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Hormone Replacement Therapy Causes a Respiratory Alkalosis in Normal Postmenopausal Women*

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ABSTRACT

Menopause is associated with an increase in venous bicarbonate concentrations that is reversible with hormone replacement therapy (HRT). However, the mechanism underlying this effect is not known. To address this question, we studied the changes in acid-base indexes in the arterialized venous blood of normal postmenopausal women commencing conjugated equine estrogen (0.625 mg/day), medroxyprogesterone acetate (MPA; 5 mg/day), their combination, or placebo, in a double blind randomized controlled study over 3 months.

Serum bicarbonate concentrations decreased significantly in the groups receiving either MPA or estrogen plus MPA ($P = 0.008$). This trend was apparent as early as 2 days and reached 2.7 and 2.3 mmol/L in the respective groups by 3 months. Similar changes were seen with partial pressure of carbon dioxide ($P = 0.04$); a change of -0.7 kPa occurred in the estrogen plus MPA group at 3 months. There were no changes in bicarbonate concentrations or partial pressure of carbon dioxide in those receiving estrogen alone or placebo. Accompanying changes in blood pH were apparent in the estrogen plus MPA group, where there was an upward trend at 1 week ($P = 0.056$) and a significant change from baseline ($+0.013$) at 3 months ($P = 0.03$). In the whole group, the changes in pH were inversely correlated with those in urinary excretion of hydroxyproline ($r = -0.44$; $P = 0.01$).

We conclude that HRT using conjugated estrogens and MPA produces small, but sustained, changes in acid-base status. These may contribute to the effects of HRT and menopause on many tissues and disease processes, including the development of osteoporosis. (*J Clin Endocrinol Metab* **84:** 1997–2001, 1999)

MENOPAUSE produces profound changes in many metabolic processes, some of which lead eventually to significant medical problems, such as osteoporosis and atherosclerosis. One metabolic consequence of the menopause is a rise in the venous bicarbonate concentration (1–4), and this change is reversed by hormone replacement therapy (HRT) (3, 5). Surprisingly, these observations have not been further explored to determine their underlying mechanism. An increase in bicarbonate concentration could be attributable to either metabolic alkalosis (*e.g.* as a result of a decline in acid production) or respiratory acidosis, resulting from a primary decrease in alveolar ventilation. The mechanisms underlying these two states are quite distinct, and their potential consequences, on calcium metabolism, for instance, are diametrically opposite.

To address this issue, we have studied the changes in acid-base metabolism in normal postmenopausal women commencing HRT. The two elements of conventional HRT, estrogen and progestin, have been assessed separately and in combination to determine their respective contributions to any effects found. In light of the major effects of both menopause and acid-base status on bone and calcium metabolism (6), indexes of mineral status have also been measured.

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Subjects and Methods

Subjects

Forty normal postmenopausal women were recruited by newspaper advertisement. All were between 1–10 yr since their last menstrual period. In hysterectomized subjects, menopausal status was confirmed by measurement of circulating estradiol and FSH concentrations (<110 pmol/L and > 30 IU/L, respectively). All subjects were screened to exclude conditions or medications known to affect acid-base or calcium metabolism, and none had used HRT within the previous 6 months. One subject randomized to placebo was found to have primary hyperparathyroidism and was removed from the study. Clinical characteristics of the study subjects are shown in Table 1.

Study protocol

Subjects were randomized to receive placebo, medroxyprogesterone acetate (MPA; 5 mg/day), conjugated estrogens (0.625 mg/day), or both active medications. A double dummy design was used so that both subjects and investigators were blinded to treatment allocation. Subjects were seen at baseline, 2 days, 1 week, and 3 months. Compliance was assessed by tablet counts. Women assigned to unopposed estrogen treatment were given a 14-day course of MPA (10 mg/day) after the collection of all study samples. The study was approved by our institutional review committee, and all subjects gave written informed consent.

Measurements

All samples were collected after an overnight fast. Second voided urine samples were collected for measurement of bone resorption markers. Acid-base status was assessed using arterialized venous blood samples. These were collected by placing the subject's hand in a chamber heated to 55 C for 15 min before blood sampling, which was undertaken without the use of a tourniquet. This technique results in local vasodilation with shunting of blood from the arterial supply to the superficial veins of the dorsum of the hand, from which blood was drawn through a catheter facing in a retrograde direction. Indexes of acid-base status measured in this way are not significantly different from those in samples drawn simultaneously from the brachial artery (7, 8).

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TABLE 1. Clinical characteristics of study subjects

	Placebo	E	P	E + P
n	9	10	10	10
Age (yr)	54 ± 5	54 ± 6	55 ± 3	56 ± 6
Yr since menopause	5 ± 5	4 ± 4	4 ± 3	5 ± 3
Wt (kg)	74 ± 15	67 ± 8	72 ± 14	71 ± 11
Ht (m)	1.62 ± 0.06	1.62 ± 0.10	1.60 ± 0.06	1.61 ± 0.06
Dietary calcium (mg/day)	900 ± 380	1190 ± 640	840 ± 320	1000 ± 280
Compliance (%)	98 ± 4	94 ± 9	97 ± 4	98 ± 3

Data are the mean ± SD.

TABLE 2. Baseline values of biochemical parameters

	Placebo	E	P	E + P
Serum				
Bicarbonate (mmol/L)	26.1 ± 1.1	25.4 ± 1.6	26.4 ± 2.2	26.6 ± 1.3
P_{CO2} (kPa)	5.89 ± 0.30	5.76 ± 0.87	5.53 ± 0.66	5.89 ± 0.24
pH	7.39 ± 0.02	7.40 ± 0.01	7.42 ± 0.03	7.40 ± 0.02
Total calcium (mmol/L)	2.37 ± 0.05	2.31 ± 0.09	2.34 ± 0.10	2.32 ± 0.06
Albumin (g/L)	42.8 ± 2.3	42.2 ± 1.5	42.0 ± 2.3	41.9 ± 1.5
Adjusted calcium (mmol/L)	2.38 ± 0.07	2.32 ± 0.08	2.35 ± 0.08	2.33 ± 0.03
Ionized calcium (mmol/L)	1.22 ± 0.06	1.22 ± 0.04	1.25 ± 0.05	1.22 ± 0.03
Phosphate (mmol/L)	1.14 ± 0.21	1.06 ± 0.15	1.09 ± 0.17	1.19 ± 0.11
PTH (pmol/L)	3.28 ± 1.26	3.47 ± 1.02	3.80 ± 1.52	3.60 ± 1.10
Total alkaline phosphatase (U/L)	76.1 ± 31.7	80.0 ± 20.9	64.3 ± 17.1	74.3 ± 15.2
Bone-specific alkaline phosphatase (U/L)	18.4 ± 5.2	19.0 ± 7.6	16.0 ± 4.5	18.9 ± 4.4
Osteocalcin (μg/L)	5.4 ± 1.1	5.0 ± 2.0	5.5 ± 1.7	6.1 ± 1.6
Fasting urine				
Hydroxyproline/creatinine (μmol/mmol)	35 ± 13	32 ± 10	30 ± 7	31 ± 12
Deoxypyridinoline/creatinine (nmol/mmol)	7.0 ± 2.2	6.0 ± 2.1	6.7 ± 2.9	7.4 ± 2.6
N-Telopeptide/creatinine (nmol/mmol)	36 ± 18	42 ± 24	48 ± 33	51 ± 23
24-h urine				
Urinary calcium (mmol/day)	4.3 ± 1.9	3.2 ± 1.5	3.8 ± 1.7	4.9 ± 1.4

Data are the mean ± SD. Bicarbonate, P_{CO2}, and pH were measured in arterialized venous blood.

pH, bicarbonate, partial pressure of carbon dioxide (P_{CO2}), and ionized calcium were assessed using a Corning 860 blood gas analyzer (Corning, Inc., Corning, NY). Serum electrolytes, creatinine, calcium, phosphate, and albumin concentrations, and total alkaline phosphatase activity were measured with a Hitachi 911 analyzer (Hitachi Scientific Instruments, Inc., Hialeah, FL) using reagents from Boehringer Mannheim (Indianapolis, IN). Adjusted calcium was calculated as total calcium + 0.02(40 − albumin). Urinary hydroxyproline was assessed using a Technicon autoanalyzer (Tarrytown, NY). Serum and urine samples for other assays were stored at −70 C before measurement. Intact PTH and osteocalcin were measured by immunoradiometric assay (Nichols Institute Diagnostics, San Juan Capistrano, CA), bone-specific alkaline phosphatase and deoxypyridinoline were determined by enzyme-linked immunosorbent assay (Metra Biosystems, Mountain View, CA), and N-telopeptide was determined by enzyme-linked immunosorbent assay (Ostex International, Inc., Seattle, WA). Baseline biochemical values are shown in Table 2.

Statistical analysis

The primary end points of this study were the changes from baseline of the indexes of acid-base status at 3 months. Data were assessed by repeated measures ANOVA using the general linear models procedure of SAS (SAS Institute, Inc., Cary, NC). All tests were two tailed, and $\alpha = 0.05$. Data are given as the mean ± SEM unless indicated otherwise.

Results

Acid-base

Serum bicarbonate concentrations decreased significantly in the groups receiving either MPA or combined therapy (Fig. 1). This trend was apparent as early as 2 days after the initiation of hormone therapy, was statistically significant by day 7 (−1.4 ± 0.6 and −1.0 ± 0.3 mmol/L in the MPA and combined therapy groups, respectively) and became more marked in these two groups at 3 months. There were no changes in bicarbonate concentrations in those receiving conjugated estrogen or placebo. The between-groups differences were significant ($P = 0.008$).

P_{CO2} in the combined therapy group paralleled the serum bicarbonate, progressively falling throughout the study period (Fig. 1). This depression of P_{CO2} was significant at all time points (−0.27 ± 0.06 at 2 days; −0.36 ± 0.06 at 7 days) reaching −0.7 ± 0.1 kPa at 3 months. In subjects treated with MPA alone, there was a similar, but less marked, decline in P_{CO2}, which was statistically significant at 3 months (change from baseline, −0.5 kPa). Subjects receiving conjugated estrogen alone or placebo showed no significant changes throughout the study. The between-groups differences were significant ($P = 0.04$).

Accompanying changes in blood pH were apparent only in the group treated with combined therapy (Fig. 1). pH in this group showed an upward trend at 1 week (0.012 ± 0.005; $P = 0.056$), which was more marked at 3 months ($P = 0.03$). There were no significant changes in pH in the other groups.

Calcium metabolism (Table 3)

Only the combined therapy group consistently showed changes; there were reductions in all of the serum calcium fractions assessed, a reduction in 24-h urinary calcium excretion, and a reduction in the serum phosphate concentration. Indexes of bone turnover decreased in both the conju-

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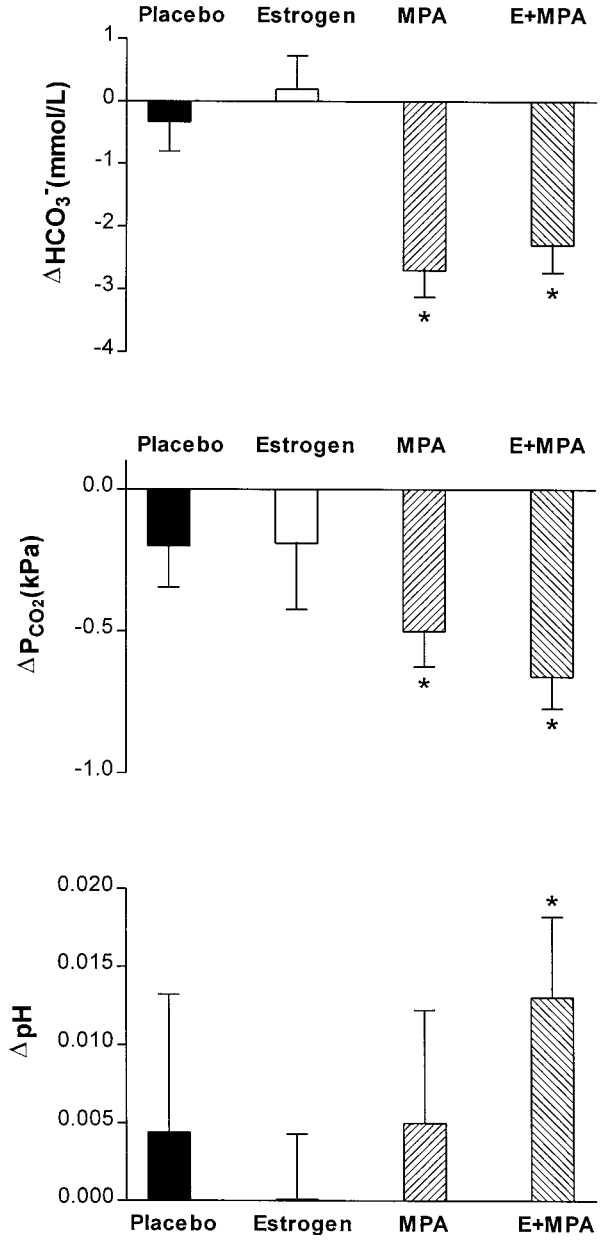

FIG. 1. Effects of conjugated equine estrogen (0.625 mg/day), MPA (5 mg/day), their combination (E+MPA), or placebo on acid-base parameters in arterialized venous blood of normal postmenopausal women. Data are shown as changes from baseline at 3 months and are the mean ± SEM. Significant changes from baseline are denoted with an *asterisk*. HCO_3^-, Bicarbonate.

gated estrogen and combined therapy groups; these changes were more consistent and tended to be larger in the latter group. Total alkaline phosphatase activity was significantly decreased in both the conjugated estrogen and the combined therapy groups from day 2 (-3.3 ± 1.3 and -2.4 ± 1.0 U/L, respectively; $P < 0.05$) and markedly so by day 7 (-9.0 ± 1.0 and -8.3 ± 0.9; $P < 0.0001$). Bone-specific alkaline phosphatase showed a similar pattern of change, although the earliest significant depression below baseline was in the conjugated estrogen group on day 7. Hydroxyproline was reduced in the estrogen group by day 7, but depression of the other bone resorption markers and serum osteocalcin was not evident until the 3 month assessment.

To assess whether the changes in bone resorption were related to those in acid-base status, correlations between the changes in the respective indexes were derived. Changes in hydroxyproline excretion were inversely related to those in pH ($r = -0.41$; $P = 0.01$). Inclusion of the treatment group as a covariable in this analysis did not significantly modify the outcome; an independent relationship was still found between change in pH and change in hydroxyproline excretion at 3 months ($P = 0.008$). N-Telopeptide and deoxypyridinoline showed similar trends ($r = -0.24$ and -0.11, respectively), which were not statistically significant. Changes in N-telopeptide were positively related to those in P_{CO2} ($r = 0.31$; $P = 0.06$).

Other biochemical measures

Serum sodium, potassium, urea, creatinine, chloride, and γ-glutamyl transferase were measured at each time point. There were no significant changes in these parameters.

Discussion

This study confirms the previous finding that combined HRT causes a decrease in circulating bicarbonate concentrations. However, it carries our understanding of this phenomenon much further by showing that the fall in bicarbonate is accompanied by a similar change in P_{CO2} and a small increase in pH. This combination of responses can only be accounted for by this form of HRT causing respiratory alkalosis. This is most likely to result from a primary stimulation of the respiratory center in the central nervous system leading to the decrease in P_{CO2}. This will directly lead to a decrease in bicarbonate concentration (as CO_2 and bicarbonate are in equilibrium with one another) and indirectly to a further decline in bicarbonate as a result of metabolic compensation for the change in pH that has occurred. As a consequence, the final change in pH is small. Treatment with MPA alone resulted in slightly smaller changes in P_{CO2} than those seen with combined therapy, and no pH change was detected. This is probably a reflection of the substantial metabolic compensation that has occurred, resulting in a pH change that is at the limits of detection of this study. The slightly greater depression of P_{CO2} by conjugated estrogen and MPA probably indicates an interaction of these hormones centrally (see below), although an interaction at the levels of tissue acid production or its renal excretion is also possible. In contrast, conjugated estrogen monotherapy did not significantly affect acid-base status, consistent with the previous observation that estrogen alone does not impact on bicarbonate concentrations (9).

Although the effects of HRT on acid-base status in postmenopausal women have not been assessed previously, there is other evidence that progestins alone or in combination with estrogens stimulate ventilation and lead to a respiratory alkalosis. Women hyperventilate during pregnancy (10, 11), a state in which both progestin and estrogen concentrations are high, although pregnancy obviously also involves other physiological changes. Qualitatively similar results have been reported in comparative studies of the

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TABLE 3. Changes in biochemical parameters at 3 months

	Placebo	E	P	E + P
Serum				
Total calcium (mmol/L)	−0.07 ± 0.03	−0.04 ± 0.02	−0.01 ± 0.03	−0.08 ± 0.02[a]
Albumin (g/L)	−1.1 ± 1.0	−2.3 ± 0.6[a]	−1.0 ± 0.6	−0.1 ± 0.3
Adjusted calcium (mmol/L)	−0.05 ± 0.02	0.00 ± 0.02	0.01 ± 0.02	−0.06 ± 0.02[a]
Ionized calcium (mmol/L)	−0.01 ± 0.02	−0.01 ± 0.01	−0.01 ± 0.02	−0.03 ± 0.01[a]
Phosphate (mmol/L)	−0.03 ± 0.07	−0.08 ± 0.06	0.04 ± 0.05	−0.18 ± 0.04[a]
PTH (pmol/L)	0.3 ± 0.2	0.0 ± 0.3	−0.1 ± 0.3	−0.2 ± 0.4
Total alkaline phosphatase (U/L)	−1.3 ± 2.6	−9.0 ± 2.5[a]	−8.9 ± 4.1	−14.9 ± 2.3[a]
Bone-specific alkaline phosphatase (U/L)	0.7 ± 0.6	−2.6 ± 0.8[a]	−0.7 ± 0.6	−3.5 ± 0.8[a]
Osteocalcin (μg/L)	−0.3 ± 0.3	−0.7 ± 0.2[a]	−0.5 ± 0.3	−1.0 ± 0.3[a]
Fasting urine				
Hydroxyproline/creatinine (μmol/mmol)	−6.3 ± 5.0	−9.5 ± 3.5[a]	−0.2 ± 4.8	−11.1 ± 3.6[a]
Deoxypyridinoline/creatinine (nmol/mmol)	−0.37 ± 0.83	−1.33 ± 0.57[a]	−0.16 ± 0.84	−3.52 ± 0.78[a]
N-telopeptide/creatinine (nmol/mmol)	6.4 ± 6.4	−10.7 ± 6.1	−13.0 ± 8.4	−20.8 ± 5.6[a]
24-h urine				
Urinary calcium (mmol/day)	−0.24 ± 0.37	−0.03 ± 0.39	−0.23 ± 0.72	−1.04 ± 0.34[a]

Data are the mean ± SEM.
[a] A significant difference from baseline, $P < 0.05$.

follicular and luteal phases of the human menstrual cycle (12) and in comparisons between men and premenopausal women (13). Pharmacological doses of progestins stimulate respiration and result in a respiratory alkalosis in men (8, 14, 15) and guinea pigs (16), but in the cat (17) and rat (18, 19) combined therapy with estrogen is necessary to produce an effect. There are progesterone receptors in the hypothalamus that mediate these effects (20), and their number is increased by exposure to estrogen (21), which may account for the greater effects of combined therapy.

Acid-base status is one of the most tightly regulated aspects of homeostasis because of its substantial impact on many biological functions. It is likely, therefore, that the effects of female sex hormones on acid-base status will have substantial consequences throughout the body. For instance, there is evidence that neuronal function (22) and the development of neurofibrillary degeneration in Alzheimer's disease (23) are both pH dependent, and it is possible that atheroma development is also influenced by acid-base status, although this appears to have received little detailed attention. Bone resorption is critically dependent on acid-base status; a 30% reduction in the carbon dioxide content of medium in which osteoclasts are cultured (from 7.5% to 5%) results in a 4-fold reduction in the number of resorption pits formed (24). A further fall in carbon dioxide content to 2.5% virtually arrests bone resorption altogether. Thus, the 11% decrease in P_{CO2} induced by HRT in the present study is likely to account for some of the reduction in bone resorption observed, even allowing for the fact that the effect of this change on pH will be less in the regulated environment that exists *in vivo*. The correlation of pH rise with the fall in hydroxyproline excretion in the present study supports this conclusion, as does the demonstration that the alkalosis associated with bicarbonate administration reduces bone resorption in postmenopausal women (25). The secondary change in bicarbonate concentration may itself contribute to reduced bone resorption, since Bushinsky *et al.* have demonstrated dependence of bone resorption *in vitro* on bicarbonate concentration even when pH is held constant (26).

Osteoblast function is also affected by pH; collagen production, alkaline phosphatase activity, and cell proliferation are higher at higher pH (27). There is also evidence that osteocalcin concentration in the serum (an index of osteoblast activity) is increased after bicarbonate administration (25). Thus, the present findings might account for the observation that serum concentrations of osteocalcin are higher in the luteal phase of the menstrual cycle (28). A correlation between the change in pH and changes in indexes of osteoblast function was not found in the present study, probably because any such relationship would be overwhelmed by the coupling of bone formation to resorption.

There is evidence that alkalosis is associated with reduced calcium excretion (25, 29), implying a direct effect of pH on renal calcium handling. However, the reduction in serum bicarbonate associated with HRT may also influence urinary calcium excretion by reducing the fraction of serum calcium that is complexed with bicarbonate, thus reducing plasma ultrafiltrable calcium (2). These mechanisms may account for the findings in the present study of a fall in urinary calcium excretion during combined therapy, whereas conjugated estrogen alone had no effect.

There are clearly mechanisms other than acid-base effects by which HRT impacts on bone and calcium metabolism, evident in the present study from the fall in markers of bone turnover in subjects treated with estrogen alone. However, it should be noted that changes in calcium metabolic indexes are consistently greater in those receiving combined treatment, suggesting that the acid-base changes might contribute. These changes might account for the greater increases in bone mass produced by combined HRT compared with estrogen alone (30) and by the greater efficacy of combined therapy in the prevention of hip fractures (31).

The potential for a small increase in pH to exert an anabolic effect on the osteoblast may apply to other cells also. Cell proliferation is highly dependent on intracellular pH, which, in turn, is influenced by the extracellular H^+ concentration (32). In muscle, low pH inhibits protein synthesis and accelerates its catabolism (33, 34), and administration of bicarbonate reduces urinary nitrogen excretion, suggesting reduced soft tissue breakdown (35). Acidosis is also associated with reduced serum concentrations of GH and insulin-like

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growth factor I (36). Thus, the acid-base effects of HRT might also contribute to its impact on soft tissues.

The present study demonstrates a suppression of some markers of osteoblast function within 2 days of initiation of HRT. These changes preceded those in bone resorption, a marked contrast to the findings with other antiresorptive therapies such as bisphosphonates (37). This indicates that the decline in bone formation after HRT cannot be simply accounted for as a coupling phenomenon secondary to the suppression of resorption. It points to the osteoblast being the primary target for estrogen in bone, consistent with it being the principal site of estrogen receptors in bone.

In conclusion, the present study has demonstrated that HRT using conjugated estrogens and MPA produces small, but sustained, changes in acid-base status. These changes correlate with the suppression of bone resorption, suggesting that acid-base changes may contribute to the positive effects of HRT on bone balance in postmenopausal women. By inference, the loss of these effects contributes to bone loss after the menopause. There are potential consequences of these effects in many other tissues that now require investigation to further understand the menopause and to determine whether combined HRT is preferable to estrogen alone. These findings will be important in assessing the adequacy of novel agents, such as the selective estrogen receptor modulators and new progestins, as substitutes for combined HRT.

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