – Q3)	Placebo (n = 33) Mean ± SD Median (Q1 – Q3)	p-value
Age (years)	54 ± 3	55 ± 4	0.333
Age at menopause (years)	50 ± 3	51 ± 4	0.663
Weight (kg)	66.7 ± 10.1	67.0 ± 10.5	0.895
Height (cm)	167 ± 5	165 ± 6	0.464
Body mass index (kg/m^2)	24.0 ± 3.5	24.6 ± 3.4	0.541
Waist circumference (cm)	88 ± 10	90 ± 10	0.494
Hip circumference (cm)	100 ± 8	104 ± 9	0.134
Mean diastolic blood Pressure (mmHg)	77 ± 8	78 ± 7	0.400
Mean systolic blood pressure (mmHg)	125 ± 15	125 ± 12	0.881
C-peptide (µg/L)	1.97 ± 0.55	2.18 ± 0.56	0.125
Fructosamine (µmol/L)	237 ± 18	241 ± 16	0.299
Glucose (g/L)	0.92 ± 0.09	0.92 ± 0.08	0.648
Insulin (mU/L)	6.86 (4.7–9.25)	7.6 (6.1–10)	0.096
Peak insulin (mU/L)	74.1 (46.0–100.0)	77.0 (57.0–100.4)	0.518
AUC 0'-150' insulin (mU/L)	6925.5 (4168.5–9585.0)	7087.5 (5619.0–10047.0)	0.298
HOMA-IR	1.6 (1.1 – 1.9)	1.8 (1.3 – 2.4)	0.119

The p-values were calculated using the Mann-Whitney *U* test for non-normally distributed variables and the independent *t*-test was used for normally distributed variables.

between the two groups. An OGTT was performed at both visits.

2.3. Laboratory investigations

Patients were instructed to fast for 12 h before the OGTT. An indwelling catheter was placed and a baseline blood sample was drawn. The patients were administered a 75 g glucose solution, which was ingested within 5 min. Blood samples were taken after 30, 60, 90, 120 and 150 min. Samples were centrifuged within 5 min and stored at −80 °C. Serum insulin and C-peptide levels were determined by electrochemiluminescence using the immunoanalyzer COBAS e411 (Roche Diagnostics, Germany), which had an intra-assay coefficient of variation (intra-CV) between 2.93 % (µ = 14.16 µU/mL) and 2.38 % (µ = 62.6 µU/mL) for insulin, and between 3.11 % (µ = 1.38 µg/L) and 2.55 % (µ = 7.07 µg/L) for C-peptide. Glucose was analyzed by the hexokinase method and its intra-assay CV ranged between 1.58 % (µ = 64.7 mg/dL) and 1.38 % (µ = 369 mg/dL). FSH, estradiol, and SHBG levels were also determined at both visits on the COBAS analyzer. Determination of the fructosamine concentration in plasma was carried out using a Roche fructosamine assay on a Modular P analyzer (Roche, Mannheim, Germany). The reaction is based on the ability of ketoamines to reduce nitro blue tetrazolium (NBT) to formazan in an alkaline solution. Results regarding the reproducibility of the OGTT in women taking placebo have been published previously [14].

2.4. Statistical analysis

The SPSS statistical program was used for statistical analysis (version 25, Chicago, IL, USA). Data are presented as mean ± standard deviation or median (25 % percentile – 75 % percentile). Test results were considered statistically significant at p-values < 0.05.

Area under the curve for glucose and for insulin was determined at both visits using the following formula:

$$AUCi = \frac{1}{2}\{(t2 - t1)(c1 + c2) + (t3 - t2)(c2 + c3) + \ldots + (tn - tn-1)(cn-1 + cn)\}.$$

ti = time and ci = measured glucose/insulin concentration at ti.

The homeostatic model assessment of insulin resistance (HOMA-IR) was determined; this quantifies insulin resistance and beta-cell function. The HOMA-IR was calculated using the following formula:

$$HOMA - IR = \frac{fasting\ glucose\ \left(\frac{mmol}{l}\right) * fasting\ insulin\ \left(\frac{\mu U}{ml}\right)}{22.5}$$

Before analysis, a normality test was performed on the data. This showed a normal distribution for all the variables except for insulin levels. A Mann-Whitney *U* test was therefore used to test for the significance of differences in insulin levels between the MHT and placebo groups. Independent sample *t*-tests were used to compare other variables between the MHT and placebo groups. A paired-samples *t*-test was performed to compare glucose levels before and after the administration of glucose at each time point. A Wilcoxon signed ranks test was performed to compare insulin levels before and after the administration of glucose at each time point in each group.

2.5. Data statement

This article contains original data which hasn't been published in previous articles. The complete dataset is provided to the editorial office. All data were collected according to General Data Protection Regulation (GDPR).

2.6. Funding

This study was funded by Grant from the Research Foundation Flanders (FWO-Vlaanderen) and the Beautiful After Breast Cancer Foundation, recognized by the King Boudewijn Foundation.

3. Results

At baseline, the HT group did not significantly differ from the placebo group in weight, height, BMI, abdominal circumference, blood pressure (BP), glycemia, parameters of insulin resistance and lipoprotein profile (Table 1). Significantly higher glucose levels at 120 min (p = 0.024) and 150 min (p = 0.030) were observed in the MHT group compared to the placebo group (Fig. 2), although all glucose levels remained within normal range.

A significantly lower peak insulin level, being the highest level of insulin measured during the OGTT, was observed (p = 0.040) at the end of the study period in the MHT group (Figs. 3 and 4). The peak insulin levels were observed at 30 and 60 min of the OGTT. A non-significant trend towards a lower AUC for insulin levels was observed in the MHT group (p = 0.192) over the study period (Fig. 4). No difference in the insulin AUC was observed in the placebo group over the study period (p = 0.549). There were no significant differences in fructosamine curves, HOMA-IR and C-peptide between the MHT group and the placebo group at baseline and at the end of the study period.

4. Discussion

An increased serum insulin level is an important risk factor for diabetes [3,4], cardiovascular disease [5] and breast cancer [6]. MHT is associated with reduced levels of insulin resistance, HbA1c and diabetes [12]. Although most studies, including six randomized trials and four observational studies as reviewed by Mauvais-Jarvis et al. [12] observe a favorable influence of MHT on glucose homeostasis, none examined glucose homeostasis as primary outcome. Differences in the study design to assess glucose homeostasis such as OGTT, IVGT or the euglycemic, hyperinsulinemic clamp may result in different findings. BMI, age at inclusion, type of MHT (oral versus transdermal), type of estrogen (Equine estrogen versus natural estrogen; low versus high dose), type of progestogen (androgenic progestogen versus natural or non-androgenic progestogen), duration of the study may all contribute to

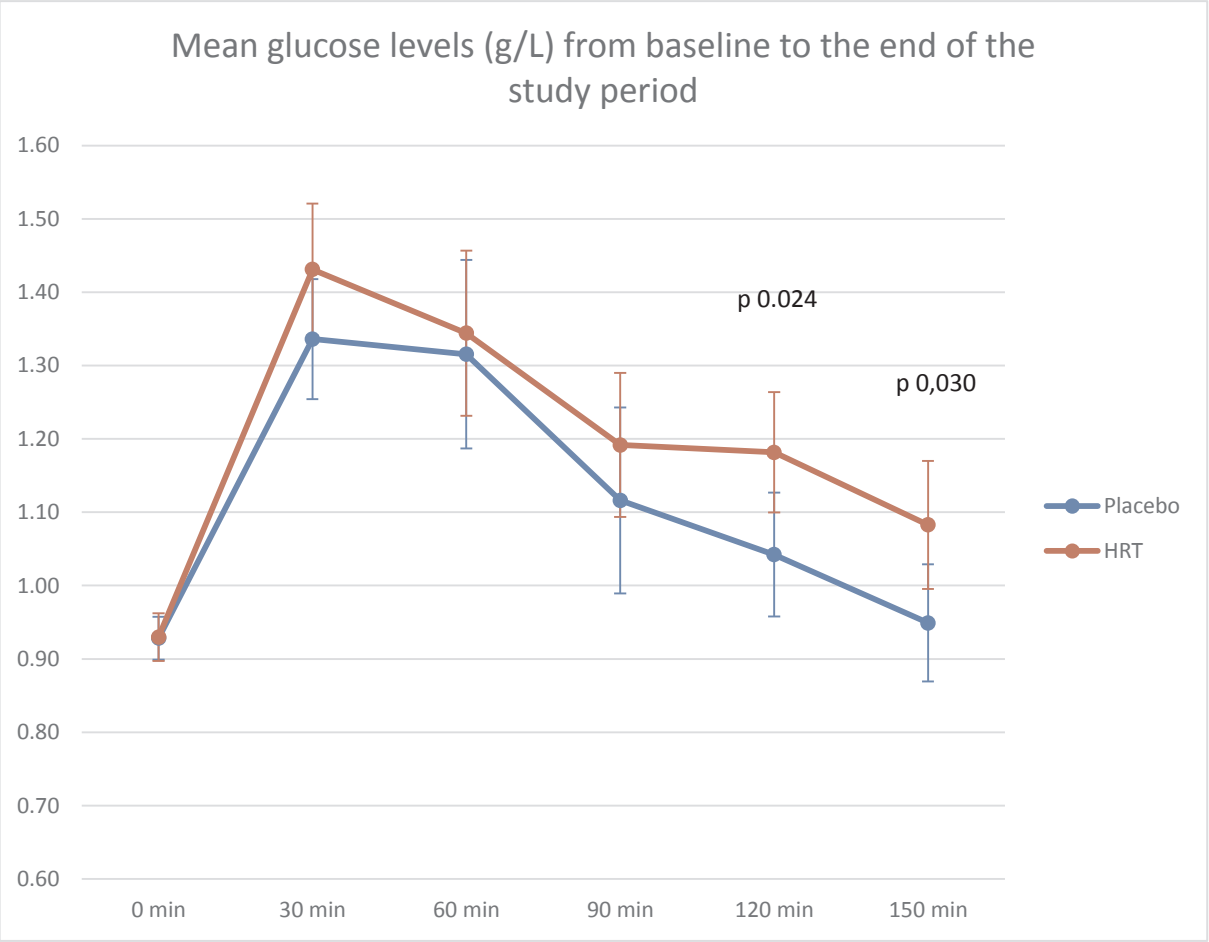

Fig. 2. Glucose levels in the MHT and placebo groups at the end of the study period. There are significant differences between the MHT group and placebo group in glucose levels at 120 min (p 0.024) and 150 min (p 0.030) after the administration of glucose.

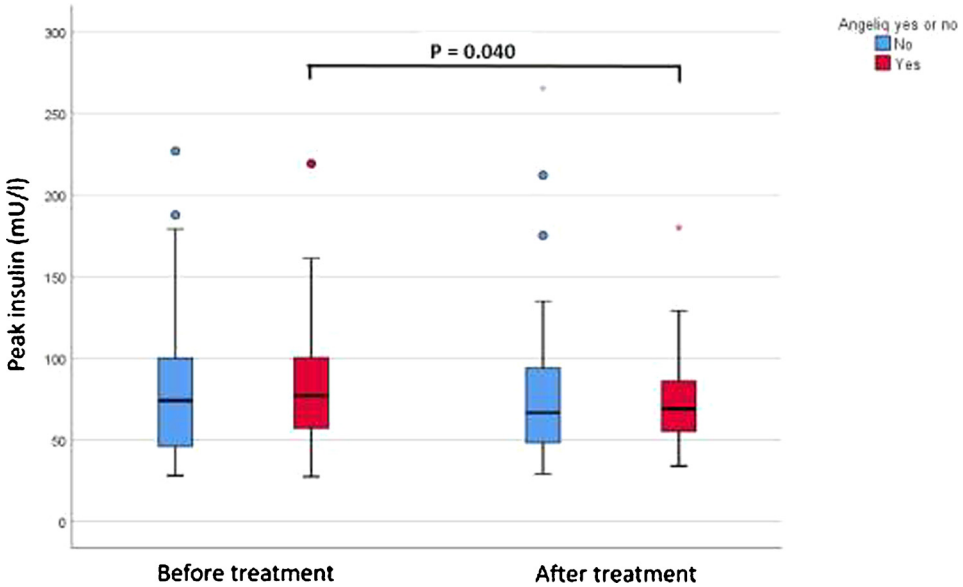

Fig. 3. Peak insulin levels during the OGTT in the MHT and placebo groups at baseline and at the end of the study period. The difference between the baseline and end-of-study values is significant in the MHT group (p = 0.040) but not in the placebo group.

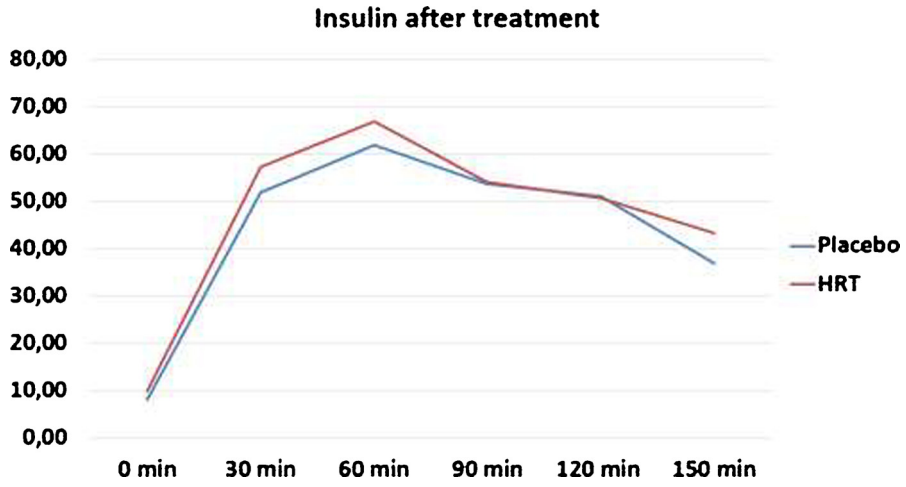

Fig. 4. Insulin curves during the OGTT in the MHT and placebo, at the end of the study period. The peak insulin levels were observed at 30 and 60 min after the start of the OGTT.

the differences observed between studies.

In long term large randomized trials such as HERS [15] and WHI [6] the reduction of diabetes was respectively 35 % and 19–21 %. These results were independent of from the reduction of BMI. However, BMI is not an accurate assessment of waist circumference and abdominal fat accumulation. As such, it is difficult to rule out that these reported reductions of diabetes in these trials may not partially have been caused by a reduction in abdominal fat accumulation due to MHT.

The aim of our study was to ascertain the short-term influence of MHT on glucose and insulin metabolism. We used the OGTT approach since it measures serum glucose and insulin levels after the normal way of ingesting sugar. The intake of 75 g of sugar by a drink may not reflect the physiological sugar burden during the intake of a meal or a soda drink. Yet if one combines a sugared donut of 100 g (containing 48.5 g of sugar) with a soda drink of 330 mL (containing around 36 g of sugar), one easily equals the sugar burden we applied in our study.

A trend towards a lower AUC for insulin levels was observed in the MHT group ($p = 0.192$), although it was not significant. MHT resulted in a significant ($p = 0.040$) reduction in peak insulin levels. This could be a reflection of the reduction in the insulin output due to MHT, or an increase in elimination by the liver, as suggested by Godsland et al. [13]. Because glucose and insulin levels naturally fluctuate widely, fructosamine and C-peptide levels were also determined in order to assess insulin resistance. The level of fructosamine reflects recent (1–2 weeks) changes in blood glucose levels. Fructosamine is comparable to the more frequently used HbA1c, i.e., glycated hemoglobin, which is a measure of average glucose levels over a longer period (approximately 3 months). C-peptide is produced by the beta cells in the pancreas when proinsulin is post-translationally cleaved to form insulin and C-peptide. Because of its 1:1 stoichiometric ratio with insulin and slower serum clearance, C-peptide is a useful marker of insulin production and, therefore, insulin resistance. There were no significant differences in fructosamine and C-peptide levels between the placebo and MHT groups at the end of the study period. There were also no significant differences in fructosamine and C-peptide levels between baseline and the end of the study period within either the placebo or the MHT group. Since we did not find any changes in C-peptide levels in the MHT group, we might deduce that MHT enhances insulin elimination.

MHT is known to improve diabetic control. Hence, it could be postulated that MHT improves insulin sensitivity in different tissues, reducing the amount of insulin required to clear the carbohydrate intake during a meal. This should result in lower insulin levels or lower serum glucose levels at the same insulin levels. We did not observe lower glucose levels in our study. In fact the glucose levels were significantly higher in the MHT group. It could be that the intake of 75 g of glucose within 5 min exceeds the capacity of the pancreas to react in an adequate manner. In everyday situations, where women normally have a more moderate glucose intake, spread over a longer time as during a meal, this increase in glycaemia may be more moderate. Consequently, lower insulin output in women on MHT may have a different impact during normal life and not result in significantly higher glucose levels as observed in our study. This increase in post challenge glucose concentrations is in accordance with the observations in in several other trials [16–18].

Our study was a short-term evaluation of the direct effects of MHT on insulin and glucose metabolism. Findings suggesting that MHT improves insulin resistance were from longer-term studies [19].

The results of the present study indicate no change in fasting glucose, insulin and C-peptide levels between baseline and the end of the study period in the MHT group, which is in contrast to the results of Godsland et al. [13]. Godsland et al. found a significant reduction in fasting glucose, insulin and C-peptide levels in their MHT group compared to their placebo group. However, insulin sensitivity was assessed using an intravenous glucose tolerance test (IVGTT), while we used oral administration of glucose, as it is more physiologically appropriate than intravenous administration. Moreover, insulin sensitivity, assessed as the glucose clearance rate, did not significantly alter in their study.

The shortcomings of our study are that only one oral estrogen/progestogen formulation was used and no comparison between oral, transdermal, different regimes was performed. Another shortcoming is the size of the study. The small size and the short term of MHT may explain why we did not find a reduction in fasting glucose in the MHT group. The strengths of the study are that all women included were healthy women not taking any other medication; the MHT contained a non-androgenic progestogen; all women had two OGT tests. The results of the control women, also willing to participate in a trial with two OGT tests were reported earlier [14].

It is possible that extended treatment does alter insulin sensitivity; further studies are needed to address this question. In our data it is clear that women with a high BMI have higher insulin levels during the OGTT (data not shown). However, since our mean BMI in the HT group was 24.6 kg/m^2 we did not have enough women with a high BMI to assess whether MHT had a different influence on insulin in lean or obese women.

5. Conclusion

We observed a trend towards lower insulin levels during an OGTT in women taking HT. The peak insulin levels in the MHT group at the end of the study period were significantly lower than at baseline, with a not

significant trend towards a lower total AUC. MHT was associated with higher glucose levels, but these levels remained within the normal range.

Contributors

H. Depypere conceived and supervised study and was responsible for patient recruitment.
A. Dierickx was responsible for laboratory work.
F. Van de Velde was responsible for laboratory work.
F. Stanczyk reviewed the article.
L. Ottoy was responsible for patient recruitment.
J. Delanghe reviewed article.
B. Lapauw reviewed article.

Conflict of interest

The authors declare that they have no conflict of interest.

Funding

Grant from the Research Foundation Flanders (FWO-Vlaanderen) and the Beautiful After Breast Cancer Foundation, recognized by the King Boudewijn Foundation.

Ethical approval

The study was approved by the medical ethics committee of the University Hospital of Ghent (EC/2008/694) in accordance with the Declaration of Helsinki. All patients signed an informed consent form before entering the study following the GDPR rules of our institution.

Provenance and peer review

This article has undergone peer review.

Research data (data sharing and collaboration)

There are no linked research data sets for this paper. Data will be made available on request.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.maturitas.2020.04.009.

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