Decreased oxidant profile and increased antioxidant capacity in naturally postmenopausal women

V. J. Victorino · C. Panis · F. C. Campos ·
R. C. Cayres · A. N. Colado-Simão ·
S. R. Oliveira · A. C. S. A. Herrera ·
A. L. Cecchini · R. Cecchini

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Abstract Recent works have shown a dual side of estrogens, and research on the relationship between oxidative stress and menopausal status remains unclear and has produced controversial results. In this work, we aimed to evaluate by sensitive methods the oxidant and antioxidant changes that develop after natural menopause. Thirty premenopausal and 28 naturally postmenopausal women volunteered for this study. Blood was collected and plasma used. 17-OH estradiol levels in plasma were estimated. Plasma levels of advanced oxidation protein products (AOPP), lipid peroxidation products (such as hydroperoxides and malondialdehyde (MDA)), and nitrites were measured, and total radical antioxidant parameter testing was performed to determine the oxidant and antioxidant profiles, respectively. Estrogen levels were significantly increased ($p < 0.02$) in

premenopausal women (54.28 ± 9.34 pg/mL) as compared with postmenopausal women (18.10 ± 1.49 pg/mL). Postmenopausal women had lower levels of lipid hydroperoxide oxidation ($p < 0.0001$), lipid hydroperoxide levels evaluated by the area under the curve (AUC; $1,366,000 \pm 179,400$ AUC; $p < 0.01$), and hydroperoxides as measured by the ferrous oxidation-xylenol orange method (31.48 ± 2.7 μ M; $p < 0.0001$). The MDA levels did not differ between pre- and postmenopausal women whether measured by thiobarbituric acid-reactive substances or high-performance liquid chromatography assays. No differences in AOPP and nitrite levels were observed between pre- and postmenopausal women. Postmenopausal women also exhibited a higher total radical antioxidant level (0.89 ± 0.08 μ M Trolox; $p < 0.0001$). Postmenopausal women demonstrated lower levels of oxidative damage and a higher antioxidant capacity than premenopausal women.

V. J. Victorino (✉) · C. Panis · F. C. Campos ·
R. C. Cayres · A. C. S. A. Herrera · R. Cecchini
Laboratory of Pathophysiology and Free Radicals,
Department of General Pathology,
State University of Londrina,
86051-990 Londrina, Paraná, Brazil
e-mail: vanjacob@gmail.com

A. L. Cecchini
Laboratory of Molecular Pathology, Department of General
Pathology, State University of Londrina,
Londrina, Paraná, Brazil

A. N. Colado-Simão · S. R. Oliveira
Clinical Analyses and Toxicology, Centre of Applied
Pathology, State University of Londrina,
Londrina, Paraná, Brazil

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Introduction

Natural menopause is defined as 12 months of amenorrhea followed by reduced ovarian hormone activity in the absence of surgical or hormonal induction. During the menopausal transition, estradiol levels decrease, leading to changes in first the follicle-

stimulating hormone and then the luteinizing hormone levels, with both hormones gradually increasing as ovulation ceases and progesterone levels decrease (Soules et al. 2001). Menopause was an important selective trait in human evolution, as women who became infertile many years before death would be better able to care for existing children. However, medical and scientific advances have negated such evolutionary pressures (Polycove et al. 2011). In fact, at present, age-related diseases that become more prevalent after menopause are of greater concern. For example, low serum estrogen concentrations cause accelerated loss of bone mass leading to osteoporosis (Gallagher and Levine 2008) and vasomotor symptoms like hot flashes (Thurston et al. 2011) and night sweats moderately increase the risk of developing coronary heart disease (Gast et al. 2011) and other cardiovascular diseases (Woodard et al. 2011), among other disorders.

Reactive oxygen and/or nitrogen species seem to be involved in aging (Beckman and Ames 1998; Feng et al. 2001; Schriener et al. 2005), and a study of chronological aging of the skin showed that the cells of the epidermis are gradually subjected to lipid peroxidation as the skin ages (Peres et al. 2011). During the female life cycle, the induction of reactive species by estrogens is thought to be important during the reproductive stage (Sipe et al. 1994; Yager 2000; Russo et al. 2003; Okoh et al. 2011), but the significance of this phenomenon during and after the menopause stage is controversial. Some studies defend hormone replacement therapy in postmenopausal women, which includes estrogen intake as protection (Bednarek-Tupikowska et al. 2004; Bednarek-Tupikowska et al. 2006; Darabi et al. 2010; Gokkusu et al. 2010) while other researchers showed no improvement in health-related quality of life (Hays et al. 2003; Sherman et al. 2003), or even an increased risk for probable dementia in postmenopausal women aged 65 years or older and no prevention of mild cognitive impairment (Shumaker et al. 2003), during estrogen plus progestin therapy. In summary, although some studies have demonstrated that estrogen has antioxidant properties (Borrás et al. 2003, 2010) and thus a protective effect on the body, an increasing number of studies have recently shown another activity of estrogens as important pro-oxidants at physiological concentrations (Sipe et al. 1995; Yager 2000; Russo et al. 2003; Okoh et al. 2011).

Reactive oxygen species are formed during both physiological and pathological processes, primarily through oxygen reduction in the mitochondrial respiratory system. When reactive species are not neutralized by the organism's antioxidant defenses, oxidative stress occurs and leads to cell structure damage (Halliwell and Gutteridge 2007). Research on the relationship between oxidative stress and menopausal status remains unclear and has produced controversial results. Contributions in this area include many *in vitro* studies (Arteaga et al. 1998; Ayres et al. 1998) and most of the *in vivo* studies that have been performed employed no sensitive methods, did not distinguish between natural and iatrogenic menopause (Signorelli et al. 2006; Pansini et al. 2008; Sowers et al. 2008; Dorjgochoo et al. 2011), and often included subjects receiving hormone replacement therapy (Bednarek-Tupikowska et al. 2006; Unfer et al. 2006; Darabi et al. 2010; Gokkusu et al. 2010). Given this context, our study aimed to evaluate by sensitive methods the oxidant and antioxidant changes that develop after natural menopause by comparing oxidant and antioxidant parameters between pre- and postmenopausal women in order to understand the consequences of the hormonal alterations of menopause for the oxidative stress status.

Methods

This research was approved by the Research and Ethics National Council (CAAE 0009.0.268.000-07); all practices were approved by the institutional board and all patients provided signed informed consent.

Subject selection

Subjects were selected between September 2010 and February 2011. Smokers, regular alcohol consumers, antioxidant supplement users, pregnant or lactating women, women performing excessive physical exercise, women receiving hormone replacement therapy, and women with chronic diseases were excluded from this study. Thirty premenopausal women with regular menstrual periods and 28 postmenopausal women with spontaneous menopause for at least 1 year were recruited for this study.

Sample collection

Heparinized fresh blood (3 mL) was collected at the Department of General Pathology, Londrina State University, PR, Brazil. Blood was centrifuged for 5 min at $1,500\times g$ at 4°C to obtain plasma. Plasma aliquots were stored in an Indrel Ultra Freezer at -86°C until further analysis.

Serum 17-OH estradiol assay

Serum 17-OH estradiol levels in plasma were determined by chemiluminescence microparticle immunoassay (Architech™, Abbott Laboratory, Abbott Park, IL, USA).

Determination of oxidative stress by highly sensitive chemiluminescence induced by *tert*-butyl hydroperoxide

A chemiluminescence-based method was employed to evaluate oxidative stress from plasma samples (Gonzales-Flecha et al. 1991; Panis et al. 2011a, b). This method uses the compound *tert*-butyl hydroperoxide to start a lipid chain reaction that can be detected by photon emission during the formation of lipid hydroperoxides. The chemiluminescence reaction was initiated by the addition of 10 μL of 3 mM *tert*-butyl hydroperoxide to 125 μL of plasma and 865 μL of 30 mM disodium phosphate/120 mM KCl buffer, pH 7.4, 37°C . Readings were performed in a Glomax luminometer (TD 20/20 Turner Designers) over 1 h at 1 reading/s. Results were expressed as relative units of light.

Determination of lipid hydroperoxide levels by the ferrous oxidation-xylenol orange method

Plasma lipid hydroperoxide concentrations were estimated by the ferrous oxidation-xylenol orange (FOX) method (Gay and Gebicki 2002; Costa et al. 2006) with modifications. This method relies on the oxidation of ferrous iron to ferric iron by plasma lipid hydroperoxides in the presence of xylenol orange. This reaction generates a ferric iron-xylenol orange complex that can be measured colorimetrically with a spectrophotometer. For sample analysis, FOX reagent was prepared by adding 100 μM xylenol orange and 250 μM ammonium

ferrous sulfate to 110 mM perchloric acid. About 50 μL of each plasma sample was mixed with 50 μL of FOX reagent. Samples were incubated for 30 min in the dark at room temperature and centrifuged at $2,400\times g$ for 3 min. The absorbance of the supernatant was measured at 550 nm, and the results expressed as micromolars of hydroperoxide (molar extinction coefficient= $4.3\times 10^4\text{ M}^{-1}\text{ cm}^{-1}$).

Lipid peroxidation—determination of MDA by TBARS and HPLC methods

The plasma levels of thiobarbituric acid (TBA)-reactive substances (TBARS), primarily malondialdehyde (MDA), were quantified spectrophotometrically as described by Oliveira and Cecchini (2008). A 400- μL volume of plasma sample was reacted with 1 mL of 1 % TBA to form MDA-(TBA)₂ complexes, and the proteins were precipitated with 1 mL of 28 % trichloroacetic acid. The samples were mixed by vortexing, incubated for 15 min in a boiling water bath, and transferred to an ice bath. The organic phase was extracted with 2 mL of butanol and centrifuged at $850\times g$ for 20 min. The supernatants were read in a spectrophotometer at 535 and 572 nm; the results were calculated based on the difference in absorption at these wavelengths and expressed as nanomolars of TBARS.

High-performance liquid chromatography (HPLC) determinations were made using an HPLC-20AT Shimadzu instrument equipped with an LC20AT pump and an SPD20A UV absorbance detector and employing a C18 reverse-phase column. MDA levels were determined based on a standard curve. A standard solution of MDA was prepared by mixing 10 mL of 0.1 M HCl with 10 mL of 1,1,3,3-tetraethoxypropane, incubated for 5 min in a boiling water bath, and transferred to an ice bath (Karatas et al. 2002).

A new method was adapted from Chirico (1994) and Karatas et al (2002) in order to improve the purity of the chromatogram and conserve the HPLC column. In this method, 160 μL of plasma sample or standard solution was mixed with 100 μL of 0.5 M perchloric acid and incubated for 10 min on ice to precipitate the proteins. Samples were then centrifuged for 5 min at $5,000\times g$ at 4°C . About 180 μL of supernatant was recovered and mixed with 100 μL of TBA. This reaction was incubated for 30 min in a boiling water bath and transferred to an ice bath to stop the reaction.

A 100- μ L volume of 1 M NaH_2PO_4 , pH 7, was added to each sample to stabilize the sample pH. Samples were centrifuged for 10 min at $5,000\times g$ at 4°C . The mobile phase consisted of 65 % 50 mM KH_2PO_4 buffer and 35 % HPLC-grade methanol. The supernatants were filtered and injected into the HPLC. The serial dilutions of the standard solution of MDA were used as calibration points to obtain the linear regression equation and calculate the MDA concentration of the samples. Readings were obtained at 535 nm over 12 min at a flow rate of 0.8 mL/minute, and results were expressed as nanomolars of MDA.

Plasmatic advanced oxidation protein products assay

Plasmatic advanced oxidation protein products assay (AOPP) was assessed using a semi-automated method based on oxidation of protein residues of tyrosine, leading to formation of dityrosine-containing protein cross-linking products detected by spectrophotometry (Lozovoy et al 2011). AOPP concentrations were expressed as micromoles per liter of chloramine-T equivalents.

Total radical-trapping antioxidant parameter

The total radical-trapping antioxidant parameter (TRAP) was measured as described by Ghiselli et al.

(1995). This assay uses 2,2'-azo-bis-(2-amidinopropane) (ABAP), a potent free radical generator that degrades itself to emit photons in a process that is amplified by the addition of luminol. This activity of ABAP is neutralized by antioxidants in the reaction mixture that inhibit its function. The photon emission profile of the ABAP solution was measured in a Glomax luminometer (Turner Designs TD 20/20) over 30 min at 5 readings/s.

ABAP emission in a reaction containing 900 μ L of 0.1 M glycine buffer at pH 8.6, 50 μ L of luminol, and 50 μ L of ABAP was measured as a pro-emission standard. An antioxidant standard solution of 25 μ M Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), an analog of hydrosoluble vitamin E, was added in order to neutralize ABAP autoxidation in a reaction containing 830 μ L of 0.1 M glycine buffer pH 8.6, 70 μ L of 25 μ M Trolox, 50 μ L of luminol, and 50 μ L of ABAP) that was used as a standard for antioxidant comparison.

Subsequently, TRAP was measured in plasma samples in reactions containing 830 μ L of 0.1 M glycine buffer at pH 8.6, 70 μ L of plasma sample, 50 μ L of luminol, and 50 μ L of ABAP. For TRAP calculation, the induction time of the sample (time for which the sample antioxidants can inhibit the ABAP action) was compared with that of the standard antioxidant (Trolox) using the following formula and the results were expressed as micromolars of Trolox:

$$\mu\text{M Trolox} = \frac{\text{Sample induction time} \times \text{Trolox concentration in the reaction}}{\text{Trolox induction time}}$$

Evaluation of nitrite levels

The plasma nitric oxide (NO) concentration was estimated by measuring nitrite as previous described (Cunha et al. 2010; Panis et al. 2011a, b). In this method, 60 μ L of plasma sample was deproteinized by adding 50 μ L of 75 mM ZnSO_4 solution (Merck). The samples were mixed and centrifuged at $9,500\times g$ for 2 min at 25°C , followed by a second deproteinization (with 55 mM NaOH; Merck) of the supernatant. The samples were mixed and centrifuged again at $9,500\times g$ for 5 min at 25°C . The supernatants were recovered and diluted 1:5 in 45 g/L glycine buffer,

pH 9.7 (Merck). Cadmium granules (Fluka) were activated for 5 min in 5 mM CuSO_4 in 15 g/L glycine-NaOH buffer, pH 9.7. The activated granules were added to the glycine-buffered diluted supernatants and incubated for 10 min. Griess reagent (Sigma) was added to 200- μ L volumes of the supernatants, and the reactions were incubated for 10 min at room temperature and centrifuged at $9,500\times g$ for 2 min at 25°C . Finally, 100 μ L of each sample was transferred to a 96-well microplate. To determine the sample nitrite concentration, a calibration curve was prepared by dilution of NaNO_2 (Merck) in distilled sterile water. The absorbance was read at 550 nm using a

standard microplate reader (Multiskan EX, LabSystems, Minnesota USA). The final results were expressed as micromolars of nitrite.

Statistical analysis

All analyses were conducted in duplicate. Statistical analysis was performed using GRAPHPAD PRISM version 5.0 (GRAPHPAD Software, San Diego, CA), Microsoft Office Excel 2007, and OriginLab8.0 software. Results were expressed as arithmetic means and errors of the means. Differences among groups were assessed by two-way analysis of variance with post-hoc Bonferroni's test for the lipid peroxidation curves and by Student's unpaired *t* test for the other parameters. Correlations among parameters were also performed. All data were analyzed using GraphPad Software to eliminate significant outliers ($p < 0.05$). A $p < 0.05$ was considered statistically significant.

Results

The study was carried out on a sample of 58 women comprising 54 Caucasian women, 4 Japanese women, and 1 woman of African descent. The postmenopausal group consisted of women presenting after at least 1 year of amenorrhea, and the mean time after menopause was 9 ± 6 years. As summarized in Table 1, the premenopausal group included 30 women with a mean age of 31 ± 2 years and body mass index (BMI) of 23.11 ± 0.69 kg/m² while the postmenopausal group comprised 28 women with a mean age of 57 ± 1 years and BMI of 25.08 ± 0.92 kg/m². No difference between BMI of premenopausal women and postmenopausal women was found ($p = 0.09$). Estrogen levels were significantly increased ($p < 0.02$) in premenopausal women (54.28 ± 9.34 pg/mL) as compared with postmenopausal women (18.10 ± 1.49 pg/mL).

As shown in Fig. 1a, significantly higher levels of lipid hydroperoxides as measured by chemiluminescence were detected in the premenopausal women than in the postmenopausal women ($p < 0.0001$), as determined by Bonferroni's test at point 46 and points 52 to 60. Figure 1b shows the integration of the area under the curve (AUC), demonstrating a significant

Table 1 Subjects' characterization

No. of women	58
Menopause status	
Premenopausal	30 (52 %)
Postmenopausal	28 (48 %)
Age (years)	
Premenopausal	$31 \pm 2^*$
Postmenopausal	57 ± 1
Time after menopause	9 ± 6
Body mass index (kg/m ²)	
Premenopausal	23.11 ± 0.69
Postmenopausal	25.08 ± 0.92
17-OH estradiol levels (pg/mL)	
Premenopausal	$54.28 \pm 9.34^*$
Postmenopausal	18.10 ± 1.49
Ethnicities	
Caucasian	53 (91.38 %)
Japanese	4 (6.9 %)
Afro-descent	1 (1.72 %)

Results are expressed as mean $\pm$ standard deviation

* $p < 0.05$

difference due to menopausal status ($p < 0.01$), with the area smaller for the postmenopausal ($1,366,000 \pm 179,400$ AUC) than for the premenopausal ($2,023,000 \pm 176,500$ AUC) women. Statistical analyses of plasma lipid hydroperoxide evaluation are presented in Fig. 1c.

The FOX assay also detected significantly lower levels of hydroperoxides ($p < 0.0001$) in the postmenopausal women (31.48 ± 2.70 μ M hydroperoxides) compared with the premenopausal women (43.86 ± 1.27 μ M hydroperoxides) (Fig. 2a).

No difference in the MDA level as detected by the TBARS assay ($p = 0.09$) was seen between pre- (184.0 ± 9.49 nM TBARS) and postmenopausal (209.2 ± 11.71 nM TBARS) women (Fig. 2b). HPLC also showed no difference in MDA levels ($p = 0.2$) between pre- (59.95 ± 4.82 nM MDA) and postmenopausal (67.91 ± 4.07 nM MDA) women (Fig. 2c).

Figure 2d shows nitrite levels, which estimate the NO levels. No significant difference ($p = 0.5$) was observed between the pre- (44.79 ± 5.13 μ M nitrite) and postmenopausal (48.94 ± 5.888 μ M nitrite) women.

As shown in Fig. 3, no difference in AOPP levels ($p = 0.1$) was observed between pre- (197.5 ± 4 μ mol/L) and postmenopausal (225.5 ± 17.03 μ mol/L) women.

Fig. 1 Plasma oxidative stress evaluated by high-sensitivity chemiluminescence. **a** Profile of peroxidation, **b** area under curve (*AUC*), and **c** statistical significance of curves are shown. *RUL* relative unities of lights, *pre* premenopausal women, *post* postmenopausal women. * $p<0.0001$, statistically significant

The total radical antioxidant capacity was greater in postmenopausal women (0.89 ± 0.08 μM Trolox; $p<0.0001$) than in premenopausal women (0.49 ± 0.02 μM Trolox), as shown in Fig. 4.

Correlations among all parameters were performed and revealed that in premenopausal women, estrogen levels were inversely proportional to BMI ($r=-0.58$ and $p=0.01$). No correlation regarding 17-OH

estradiol levels and oxidative stress parameters was found (data not shown).

Discussion

It is generally accepted that postmenopausal women have a higher risk of osteoporosis (Gallagher and Levine 2008) and increased prevalence of heart and

Fig. 2 **a** Hydroperoxide levels measured by the FOX assay, **b** malondialdehyde (*MDA*) levels measured by thiobarbituric acid-reactive substances (*TBARs*), **c** *MDA* measured by HPLC, and **d** nitrite levels. *PRE* premenopausal women, *POST* postmenopausal women. * $p<0.0001$, statistically significant

Fig. 3 Advanced oxidation protein products (AOPP). *pre* premenopausal women, *post* postmenopausal women

cardiovascular diseases (Gast et al. 2011; Woodard et al. 2011). As well, an inverse correlation between body mass index and estrogen levels has already been described (Potischman et al. 1996). There is also speculation that estrogens exert an antioxidant action due to their aromatic hydroxyphenol structure (Nathan and Chaudhuri 1998). In this context, 17 β -estradiol regulates several physiological processes including development and growth (Tagawa et al. 2009). It also presents relevant antioxidant properties in several biological systems, mainly by protecting against oxidative stress responses generated by the estrogen receptor α (Urata et al. 2006). This compound is able to prevent oxidative stress by a wide of pathways, including the prevention of oxidative-induced cell death in keratinocytes (Kanda and Watanabe 2003), cortical neurons (Numakawa et al. 2007), and retinal pigment epithelium (Giddabasappa et al. 2010).

However, the present study supports a hypothesis that may be controversial: the data indicate the novel finding that premenopausal women are more susceptible than postmenopausal women to oxidative stress. This more favorable oxidative status is supported

Fig. 4 Antioxidant profile determined by total radical-trapping antioxidant parameter (TRAP). *pre* premenopausal women, *post* postmenopausal women. * $p < 0.0001$, statistically significant

by significantly lower levels of oxidative stress and hydroperoxides in postmenopausal women as evidenced by CL and FOX measurements, respectively. The high antioxidant status revealed by TRAP analysis also corroborates the higher oxidative status of postmenopausal women.

The study was performed on a population sample that included premenopausal and naturally postmenopausal women in numbers that, although limited, were much larger than those recruited for some prospective studies (Gambacciani et al. 1997). To the extent possible, we took care to remove confounding factors related to lifestyle (e.g., antioxidant use, smoking, regular alcohol consumption, excessive physical exercise, pregnancy, lactation, treatment with hormone replacement therapy, and chronic disease).

Most current studies of oxidative stress are focused primarily on LDL oxidation and 8-isoprostane quantification and are too few in number to clarify the role of the redox status of postmenopausal women. Oxidative stress and antioxidant parameters in postmenopausal women have already been investigated by some authors in the context of spontaneous menopause (Signorelli et al. 2006; Pansini et al. 2008; Sowers et al. 2008; Dorjgochoo et al. 2011), induced menopause (Bednarek-Tupikowska et al. 2006), or even hormone replacement therapy (Gokkusu et al. 2010) and also in vitro (Arteaga et al. 1998; Ayres et al. 1998). Nevertheless, these studies have not yet reached a consensus, and high-sensitivity techniques should be employed in order to better clarify the relationship between menopausal status and oxidant/antioxidant parameters.

In the present study, the detection of plasma lipid peroxidation was performed by a highly sensitive chemiluminescence method, allowing the quantification of the very low levels of lipid peroxides performed in vivo. This method also provides information on non-enzymatic antioxidant defenses based on the increased photon emission caused by oxidative stress previously suffered by the cells, as we previously reported for several in vitro models (Colado-Simão et al. 2006; Casado et al. 2007; Peres et al. 2011).

The results show a significant decrease in the level of pre-formed lipid hydroperoxides in postmenopausal women compared with that in premenopausal women. The lipid chain reaction induced by the *tert*-butyl hydroperoxide technique evaluates the integrity of non-enzymatic antioxidant defenses and the plasma

hydroperoxide levels (Colado-Simão et al. 2006). Lipid peroxidation is a process involving three steps (initiation, propagation, and termination) that in the case of plasma affect mainly lipoproteins (Zwart et al. 1999; Halliwell and Gutteridge 2007). Initiation is the most significant stage of lipid peroxidation observed in human plasma; it is characterized by the ascendant part of the curve and depends on the antioxidant content of the sample. High antioxidant content is able to retard the initiation process and reduce the initial velocity of the reaction, but a decreased area under the curve specifically represents reduced lipid hydroperoxides within the sample. Our data demonstrate that the TRAP was significantly higher in postmenopausal women, which is consistent with the decreased lipid hydroperoxide levels of postmenopausal women compared with those of premenopausal women. The low levels of hydroperoxides detected by the FOX assay corroborate these results.

The TRAP of the plasma, which represents low molecular weight molecules, was augmented in the postmenopausal women, suggesting that these molecules present a systemic antioxidant defense against other oxidative processes.

The fact that the MDA, AOPP, and NO levels did not differ between post- and premenopausal women is a novel finding, as Trevisan et al. (2001) reported increased plasma levels of MDA in postmenopausal women. However, our results are consistent with the increased total plasma antioxidant capacity and decreased lipid markers of stress observed in these women.

Non-enzymatic lipid peroxidation can produce several small metabolites, including MDA, isoprostanes, and hydroperoxides (Zwart et al. 1999). Scientific hypotheses defending the importance of lipid peroxidation products (Gago-Dominguez et al. 2007) and mild oxidative stress (Halliwell 2007) as modulators have recently emerged. Here, the levels of the lipid peroxidation product MDA were not affected by menopausal status even when assessed by HPLC and TBARS assays. On the other hand, low levels of hydroperoxides were observed in postmenopausal women, suggesting that in the absence of estrogen the lipid peroxidation chain shunts its metabolism to form hydroperoxides rather than MDA. These findings provide new data to inform further investigations of the role of oxidative stress in menopause and its relationship to age-related diseases. Therefore, our data could suggest that postmenopausal women

compensate for the absence of the antioxidant activity of estrogen by producing higher levels of low molecular weight antioxidant molecules as an adaptive process. In addition, as postmenopausal women exhibited low lipid peroxidation levels, estrogen could be associated with lipid peroxidation events under physiologic conditions.

The quantification of NO did not differ between the groups and is thus in agreement with Felty et al. (2005) who reported that nitric oxide does not participate in the establishment or enhancement of oxidative stress in estrogen receptor positive cells line.

Several studies have investigated the antioxidant properties of estradiol. Recently published in vitro studies have demonstrated decreased induction of peroxide production by estradiol in isolated mitochondria (Borrás et al. 2010) and higher activity and expression of genes encoding Mn-superoxide dismutase and glutathione peroxidase in mitochondria from females mitochondria compared with those of males (Borrás et al. 2003). However, other studies have demonstrated a pro-oxidant activity of estrogen. For example, in the mammary environment, the metabolism of 17β -estradiol by lactoperoxidase can produce reactive phenoxyl radical intermediates. These intermediates give rise to superoxide radicals and their dismutation with consequent formation and intracellular accumulation of hydrogen peroxide, supporting a role for estrogen-related oxidative stress in the development of breast cancer (Sipe et al. 1994). Endogenous or synthetic estrogens can be converted to catechol metabolites through aromatic hydroxylation and give rise to reactive estrogen semi-quinones and quinines (Okoh et al. 2011). Catechol metabolites of endogenous estrogens have previously been shown to be involved in the redox cycle in breast epithelial cells through generation of reactive oxygen species such as hydrogen peroxide and hydroxyl radicals (Fussell et al. 2011).

Taken together, our findings indicate diminished pro-oxidative parameters and point the augmented total antioxidant capacity in postmenopausal women as measured by high sensitive techniques. This is the first report in humans corroborating the recent in vitro studies that have implicated estrogens as inducers of reactive species even at physiological concentrations. Therefore, the present results indicate decreased oxidative stress and increased antioxidant capacity in postmenopausal women relative to their premenopausal counterparts.

Conclusions

This study demonstrates for the first time decreases in the lipid peroxidation profile and increased antioxidant activity in the plasma from postmenopausal women. Shortcomings of this study include the small size sample and the lack of patients' follow-up for a long period to better evaluate the relationship between of oxidative stress levels and estrogen deprivation. In spite of this, this research provides new data in the field of menopausal studies and informs further analysis of the role of lipid peroxidation products in diseases for which the risk increases after menopause, such as cancer development. It also raises questions about the efficacy of routine clinical use of hormone replacement treatment after menopause.

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