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Short-term effects of low-dose estrogen/drospirenone vs low-dose estrogen/dydrogesterone on glycemic fluctuations in postmenopausal women with metabolic syndrome

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Abstract This study aims to compare the effects of low-dose emidrate estradiol/drospirenone (E2/DRSP) vs low-dose emidrate estradiol/dydrogesterone (E2/DG) combination on the *mean amplitude of glycemic excursions* (MAGE) value in postmenopausal women affected by metabolic syndrome (MS). One hundred sixty postmenopausal women were recruited to receive a treatment with oral doses of E2/1 mg plus drospirenone/2 mg (E2/DRSP group) or oral dose of E2/1 mg plus dydrogesterone/5 mg (E2/DG group) for 6 months. At enrollment and after 6 months, anthropometric, metabolic, and inflammatory parameters have been assessed. MAGE, evaluated during 48-h continuous subcutaneous glucose monitoring (CSGM), allowed us to assess daily glucose fluctuations at baseline and after 6 months. After hormone therapy, both groups showed a significant decline in fasting plasma glucose levels ($p<0.05$), while only E2/DRSP group showed a statistically significant decline in waist circumferences, post-prandial glycemia, LDL, plasma triglycerides, MAGE, HOMA index, and plasma IL-6 ($p<0.05$) levels. In the whole population ($n=160$), after 6 months of indicated therapy, changes in fasting plasma glucose and PAI-1 levels correlated with the changes in MAGE values, while only in E2/DRSP group that MAGE reduction was positively associated with a stronger decrease in waist circumferences, triglycerides, and TNF-α plasma levels. The independent effect of hormone therapy (HT) on reduction in MAGE value has been tested in three different multiple linear regression models. HT resulted to be associated with MAGE, independent of other confounding variables. Although both groups had a decline in fasting plasma glucose, only drospirenone treatment revealed positive effects on glycemic excursions and insulin sensitivity, induced favorable changes in lipid profile, and showed an improvement of inflammatory indices in postmenopausal women with MS.

Keywords Glycemic fluctuations · Menopause · Drospirenone · Metabolic syndrome

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Introduction

Menopausal transition is associated with an increasing risk of metabolic syndrome (MS). Many features of MS

occur with estrogen deficiency in postmenopausal women and may explain the acceleration of cardiovascular diseases (CVD) in women after menopause: in fact, it has been estimated that half of all cardiovascular events in women, older than 50 years, are related to MS (Carr 2003).

Impairment of carbohydrate metabolism is the most relevant feature of MS. It has been suggested that women who develop insulin resistance with small dense LDL and elevated plasminogen activator inhibitor-1 (PAI-1) levels after menopause may be a carrier of a genetic predisposition to CVD, which is masked by the effects of estrogen and unmasked after menopause (Sites et al. 2002). Moreover, glycemic disorders sustain oxidative stress activation and pro-inflammatory status both involved in the pathophysiological mechanism of endothelial dysfunctions and related to cardiovascular complications (Mellen et al. 2006). Several studies show that glucose fluctuations during postprandial periods and more generally during glucose fluctuations exhibit a more specific triggering effect on oxidative stress than chronic sustained hyperglycemia (Monnier et al. 2006). Therefore, glucose fluctuations have been proposed as a target for the glucose assessment in type 2 diabetes patients (Monnier and Colette 2008). Several other methods such as mean absolute glucose change (MGC) have been proposed to quantify the amplitude of the glycemic fluctuations but have not gained widespread use. We acknowledge that the different indexes do not seem to be interchangeable and should be used in appropriate conditions; indeed, most of these indices are highly correlated (Hill et al. 2011) and provide the same information, making a reasonable reduction in the number of indices that needs to be considered. Indeed, the "gold standard" is considered the *mean amplitude of glycemic excursions* (MAGE) value which is obtained by measuring the arithmetic mean of the differences between consecutive peaks and nadirs with a measurement in the peaks-to-nadir direction by the first qualifying excursion (Monnier et al. 2007). MAGE estimation requires the use of new devices allowing continuous glucose monitoring on ambulatory basis (Hirsch et al. 2008).

Furthermore, hormone therapy (HT) is considered the gold standard therapy for the alleviation of menopausal symptoms, at the lowest effective dose for the shortest possible time (Genazzani et al. 2000). The potential effects of HT on cardiovascular disease remain controversial: a correct management requires an accurate selection of participants (Collins et al. 2007) as well as an accurate selection of appropriate molecules, especially regarding progestin. Usually, the progestin used in HT is derived from testosterone with side effects related to its androgenic properties or its glucocorticoid effects (Sitruk-Ware 2008). Dydrogesterone (DG), a retroprogesterone with good oral bioavailability, is a biologically active metabolite of the progesterone showing anti-estrogenic effect on the endometrium causing, in turn, a secretory transformation (Chakravarty et al. 2005). It is devoid of androgenic or anti-androgenic action (Kronawitter et al. 2009), it has a neutral effect on glucose metabolism (Morin-Papunen et al. 2004), and it is administered in combination with a low estrogen dose (E2) (1 mg). Another drug developed for HT in combination with a low estrogen dose (E2) is the novel progestin drospirenone (DRSP). It is derived from 17-α-spironolactone and shows pharmacological properties closely related to the endogenous progesterone, antimineralocorticoid effects through aldosterone receptor antagonism (Palacios et al. 2006), and anti-androgenic properties, and lacks any estrogenic, glucocorticoid or antiglucocorticoid activity (Preston et al. 2005). Since the glycemic fluctuations are considered determinant of an overall metabolic cardiovascular risk, the main target of this study has been to compare the effects of low E2/DRSP dose vs low E2/DG dose on glycemic variability in postmenopausal women affected by MS.

Methods

Participants

One hundred seventy-two menopausal women admitted from October 2010 to October 2011 in the outpatient Menopausal Centre of the Second University of Naples were enrolled in the study. Twelve women dropped out from the study mainly because of low compliance to the treatment. Eligibility for the study was based on a diagnosis of menopause state (1 year of amenorrhea, FSH >30 IU/L, E2 <20 pg/ml) and on diagnosis of MS as defined by the International Diabetes Federation (Alberti et al. 2005) as central obesity (waist circumference ≥88 cm in women) plus two of the following four risk factors: raised blood pressure (systolic ≥130 or diastolic ≥85 mmHg), raised triglycerides (≥150 mg/dL), reduced high density lipoprotein

(HDL) cholesterol (<50 mg/dL in females), and raised fasting plasma glucose (≥100 mg/dL) (Edwardson et al. 2012).

Exclusion criteria included triglycerides >350 mg/dl, blood pressure >150/90 mmHg, previous steroid or nonsteroidal anti-inflammatory therapy, HT contraindications, and thyroid disorder. At enrolment and after 6 months, the following have been evaluated: anthropometric measurements (weight, waist-to-hip ratio WHR, body mass index BMI, and blood pressure), metabolic, hormonal, and inflammatory parameters (fasting plasma glucose, 2 h postprandial plasma glucose, plasma insulin, HOMA index, plasma triglycerides, HDL, LDL, and total plasma cholesterol, estrogens and testosterone plasma levels, DHEA-S, PAI-1, PCR, IL-6, and TNF-α plasma levels). After a clear explanation of the study and after each patient had given oral agreement, all participants signed the informed consent to participate in the study, which was approved by the ethical committee of our institution.

Study protocol

The study was designed as a prospective, randomized, open-label parallel group with a blinded-endpoint (PROBE design) study of emidrate estradiol (E2, 1 mg) plus drospirenone (2 mg) versus oral dose of emidrate estradiol (E2, 1 mg) plus dydrogesterone (5 mg). Postmenopausal women were equally randomized, in one to one ratio, according to the order of access at outpatient Menopausal Centre of the Second University of Naples, and submitted to receive a treatment with oral doses of emidrate estradiol (E2, 1 mg) plus drospirenone (2 mg) (E2/DRSP group) (n=80) or oral dose of emidrate estradiol (E2, 1 mg) plus dydrogesterone (5 mg) (E2/DG group) (n=80); both the patients and doctors are aware of the regimen being administered. Due to its open-label nature, in order to avoid bias and to achieve accurate results, the study had a blinded end point.

At enrolment and after 6 months, subcutaneous interstitial glucose levels were monitored on an ambulatory basis at the geriatric department of the Second University of Naples over a period of three consecutive days by using CSGM (Glucoday, Menarini-Italy). The sensor was inserted on day 1 and removed on day 3 at mid-morning. The data were downloaded to a computer for evaluation of glucose variations, but calculations of glucose variations were limited to data obtained on days 2 and 3 to avoid bias due to both insertion and removal of the sensor and, thus, to insufficient stabilization of the monitoring system.

During the whole period of the study protocol, patients were recommended to follow a regular diet regime verified by a daily diary. The meal taken on the CSGM was standardized: breakfast contained 421 kcal (58 % carbohydrate, 15 % protein, and 27 % fat), lunch contained 689 kcal (56 % carbohydrate, 18 % protein, and 26 % fat) and dinner contained 490 kcal (51 % carbohydrate, 24 % protein, and 25 % fat). The MAGE which has been described by Service et al. (1970) was used in the present study for assessing glucose fluctuations during 48 h. We used the glucose profiles obtained from CSGM for the calculation of the MAGE by measuring the arithmetic mean of the differences between consecutive peaks and nadirs; the measurement in the peak-to-nadir or nadir-to-peak direction was determined by the first qualifying excursion. This parameter was designed to quantify major swings of glycemia and to exclude the minor ones. For this reason, only increases of more than 1 SD of the mean glycemic values were taken into account.

Physical activity data were collected by the administration of the IPAQ-SF test (International Physical Activity Questionnaire-Short Form) (Canário et al. 2012) both at baseline and after 6 months. The questionnaire took approximately 5 min to be completed. The level of physical activity was classified as low (about 30 min/day), moderate (about 60 min/day), or vigorous (about 90 min/day). All women were encouraged to maintain constant physical activity during the study period.

Laboratory measurements

Plasma insulin level was determined by a commercial double-antibody, solid-phase RIA (Sorin Biomedica, Milan, Italy) (intra- and inter-assay coefficients of variation were 4.6 and 5.9 %, respectively). Plasma glucose level was determined by enzymatic colorimetric assay using a modified glucose oxidase–peroxidase method (Roche Diagnostics, GmbH, Mannheim, Germany) (intra- and inter-assay coefficients of variation were 0.6 and 1.6 %). Plasma lipid and plasma lipoprotein levels were quantified from fresh samples drawn after participants had been fasting for at least 12 h (for the plasma total cholesterol levels, intra- and inter-assay coefficients of variation were 0.84 and

1.3 %, respectively; for the plasma triglycerides levels, intra- and inter-assay coefficients of variation were 0.4 and 1.6 % respectively).

PAI-1 levels were measured by enzyme-linked immunoabsorbent assay (ELISA) technique (Byk Gulden, Milan, Italy) (intra- and inter-assay coefficients of variation were 4.5 and 3 %, respectively). IL-6 plasma levels were determined in duplicate using a highly sensitive quantitative sandwich enzyme assay (Quantikine HS PharmPak R&D System) (intra- and inter-assay coefficients of variation were 7.4 and 6.5 % respectively). High-sensitivity TNFa was assayed by immunonephelometry on a Behring Nephelometer II (Dade Behring, Marburg, Germany) (intra- and inter-assay coefficients of variation were 5.4 and 4.2 % respectively). Plasma C-reactive protein (PCR) was determined using automated turbidimetry (intra- and inter-assay coefficients of variation were 5.2 and 6.2 % respectively).

We used the 2-h postprandial glucose levels (lunch and dinner) value to obtain the mean value of 2-h postprandial glucose levels for each participant. Insulin resistance was estimated by homeostasis model assessment (HOMA), calculated using the following formula: fasting plasma insulin (micro−international units/ml)×fasting plasma glucose (mmol/l)/22.5.

Statistical analysis

All statistical analyses were performed with the use of SPSS software (version 17). All data are presented as mean ± SD. A value of $p<0.05$ was considered significant. Sample size calculation was estimated on an IBM PC computer by GPOWER software. Because the resulting sample size, estimated according to a global effect size of 25 % with a type I error of 0.05 and a power of 88 %, which was 158 participants, the number of required participants was set at 160.

Paired sample *t* test was used to compare the differences across the two study groups at baseline and after 6 months. Both univariate and multiple stepwise logistic regression analyses were used to assess the association between a dependent variable (MAGE value) and independent metabolic, hormonal, and inflammatory variables. Multivariate stepwise regression analysis was performed to identify the set of variables that were statistically significant predictors of impaired glycemic control independent of other related covariates. In the multivariate model, we entered all variables that were significant at the $p<0.05$ level in the univariate analyses. All statistical analyses were performed by a single operator who was blinded to the treatment group.

Results

The two groups were well matched at baseline, without significant differences in anthropometric and metabolic parameters (Table 1). At baseline, participants were slightly overweight (BMI=27.7±1.7 kg/m^2); basal gluco-metabolic data (fasting and post-prandial glucose, MAGE, HOMA index, and lipidemic profile) and values of inflammatory indices levels were similar among groups (Table 1). The frequency and duration of walking and other leisure-time physical activities were about 30 min/day (low physical activity), without significant differences at baseline and after 6 months (data not shown).

In the whole population (n=160), baseline univariate analysis showed that triglycerides levels correlated with PCR (r=0.155, $p<0.05$) and PAI-1 (r=0.164, $p<0.04$) levels, while HOMA index correlated with PCR levels (r=0.170, $p<0.03$). In addition, MAGE correlated with PAI-1 levels (r=0.352, $p<0.001$) and estrogen levels (r=−0.170 $p<0.031$), while no significant correlation with fasting (r=−0.120, p=NS) and postprandial plasma glucose levels (r=0.055, p=NS) was found. Compared to baseline values, both E2/DRSP group and E2/DG group resulted in a significant decrease in body weight ($p<0.05$), while only E2/DRSP resulted in a significant decline in BMI ($p<0.02$) and in waist ($p<0.01$) circumference.

Systolic blood pressure (SBP) significantly decreased only in E2/DRSP group ($p<0.05$) while diastolic blood pressure (DBP) remained unchanged in both treated groups.

Both E2/DRSP and E2/DG groups resulted in a significant decline in fasting plasma glucose (FPG) ($p<0.05$) levels, while mean values of 2 h postprandial glucose and MAGE significantly decreased only in E2/DRSP group ($p<0.005$) (Table 1).

After 6 months therapy, a significant improvement in the lipid profile was observed only in E2/DRSP group. Indeed, CSGM measurements provided evidence of larger MAGE decrements in E2/DRSP group compared with E2/DG group ($p<0.001$).

Table 1 Clinical characteristics and metabolic profile in E2/DRSP group and E2/DG group

	E2/DRSP group *n*=80			E2/DG group *n*=80		
	Baseline	After 6 months therapy	*p*	Baseline	After 6 months therapy	*p*
Antropometric variables						
Age (years)	51±1	–	–	50±2	–	–
Weight (kg)	74.5±6.2	72.7±6.1	0.05	74.2±5.6	72.5±3.9	0.03
BMI (kg/m^2)	27.4±1.9	26.7±1.7*	0.02	27.9±1.3	27.9±1.4	NS
Waist (cm)	90.2±1.5	88.51±1.0*	0.01	90.1±1.6	89.7±1.2	NS
SBP (mmHg)	133±8	130±7	0.05	131±6	129±7	NS
DBP (mmHg)	86±5	85±3	NS	84±4	84±4	NS
Laboratory variables						
FPG(mg/dl)	111±5	109±7	0.01	110±10	107±7	0.04
Insulin (mIU/ml)	12.8±2.5	12.3±2.4	NS	12.1±2.5	12.7±2.4	NS
Mean 2 h PPG (mg/dl)	143±27	133±13*	0.005	146±23	141±21	NS
Total cholesterol (mg/dl)	241±16	236±38	NS	237±23	233±22	NS
HDL (mg/dl)	42±6	43±9	NS	44±7	43±9	NS
LDL (mg/dl)	158±11	153±14	0.009	152±26	146±27	NS
Triglycerides (mg/dl)	200±47	148±29*	0.001	199±36	196±33	NS
MAGE (mg/dl of glucose)	51±11	44±8*	0.001	50±10	48±8	NS
HOMA index	3.55±0.7	3.31±0.6	0.03	3.33±0.7	3.39±0.6	NS
Hormonal assessment						
Estrogen (pg/ml)	13.4±1.8	46.9±6.6	0.001	13.6±1.7	45.8±2.9	0.001
Testosterone (ng/ml)	0.33±0.1	0.26±0.2*	0.01	0.32±0.2	0.33±0.1	NS
DHEA (pg/ml)	5.89±1.3	5.67±0.9	NS	6.0±1.3	5.9±1.2	NS

Data are expressed as means ± DS

*$p<0.05$ compared to E2/DG group

BMI body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *FG* fasting plasma glucose, *PPG* postprandial glucose, *HDL* high density lipoprotein, *LDL* low density lipoprotein

Focusing on hormone profiles, as expected, the estrogen levels increased during both treatments, while androgen levels such as testosterone decreased significantly only in E2/DRSP group (Table 1).

Compared to baseline, a significant decline in IL-6 plasma levels was observed only in E2/DRSP group. All the other inflammatory parameters declined without reaching statistical significance. After 6 months therapy, PAI-1 levels in E2/DRSP group were lower than E2/DG group (Table 2).

In the whole population (n=160) after 6 months of therapy, changes in MAGE values significantly correlated with the changes in fasting plasma glucose (r=0.152, $p<0.05$) and PAI-1 levels (r=0.323, $p<0.001$). The change in MAGE was significantly correlated with waist circumferences (r=0.237, $p<0.034$), plasma triglycerides (r=−0,294, $p<0.008$), and TNF-α plasma levels (r=0.220, $p<0.050$) only in E2/DRSP group.

The independent effect of HT on reduction in MAGE value has been tested in three different, multiple stepwise regression models (Table 3), waist circumference, fasting plasma glucose levels, mean 2-h postprandial plasma glucose, HOMA index, LDL cholesterol, and triglycerides levels, while HT (model 1), model 1+estrogens and testosterone plasma levels (model 2), and model 2+PAI-1, PCR, IL-6, TNF-α plasma levels (model 3) were used as covariates.

In all the models tested, HT resulted to be independently associated with MAGE. In the more complex model, including also inflammatory parameters as

Table 2 Inflammatory indices in E2/DRSP group and E2/DG group

	E2/DRSP group *n*=80			E2/DG group *n*=80		
	Baseline	*p*	After 6 months therapy	Baseline	*p*	After 6 months therapy
PCR (mg/l)	2.72±1.02	NS	2.39±1.25	2.53±0.99	NS	2.46±0.92
PAI-1 (ng/ml)	40.5±11.5	NS	37.2±14.6*	42.1±11.4	NS	41.5±11.4
IL-6 (pg/ml)	2.37±0.21	0.04	2.31±0.18	2.36±0.15	NS	2.32±0.15
TNF-α (pg/ml)	1.91±0.28	NS	1.84±0.37	1.90±0.49	NS	1.89±0.52

Data are expressed as means ± DS

* $p<0.05$ compared to E2/DG group

confounding factors, only HT and TNFa plasma levels resulted to be independently associated with MAGE (Table 3, model 3). This latter model explained 44 % of variability of the MAGE.

Discussion

Our study shows new evidence that E2/DRSP therapy provides more positive effects on carbohydrate metabolism, improves glycemic excursions and insulin sensitivity, and induces favorable changes in lipid profile and a reduction in inflammatory indices in postmenopausal women affected by MS when compared to E2/DG treatment. In E2/DRSP group in particular, compared to E2/DG group, the control of glycemic excursions (MAGE) over a daily period was associated with pro-inflammatory cytokines reduction.

The conversion from pre- to post-menopause is associated with the emergence of many features of the metabolic syndrome, including an increased central body fat and blood pressure, a shift toward a more atherogenic lipid profile, and increased glucose and insulin levels with an increasing type 2 diabetes risk (Carr 2003). Numerous recent studies highlighted the efficacy and the absence of important adverse effects of low-dose estrogen in combination with progestin in the treatment of menopausal symptoms, while the effects of HT on the cardiovascular risk remain controversial as the result of pharmacological combinations administered. As far as carbohydrate metabolism, the use of HT determines a change in the features of MS including insulin resistance and a new-onset type 2 diabetes. Again, observational and randomized studies demonstrate that HT reduces the risk to develop diabetes (Rossouw et al. 2002). Menopausal estrogen deficiency may be also responsible for decreased pancreatic insulin secretion and alteration of its metabolic clearance rate changes, conditions that can be reversed toward improved insulin secretion and sensitivity by estrogen treatment in small dosages. By contrast, synthetic androgenic progestin counteracting these effects of estrogen, more than progesterone derivatives do, may partly help to promote insulin resistance and hyperinsulinism (Gaspard et al. 1995). It is well established that many of the progestins used in HT are derived from testosterone, and their main side effects are related to its androgenic properties or to its glucocorticoid effects (Kwok et al. 2004). As previously shown, both DRSP and DG are not derived from testosterone and both are available with E2 in HT formulations.

By considering neutrally the effects of estrogen on metabolic parameters especially on glycemic variability due to the same hormonal replacement after therapy in the two groups, the aims of our study were both to highlight the effects of low-dose E2/DRSP combination vs low-dose E2/DG on parameters of MS and, more importantly, to evaluate the impact on carbohydrate metabolism through daily glucose fluctuations in postmenopausal women. To the best of our knowledge, no previous data in the literature have evaluated the trend of glycemic fluctuations by MAGE during menopause and the impact of HT on such parameters. Despite that MAGE displays some significant limitations (Standl et al. 2011), it is the most common measure used for evaluating the association between glycemic variability and oxidative stress and inflammation parameters (Monnier et al. 2006). Recent studies strongly suggest that glucose fluctuations over a daily period exhibited a more specific triggering effect on oxidative

Table 3 Linear multivariate analyses with MAGE as dependent variable

		MAGE				
		B	SEM	*β*	*t*	*p* Value
Model 1	Waist	−0.432	0.608	−0.062	−0.711	0.478
	FPG	0.062	0.096	0.050	0.639	0.524
	PPG	−0.036	0.037	−0.076	−0.971	0.333
	HOMA index	1.795	1.040	0.133	1.725	0.087
	LDL cholesterol	−0.046	0.031	−0.117	−1.488	0.139
	Triglycerides	−0.032	0.022	−0.143	−1.463	0.146
	HT[a]	*6.458*	*1.893*	*0.369*	*3.411*	*0.001*
Model 2	Waist	−0.416	0.610	−0.060	−0.681	0.497
	FPG	0.056	0.097	0.045	0.576	0.565
	PPG	−0.038	0.037	−0.079	−1.004	0.317
	HOMA index	1.752	1.041	0.130	1.683	0.094
	LDL cholesterol	−0.048	0.031	−0.120	−1.521	0.130
	Tryglicerides	−0.030	0.022	0.137	−1.397	0.165
	HT[a]	*6.637*	*1.921*	*0.380*	*3.455*	*0.001*
	Estrogen	0.189	0.132	0.110	1.434	0.154
	Testosterone	−0.774	3.624	−0.017	−0.213	0.831
Model 3	Waist	−0.468	0.596	−0.067	−0.785	0.434
	FPG	0.019	0.097	0.015	0.196	0.278
	PPG	−0.040	0.037	−0.084	−1.090	0.317
	HOMA index	1.687	1.015	0.125	1.662	0.099
	LDL cholesterol	−0.057	0.031	−0.145	−1.875	0.063
	Tryglicerides	0.025	0.021	0.111	−1.153	0.251
	HT[a]	*5.822*	*1.912*	*0.333*	*3.046*	*0.003*
	Estrogen	0.100	0.132	0.058	0.756	0.451
	Testosterone	−0.876	3.545	−0.019	−0.247	0.805
	PAI-1	0.045	0.051	0.069	0.887	0.377
	PCR	−0.393	0.607	−0.049	−0.648	0.518
	IL-6	4.673	0.607	−0.049	−0.648	0.518
	TNF-α	*4.452*	*1.510*	*0.230*	*2.948*	*0.004*

For MAGE R^2 =0.35 (model 1); R^2 =0.36 (model 2)

FPG fasting plasma glucose, *PPG* 2-h postprandial glucose, *HT* hormone therapy

[a] E2/DRSP therapy calculated as 1 and E2/DG calculated as 2

stress than chronic sustained hyperglycemia. It has been demonstrated that oxidative stress was highly and positively correlated with glycemic variability over a daily period assessed from the MAGE (Monnier et al. 2006). As a consequence, the concept that postprandial hyperglycemic spikes are "dangerous waves" should be extended to both upward (postprandial) and downward (interprandial) periods as well as to nocturnal fluctuations of glucose around a mean value (Coutinho et al. 1999).

Furthermore, in the previous study, when evaluating the impact of E2/DRSP on glucose metabolism, no negative effect on carbohydrate metabolism has been found to act in a neutral way on insulin sensitivity in healthy normotensive menopausal women (Villa et al. 2011). The effect on carbohydrate metabolism in healthy postmenopausal women was also evaluated in another study that demonstrated an unchanged glucose metabolism, fasting plasma insulin level, and insulin response to repeated glucose loads (Gaspard et al. 1999).

In the present study, the estrogen effect on metabolic parameters was comparable in both treatment groups, thus, we hypothesized that metabolic modifications were influenced by the different progestins administered.

A potential effect of the dietary intake on body weight and fasting plasma glucose should have been taken into account. Indeed, despite the significant decline in body weight and fasting plasma glucose observed in both groups, no significant differences between the two study groups were found, and a multivariate analysis clearly demonstrated that our results were independent of anthropometric and metabolic parameters thus suggesting that the potential impact of dietary intake on glucose levels and weight should be considered as a minor factor confounding our results.

Metabolic parameters such as fasting and postprandial plasma glucose and HOMA index, over a 6-month study period, were significantly reduced in both DRSP group and DG group; nevertheless, the positive effects on glucose fluctuations, as estimated from MAGE value that reflects both upward and downward glucose changes, were more pronounced in E2/DRSP group than in E2/DG group.

However, statistically significant difference of MAGE value between the two groups did not show a wide numeric difference of glycemic variability. In this regard, a recent study (Churruca et al. 2008) suggests that the evolution from health, through the MS to type 2 diabetes mellitus, seems to be marked by a progressive loss of complexity in the glycemic profile that usually precedes hyperglycemia development, suggesting that the path to type 2 diabetes mellitus is a quantitative change and not a qualitative change of glucose profiles.

The effect of glycemic variability may be due to different DRSP and DG actions. In particular, DRSP showed anti-aldosterone activity through the blockade of mineralocorticoid receptor (Palacios et al. 2006). Both experimental and clinical studies implicate also aldosterone in the development of insulin resistance, hypertension, endothelial dysfunction, cardiovascular tissue fibrosis, inflammation, and oxidative stress. Blockade of renin–angiotensin–aldosterone system (RAAS) components, in particular aldosterone receptor, results in attenuation of insulin resistance, glucose homeostasis, as well as decreased cardiovascular disease morbidity and mortality (Lastra-Lastra et al. 2009; McGuire et al. 2008). In agreement with our study, E2/DRSP therapy induced an improvement in insulin sensitivity, as suggested by the decrease in HOMA index.

In addition, the anti-aldosterone effect of DRSP can reduce estrogen-related sodium and water retention in postmenopausal women receiving HT via the RSSA which regulates sodium and water balance. This may translate into weight benefits and into a reduction in systolic blood pressure (White et al. 2006). According to the literature and in our study, a significant reduction in waist circumference, as well as a decrease of systolic blood pressure values, was found in E2/DRSP group. It is known that drospirenone is associated with an improvement of endothelial dysfunction. Markers of impaired fibrinolysis, PAI-1, tissue plasminogen activator (tPA), subclinical inflammation, C-reactive protein (CRP), and IL-6, are also associated with insulin resistance and hypertension and appear to play a role in the CVD pathogenesis (Carr 2003 E2/DRSP reduced significantly the aldosterone-induced stimulation of PAI-1 and adipokines, such as IL-6 implicated in the atherogenic process (Seeger et al. 2009; Casanova et al. 2009). Interestingly in our study, the low-dose estrogen/DRSP combination induces favorable changes in lipid metabolism confirming the results of others studies (Shulman 2006; Foidart and Faustmann 2007), while all lipid parameters remained unchanged during therapy with low-dose E2/DG.

In addition, DRSP has been documented to display anti-androgenic activity at the peripheral level by repression of androgen receptor-mediated transcription In fact, previous studies based on *in vitro* transactivations assays have demonstrated a competitive binding for DRSP to the androgen receptor that is intrinsic to its molecular structure and is dose dependent (Fuhrmann et al. 1996). In this study, DRSP reduced testosterone levels and it is known that free testosterone positively correlated with insulin resistance as well as with metabolic syndrome components (Golden et al. 2004).

These observations provide a possible explanation for the independent role of E2/DRSP therapy on MAGE, instead of on the main hyperglycemic and inflammatory markers, underlining that the appropriate glucose concentration may have a major pivotal role on metabolic activity. Because the glycemic fluctuations, as estimated from MAGE value, reflect both upward and downward glucose changes, whereas post prandial glucose (PPG) and FPG values are only markers of upward variations, we hypothesize

that MAGE value is a wider glycemic variations integrator than the PPG and FPG values.

Conclusions

In conclusion, low-dose E2/DRSP treatment revealed positive effects on carbohydrate metabolism, improved insulin sensitivity and glycemic excursions, induced favorable changes in lipid profile, and showed an improvement of inflammatory indices in postmenopausal women with MS, conditions which were generally viewed to be more beneficial as concern for cardiovascular disease prevention than the administration of low-dose E2/DG. Such results may be due to the specific anti-aldosterone activity of DRSP rather than to the drug group effect.

Our study which was carried out in a selected and limited population and lasting only a brief period indicated an overall remarkable beneficial effect on several cardiovascular disease determinants and encouraged further investigations and further studies with more participants.

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