

Trace elements and vitamin levels in menopausal women receiving hormone replacement therapy

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Summary

Trace elements are extremely important in human metabolism, growth, and tissue repair. The risk of nutritional disturbances, in particular, vitamin and trace element deficiencies, are striking during menopause.

The aim of this longitudinal study was to evaluate the effect of estrogen treatment on serum levels of copper, zinc, magnesium, calcium, vitamin E, and vitamin A in menopausal women. Thirty-eight menopausal women were included in the study, and were administered a continuous hormone replacement therapy (HRT) of 0.625 mg conjugated equine estrogen (CEE) and 2.5 mg medroxyprogesterone acetate (MPA). Blood samples were obtained before and six months after HRT.

There was a statistically significant difference between levels of serum copper, zinc, magnesium, calcium, vitamin E and vitamin A before and after HRT ($p < 0.001$).

In conclusion we observed a beneficial effect of HRT on serum levels of trace elements, vitamin A, and vitamin E in addition to the well known other benefits.

Key words: Menopause; HRT; Trace elements; Vitamin.

Introduction

Trace elements play important roles in the human body [1, 2]. Zinc and copper are important in tissue development by being constituents of DNA and protein synthesis and nerve myelination. A deficiency of zinc and copper may result from inadequate intake in the diet, decreased absorption or a metabolic congenital disease [1-4]. Insufficient intake in the diet has been observed especially in poor living conditions as in underdeveloped countries [5]. Magnesium is a cofactor for many enzyme systems that are critical for intracellular metabolism, particularly tyrosine kinase activity [1, 6].

The most clearly established and critical functional role for ascorbic acid is as a cofactor for procollagen hydroxylase, the enzyme responsible for hydroxylation of prolyl and lysyl residues within ascent peptides in connective tissue proteins. Among the functions of vitamin A are the participation of retinal in vision, reproduction and growth. Systemic effects that reflect an optimal level of vitamin A are the stabilization of cellular and intracellular membranes, the maintenance of the integrity of epithelial tissue, and the synthesis of glycoproteins [7-9].

Vitamin E is present on cellular membranes, helping to stabilization against the process of lipid peroxidation. It protects cells by scavenging free radicals during the peroxidation of unsaturated fatty acids through a reaction that leads to the formation of the stable vitamin E radical, which itself can be reduced by vitamin C [7].

The risk of nutritional disturbances, in particular vitamin and trace element deficiencies, is high during menopause. Such deficiencies can be revealed by nutritional survey results for lifestyle related to the natural

events of aging, together with hormonal disturbances. The consequences of these deficiencies affect sensitivity to estrogens, structure of the skin and its accessory structures, bone metabolism, immune function and increased risk of degenerative pathology, in particular cardiovascular. It has been described in many studies that menopausal women have lower trace element levels [10-12].

The aim of this study was to evaluate the efficacy of estrogen treatment by assessing the serum copper, zinc, magnesium, calcium, vitamin E, and vitamin A levels in menopausal women.

Materials and Methods

Thirty-eight menopausal women were included in the study, and were administered a continuous hormone replacement therapy (HRT) of 0.625 mg conjugated equine estrogen (CEE) and 2.5 mg medroxyprogesterone acetate (MPA). Fasting blood samples were collected before and six months after HRT. Serum was obtained from clotted blood and stored in tubes washed with deionized water. Samples were stored at -20°C and analysed by flame atomic absorption spectrophotometry. Samples for copper, zinc and magnesium were diluted with deionized water at ratios of 1:2, 1:5, 1:50, respectively. Samples for calcium were diluted with 1% lanthanum chloride at a ratio of 1:50.

Calibration standards and quality control specimens were also diluted in the same way. Measurement was performed with a Perkin Elmer 2380 atomic absorption spectrophotometer at wavelengths 324.8, 213.9, 285.2 and 422.7 nm for copper, zinc and magnesium, respectively; 0.2 ml serum was mixed with 0.4 ml propanol for 1 minute and ultra-centrifuged; 50 μl of the clear supernatant was collected and was assessed using high pressure liquid chromatography (HPLC) of wavelength filters in 325 nm for vitamin A, in 280 nm for vitamin E [13]. The HPLC condition was the mobile phase consisting of methanol mixed with water at a ratio of 54:4 with a flow rate of 1.5

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ml/minute. Vitamin A and E were measured at 325 nm and 280 nm, respectively, with acceptable sensitivity standards. Controls were also measured together with the test samples. Normal values for vitamin A and E were taken as 200-1500 ng/ml and 5-16 µl/ml, respectively.

Statistics

SPSS 9.05 was used for the statistical calculations. The results were evaluated using the Student's *t*-test; *p* values less than 0.05 were considered significant.

Results

Increased serum levels of copper, zinc, magnesium, calcium, vitamin A and vitamin E were observed in menopausal women after six months of treatment with conjugated estrogen 0.625 and medroxyprogesterone acetate 2.5 mg (after HRT group) when compared to before treatment status. (before HRT group). The increases in serum levels of copper, zinc, magnesium, calcium, vitamin E and A were 29.45%, 23.5%, 10.22%, 27.2%, 57.2%, 70.2%, respectively.

The levels of serum copper, zinc, magnesium, calcium, vitamin A and vitamin E before and after HRT in menopausal women are shown in Table 1. Differences between serum levels of copper, zinc, magnesium, calcium, vitamin A, and vitamin E before and after HRT were statistically significant (*p* < 0.001).

Table 1. — Serum Cu, Zn, Mg, Ca, vitamin E and A before and after HRT.

Groups	Cu µmol/l	Zn µmol/l	Mg mmol/l	Ca mmol/l	Vitamin E µg/ml	Vitamin A ng/ml
Before HRT	12.9±2.4	11.4±1.8	0.62±0.14	1.7±0.2	2.1±0.8	220±25
After HRT	16.7±3.6	14.6±2.2	0.71±0.16	2.1±0.3	4.9±0.9	280±28
<i>p</i> value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

HRT: Hormone replacement therapy.

Discussion

Menopause is the permanent cessation of menstruation following the loss of ovarian activity. Menopause is best understood not as a single event but as a cumulative process that begins during fetal development and continues for many years following the cessation of menses [14-16].

These changes typically include shortening of the intervals between menses during the years prior to menopause, a decline in serum estradiol levels. Estrogen affects a wide range of tissues and organs within the female body. Menopausal changes result from fluctuating hormonal levels and ratios. This includes decreased thyroid and renal function and neurologic changes, among others [14-16]. Loss of estrogen due to ovarian failure can cause a variety of distressing symptoms associated with atrophy of the tissues of the urogenital system. Estrogen has a direct effect on cardiac cells and the cells of the coronary arteries. The hormone acts as a vasodilator which can decrease blood pressure. In addition,

estrogen favorably affects the ratio of high density to low-density lipoproteins. Hormonal changes in menopause increase the incidence of cardiovascular disease. The risk of cardiovascular disease increases in women after menopause due to effects of the natural aging process and also to estrogen deficiency that begins in the premenopausal period [14, 16].

The necessity of estrogen for the preservation of bone mass was recognized a long time ago. However, the exact mechanism by which estrogen contributes to bone formation and preservation is not yet known. Estrogen receptors have been found on osteoblastic cells, which thus provides evidence of a primary effect [14, 16]. Estrogen may also contribute to bone formation with secondary effects by regulating the action of certain growth factors or by inhibiting the action of substances that contribute to bone turnover. Several researches revealed that calcium homeostasis deteriorates after menopause due to decreased calcium absorption and increased calcium loss via the kidney [14, 16].

Estrogen replacement therapy has been shown to reduce the risk of cardiovascular disease in menopausal women. Estrogen replacement therapy increases high density lipoprotein (HDL) cholesterol and decreases low density lipoprotein (LDL) cholesterol by as much as 10-15% [14, 16]. Moreover, there is strong evidence that estrogen replacement therapy protects against postmenopausal osteoporosis. Menopausal estrogen replacement therapy prevents bone loss. Several studies have demonstrated that estrogen even increases bone in postmenopausal women. Long-term estrogen use resulting in an approximately an 80% reduction in vertebral fracture rates has been observed in a study [14, 16].

Progestin is added to protect the uterus from hyperplasia. Combination therapy is used primarily for the risk of endometrial cancer associated with the use of unopposed estrogen [14, 16].

On the basis of current data, it is not possible to define the precise period of estrogen use that results in a reduction in the risk of fracture [14, 16]. The majority of research into the cardio-protective effects of estrogen is based on 0.625 mg oral conjugated equine estrogens taken daily [16].

The evidence is now convincing that vitamin E plays a role in prevention of atherosclerosis, in part through inhibition of oxidation of LDL. Metabolic balance studies showing that the dietary ratio of calcium to magnesium is best maintained at 2:1 [17, 18]. Vitamin E is an excellent lipid soluble, chain-breaking antioxidant in the presence of other cooperative antioxidants [17, 19].

Magnesium deficiency caused by a poor diet or defects in magnesium metabolism, may prove to be a missing link between various cardiovascular risk factors, atherosclerosis and osteoporosis. Deficiency of this mineral is believed to contribute to coronary artery disease (CAD) by increasing LDL levels and decreasing HDL [17, 20, 21]. Adequate intake of magnesium is also crucial for prevention of osteoporosis. Deficiency of this mineral can contribute to a decrease in bone density, in part

because of its effect on the parathyroid glands. Magnesium deficiency impairs secretion of parathyroid hormone, an action that can reduce calcium absorption and retention [17, 21].

Several other nutritional deficiencies have been associated with cardiovascular disease and osteoporosis, including selenium, calcium, chromium, vitamin C, and beta-carotene [17, 19]. A woman who can not use or refuses hormone replacement therapy, must consider other ways (alternative therapies) to address two issues related to menopause: reducing her risk of developing cardiovascular disease, osteoporosis, and other health problems that increases with age, and symptomatology [17]. Risk reduction of an array of health problems can be achieved through diet, exercise, and stress management. The nutraceuticals of specific reduced vitamins, minerals, phytoestrogens and essential fatty acid supplementations are a vital component of the risk reduction health program [17].

Treatment with HRT is suggested for menopausal women disturbed by the symptoms of hormone deprivation. Moreover, there are many beneficial effects of HRT on the health of menopausal women, like prophylaxis against osteoporosis and reduction of cardiovascular disease risk. The underlying mechanisms of these beneficial effects are strongly associated with trace elements and vitamins. As we observed in this study, HRT results in increased serum levels of trace elements and vitamins, implying their important roles in the beneficial processes.

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