Micronized Versus Nanoparticles in Transdermal Hormone Replacement Therapy: Effects on Blood Pressure and Inflammatory Parameters in Brazilian Postmenopausal Women

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Micronized Versus Nanoparticles in Transdermal Hormone Replacement Therapy: Effects on Blood Pressure and Inflammatory Parameters in Brazilian Postmenopausal Women

by

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Abstract

**Purpose:** To assess the effects of micronized (MIC) and nanoparticulate (NANO) transdermal hormone therapy (THT) on blood pressure, fasting insulin and ultra-sensitive C-reactive protein (CRP) in postmenopausal women.

**Methods:** In this open label study, 27 postmenopausal women, with no clinical evidence of cardiovascular disease, were randomly divided in two groups. During 12 weeks, fifteen patients received on the left forearm micronized (MIC) THT (micronized 17β-estradiol 2.5 mg/day + progesterone 100 mg/day). Fourteen patients received a nanoparticulate (NANO) THT (nanoparticulate 17β-estradiol 2.5 mg/day + progesterone 100mg/day). After treatment patients were evaluated. Baseline and Post-THT measures were determined: Insulin, body mass index, waist circumference, blood pressure, CRP-stratified levels, total progesterone, TSH and FSH levels. The study was registered at clinical trials.gov (NCT02467673).

**Results:** The mean age was 61.4 years. CRP-stratified high levels decreased in a higher number on NANO THT patients, who moved from high to intermediate and low levels (P<0.05). No effect was observed on TSH and FSH serum levels in both groups. Insulin levels decreased significantly on NANO group (P<0.05) patients and did not change on MIC group.

**Conclusions:** Micronized and Nanoparticulate THT for 12 weeks had beneficial effects in postmenopausal women with no clinical evidence of cardiovascular disease.
Keywords: Menopause; Nanotechnology; Transdermal delivery; Hormone therapy; Nanoparticles

Introduction

Hormone replacement Therapy (HRT) has been used to ameliorate the symptoms of menopause. The first treatments to replace hormones used to prescribe estrogens alone, however recent studies has shown that the use of progesterone was related with different benefits for women health [1,2]. The Women’s Health Initiative (WHI) reported that molecules with different structures from the human steroids increased breast cancer, affecting the credibility of bioidentical hormone replacement therapy [3-6].

Recently, different studies have been proving that exposure to transdermal analogous steroid hormones plays an important role in the prevention of breast cancer and a better quality of life for men and women relieving menopausal symptoms [7,8].

Transdermal drug delivery has several advantages when compared to other routes of drug administration [9]. This strategy, remains the most safe and effective treatment for relieving menopausal symptoms, especially in those women for whom other routes are an impeditive factor [10]. The hormone replacement therapy by micronized transdermal route is frequently related with different adverse events [11]. Recently, the use of Nanotechnology has been providing beneficial evidences treating menopausal symptoms [12]. Nanoparticles exert unique chemical and physical properties that enable the delivery of different compounds directly through the skin layers [13]. The use of Nanodrugs became a novel strategy in different medical specialties [14].

The effects of hormone therapy on cardiovascular risk have been shown to be different depending on the route of hormone administration. Recently, there has been an increasing interest in determine the effects of the routes over hormone therapy [15,16]. There is an on going debate for establishing a protocol for menopause management with minimum side effects [17].

Recent studies have been demonstrating beneficial effects of transdermal route over the intramuscular, vaginal and oral route in different aspects [18,19]. However there are few data regarding the comparative effects between micronized and nanoparticulate formulations in hormone therapy in postmenopausal women.

The present study aimed to assess and compare the effects of micronized and nanostructured transdermal hormone therapy (THT) administered daily by a metered-dose pump after 12 weeks follow-up on Blood pressure, C-reactive protein (CRP) and insulin in a sample of postmenopausal women.

Methods

Ethics

Initially, a written informed consent was provided for individuals willing to participate in a protocol approved by the Institutional Review Board and the local Ethics Committee. The study was registered at Clinicaltrials.gov: NCT02467673.

Study subjects

The study involved postmenopausal women (based on the time of the last menstrual cycle and estradiol less than 35ng/dL) with the following characteristics: a body mass index between 18 and 27 kg/m², diminished libido, sexual behavior complaints and no evidence of cardiovascular disease and severe clinical depression. All participants were in general good health based on history and physical examination.

This prospective, open label study was carried out between September 2012 and June 2013, with women consulting for climacteric symptoms at the Medical Center in Teresina, Brazil (part of a PhD thesis in the Post-graduation Program in Biotechnology). Non-hysterectomized, symptomatic, postmenopausal women fulfilling the following inclusion criteria were consecutively enrolled in the study: (1) last menstrual period between 6 months and 1 years before the beginning of the study plus follicle stimulating hormone (FSH) levels > 35 IU/l; (2) age between 42 and 59 years; (3) no use of any medication known to interfere with hormonal, glucose or lipoprotein levels in the past 12 weeks; (4) no use of steroidal or non-steroidal anti-inflammatory drugs in the last 15 days.

Patients presenting recent psychiatric or systemic illness, uncontrolled hypertension (blood pressure>160/95mmHg), unstable cardiovascular disease, genital bleeding, use of psychoactive medications, alcohol excess consumption, diabetes, endometrial thickness > 0.5cm, clinically relevant abnormal mammogram, history of cancer or thromboembolism were excluded.

Study design

This was a prospective short-term clinical trial study of 29 female patients aged 43-75 years old treated for menopausal symptoms. Eligibility and screening procedures, including breast examinations and collection of blood samples were performed at the pretreatment visit (4 weeks before study entry). Women were included for a 2.5 mg of 17β-estradiol and 100 mg of progesterone both hormones delivered by transdermal route by metered dose system (0.8g per dose) for 12 weeks, where participants were seen at the medical study center at baseline and after 12 weeks of treatment.
We consecutively enrolled 185 postmenopausal women fulfilling all the inclusion criteria. They were randomized into two groups of treatment. In order to generate a random sequence, a generate computer list was used. Participants were allocated for treatment following the numbered list. They were randomized into two groups of treatment (NANO) and (MIC).

Some of the participants had been included in a previous study. For the present study, the following transdermal protocol was used: the micronized THT, MIC group (n=26) participants received micronized 17β-estradiol (2.5 mg/day) and progesterone (100 mg/day) for 12 weeks. The nanoparticulate THT, NANO group (n=28) nanoparticulate received 17β-estradiol-Biolipid/B2® (2.5 mg/day) and progesterone-Biolipid/B2® (100 mg/day - Evidence Pharmaceuticals, Sao Paulo, Brazil). At the end of this first 12 weeks period, the patients were evaluated.

Women were excluded from the study if they had a clinically significant abnormal physical finding or if any adverse event had occurred that made continuation unsafe (as determined by the investigator). Women who had commenced any other systemic hormonal therapy were also excluded. All participants who completed the third month were invited to continue receiving their assigned treatment. Findings from the extension phase will be reported separately.

Treatment

During the 12 week period, participants self-administered a daily metered-dose of 0.8g of a micronized or a nanoparticulate formulation basically composed by 2.5 mg of 17β estradiol + 100 mg of progesterone in the left volar arm.

Clinical procedures

The first clinical consultation consisted on a brief explanation about benefits and risks of THT. At each visit any adverse events were recorded. In addition, during the treatment volunteers were asked to respond questions about THT complaints or any side effects.

Safety assessments

Safety evaluations included review of adverse events since the last visit, recording of vital signs. The volar arm application sites were examined for any dermal reaction. Physical examinations, including breast and pelvic examination, were periodically performed. Vital signs, weight, and any vaginal bleeding were recorded at each visit. Blood samples were collected at baseline for serum chemistry analysis, including coagulation factors after 12 weeks of treatment.

Anthropometric variables

Anthropometric measurements were taken by a single experience physician researcher (AF) who was thoroughly trained according to the study protocols. The weight and height were performed with participants wearing a robe without shoes. The patients were measured to the nearest 0.1kg and 0.1 cm respectively at each visit. Portable electronic scale (Otobonni, Sao Paulo, Brazil) placed on a firm, flat surface and calibrated to ensure accuracy. Height was assessed using a portable stadiometer (Otobonni, Sao Paulo, Brazil) and BMI was calculated as weight divided by the square of height. Waist Circumference was measured between the lowest rib and iliocrest on bare skin. Participants were instructed to breathe in, and then out, and to hold their breath while measurement was made to the nearest 0.1 cm using a measuring tape (Vonder, Sao Paulo, Brazil). Blood pressure was measured by a sphygmomanometer (Premium, Sao Paulo, Brazil).

Serum hormone measurements

Serum levels of follicle stimulating hormone (FSH), Thyroid stimulating hormone (TSH) and estradiol (E2) were measured at baseline and after 12 weeks of treatment. Serum levels of FSH and TSH were obtained by specific radio-immunoassay. The FSH results were expressed as units per liter (IU/L).

The Blood samples were collected from the subjects early in the morning (drawn before 9 AM) after an overnight fast. All samples were immediately centrifuged. Estradiol, TSH and FSH hormones were analyzed by immunoassays performed on VITROS® 5600 Integrated System.

Serum insulin levels were measured by Chemiluminescence Immunoassay using ECLIA (Roche Diagnostics), with a sensitivity of 0.200 μ IU/ml and intra and interassay CVs of 2.0% and 4.3%, respectively. CRP was assayed with a validated high-sensitivity nephelometric method (Dade Behring Marburg, Marburg, Germany). Sensitivity was 0.17 mg/l and intra and inter-assay CVs were 4.4% and 5.7%, respectively. For data analysis, individual results below the limit of sensitivity were considered as equal to 0.17 mg/l. All samples were measured simultaneously in duplicate. The intra-assay variation of the kit was < 10%.

Emulsion preparation

Two permeation enhancers were used in this study. The Pentravan® was used to enhance transdermal permeation in the MIC group and Biolipid/B2® was used in the NANO group. Formulation was prepared and the following mass ratio was obtained and distributed for both groups as follows: Micronized group (MIC) received micronized progesterone 100µg + Pentravan® and micronized 17β-estradiol 2.5mg + Pentravan® (Fagron Pharmaceuticals, Sao Paulo, SP, Brazil). The NANO group received nanoparticulate progesterone 100µg + Biolipid/B2® and nanoparticulate 17β-estradiol 2.5mg + Biolipid/B2® (Evidence Pharmaceuticals, Sao Paulo, SP, Brazil).
Particle size measurements

Particle size analysis was performed by dynamic light scattering (DLS), also known as photon correlation spectroscopy, using a particle size analyzer (Malvern NanoZS90 Zetasizer England, UK). Prior to the measurements, all samples were diluted (1:360) using Milli-Q water to yield a suitable scattering intensity. DLS data were analyzed at 25 °C and with a fixed light incidence angle of 90°. The mean hydrodynamic diameter (Z-average) and the polydispersity index (PDI) were determined as a measure of the width of the particle size distribution. The Z-average and PDI of the analyzed samples were obtained by calculating the average of 13 runs. The measurements were performed in triplicate.

Size and Zeta-potential measurements

The size and zeta-potential of the progesterone particles were measured by a Zetasizer Nanoseries-ZS90 (Malvern, UK). The size measurements were performed in disposable sizing cuvettes at a laser wavelength of 633 nm and a scattering angle of 90 °C, while the zeta-potential measurements were performed in disposable zeta-potential cells. Before the measurements, the progesterone particles were diluted 1:360 in Milli-Q water. Each measurement was repeated for 3 runs per sample at 25°C.

Scanning Electron Microscope (SEM) Assay images

The electron microscopy analysis of the nanoparticles was obtained by an equipment TESCAN SEM (Model VEGA/XMU, Brno, Czech Republic) using accelerating voltage of the 30Kv. All samples analyzed for SEM were previously sputtered with a ~20nm gold layer in order to obtain the images of the hormone nanoparticles.

Statistical Analysis

All statistical analyses were performed using SPSS statistical package for Windows version 22 (SPSS Inc., Chicago, IL, USA). The data are presented as the mean ± SEM or as the medians. Differences between baseline and after treatment were evaluated by Student’s T test to compare medians. The sample size was estimated based on an interim analysis with the first 20 patients in each treatment group, considering a power of 80% and α of 5%. To detect a difference of 0.5 mg/L in CRP between baseline and post-treatment, 80 women would be required. Log10 transformation was used to normalize the distribution of non-Gaussian variables. Two-way analysis of variance (ANOVA) with repeated measures was carried out for comparing basal conditions of micronized and nanoparticulate hormone therapy. Changes in cardiovascular risk before and after THT, according to categorical CRP values, were estimated by the Student’s T test for Non-paired samples. Pearson correlation coefficient was calculated to determine the relationship between insulin levels and blood pressure after THT.

Results

Patients

A total of 185 women were screened, of which 54 underwent randomization. The mean age of participants was 52.6±7.4 years, and 92% were of Caucasian descent (the remaining 8% were of African descent). Eleven patients in the MIC group and fourteen patients in the NANO group dropped out in the first treatment round (Figure 1). Therefore, 29 patients completed the study. At baseline characteristics were similar among the groups (Table 1).

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**Figure 1:** Enrolment and outcomes on the trial.
Table 1: Anthropometric, hormonal and metabolic marker of inflammation at baseline and after transdermal hormone therapy \((n=107)\). Parametric variables: values given as means ± standard deviation. \(P\) Values: one-way analysis of variance with adjustment with Bonferroni multiple-comparison correction \((\alpha < 5\% )\).

<table>
<thead>
<tr>
<th></th>
<th>NANO ((n=53))</th>
<th>MIC ((n=54))</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age ±SD, (y)</td>
<td>55.8 ± 8.4</td>
<td>56.8 ± 5.5</td>
<td>(P&gt;0.5^a)</td>
</tr>
<tr>
<td>Mean weight ±SD, (kg)</td>
<td>61.2 ± 9.2</td>
<td>63.8 ± 7.9</td>
<td>(P&gt;0.5^a)</td>
</tr>
<tr>
<td>Body Mass Index ±SD, ((M2))</td>
<td>23.1 ±2.5</td>
<td>25.9 ± 2.9</td>
<td>(P&gt;0.5^a)</td>
</tr>
<tr>
<td>Waist Circumference ±SD, ((Cm))</td>
<td>82.9 ± 5.9</td>
<td>88.9 ± 6.8</td>
<td>(P&gt;0.5^a)</td>
</tr>
<tr>
<td>Estradiol (pg/mL)</td>
<td>43.6 ±71.5</td>
<td>48.9 ± 93.6</td>
<td>(P&gt;0.5^a)</td>
</tr>
<tr>
<td>TSH (pg/mL)</td>
<td>1.27 ± 1</td>
<td>1.98 ± 2.7</td>
<td>(P&gt;0.5^a)</td>
</tr>
<tr>
<td>FSH (pg/mL)</td>
<td>79.1 ±27.7</td>
<td>76.2 ± 30.6</td>
<td>(P&gt;0.5^a)</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>2.14 ±1.6</td>
<td>1.08 ± 1.2</td>
<td>(P&gt;0.5^a)</td>
</tr>
<tr>
<td>Fasting Insulin (mg/dL)</td>
<td>4.5 ±2.7</td>
<td>5.8 ± 4.4</td>
<td>(P&gt;0.5^a)</td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>98.6 ±10.5</td>
<td>92.8 ± 1.2</td>
<td>(P&gt;0.5^a)</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>115.6 ± 8.9</td>
<td>125 ± 13</td>
<td>(P&gt;0.5^a)</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>61.2 ± 9.2</td>
<td>90.6 ± 20.2</td>
<td>(P&gt;0.5^a)</td>
</tr>
</tbody>
</table>

SD: standard Deviation; ^aStudent’s t-test comparison between NANO and MIC groups;

Efficacy outcomes

The figure 2 presents the values after 12 weeks of treatment divided by groups. Both treatments were equally effective in increasing serum estradiol levels. No changes were observed in BMI, weight and waist circumference after 12 weeks in both treatment groups. In the NANO group insulin and C-reactive protein and \((p<0.05)\) significantly and diastolic blood pressure decreased only after nanoparticulate therapy.

At baseline, approximately 82% of all participants reported unpleasant menopause symptoms. The decrease in the episodes from baseline to week 12 was significantly greater in the NANO group than in the MIC group \((a \text{ decrease of} 24 \text{ episodes vs.} 8, \(P<0.05)\). A significant increase in the estradiol in both groups was evident throughout the 12-week evaluation period.

When CRP levels were stratified according to low, intermediate or high levels \((<1, 1-3 \text{ or } >3\text{mg/L}, \text{respectively})\) no differences were found in levels of CRP in MIC THT users. In patients treated with nanoparticulate THT, fewer patients remained in the high levels of CRP. These patients moved to the intermediate and low CRP levels (Figure 2).

Table 2 shows fasting Insulin levels and blood pressure at baseline and after micronized and nanoparticulate THT. Blood pressure remained constant after THT.

Figure 2: Plasma C-reactive protein (CRP) levels at baseline and after micronized and nanoparticulate transdermal hormone therapy \((n=76)\) in postmenopausal women.

Low CRP levels: CRP < 1mg/L; intermediate CRP levels: CRP 1-3mg/L; high CRP levels: CRP > 3mg/L. NANO B=Baseline, before hormone therapy nanoparticulate estradiol + nanoparticulate progesterone; MICRO B=Micronized estradiol + progesterone; **, \(P<0.04\) for differences between baseline and nanoparticulate THT. Student’s T test
Table 2: Correlations between levels of fasting insulin and blood pressure (BP) (n=76). Pearson correlation test.

<table>
<thead>
<tr>
<th>Fasting Insulin levels (µU/ml)</th>
<th>Baseline</th>
<th>After THT</th>
<th>r</th>
<th>p</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>0.33 0.07</td>
<td>0.21 0.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>0.20 0.29</td>
<td>0.27 0.14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Zeta potential measurements

The nanoemulsion of estradiol and progesterone presented a high negative average zeta potential of -63.5mV and -46mV respectively. The zeta potential is an important factor for evaluating the stability of a nanoemulsion. In general, particles are considered stably dispersed when the zeta potential is below -30 mV or above 30 mV due to the electric repulsion between the nanoparticles.

Table 1: Anthropometric, hormonal and metabolic marker of inflammation at baseline and after transdermal hormone therapy (n=107). Parametric variables: values given as means ± standard deviation. P Values: one-way analysis of variance with adjustment with Bonferroni multiple-comparison correction (α < 5%).

Table 3: Anthropometric, hormonal and metabolic marker of inflammation at baseline and after transdermal hormone therapy (n=107). Parametric variables: values given as means ± standard deviation. P Values: one-way analysis of variance with adjustment with Bonferroni multiple-comparison correction (α < 5%).

Effect on serum hormone levels

The estradiol and progesterone serum concentration profile are presented in Figure 3A & 3B. After a daily single-dose application of 17β-estradiol 2.5mg to the left upper arm on week 12, the mean of estradiol was found to be 94.52ng/dL (range, 10-381.7). The mean baseline concentration of estradiol was 34.8ng/dL (range, 4.3-166).
At baseline, the mean of estradiol was below of the normal female reproductive range, for the 15 women who completed the study protocol. The mean serum estradiol in NANO group increased by 59.72ng/dL (95% CI, -2.8 to 78.79) and estradiol in MIC group increased by 49.88pg/dL (95% CI, 7.94 - 40.77) after 12 weeks of treatment with transdermal nanoemulsion. The mean values for TSH and FSH remained within the menopause range (Table 2).

**Adverse events**

No adverse effects were reported by the volunteers at any time during the study. Application of the micro or nanoemulsion was well tolerated, with no women reporting any skin reactions and no other serious adverse events occurred.

**Discussion**

This study was undertaken to compare and evaluate the effects of micronized and nanoparticulate transdermal hormone therapy (THT). After 12 weeks of treatment the conventional micronized and nanoparticulate THT did not induce any harmful effect on anthropometric and hormonal variables in postmenopausal Brazilian women. However, the conventional micronized THT induced a slight increase on diastolic blood pressure and increased the levels of CRP, TSH, but did not reach statistical significance.

Interestingly, the nanoparticulate THT has been shown to be beneficial for postmenopausal women with a mean age higher than 60 years of age. This result corroborates our previous finding using this kind of technology [1,20]. Thus, it is reasonable to speculate that physiologic mechanisms involved with the nanoparticulate system support this strategy for treating symptomatic postmenopausal women, which seems to be related to the beneficial changes in the cardiovascular system [20].

In the present study, participants had no previous cardiovascular disease and were in good general health. Weight, BMI, waist circumference and thyroid stimuli-hormone was the only variables that remained unchanged for both groups during THT. Interestingly, 17β-estradiol was the only variable that reached statistical significance in both treatments increasing significantly.

Interestingly, in both treatments groups participants have a slight decrease on weight, body mass index and waist circumference, however this change was not statistical significant. In fact, the menopause condition is related with an increase of central adiposity than with weight gain [21-23]. Recent studies have been proving that THT does not contribute to weight gain [18]. Previous studies have assessed the influence of THT on waist circumference [24-26]. Taken together, the present results and these previous reports support the idea that micronized and nanoparticulate THT are associated with decreased waist circumference in postmenopausal women [25,26]. Transdermal THT has been proving to be more beneficial for menopausal women, since the absence of the first pass metabolism seems to be related to positive effects leading to beneficial changes especially in those related with the blood pressure in menopause transition [27].

In the present study, we found amelioration of the insulin profile, with statistical differences between treatments. Nanoparticulate THT promoted a significant decrease in CRP and fasting insulin and, in contrast to micronized transdermal estrogens, the CRP presented a slightly increase after treatment, probably because of the size of the particle can have an relevant impact on hormone absorption through the skin layers. There are many studies that have shown that the size of the particle plays an important role over the clinical evidences and serum variables results [20,28].

Different studies using confocal Raman spectroscopy have shown that nanoparticles are more effective for drug delivery over the skin layers. In the present study when the size of particles were compared there was a significant difference (p<0.01). The mean size used in the nanoparticulate group (NANO) have a mean size of 323 nanometers (NANO) while those used in the MIC group presented a mean size of 800 micrometers (MIC).

Other important aspect to be considered in THT is the zeta potential. Nanoparticles with zeta potentials greater than +30 mV or less than -30 mV are considered strongly cationic and strongly anionic, respectively. Since most cellular membranes are negatively charged, zeta potential can affect the tendency of the nanoparticles to permeate membranes, with cationic particles generally displaying more toxicity associated with cell wall disruption. In the present study it was demonstrated that nanoparticles of progesterone and estradiol presented a strong anionic values -54mV and -42mV respectively, probably this is the key to understand the facilitate a better skin absorption. Other interesting finding of the study is the uniformity of shape and size of the nanoparticulate hormones the figures taken by Scanning electron microscopy shows a uniform and regular shape and distribution for the Nanoparticulate in contrast with the micronized particles.

The present data confirm and expand our previous results obtained with long and short-term treatment [2]. Some studies suggest higher prevalence of thromboembolic events risks related with oral route used for hormone therapy due to the activation inflammatory mechanisms with significant changes in lipid profile [29,30].

Insulin is a marker for cardiovascular risk [31], this parameter is strongly associated with coronary heart disease [7]. In the present study, while no changes were found in insulin in micronized group there was a beneficial decrease of insulin serum levels in those patients treated with nanoparticulate hormones, also there was significant a decrease in diastolic blood pressure after nanoparticulate THT. The present findings confirm those from previous studies and seem to
be related to the route of administration [32,33]. According to previous studies, we also found no association between insulin and blood pressure [34].

C-reactive protein is considered to be a precise marker of chronic inflammation. The CRP concentration has continuous associations with cancer, risk of coronary heart disease and ischemic stroke [19]; there are many studies that indicate a positive correlation between levels of CRP during other routes of hormone replacement therapy and cardiovascular risk [7]. Regarding levels of CRP after THT, more women moved from the high levels of CRP to intermediate and low-level strata after nanoparticulate THT. It is reasonable to speculate that is related with the absence of the first pass hepatic metabolism, preventing the synthesis of fibrinogen and cytokines [31]. Moreover, it was possible to observe more beneficial changes on those women treated with nanoparticulate THT. There was a statistically significant decrease in CRP-related cardiovascular risk, especially in women at the highest cardiovascular risk stratum according to CRP stratification.

As far as we concern this is the first clinical evidence on this matter, since few studies have previously assessed the relationships between nanoparticulate THT and CRP levels.

Another important point related to THT concerns the influence of different size of progesterone particles on clinical variables. Supplementation of micronized progesterone, depending on the route of administration can interfere with the lipid profile and glucose tolerance and could affect the mechanisms of estrogen-induced CRP stimulation [35]. Recently, we have shown that the addition of nanostructured progesterone to estrogen did not worsen waist circumference or body mass index [36]; supporting the notion that nanoparticulate progesterone has a beneficial effect on variables of cardiovascular risk [37]. We observed some interesting differences between micronized and nanoparticulate THT in terms of CRP levels in this sample of postmenopausal women. Conflicting results have been previously obtained when CRP levels were measured in postmenopausal women before and after different routes of hormone therapy. To the best of our knowledge, no studies so far have evaluated the effects of nanoparticulate THT on CRP in postmenopausal women. This suggests that the decrease in CRP reflects a restoration by nanoparticulate THT of altered adaptive responses involved in blood pressure control by menopause [38,39].

One limitation of the present study was the impossibility to perform double blinding for investigators and participants [40-42]. However, our findings are presented by laboratory results performed by technicians that were blind regarding the treatment used [43-15].

The findings of the study shows that both treatments micronized and nanoparticulate THT for 12 weeks had similar beneficial or neutral effects on anthropometric [46-53], markers of inflammation and hormonal variables in postmenopausal women with no clinical evidence of cardiovascular disease. Significant effects were found regarding CRP levels in Nanoparticulate group users. Further studies are needed in order to better understand the interaction between the nanoparticulate THT in menopause transition.

Acknowledgments

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Botelho MA, Queiroz DB and Freitas A collected the data. Rego A and Araújo-Filho were responsible for the bibliographic review and data analysis. Botelho MA performed the statistical analysis. Queiroz DB was responsible for acquiring the SEM images and performing the nanosizer analysis. Botelho MA, conceived the project and wrote the manuscript. Freitas A and Ivaldo Silva critically revised the content of the manuscript.

References

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28. Casanova G, Spritzer PM (2012) Effects of micronized progesterone added to non-oral estradiol on lipids and
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