
Journal of Toxicology and Environmental Health, Part A: Current Issues
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/uteh20

# EFFECTS OF HORMONE REPLACEMENT THERAPY ON LIPID PEROXIDES AND OXIDATION SYSTEM IN POSTMENOPAUSAL WOMEN

Tulay Akcay, Yildiz Dincer, Refik Kayali, Umur Colgar, Engrin Oral, Ufuk Cakatay
Published online: 30 Nov 2010.

To cite this article: Tulay Akcay, Yildiz Dincer, Refik Kayali, Umur Colgar, Engrin Oral, Ufuk Cakatay (2000) EFFECTS OF HORMONE REPLACEMENT THERAPY ON LIPID PEROXIDES AND OXIDATION SYSTEM IN POSTMENOPAUSAL WOMEN, Journal of Toxicology and Environmental Health, Part A: Current Issues, 59:1, 1-5, DOI: 10.1080/009841000157023

To link to this article: http://dx.doi.org/10.1080/009841000157023

Journal of Toxicology and Environmental Health, Part A, 59:1–5, 2000

0098-4108/00 $12.00 + .00



# EFFECTS OF HORMONE REPLACEMENT THERAPY ON LIPID PEROXIDES AND OXIDATION SYSTEM IN POSTMENOPAUSAL WOMEN

**Tülay Akçay, Yildiz Dinçer, Refik Kayali**

Biochemistry Department, Cerrahpaşa Medical Faculty, University of Istanbul, Istanbul, Turkey

**Umur Çolgar, Engin Oral**

Gynecology and Obstetrics Department, Cerrahpaşa Medical Faculty, University of Istanbul, Istanbul, Turkey

**Ufuk Çakatay**

Central Laboratory, Istanbul Medical Faculty, University of Istanbul, Istanbul, Turkey

*A short-term evaluation of 6 months of estrogen therapy on oxidant status in 38 postmenopausal women was conducted. The levels of serum lipid peroxidation products, glutathione (GSH) status, and glutathione-related enzymes were evaluated before and after 6 months of hormone replacement therapy. After 6 months of estrogen treatment there was a significantly increased concentration of thiobarbituric acid-reactive substances (TBARS), which are an end product of lipid peroxidation. This was accompanied by a significant increase in the activity of glutathione peroxidase (GSH-Px). However, the activities of glutathione reductase (GSSG-R) and superoxide dismutase (SOD) were significantly decreased and total protein thiols were reduced. Data suggest that hormone replacement therapy in postmenopausal women is associated with oxidant mechanisms.*

It is known that oxidant stress plays an important role in cancer and atherosclerosis, which are among the leading causes of death (Sun, 1990; Dreher & Junod, 1996; Halliwell et al., 1992; Plachta et al., 1992).

High concentrations of low-density lipoprotein (LDL) and low levels of high-density lipoprotein (HDL) are associated with an increased incidence of osteoporosis (Goldstein et al., 1973). Estrogens can decrease the concentrations of LDL and increase HDL levels (Burkman, 1988), indicating a potential beneficial use in atherosclerosis. However, the underlying mechanisms of estrogen action are not established. Maziere et al. (1991) showed that the levels of estrogens at much higher than physiological concentrations prevented in vitro LDL oxidation. However, this finding was not supported by the in vivo observations of McManus et al. (1997), where oxidation of LDL was not markedly inhibited by estrogens in post-

Address correspondence to Professor Dr. Tülay Akçay, Kayişdağı Caddesi No. 156/4, Kadiköy, Istanbul, Turkey.

menopausal women. The incidence of atherosclerosis is lower in females than in males, and this phenomenon is associated with reduced lipid peroxide levels (Knight et al., 1987; Yagi & Komura, 1986). Thus, it was of interest to determine whether estrogen hormone replacement affected lipid peroxidation in a postmenopausal female population.

## MATERIAL AND METHODS

Eight-milliliter heparin blood samples were taken from nondiabetic and nonsmoker postmenopausal females aged 45–55 yr. These women were healthy and not taking any medications. Gynecology and obstetric examinations were conducted at the main-science branch of Cerrahpaşa Medical School. The number of women prior to hormone replacement was 37; the number receiving hormone for 3 and 6 months was 22 and 20, respectively. Plasma was obtained by centrifugation of blood at 700 × g. As the hormone replacement therapy (HRT), the postmenopausal females were given "konjuge estrogens Equine" (Premarin) at a dose of 0.625 mg/d for 25 d and medroxyprogesterone acetate (MPA) (Provera) at a dose of 5 mg/d for 10 d.

Plasma TBARS level was estimated according to thiobarbituric acid method of Stocks and Dormandy (1971). Erythrocyte GSH levels were determined according to the method of Beutler et al. (1963). Erythrocyte GSH-Px activity was estimated according to Paglia and Valentine (1967). One unit of enzyme activity was defined as 1 μmol NADPH oxidized per minute. Activity was expressed as units per gram hemoglobin (U/gHb). Erythrocyte GSSG-R activity was estimated according to the method of Goldberg and Spooner (1983). The unit of enzyme activity was defined as 1 μmol NADPH oxidized per minute. Activity was expressed as units per gram hemoglobin (U/gHb). Erythrocyte superoxide dismutase (SOD) was estimated with a diagnostic kit from Randax Laboratories Ltd. (catalog number SD125). One unit of SOD is defined as the amount of protein that inhibits the rate of formazan dye formation by 50%. Total plasma thiol concentration was determined by using 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB) as described by Hu (1994). Absorbances were measured at 412 nm against blank samples without DTNB.

### Statistics

Data are given as mean ± SEM. Data were analyzed using a one way analysis of variance (ANOVA). The significance of differences was tested using Dunnett's test. The criterion for significance was $p < .05$.

## RESULTS

TBARS, GSH, GSH-Px, GSSG-Px, and SOD levels in postmenopausal females before hormone replacement therapy (HRT) and at 3 and 6 months

thereafter are indicated in Table 1. Although no significant differences were found in the levels of TBARS and total protein thiol after 3 months of treatment, a significant increase occurred after 6 months. After 3 months of HRT the activity of GSH-Px rose significantly while SOD activity fell (Table 1). HRT administration for 6 months significantly elevated the activity of GSH-Px, accompanied by a reduction in total protein thiol levels and activity of SOD and GSSG-R. GSH levels remained unchanged throughout HRT.

## DISCUSSION

At present, standard hormone replacement therapy (HRT) includes estrogen and progesterone. The reason for the combination is to prevent the increase of endometrial cancer risk caused by estrogens (Speroff et al., 1989; Guetta & Cannon, 1996) and is the basis for using estrogen and progesterone in our therapeutic regimen.

The route of administration and type of estrogen are factors in HRT effectiveness. McManus et al. (1996) showed that transdermal estrogen has less effect on the lipid parameters than oral preparations. Taniguchi et al. (1994) found that conjugated estrogens produced more potent antioxidant effects than parent compound. Subbiah et al. (1993) demonstrated that conjugated equine estrogens were effective antioxidants. Thus, conjugated estrogens from equine sources were administered by the oral route.

Lipid peroxidation, as determined by TBARS levels, was significantly increased after 6 months of HRT. Inal et al. (1977) found that transdermal application of estradiol and medroxyprogesterone did not markedly alter TBARS. However, addition of vitamin E significantly lowered TBARS. Ciavatti et al. (1989) also showed that oral contraceptives elevated total lipid content and lipid peroxidation. Data support our findings that HRT

**TABLE 1.** Oxidation and Antioxidation System Lipid Peroxidation Indicators After Hormone Replacement Therapy

| | Measurement time | | |
|---|---|---|---|
| Parameter[a] | Before treatment ($n$ = 38) | After 3 months of treatment ($n$ = 22) | After 6 months of treatment ($n$ = 20) |
| TBARS (nmol/ml) | 1.43 ± 0.45 | 1.45 ± 0.33 | 1.70 ± 0.46[b] |
| Total protein thiol (μ*M*) | 393.62 ± 159.24 | 365.02 ± 120.92 | 286.49 ± 65.65[b] |
| GSH (μmol/gHb) | 2.01 ± 0.49 | 2.07 ± 0.32 | 2.01 ± 0.39 |
| GPx (U/gHb) | 56.65 ± 18.7 | 74.40 ± 14.86[b] | 84.50 ± 11.97[b] |
| GSSG-Px (U/gHb) | 10.41 ± 2.42 | 9.29 ± 1.97 | 8.99 ± 1.81[b] |
| SOD (U/gHb) | 1453 ± 834 | 773 ± 196[b] | 713 ± 272[b] |

*Note.* Data are mean ± SE for $n$ given in parentheses.

[a]See text for definition of units.

[b]Significant at $p < .05$ for difference from pretreatment values.

elevated TBARS formation. In contrast, Tranquilli et al. (1995) showed that transdermal HRT decreased free radical production in platelets of postmenopausal women. However, the current study and McManus et al. (1996) using oral treatment failed to confirm an antioxidant action for HRT. The fact that estrogens were effective in vitro could be due to the small size of LDL particles (Wagner et al., 1992; Manning et al., 1996), which is known to enhance susceptibility to oxidants (Tribble et al., 1992).

Tamoxifen, which is widely used in the treatment of breast cancer, exerts antiestrogenic effects. Jordan (1993) reported that the antitumorigenic effects of tamoxifen were associated with drug binding to estrogenic receptors, thereby preventing estrogens from producing effects. Wiseman et al. (1990) demonstrated that tamoxifen inhibited lipid peroxidation. Further, Thangaraj et al. (1974) found that in postmenopausal women treated for breast cancer with tamoxifen a significant fall in TBARS was noted. Clearly, HRT is associated with elevated lipid peroxidation as evidenced by a rise in TBARS levels and fall in thiols, SOD, and GSSG-R. An antiestrogenic agent thus acts as an antioxidant.

Laloraya et al. (1996) found that estrogens lowered SOD activity, a finding supported in this study. The elevation in GSH-Px seen after HRT suggests an increased usage of GSH (Guemouril et al., 1991); however, GSH levels were similar to pretreatment levels. It should be noted though that total protein thiols were significantly reduced. Data suggest that in postmenopausal women receiving HRT, there is increased lipid peroxidation.



## REFERENCES

Beutler, E., Duran, O., and Duarte, M. B. K. 1963. Improved method for the determination of blood glutathione. *J. Lab. Clin. Med.* 51:822–888.

Burkman, R. T. 1988. Lipid and lipoprotein changes in relation to oral contraception and hormonal replacement therapy. *Fertil. Steril.* 49:398–508.

Ciavatti, M., Blache, D., and Renaud, S. 1989. Hormonal contraceptive increases plasma lipid peroxides in female rats. *Atherosclerosis* 9:84–89.

Dreher, D., and Junod, A. F. 1996. Role of oxygen free radicals in cancer development. *Eur. J. Cancer* 32A:30–38.

Goldberg, D. M., and Spooner, R. J. 1983. In *Methods of enzymatic analysis,* ed. H. V. Bergmeyer, 3rd ed., vol. 3, pp. 258–265. Deerfield Beach, FL: Verlag Chemie.

Goldstein, J. L., Schrott, H. G., Hazzard, W. R., Bierman, E. L., and Motulsky, A. G. 1973. Hyperlipidemia in coronary heart disease. II. Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidemia. *J. Clin. Invest.* 52:1544–1568.

Guemouril, L., Artur, Y., Herbeth, B., Jeandel, C., Cuny, G., and Siest, G. 1991. Biological variability of superoxide dismutase, glutathione peroxidase and catalase in blood. *Clin. Chem.* 37/11: 1932–1937.

Guetta, V., and Cannon, O. R. 1996. Cardiovascular effects of oestrogen and lipid-lowering therapies in postmenopausal women. *Circulation* 93:1928– 1937.

Halliwell, B., Gutteridge, J. M. C., and Cross, C. E. 1992. Free radicals. Antioxidants and human disease: Where are we now? *J. Lab. Clin. Med.* 119:598–620.

Hu, M. L. 1994. Measurement of protein thiol groups and glutathione in plasma. *Methods Enzymol.* 233:381–385.

Inal, M., Sunal, E., Kanbak, G., and Zeytinoğlu, S. 1977. Effects of postmenopausal hormone re-

placement and α-tocopherol on the lipid profiles and antioxidant status. *Clin. Chim. Acta* 268: 21–29.

Jordan, V. C. 1993. A current view of tamoxifen for the treatment and prevention of breast cancer. *Br. J. Pharmacol.* 110:507–517.

Knight, J. A., Smith, S. E., Kiader, V. E., and Anstall, H. B. 1987. Reference intervals for plasma lipoperoxides: age, sex and specimen-related variations. *Clin. Chem.* 31:2289–2291.

Laloraya, M., Jain, S., Thomas, M., Kopergaonkar, S., and Pradeep, K. G. 1996. Estrogen surge: A regulatory switch superoxide radical generation at implantation. *Biochem. Mol. Biol. Int.* 39:933–940.

Manning, J. M., Campos, G., Edwards, I. J., Wagner, W. D., Wagner, J. D., Adams, M. R., and Parks, J. S. 1996. Effects of hormone replacement therapy on low density lipoprotein composition and distribution in ovariectomized cynomolgus monkeys. *Atherosclerosis* 121:217–229.

Maziere, C., Auclair, M., Ronveaux, M., Salmon, S., Santus, R., and Maziere, J. C. 1991. Oestrogens inhibit copper and cell-mediated modification of low density lipoprotein. *Atherosclerosis* 89: 175–182.

McManus, J., McEneny, J., Young., I. S., and Thompson, W. 1995. The effect of various estrogens and progestogens on the susceptibility of LDL cholesterol to oxidation. *Menopause* 2:275.

McManus, J., McEneny, J., Young, I. S., and Thompson, W. 1996. The effect of various estrogens and progestogens on the susceptibility of low density lipoproteins to oxidation in vitro. *Maturitas* 25:125–131.

Paglia, D. E., and Valentine, W. N. 1967. Studies on the quantitative and qualitative characterisation of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.* 70:158–169.

Plachta, H., Bartnikovska, E., and Obara, A. 1992. Lipid peroxides in blood from patients with atherosclerosis of coronary and peripheral arteries. *Clin. Chim. Acta* 211:101–112.

Speroff, L., Glass, R. H., and Kase, N. G. 1989. *Clinical gynecologic endocrinology and infertility,* 4th ed. Baltimore, MD: Williams & Wilkins.

Stocks, J., and Dormandy, T. L. 1971. The autoxidation of human red cell lipids induced by hydrogen peroxide. *Br. J. Haematol.* 20:95–111.

Subbiah, R. M. T., Kessel, B., Agrawal, M., Rajan, R., Abplanalp, W., and Pymaszewsk, Z. 1993. Antioxidant potential of specific estrogens on lipid peroxidation. *Clin. Endocrinol. Metabol.* 77: 1095–1097.

Sun, Y. 1990. Free radicals, antioxidant enzymes and carcinogenesis. *Free Radical Biol. Med.* 8:583–599.

Taniguchi, S., Yanase, T., Kobayashi, K., Takayanagi, R., Haji, M., Umeda, F., and Nawata, H. 1994. Catechol estrogens are more potent antioxidants than estrogens for the $Cu^{2+}$-catalyzed oxidation of low or high density lipoprotein: Antioxidative effects of steroids on lipoproteins. *Endocrinol. J.* 41:605–611.

Thangaraju, M., Vijayalakshmi, T., and Sachdanandam, P. 1974. Effect of tamoxifen on lipid peroxide and antioxidative system in postmenopausal women with breast cancer. *Cancer* 74:78–81.

Tranquilli, A. L., Mazzanti, L., Cugini, A. M., Cester, N., Garzetti, G. G., and Romanini, C. 1995. Transdermal estradiol and medroxprogesterone acetate in hormone replacement therapy are both antioxidants. *Gynecol. Endocrinol.* 9:137–141.

Tribble, D. L., Holl, L. G., Wood, P. D., and Krauss, R. M. 1992. Variations in oxidative susceptibility among six low-density lipoprotein subfractions of differing density and particle size. *Atherosclerosis* 93:189–199.

Wagner, J. D., St. Clair, R. W., Schwnkle, D. C., Shively, C. A., Adams, M. R., and Clarkson, T. B. 1992. Regional differences in arterial low density lipoprotein metabolism in surgically postmenopausal cynomolgous monkeys. *Arterioscler. Thromb.* 12:717–725.

Wiseman, H., Laughton, M. J., and Armsteinn, H. R. V. 1990. The antioxidant action of tamoxifen and its metabolites. *FEBS Lett.* 263:192–194.

Yagi, K., and Komura, S. 1986. Inhibitory effect of female hormones on lipid peroxidation. *Biochem. Int.* 13:1051–1056.