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Original Research

Hormone therapy is not associated to pain thresholds in healthy postmenopausal women

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Summary

Purpose of Investigation: To evaluate if hormone replacement therapy modifies pain thresholds in healthy postmenopausal women. *Study Design:* A cross-sectional study including 78 healthy postmenopausal women. *Materials and Methods:* Pain thresholds were determined using pressure algometry and electrical stimulation. Participant interviews were followed by the application of a semistructured questionnaire including psychometric assessment with Beck Depression Inventory and State-Trait Anxiety Inventory. A 10-mL peripheral blood sample was collected after the interview. Serum concentrations of estradiol, progesterone, and testosterone were determined by chemoluminescence. *Results:* Users of the medication presented significantly lower sensory electrical thresholds (3.60 ± 0.85) than non-users (4.58 ± 1.30). No significant difference in pain threshold was observed between groups. Weak positive correlation between estradiol levels and sensory thresholds was observed ($r = 0.26$, $p = 0.04$). *Conclusion:* Hormone replacement therapy is not associated with modifications in pain thresholds, neither mechanical nor electrical ones, in healthy postmenopausal women.

Key words: Hormone therapy; Postmenopausal women; Pain threshold.

Introduction

Chronic pain is an enormous medical problem worldwide and should be seen as a health priority. At least 20% of individuals are affected by persistent painful conditions, with not only negative repercussions on health, but also with significant social and economic consequences [1]. Women are known to be more affected than men by diseases involving persistent pain [2, 3]. Indeed, even from a laboratory viewpoint (sensitivity to experimentally induced pain), there is also evidence that the pain threshold of women is lower than that of men [4]. Several studies have consistently reported that the prevalence of painful conditions increases with increasing population age [5, 6], especially among women older than 40 years [7].

There is evidence that, beyond the menstrual cycle [8-10], menopause [11, 12] affects the perception of pain. In fact, many factors are known to interfere with the perception of pain stimuli [13-15], all of them possibly interacting to explain the difference in pain sensitivity observed between genders [16]. As expected, the hormonal factor is one of the most extensively [17] . There is evidence that gonadal steroid hormones may interfere with various pathways of pain mechanisms [18, 19]. The role of these hormones has been more extensively documented in animal models [9, 19, 22], whereas the information regarding humans is not yet consensual [23, 24]. The role of estrogens has been more extensively studied, and numerous mechanisms have been postulated for the interference of estrogens with the pain perception system, with both an excitatory and/or inhibitory action [25]. On the other hand, there are few studies considering the role of progesterone and progestins, although we have recently observed that some progestins can influence the pain perception of healthy women taking contraceptives [26].

In postmenopausal women with persistent painful conditions, hormone replacement therapy (HRT) based on estradiol supplementation seems to increase clinical pain perception [27-29], although these findings have not been confirmed in all conditions [30]. In turn, it has been reported that in healthy postmenopausal women HRT is associated with greater experimental pain sensitivity [31]. Curiously, these studies do not specify the indication for use, the class, the dose or the route of administration of specific formulations, a fact that in itself, compromises the elaboration of a hypothesis regarding the possible influence of this drug class on pain sensitivity. Furthermore, another class of drugs used for HRT has a particular mechanism of action, as is the case for tibolone. This drug has both estrogenic, androgenic, and progestagenic effects and its metabolites can be found in various brain regions, as also observed for estrogen [32]. Considering that the quantitative assessment of experimental pain perception is relevant in various clinical situations, such as detection of individual differences in the pain processing mechanisms in the

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central nervous system (SNC) [33], prediction of postoperative pain [34] and prediction of clinical response [35] to analgesics, in addition to being potentially useful for a coping strategy training [36], the objective of the present study was to determine the pain thresholds of healthy postmenopausal women taking different HRT formulations.

Materials and Methods

This was a cross-sectional study conducted from August 2012 to December 2013 at the Teaching Health Center of the Ribeirão Preto Medical School, University of São Paulo (CSE-FMRP-USP per the Portuguese acronym), a primary care unit. The study was approved by the Research Ethics Committee of CSE-FMRP-USP (protocol number 453/2011) and was conducted in accordance with the Declaration of Helsinki. All women gave written informed consent to participate.

Eligibility criteria were: healthy women aged over 45 years and in menopause (considering 12 full months without menstruation) with a body mass index ≤ 30 kg/m^{-2}, with no clinical history or record of comorbidities such as neoplasias, diabetes, hypertension, mood disorder, or recent acute painful events registered in the health system of the municipality or informed during interview, taking no other type of medication (mainly analgesic, antidepressive or anti-inflammatory agents). Women without symptoms of hot flashes were allocated to the control group (apparently healthy) and women using HRT for at least six months exclusively indicated due to hot flashes symptoms were allocated to the study group. Two smokers were excluded during selection. Women were invited to participate in the study by telephone contact or by personal contact after an appointment at the health unit.

An initial interview was held with all women, during which all procedures were first explained, followed by the application of a semistructured questionnaire, including psychometric assessment with the Brazilian version of the Beck Depression Inventory (BDI) and the State-Trait Anxiety Inventory, State Scale (STAI-S) [37, 38].

After the interview, a 10-mL peripheral blood sample was collected for the determination of estradiol, progesterone, and testosterone (free fraction) levels. The samples were collected at about 10:00 a.m. and centrifuged at 3,540 *g* for ten minutes. After separation, serum was stored frozen at -80ºC until the time for analysis. Next, all volunteers received an initial training in order to familiarize themselves with the procedure and were instructed regarding the ideal threshold rating [39]. The measurement obtained at training time was not used for data analysis.

Serum concentrations of estradiol, progesterone and testosterone (free fraction) were determined by chemoluminescence using a commercial system and analyzer. The detection limits and analytical sensitivity for estradiol, progesterone, and testosterone were: 20.0 to 2,000.0 pg/mL (15 pg/mL), 0.20 to 40.0 ng/mL (0.1 ng/mL), and 20.0 to 1,600.0 ng/dL (10 ng/dL), respectively.

The authors used a model DD-500 portable digital dynamometer with 20 kgf × 5 gf capacity which consists of a pressure-sensitive rod fitted with a 1-cm^2 rubber base on the distal extremity. Two points were tested on the abdominal wall (right and left in an area corresponding to the uterine viscerotome) [40] and one point on the forearm of the non-dominant limb. The resting periods were similar to those for the previous stage, with the difference that the abdominal measurements were made with the volunteer in dorsal decubitus and with the lower limbs extended. The volunteers were instructed to inform the examiner as soon as the pressure sensation became painful (pain threshold) and the pressure appearing on the dial at that moment was recorded. The rate of force application perpendicular to the site of evaluation was approximately 1kg × s^{-1} × cm^{-2}. Both measures were taken three times, with a interval of 15 minutes, and their mean value was considered for analysis.

Pain thresholds to electrical stimuli were obtained using a constant current device delivering a pulsed electric current at a fixed frequency of 50 Hz [41] with a biphasic square waveform and a pulse duration of 200 ms. Silicon-carbon electrodes measuring 5 × 3 cm were coupled to the skin with 1-mL of water-soluble gel for each electrode and were placed on the ventral side of the flexor muscles of the wrist and the fingers of the non-dominant limb. Their placement followed the longitudinal direction of the muscle fibers, with the first electrode being coupled at a distance of 4 cm from the elbow joint interline and the second electrode affixed 4 cm distal from the first. Before the formal measurement for the study, the volunteers underwent training to familiarize themselves with the procedure and were instructed with regards to the ideal judging of the thresholds [42]. During execution of the procedure, the subjects remained seated with their non-dominant forearm in a supine position supported on the examination table. The device was activated and the intensity was gradually increased by the examiner, 1 mA every two seconds. The volunteer was instructed to report the first sensation of current flow and that moment was identified as the sensory threshold. Later, the intensity continued to be increased to identify (by visual inspection) the initial muscle contraction, which was deemed to be the motor threshold. Finally, the intensity was increased incrementally until the first painful sensation was observed, which was identified as the pain threshold. The number of measurements, intervals, and considerations for analysis were as described earlier.

The initial interview, the application of the semistructured questionnaire and blood collection, were the responsibility of one investigator (MMM) and the threshold measurements were performed by two other investigators (PSS and TMG). The age of both female investigators was similar to that of the study subjects. Both were blind to the information obtained in the other stage of the study. Data were coded independently by two investigators (JCRS and FJCR). In parallel, the authors insured safety of the information by defining access rules through private virtual networks and users and passwords with privileged access to the database. The Filemaker Server 11 was used as the database management system. Data analysis was conducted by another investigator (OBPN) and an independent statistician who was unaware regarding the groups. In an attempt to avoid potential bias the authors adhered to recommendations about how to perform the study based on a consensus panel [43].

Before including hormonal replacement users in the study, sample size was estimated based on a pilot study with ten apparently healthy control women. Sample size estimation was based on the differences between two means of the pain thresholds. The authors considered a standard deviation of 0.9 kg × cm^{-2} and a significant difference to detect of 0.70 that they considered clinically significant. In order to obtain 80% of power to detect this change with an overall two-sided type I error rate of 5%, this study required at least 26 subjects per group. This sample size was estimated using an online calculator (lee.dante.br).

Healthy women non-users of HRT (n = 26) and users of HRT (n = 52), taking 1 mg estradiol plus 0.15 mg norethisterone acetate (n = 26) or 2.5 mg tibolone (n = 26), were all taken orally.

All analyses were carried out by a professional who was blinded to the clinical data. The results were obtained with the aid of the SAS 9.2 software, considering a 5% level of significance for analysis.

A mixed-effects linear regression model (fixed and random effects) was used for comparison of the three measurements of elec-

Table 1. — *Clinical characterization of the women included in the study.*

	Healthy group (n=26)	HRT group (n=52)	E + NET (n=26)	TIB (n=26)	p
Age	56.0±6.7	56.4±5.8	54.9±5.5	57.8±6.2	0.797
Duration of menopause	6.3±3.0	6.6±2.5	6.3±1.3	6.9±3.9	0.469
BMI	25.6±2.8	26.0±4.1	26.2±4.0	25.8±4.4	0.678
Parity (median, range)	2 (0-5)	2 (0-8)	2(0-4)	2.5(0-8)	0.862
Schooling					0.901
Elementary	13 (50.0)	22 (42.3)	10 (38.5)	12 (46.2)	
Middle school	7 (26.9)	18 (34.6)	10 (38.5)	9 (34.6)	
Higher education	6 (23.1)	11 (21.2)	6 (23.1)	5 (19.2)	
Marital status					0.912
Single	2 (7.7)	3 (5.8)	1 (3.9)	2 (7.7)	
Stable union	17 (65.4)	38 (73.1)	20 (76.9)	18 (69.2)	
Widowed or divorced	7 (26.9)	11 (21.2)	5 (19.2)	6 (23.1)	
BDI (mean±sd)	6.0±3.5	6.3±4.0	6.9±4.2	5.7±3.9	
S-TAI (mean±sd)	41.0±12.4	44.2±12.4	43.5±11.5	44.7±13.3	0.122
Estradiol	20.6* (20.0-31.7)	31.4 (20-52.7)	47.9* (31.5-64.4)	21.8 (20-34.5)	0.031*
Progesterone	0.2 (0.2-0.3)	0.2 (0.2-0.2)	0.2 (0.2-0.2)	0.2 (0.2-0.2)	0.213
Testosterone	20 (20.0-27.7)	20 (20.0-20.0)	20 (20.0-20.0)	20 (20.0-20.0)	0.157

BMI: body mass index, kg/m^2; BDI: Beck Depression Inventory; S-TAI: State-Trait Anxiety Inventory;. HRT: hormone replacement therapy; E+NET: estradiol + norethisterone; TIB: tibolone; Age and duration of menopause are reported in years; Parity is reported as median and range; estradiol, progesterone, and testosterone are reported as median (interquartile); The other quantitative variables are reported as mean and standard deviation.

Table 2. — *Pain thresholds and category of hormone replacement therapy.*

Hormone therapy	Control group (n=26)	E+NET (n=26)	ED	95% CI	TIB (n=26)	ED	95% CI
Algometry thresholds							
Forearm	2.57±0.98 [0.38]	2.34±0.91 [0.39]	-0.23	-0.97 to 0.51	2.46±1.35 [0.55]	-0.10	-0.86 to 0.65
Right abdomen	2.00±0.92 [0.46]	1.88±0.98 [0.52]	-0.13	-0.83 to 0.58	2.11±0.97 [0.46]	0.11	-0.61 to 0.83
Left abdomen	2.08±0.96 [0.46]	1.75±0.83 [0.47]	-0.33	-0.94 to 0.28	2.09±0.94 [0.45]	0.01	-0.61 to 0.64
Electrical thresholds							
Sensory	4.58±1.30 [0.28]	3.76±0.77 [0.20]*	-0.82	-1,56 to -0.07*	3.43±0.93 [0.27]*	-1.15	-1.92 to -0.38*
Motor	10.29±2.85 [0.28]	10.37±2.47 [0.24]	0.08	-1.78 to 1.95	9.81±2.37 [0.24]	-0.48	-2.39 to 1.43
Painful	15.87±5.33 [0.34]	16.96±6.38 [0.38]	1.09	-2.56 to 4.75	15.48±4.40 [0.28]	-0.39	-4.14 to 3.36

Thresholds are reported as mean ± standard deviation and [coefficient of variation]. The comparisons were made between users of HRT and a healthy control group. They are represented by estimated difference (ED) and adjusted 95% confidence interval (95% CI). The significantly different measurements are marked by an asterisk (*). E: estradiol 1 mg; NET: norethisterone 0.15 mg; TIB: tibolone 2.5 mg.

Table 3. — *Correlation between pain thresholds and hormonal levels.*

	Estradiol	Progesterone	Testosterone
Algometry thresholds			
Forearm	0.02 (0.87)	-0.09 (0.43)	-0.03 (0.82)
Right abdomen	0.04 (0.85)	0.06 (0.77)	0.08 (0.69)
Left abdomen	0.06 (0.71)	0.10 (0.53)	0.17 (0.25)
Electrical thresholds			
Sensory	0.26 (0.04)*	0.18 (0.12)	0.04 (0.77)
Motor	0.08 (0.52)	0.00 (0.99)	-0.03 (0.81)
Painful	0.08 (0.53)	-0.05 (0.67)	-0.02 (0.88)

Note: the values are presented by Person correlation coefficient and (p value).

trical thresholds and algometry. Mixed-effects linear models were used for the analysis of data in which the responses were grouped and the suspicion of independence between observations in the same group is inappropriate.

Analysis of covariance (ANCOVA) with the Tukey post-test, which permits the comparison of three or more groups and the adjustment of covariables, was proposed for the comparison of groups and types of HRT regarding all thresholds. The Pearson correlation coefficient was used to analyze the correlation between thresholds and serum levels of estradiol, progesterone, and free testosterone.

Results

The characterization of healthy women users and non-users of HRT is presented in Table 1.

The authors observed that users of HRT presented significantly lower sensory electrical thresholds (Table 2). On the other hand, there was no significant difference in pain threshold among the groups, even considering the combination of estradiol plus norethisterone or tibolone. No correlation between electrical pain thresholds or algometry and serum estradiol, progesterone or free testosterone levels was observed. Nevertheless, the authors observed a weak positive correlation between estradiol levels and sensory thresholds (Table 3).

Discussion

In the present study the authors did not find a reduction of pain thresholds associated with HRT. The only difference they observed was the lower threshold of initial perception during electrical stimulation (sensory perception, sensory threshold, or perception threshold) in HRT users, regardless of the type of drug used, compared to non-HRT users. The only study they identified specifically designed to evaluate the thresholds in menopause reported that HRT was associated with a lower pain threshold [31]. In contrast to the present study, the population from the cited one was significantly older, and we know that increasing age is associated with changes in pain thresholds [44, 45] and a progressive delay in the perception of stimuli [46]. In addition, the cited authors did not clarify if all participants were on HRT at the time of the study, and did not mention the reason for HRT use or what drugs were being used. All of these variables have the potential to interfere with the measurement of pain thresholds. Also, they used as a methodology the measurement of temperature threshold. Although this is an appropriate method [47], the integrity of the non-noxious thermal systems [48] is essential because the perception of pain induced by variation in temperature depends on the integration of nociceptive and non-nociceptive systems. This is important because many postmenopausal women have hot flashes which, in turn, represent a problem in the thermoregulatory mechanisms, partly attributed to estrogen withdrawal [49, 50]. In addition, skin temperature can influence the heat pain thresholds, but not the thresholds of mechanically induced pain [51].

Regarding the sensory threshold (sensory perception or perception threshold), the difference between groups in the present study can be explained by HRT use. It is known that the estrogen deficiency that occurs after menopause is one of the factors responsible for skin dryness [52]. The increase in epidermal thickness and dermal hydration induced by HRT based on the use of estradiol [53. 54], or even due to the increased amount of total body water associated with the use of tibolone [55] can culminate in less resistance to electric current [56]. Consequently, electrical current flows more easily and stimulation can thus be performed faster. Moreover, some relationship may also exist between a low perception threshold and any disruption in the CNS previously present in women with significant vasomotor symptoms, irrespective of HRT. However, since the present study was not commenced before the beginning of HRT use, it is not possible to confirm this hypothesis.

The present authors recognize some limitations of this study, although they attempted to control all possible elements with a potential interference in the measurement of experimental pain thresholds. The first was that unfortunately, postmenopausal women had a series of comorbidities that impaired generalization of the present results to this general population. Second, despite the homogeneous characterization of the study, the participants had previously experienced significant hot flushes that motivated the use of HRT. Such a condition may reflect some neurochemical imbalances in the CNS that also has the potential to interfere with the process of modulation of pain perception. Another point of view is the influence of circulating sex steroids. Although the weak correlation found, the chemoluminescence method is not sufficient to provide the proper low-end sensitivity for analyzing the expected low concentrations in postmenopausal women. Moreover, the measurement of free testosterone by this technique is highly inaccurate. Also tibolone, not being a sex steroid, is not expressed by measuring circulating estradiol or progesterone or testosterone. Norethisterone is also not expressed by measuring progesterone; nonetheless this correlation seems unlikely.

Conclusions

The present results suggest that HRT does not seem to be associated with higher or lower pain thresholds, at least when it includes estradiol plus norethisterone acetate or tibolone, both taken orally. The occurrence or clinical worsening of pain symptoms should not be a concern for these women when HRT is indicated. The present authors believe that further studies need to be designed to assess whether thermoregulation phenomena may be associated with altered sensory perception in women with vasomotor symptoms.

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