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Comparison of the effect of oral and transdermal hormone therapy on fasting and postmethionine homocysteine levels

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Objective: To compare the modifications on basal and post-methionine homocysteine (Hcy) levels induced by transdermal vs. oral continuous combined hormone therapy (HT).

Design: Prospective randomized study.

Setting: Outpatient service at university hospital.

Patient(s): Twenty-four healthy postmenopausal women.

Intervention(s): Six-month administration of transdermal (50 μg/d of E_2 and 140–170 μg/d of norethisterone [NET] acetate; n = 12) or oral (2 mg of E_2 and 1 mg of NET acetate; n = 12) HT.

Main Outcome Measure(s): Fasting levels of Hcy, cysteine (Cys), folate, and vitamin B12. Post-methionine Hcy concentrations.

Result(s): During HT, a slight decrease of fasting Hcy (8.9 [6.7; 15.2] μmol/L vs. 8.3 [4.9; 12.0] μmol/L) and fasting Hcy/Cys, a possible index of Hcy trans-sulfuration (0.061 [0.039; 0.107] μmol/L vs. 0.048 [0.032; 0.093] μmol/L) was observed. Modifications were similar in the transdermal and oral group. Net decreases of Hcy and Hcy/Cys observed during HT were related linearly to pretreatment values (r = 0.821 and r = 0.775, respectively), and were significant for Hcy above, but not below, 9 μmol/L. Transdermal (33.5 [27.5; 75.9] μmol/L vs. 28.4 [17.4; 48.9] μmol/L) or oral HT (36.1 [17.7; 74.8] μmol/L vs. 29.9 [17.5; 50.3] μmol/L), decreased, similarly, post-methionine Hcy levels.

Conclusion(s): Similarly to oral, transdermal HT reduces post-methionine Hcy and fasting Hcy when it is elevated. (Fertil Steril® 2004;81:99–103. ©2004 by American Society for Reproductive Medicine.)

Key Words: Homocysteine, hormonal replacement therapy, E_2, norethisterone, cardiovascular risk, menopause

Homocysteine (Hcy) is a sulfur-containing amino acid formed during the metabolism of methionine. Under conditions in which an excess of methionine is present or cysteine (Cys) synthesis is required, Hcy enters the trans-sulfuration pathway and is transformed into Cys, by way of cystathionine, by the vitamin B6-dependent enzyme cystathionine β-synthase (1). In the remethylation cycle, methionine resynthesis is obtained by Hcy acquisition of a methyl group, in a reaction catalyzed by methionine synthase (2). Vitamin B12 and folate are essential co-factors for methionine synthase and to maintain Hcy levels in the physiological range (3).

Individuals with congenital (4, 5) or acquired (3) Hcy elevation, are at increased risk of artery and vein diseases (6–9), as confirmed by prospective intervention trials (10, 11). Homocysteine increases after the intake of proteinic food rich of methionine. An excessive post-methionine increase is present in about 27% of subjects with normal fasting Hcy (12) and it represents an additional independent risk factor for cardiovascular diseases (12) and deep vein thrombosis (9).

Homocysteine is influenced by sex hormones. It is lower in women than in men (3, 13–15), it decreases during pregnancy (16), and it is lower in premenstrual that in postmenopausal women (17–19). Homocysteine increases in female-to-male transsexuals treated with androgens (20) and decreases in male-to-female transsexuals (20) or in elderly

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men (21) treated with estrogens (E). In postmenopausal women, hormonal therapy (HT) decreases fasting Hcy in all (22–27) but one study (28), and was reported to either decrease (27) or increase (29) post-methionine levels. Transdermal HT, investigated only in two studies (28, 30), was reported not to affect fasting Hcy. In the present study, we compared the effect of transdermal with that of oral HT on fasting and post-methionine Hcy levels.

MATERIALS AND METHODS

Twenty-four women in natural menopause for 1 to 5 years with FSH values >40 IU/L and E_2 values <20 pg/mL were recruited at the Menopause Center of our institute. Subjects voluntarily gave their informed consent to participate in the study, which was previously approved by the local ethical committee and institutional review board. None of the women was suffering from endocrine, renal, or liver disturbances. Women taking vitamin supplements were excluded from the study. Furthermore, each subject was requested not to take vitamin supplements for the entire period under investigation.

After a computer-generated list of randomization, each subject was allocated to receive either a continuous combined transdermal HT, with 50 μg/d of E_2 and 140–170 μg/d of norethisterone (NET) acetate (Combestril, Rottapharm, Monza, Italy; n = 12), or a continuous combined oral HT, with 2 mg of E_2 and 1 mg of NET acetate (Kliogest; Novo Nordisk Pharmaceutics, Roma, Italy; n = 12). Before and after 6 months of therapy, each subject received an oral methionine load (0.1 g/kg body weight). Methionine was administered at 8:00 A.M., after a 24-hour low protein diet and overnight fasting. Blood samples were collected just before methionine administration (time 0) and after 4 hours (27). After centrifugation, serum was stored at −20°C until assayed. Serum Hcy and Cys levels were assayed by a high-performance liquid chromatography (HPLC) method (BIO-RAD, Munich, Germany) with a fluorometric detection. Internal standard (100 μL) and reduction reagent (50 μL) was added to 50 μL of serum. After addition of 100 μL of derivatization reagent, the sample was incubated for 5 minutes at 50°C. After cooling, the proteins were precipitated by adding 100 μL of precipitation reagent and removed by centrifugation. The supernatant was injected into an isocratic HPLC system. The assay had a sensitivity of 0.5 mmol/L and intra- and interassay coefficient of variation (CV) of 3.8% and 6%, respectively. Serum folate and vitamin B12 were determined in fasting samples, by the SimulTRAC-SNB kit for the simultaneous radiodosage of vitamin B12 [57 Co] and folate [125 I] (ICN Pharmaceuticals, Orangeburg, NY). The sensitivity was 75 pg/mL for vitamin B12 and 0.6 ng/mL for folate. Intra-assay CV was 11.2% for vitamin B12 and 4.1% for folate, whereas inter-assay CV was 12.3% for vitamin B12 and 7.1% for folate.

Statistical analysis was performed with the statistical package StatView 5.0.1 for Apple Macintosh (SAS Institute, Inc., Cary, NC). Baseline and net modifications in the two groups were compared by the Mann-Whitney *U* test. The Wilcoxon signed-rank test was used to test the changes of fasting and post-methionine Hcy values induced, within each group, by HT. Regression analysis was used to evaluate the relation between folate or vitamin B12 and Hcy, as well as between the net Hcy modifications induced by HT and its pretreatment values. For all analyses the null hypothesis was rejected at $P<.05$.

All the results are expressed as the median with confidence interval (10th–90th percentile).

RESULTS

Characteristics of subjects at baseline and after 6 months of oral or transdermal HT are reported in Table 1. At baseline, no significant difference was observed between the two groups. After 6 months, a significant increase in serum glucose ($P<.04$) and body mass index ($P<.008$) was observed in women receiving oral HT. In all women considered together (n = 24), fasting Hcy was significantly and inversely related to levels of folate ($y = -0.825x + 15.09$; $r = 0.607$, $P=.0098$) and vitamin B12 ($y = -0.01x + 15.79$; $r = 0.679$, $P=.002$). After 6 months of either transdermal or oral HT, no change was observed in the levels of folate or vitamin B12 (Table 1). The HT slightly decreased fasting Hcy from 8.9 (6.7; 15.2) μmol/L to 8.3 (4.9; 12.0) μmol/L ($P<.02$). The effect was not different between the two HT groups, although in the transdermal group, but not in the oral group, it reached statistical significance (Table 1). The net Hcy decrease induced by either transdermal or oral HT was linearly related to pretreatment values of fasting Hcy. The regression slopes of the transdermal and oral group were not different from each other, and the data were considered together (Fig. 1). The regression line encounters the zero line at basal pretreatment Hcy levels of 9 μmol/L.

When women were divided on the basis of their pretreatment baseline values, HT reduced Hcy in those 11 women whose baseline values were above 9 μmol/L. In these women Hcy decreased from 13.0 (9.3; 19.1) μmol/L to 10.1 (5.6; 12.0) μmol/L ($P<.005$), with no difference between the oral (−2.6 [−4.3; 0.17] μmol/L; n = 6; $P<.05$) and transdermal (−1.5 [−18.7; −0.4] μmol/L; n = 5; $P<.05$) route of HT administration. In contrast, HT was without effect in the 13 women whose baseline values were below 9 μmol/L. In these women Hcy did not significantly vary from 7.8 (6.0; 8.9) μmol/L to 7.7 (4.3; 9.3) μmol/L.

The Hcy/Cys, a possible index of Hcy metabolization through the trans-sulfuration pathway, was significantly reduced after HT (0.061 [0.039; 0.107] vs. 0.048 [0.032; 0.093]; $P<.01$) with no difference between the two routes of administration. Also in this case, when the pretreatment

TABLE 1

Characteristics of women evaluated before and after 6 months of the transdermal (n = 12) or the oral (n = 12) administration of continuous combined estradiol and norethisterone acetate.

	Transdermal		Oral	
	Before	During	Before	During
Age (y)	53.5 (51;58)		55 (51;60)	
BMI (kg/m^2)	23.8 (21.3;29.3)	24.2 (21.3;30.8)	24.0 (21.0;28.2)	24.5 (21.5;29.1)[b]
T-cholerol (mg/dL)	219 (172;253)	194 (150;235)	237 (191;275)	205 (183;254)
LDL (mg/dL)	118 (84;180)	103 (72;162)	159 (83;210)	142 (117;178)
HDL (mg/dL)	71 (49;88)	73 (59;85)	53 (38;84)	45 (37;58)
Triglycerides (mg/dL)	86 (44;122)	75 (40;84)[a]	85 (58;124)	96 (44;141)
Glucose (mg/dL)	97 (79;101)	97 (75;104)	85 (78;102)	94 (79;108)[a]
Hcy (μmol/L)	9.4 (6.3;16.7)	8.1 (4.2;9.3)[a]	10.1 (6.6;15.2)	9.7 (5.5;12.3)
Cys (μmol/L)	104 (93;136)	95 (81;126)	203 (96;350)	248 (116;384)
B12 (pg/L)	433 (174;566)	384 (259;839)	325 (215;1,140)	318 (186;1,075)
Folate (ng/L)	5.4 (2.0;11.2)	5.3 (1.8;9.7)	4.0 (2.1;6.7)	4.3 (2.4;7.7)
Post-load Hcy (μmol/L)	33.5 (27.5;75.9)	28.4 (17.4;48.9)[a]	36.1 (17.7;74.8)	29.9 (17.5;50.3)[b]

Note: Results are expressed as the median values. Confidence limits representing the 10th and the 90th percentile are reported in parenthesis.
[a] $P<.05$.
[b] $P<.01$ vs. before.

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Hcy/Cys levels were higher the decrement induced by either one of the two HT regimens was greater (Fig. 1). The levels reached by Hcy after methionine administration were similarly reduced after 6 months of either transdermal or oral HT (Table 1).

The net increase of Hcy after methionine administration was more pronounced before than after transdermal (24.4 [20.1; 63.1] μmol/L vs. 20.2 [12.0; 40.0] μmol/L; $P<.05$) or oral HT (26.2 [9.8; 60.3] μmol/L vs. 21.8 [10.6; 38.3] μmol/L; $P<.02$) administration. No difference was observed between the two routes of HT administration (Fig. 2).

DISCUSSION

The effect of gonadal steroids on Hcy metabolism is not fully understood. It is generally accepted that levels of Hcy are increased by androgens and decreased by E (20). This opposite effect is of particular relevance for HT, in which E given by different routes of administration are often associated with progestins possessing minor or major androgenic properties (31). Most of the studies heretofore performed focused on oral HT. Oral E associated with nonandrogenic progestins as dydrogesterone (22–24), trimegestone (24), or medroxyprogesterone acetate (25, 26, 32) decreased fasting Hcy, whereas oral E associated with androgenic progestins, as NET acetate, did not modify in one study (28) and decreased in another study (27), both fasting and post-methionine Hcy. The present study confirms that oral E_2 continuously combined with oral NET decreases post-methionine Hcy levels. In contrast, mean levels of fasting Hcy were not significantly reduced.

FIGURE 1

Regression analysis between levels of homocysteine (Hcy; **top panel**) or the ratio homocysteine/cysteine (Hcy/Cys; **bottom panel**) and the net modifications induced by continuous combined E_2 plus norethisterone administered for 6 months by a transdermal (*open circles*) or an oral (*closed circles*) route.

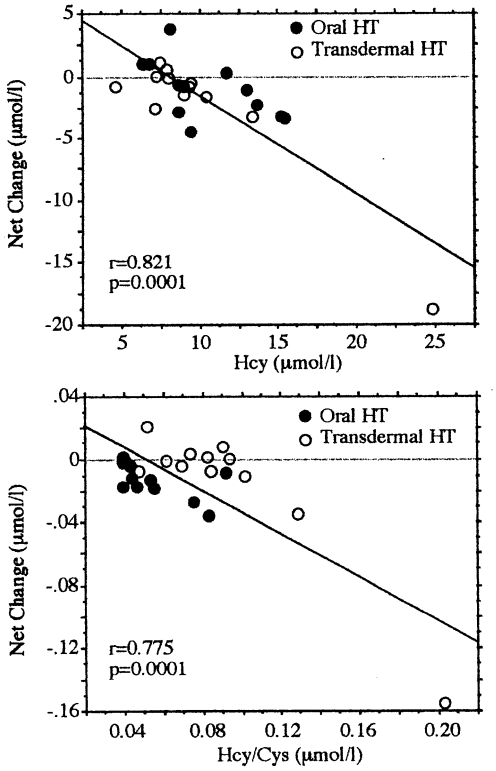

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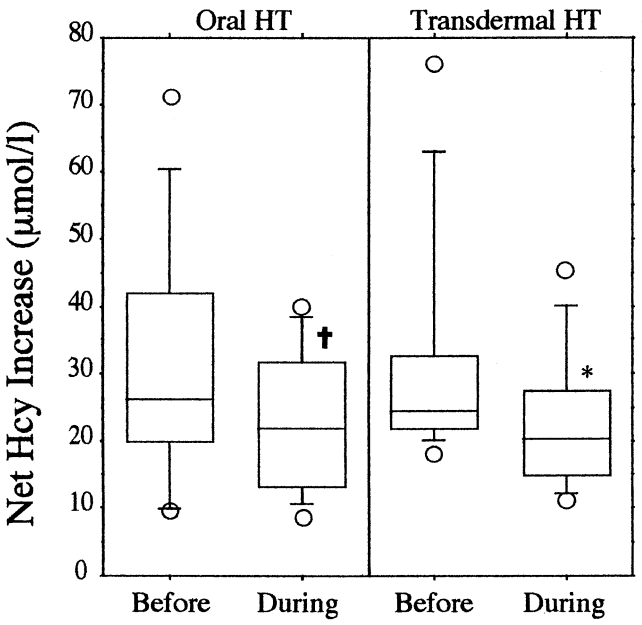
FIGURE 2

Difference between postoral methionine load and basal Hcy values observed in basal condition and after continuous combined E_2 plus norethisterone administered for 6 months by an oral (**left panel**) or transdermal (**right panel**) route. Values are reported as the median of the values. The *boxes* show the 25th to 75th percentiles and the *lines* the 10th to 90th percentiles. *$P<.05$; †$P<.02$ vs. the corresponding before, by Wilcoxon signed-rank test.



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Transdermal HT is characterized by a more physiological E environment, and by a markedly reduced metabolic impact particularly on liver and lipoproteins (33). Whether the effect of transdermal HT on Hcy metabolism is similar to that exerted by oral HT was herein investigated by administering the same compounds by both routes. A placebo group was not included, and this represents a limit of our study. The results indicate that similarly to oral administration, transdermal HT reduces the increase of Hcy induced by methionine administration. Because the effects of transdermal HT seems to be comparable to those of oral HT, the hepatic first pass of E does not seem to be necessary for HT to induce a positive effect on Hcy metabolism.

Oral and transdermal HT, when combined also decreased fasting Hcy. The effect was similar with both treatments, but it was statistically significant only in the transdermal group. The effect of E_2 and NET on fasting Hcy have been investigated with conflicting results (27, 28). Similarly, transdermal E_2 was reported not to affect basal Hcy levels (28, 30). Slight differences in baseline Hcy levels of women included in the different studies may explain these differences. We have shown that the effect of either transdermal or oral HT on fasting Hcy is related to preexisting Hcy levels, high but not low Hcy being reduced by HT. A value of 9 μmol/L, by regression analysis, was the limit above which HT reduces Hcy. When data above this limit were separately evaluated, a significant decrease in Hcy was observed, independent of the route of HT administration. Vice-versa, HT was ineffective in those cases in which baseline Hcy levels were below this cutoff point. Previous published results (22) have reported the capability of oral HT to decrease Hcy only in women with elevated fasting Hcy levels.

The mechanism through which HT, either oral or transdermal, decreases Hcy is unclear. The effect does not seem to be mediated by modifications in liver or lipoprotein metabolism. Previous data have also reported that these modifications cannot be explained by modifications in vitamin B6, vitamin B12, and folate levels (27). Also in the present study, levels of vitamin B12 and folate were not modified by HT. Vitamin B6 was not measured. However, a widespread use of vitamin B6 alone is rather uncommon and none of the women reported taking vitamins during the study period.

The preferential effect of HT on elevated Hcy and on post-methionine Hcy levels, along with the reduction of the Hcy/Cys ratio, seems to sustain the activation by HT of trans-sulfuration Hcy catabolism to cysteine. This view is supported by a recent study in which elevated fasting Hcy was reduced by HT in patients in which the remethylation pathway was impaired by a thermolabile form of methylentetrahydrofolate reductase (32).

In conclusion, similar to oral administration, transdermal E_2 continuously combined with transdermal NET acetate reduces elevated and post-methionine Hcy levels. In the long term, these modifications may exert possible beneficial effects on the circulatory system of postmenopausal women.

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