Hypothesis/Commentary

Role of Lipid Peroxidation in the Epidemiology and Prevention of Breast Cancer

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Abstract

We have recently proposed a common mechanistic pathway by which obesity and hypertension lead to increased renal cell cancer risk. Our hypothesis posits lipid peroxidation, which is a principal mechanism in rodent renal carcinogenesis, as an intermediate step that leads to a final common pathway shared by numerous observed risks (including obesity, hypertension, smoking, oophorectomy/hysterectomy, parity, preeclampsia, diabetes, and analgesics) or protective factors (including oral contraceptive use and alcohol) for renal cell cancer [Cancer Causes Control 2002;13:287–93]. During this exercise, we have noticed how certain risk factors for renal cell carcinoma are protective for breast cancer and how certain protective factors for renal cell carcinoma increase risk for breast cancer. Parity and oophorectomy, for example, are positively associated with renal cell carcinoma but are negatively associated with breast cancer. Similarly, obesity and hypertension are positively associated with renal cell carcinoma, but obesity is negatively associated with breast cancer in premenopausal women and hypertension during pregnancy is negatively associated with breast cancer. Furthermore, alcohol intake, negatively associated with renal cell carcinoma, is also positively associated with breast cancer. We propose here the possibility that lipid peroxidation may represent a protective mechanism in breast cancer. Although this runs counter to the conventional view that lipid peroxidation is a process that is harmful and carcinogenic, we present here the chemical and biological rationale, based on epidemiologic and biochemical data, which may deserve further consideration and investigation. (Cancer Epidemiol Biomarkers Prev 2005;14(12):2829–39)

Introduction

Cellular oxidants, called reactive oxygen species (ROS), are constantly produced in animal and human cells. Numerous in vitro experiments show that ROS damages DNA, inducing premutagenic modifications of nucleotides and promoting oxidation of proteins and lipid peroxidation. Data support the notion that increased formation of ROS may play an important role in carcinogenesis, atherosclerosis, diabetes, emphysema, cataracts, and neurodegenerative diseases (1). We recently proposed lipid peroxidation to be a main mechanism of renal carcinogenesis (2-4). However, accumulating data suggest that ROS not only act as damaging entities but also may carry out important beneficial functions. ROS seem to be mediators or triggers of protective mechanisms, such as apoptosis, phagocytosis, and detoxification reactions. Among these mechanisms, apoptosis, which eliminates pre-cancerous and cancerous cells, is particularly important (1).

Lipid peroxidation is probably the most extensively investigated free radical–induced process (5). Polyunsaturated fatty acids, containing two or more double bonds, are particularly susceptible to peroxidation, and once the process is initiated, it proceeds as a free radical–mediated chain reaction involving initiation, propagation, and termination (5). Initiation of lipid peroxidation is caused by an attack of any species that has sufficient reactivity to abstract a hydrogen atom from the polyunsaturated fatty acid moiety of membrane phospholipids (5). Peroxidation of cell membranes, which contain a high concentration of polyunsaturated fatty acids, is a critical mechanism leading to growth inhibition and cell death (6). Death can occur by necrosis, but lipid peroxidation can trigger the process of apoptosis, activating the intrinsic suicide pathway present within all cells (5).

A consideration of the animal and in vitro literature suggests that an influence on breast cancer protection may relate to the generation of lipid peroxidation products. Several lines of evidence also suggest that lipid peroxidation may play an important role in human breast cancer. In this communication, we review the epidemiologic, experimental, and clinical evidence regarding lipid peroxidation as a possible mechanism in breast cancer protection.

Evaluation of the Experimental Evidence in Breast Carcinogenesis: Role of Lipid Peroxidation

Some human studies have provided evidence of the potential role of oxidative stress and lipid peroxidation in breast cancer etiology. For example, elevated levels of lipid peroxidation products have been detected in breast cancer patients and in women at high risk for breast cancer as opposed to controls (7-11), although other studies have found them to be significantly decreased (12-14). With respect to experimental studies, there is ample evidence implicating lipid peroxidation and oxidative stress in degenerative diseases and selected cancers, such as kidney, liver, and skin (1, 2, 15-17). With regard to breast cancer, however, the role of lipid peroxidation is controversial.
The role of lipid peroxidation products in the stimulation of mammary gland tumorigenesis is uncertain (18). Some studies are suggestive of an effect, but others are not (18). In chemically induced mammary tumor animal models, high-fat diets are associated with increased tumor incidence, and this effect is diminished by antioxidants (vitamin E and selenium), observations that would support a role for lipid peroxidation (18, 19). However, Horvath and Ip (19) reported that, although supplementation of the diet with two antioxidants, vitamin E and selenium, inhibited 7,12-dimethylbenz(a)anthracene tumor development, the indices of lipid peroxidation did not decrease in parallel with tumor incidence in this model (20). This suggests that systemic suppression of lipid peroxidation by vitamin E alone was not sufficient to inhibit mammary tumor formation. Likewise, the anticarcinogenic effect of selenium was found unrelated to its function in the regulation of selenium-dependent glutathione peroxidase. Using also the 7,12-dimethylbenz(a)anthracene model, Lane et al. (21) found no association between high fat intake, lipid peroxidation, and mammary tumor development (20). Furthermore, in addition to its antioxidant activity, selenium is known to increase ROS at high levels, which is proposed to be one of its possible anticarcinogenic mechanisms (22). In a recent study, dietary depletion of vitamin E and vitamin A inhibited mammary tumor growth and metastasis in transgenic mice (23).

In contrast, there is evidence favoring lipid peroxidation as an anticarcinogenic mechanism in breast cancer. A consideration of the animal and in vitro literature suggests that an influence on breast cancer protection relates to the generation of lipid peroxidation products. The following lines of experimental evidence in rodents and cultured breast cancer cells suggest that increased cytotoxic lipid peroxidation products may play an important role in breast cancer protection.

(a) Polyunsaturated fatty acids, including marine n-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid (18, 24-28), α-linolenic acid (28, 29), conjugated linoleic acid (30-32), and γ-linolenic acid (28, 33-36), have been shown to inhibit the growth of breast cancer in vivo and in vitro, and this inhibition is correlated with the extent of lipid peroxidation generated in tumor cells. (b) This suppression in cancer growth is enhanced by pro-oxidants (iron and drugs that increase lipid peroxidation; refs. 24, 29, 35-38). (c) The suppression in cancer growth is eliminated by antioxidants, and this elimination is proportional to the inhibition of lipid peroxidation products by antioxidants (18, 24-26, 28, 29, 33, 35, 36). (d) Although therapeutic mechanisms are not always relevant to etiology, marine n-3 fatty acids have been shown to enhance the cancer-killing effect of several chemotherapeutic drugs, and this is thought to be achieved through increased lipid peroxidation or lipid peroxidation–related mechanisms (37, 39-45). We also have supporting evidence in humans implicating the peroxidation products of marine n-3 fatty acids as the proximal anticarcinogens (46). n-3 Fatty acids may modify the carcinogenic process via several other molecular mechanisms in addition to increased lipid peroxidation, such as suppression of arachidonic acid–derived eicosanoid biosynthesis, influences on transcription factor activity, gene expression, alteration of estrogen metabolism, and mechanisms involving insulin sensitivity and membrane fluidity. The fact that inhibition of cancer growth is correlated with extent of lipid peroxidation and elimination is proportional to inhibition of lipid peroxidation products suggests that one causal mechanism may be related to lipid peroxidation.

It is of interest that accumulating evidence suggests that oxidative stress-induced apoptosis plays an important role in the anticarcinogenic effect of several chemopreventive agents, including retinoids, nonsteroidal anti-inflammatory drugs, polyphenols, tamoxifen, vanilloids (including capsaicin, curcumin, and resiniferatoxin), and rotenoids (rotenone and deguelin; ref. 47). Several of these chemopreventive compounds contain agents that can promote ROS generation or trigger oxidative stress [e.g., N-(4-hydroxyphenyl)retinamide, celecoxib, indomethacin, epigallocatechin gallate, curcumin, tamoxifen, capsaicin, resiniferatoxin, rotenone, and deguelin], which seems to be associated with apoptosis induction in various cell types (47).

Epidemiology of Breast Cancer: Role of Lipid Peroxidation

Some epidemiologic evidence suggests that the lipid peroxidation mechanism may play a role in human breast cancer. (a) Although estrogen metabolism may serve as a source of oxidative stress (48, 49), estrogens, known to increase breast cancer risk, have been found to inhibit lipoprotein peroxidation in vivo and in vitro (50, 51). (b) There is a long-term protective effect of pregnancy in breast cancer, and pregnant women exhibit significantly higher lipid peroxidation levels than nonpregnant women (52). (c) Breast cancer risk is reduced by oophorectomy and menopause, conditions both associated with significant increases in lipid peroxidation (53-55). (d) Hormone replacement therapy (combined estrogen plus progestin) increases breast cancer risk and significantly decreases lipid peroxidation in postmenopausal women (56-58). (e) Although tamoxifen has been found both to inhibit and to enhance oxidative stress markers, experimental evidence suggests that the pro-oxidant properties of tamoxifen may be the ones responsible for its anticancer effect predominantly in estrogen receptor (ER)−negative breast tumors (59-61). (f) There is a recently confirmed finding that a history of preeclampsia/pregnancy−induced hypertension is associated with a reduced risk of breast cancer (62); although other mechanisms have been suggested to explain this association, such as reduced levels of estrogens and insulin-like growth factor-I (62), the statistically significant higher lipid peroxidation levels found in women suffering from this condition with respect to those associated with a normal pregnancy (63-66) may be responsible, at least in part, for this protection. (g) Physical activity is associated with a decreased risk of breast cancer and has been consistently shown to increase lipid peroxidation in human and animal studies (67-70). (h) Alcoholic beverages have been consistently shown to increase breast cancer risk and have been found to inhibit lipid peroxidation in several studies (71-75).

Estrogens. The connection between breast cancer and estrogen has been recognized for >100 years, since Beatson showed that bilateral oophorectomy resulted in the remission of breast cancer in premenopausal women (76, 77). Subsequent evidence has implicated both endogenous and exogenous estrogen in the pathogenesis of breast cancer, although the exact mechanisms remain to be fully elucidated. There are three mechanisms considered responsible for the carcinogenicity of estrogens in the human breast: (a) receptor-mediated hormonal activity, which stimulates cellular proliferation, resulting in more opportunities for accumulation of the genetic damage that leads to carcinogenesis; (b) a cytochrome P450−mediated metabolic activation, which elicits direct genotoxic effects by increasing mutation rates; and (c) induction of aneuploidy by estrogen (78). However, the underlying molecular mechanism of estrogen-associated breast carcinogenesis is still unclear. We wonder if one mechanism may be related, in part, to decreased generation of lipid peroxidation products. Estrogens inhibit lipid peroxidation (see below) and it has been shown that decreased lipid peroxidation is associated with increases in cell division, with the higher the extent of lipid peroxidation in the cells, the lower the rate of cell division (79, 80).
Estrogens have been shown to inhibit lipoprotein peroxidation in vivo and in vitro (50, 51, 81-88). Some clinical studies have also found that estrogen treatment inhibited the susceptibility of low-density lipoprotein to oxidative modification (50, 86), whereas others found that oxidation of low-density lipoprotein particles was not generally influenced by estrogen (89, 90). Reasons for these differences are not clear. Estradiol may inhibit peroxidation by a series of mechanisms that include stimulated cell antioxidant defenses and structural modification of apolipoproteins and membranes, making them more resistant to lipid peroxidation (91). Although the majority of studies point to estrogen as an antioxidant, others have found that estrogen metabolites may serve as a source of oxidative stress (48, 49). What is important in considering the possible effect of lipid peroxidation is to identify which of these properties is responsible for the carcinogenic effect of estrogen in the breast.

In the estrogen-induced Syrian hamster model, estrogen treatment increases lipid peroxidation/oxidative stress and subsequently the incidence of renal cell cancer (2, 3). Because estrogen is a risk factor for breast cancer, it is tempting to conclude, based on this model, that lipid peroxidation may be one mechanism whereby estrogen increases breast cancer. However, estrogen induces renal cancer in this model, not breast cancer. Indeed, we have proposed lipid peroxidation to be a relevant mechanism for renal carcinogenesis, a notion that is supported by experimental and epidemiologic data (2, 3).

Experimental evidence suggests that the carcinogenic effect of estrogens in breast cancer may be related to its cell proliferative, antiapoptotic functions derived from its antioxidant activities. Previous studies have documented the direct antioxidant effects of estradiol, and it is tempting to ascribe the antiapoptotic effects of estradiol to its scavenging of ROS (ref. 92; although this is by no means proven). Recent reports have also shown an indirect antioxidant effect, in which long-term exposure of MCF-7 human breast cancer cells to estradiol results in ER-dependent and estradiol dose-dependent overexpression of the antiapoptosis gene Bcl-2 (92).

In addition, although estrogen is known to stimulate cell proliferation, the breast epithelium of sexually mature and normally cycling women does not exhibit maximal proliferation during the follicular phase of the menstrual cycle, when estrogens reach peak levels of 200 to 300 pg/mL and progesterone is <1 ng/mL (93, 94). Instead, the breast epithelium exhibits its maximal proliferative activity during the luteal phase, when progesterone levels increase and are at their highest and estrogen levels are 2- to 3-fold lower than those observed during the follicular phase (93, 94). Again, a consideration of lipid peroxidation may shed light into these observations. Several rodent and human studies have shown that increased lipid peroxidation/ROS inhibits progesterone production in luteal cells and is a trigger for luteal regression and luteolysis (refs. 95-108; see Potential Anticancer Effects of Lipid Peroxidation on Stages of Breast Differentiation and Development).

Parity. More than 30 years ago, MacMahon et al. (109) described the decreased risk of breast cancer associated with parity and with early versus late first full-term pregnancy. Some molecular events take place in the breast during pregnancy that seems to “immunize” the breast against development of neoplasia. Unfortunately, 30 or 40 years after the first observation, we still do not know what these changes are, how they take place, and how they can be reproduced with a predictable intent. Although there are several possible ways in which pregnancy might influence breast cancer risk, including a pregnancy-induced maturation of mammary cells, making them less susceptible to carcinogenic transformation, or a long-lasting hormonal change, or both (110), the underlying molecular mechanism is still unclear.

The majority of mammary growth occurs during pregnancy. Before pregnancy, the majority of mammary tissue is adipose; when pregnancy occurs, the adipose tissue and stroma of the gland progressively thins as the glandular components of breast enlarge. Mammary glandular growth occurs at the expense of the surrounding adipose tissue, and when the proliferation is complete (usually about half of the gestational period), there is very little adipose tissue apparent. We raise the possibility that pregnancy-associated lipid peroxidation may be responsible, at least in part, for the thinning of the adipose tissue and subsequent mammary gland growth and proliferation.

It is possible that pregnancy is a physiologic stimulus for lipid peroxidation (63). Numerous studies compared healthy pregnant and nonpregnant women (reviewed in ref. 52); all consistently found greater blood lipid peroxide levels in the pregnant women. Reported ratios of pregnant to nonpregnant values ranged from 1.08 to 3.04 (52). Lipid peroxides are also increased in the placenta. Concentration of lipid peroxides in placenta is reportedly higher than in blood (reviewed in ref. 52). It is clear from studies of preeclamptic patients that the placenta is the most likely source of lipid peroxides secreted (or excreted) into the maternal circulation (111-113).

Lipid peroxide levels in the first trimester of pregnancy were sometimes higher and sometimes lower than the level of nonpregnant control group. By the second trimester, increases of 10% to 50% over first trimester values were usually seen (52). For example, in one study, the level of conjugated dienes (lipid peroxidation markers) in serum rose >45% when pregnancy advanced from the first to the second trimester (114). Third trimester levels sometimes but not always declined (52). All studies that provided data on lipid peroxide markers during pregnancy and postpartum showed a decrease in at least one marker after delivery (52). It is possible that, by attaining elevated levels during the second trimester (114), lipid peroxidation products may reflect an important change in the pregnant state wherein breast differentiation occurs, and the majority of mammary gland growth takes place.

Lambe et al. provided evidence that a livebirth has dual effects on maternal breast cancer risk, a transient increase followed by a long-term more marked decrease (115). They showed that, compared with nulliparous women, uniparous women have elevated odds ratios for breast cancer soon after delivery but that the odds ratios decline later. Rosner et al. (116), modeling data from the Nurses’ Health Study, also found a significant short-term increase in the risk of breast cancer immediately after the first delivery. Chie et al. (110) also found that there is a modest and transient increase in breast cancer risk after childbirth.

Lipid peroxide markers decrease immediately postpartum. The expulsion of the placenta, an important source of lipid peroxides, is a determinant of this rapid decrease. The peroxide markers begin to return to normal within 24 hours of delivery (117). Across studies, the decreases during the first 3 days after delivery ranged from 9% to 42% of the highest pregnancy measurement (reviewed by ref. 52). The levels of lipid peroxides measured postpartum were similar to those of nonpregnant women within the first 5 days after delivery (52, 118, 119). This abrupt decline in lipid peroxides postpartum may be a determinant of the transient increase of breast cancer detected immediately after giving birth. There are other mechanisms that have been hypothesized for this transient increased risk, for example that pregnancy increases the short-term risk of breast cancer by stimulating the growth of cells that have undergone the early stages of malignant transformation but that confers long-term protection by inducing the differentiation of normal mammary stem cells that have the potential for neoplastic change (115).

Preeclampsia. A negative association between preeclampsia (or pregnancy-induced hypertension) and breast cancer risk...
has been found in many studies (reviewed in ref. 62). Data suggest that a woman who experiences preeclampsia or who was herself born to a preeclamptic pregnancy is at reduced risk for breast cancer later in life (62). The reduction in risk ranges from 25% to 75%.

In a recent study (120), an increase in blood pressure from second to third trimester seemed to confer a reduction of up to 51% in the breast cancer rate (120). In addition, certain placental characteristics commonly seen in preeclampsia, such as small placental diameter and placental infarction, were independently associated with a reduced breast cancer rate; the association with small placental diameter increased with age at first pregnancy ($P = 0.008$). Maternal floor infarction of the placenta was associated with a 60% reduction in breast cancer rate (95% confidence interval, 12-82%). In combination, placental risk factors were associated with a reduction in the breast cancer rate of at least as high as 94% (95% confidence interval, 80-98%; ref. 120).

It has been shown that preeclampsia is associated with increased lipid peroxidation in the maternal circulation and in the placenta (63, 121). Multiple studies have found lipid peroxidation to be significantly elevated (20-60%) in the blood from women with preeclampsia compared with normal pregnancy (64-66, 117, 122-125) and in preeclamptic placenta compared with normal placenta (64, 65, 112, 126-129). It is clear from studies of preeclamptic patients that the placenta is an important source of lipid peroxides secreted (or excreted) into the maternal circulation (111, 129, 130).

There are other mechanisms that have been suggested to explain the preeclampsia-induced breast cancer protection, provided previously and discussed by Innes and Byers (62). However, increased levels of placental apoptosis have been found in uncomplicated pregnancy (131) and in preeclampsia (132), and limited evidence suggests the involvement of ROS generation in the increased apoptosis that occurs during preeclampsia (133).

**Oophorectomy and Menopause.** Over a century ago, Beatson (76, 77) reported on the positive effect of oophorectomy in premenopausal women with metastatic breast cancer. Beatson indicated that his rationale for the oophorectomy treatment was that oophorectomy would cause fatty degeneration of the malignant cells (76). Oophorectomy combined with tamoxifen seems to be equivalent to, or perhaps even superior to, standard chemotherapy regimens and is now recognized as an optional, first-line adjuvant treatment for patients with axillary node-negative and node-positive disease (134). Similarly, menopause is a very important protective factor for breast cancer. The prominent reductions in female sex hormones, which are clearly implicated in the pathogenesis of breast cancer, after oophorectomy and menopause are thought to be responsible for this protection, but the underlying molecular mechanism is still unclear. We hypothesize that this underlying mechanism may be related, at least in part, to increased generation of lipid peroxidation products.

An increase in the level of lipid peroxides after bilateral ovariectomy has been found in serum or plasma of female mice (53, 135) and rats (54). This increase is likely a reflection of the antioxidant activities of female hormones and is abolished on its administration. Similar increase in the level of lipid peroxide was observed in the serum of women who had undergone bilateral ovariectomy (136).

Similarly, several studies compared serum/plasma lipid peroxide levels of normal premenopausal and postmenopausal women and have found a significant increase in serum/plasma lipid peroxides after natural menopause (55, 136, 137).

**Combined Hormone Replacement Therapy.** Hormone replacement therapy (estrogen plus progestin) increases breast cancer risk (138) and significantly decreases lipid peroxidation in postmenopausal women (56-58). A significant reduction of peroxide level in platelet membranes from menopausal women was observed after oral medroxyprogesterone acetate administration. During hormone replacement therapy, a similar reduction in lipid oxidation was observed at the peak of the estrogen effect and a further decrease with the administration of medroxyprogesterone acetate. Reduction of lipid peroxidation during hormone replacement therapy is not only due to estrogens but also depends on the combined action of sex steroids (57).

**Tamoxifen.** In the MCF-7 in vivo nude mouse model of tamoxifen resistance, lipid peroxidation was statistically significantly higher in the tamoxifen-sensitive breast tumors ($P = 0.016$) but then returned to baseline levels after resistance emerged (139). In another study, tamoxifen induced oxidative stress and apoptosis in ER-negative human cancer cell lines (140). In contrast, tamoxifen has been shown to inhibit lipid peroxidation in some studies (141, 142). Tamoxifen has both estrogenic and antiestrogenic properties, and as such, it may exhibit both antioxidant and oxidant activities. Experimental evidence suggests that the prooxidant properties of tamoxifen are the ones that may be related to its anticarcinogenic effect (59-61).

Although the primary mechanism of action of tamoxifen is believed to be through the inhibition of ER, research over the years has indicated that additional, non-ER-mediated mechanisms exist. It is thought that oxidative stress mediates the actions of tamoxifen in ER-negative breast cancer cells (59-61). Tamoxifen induces apoptosis in these cells through caspase-3 and c-Jun NH2-terminal kinase 1 pathways, which are probably initiated at the cell membrane by an oxidative mechanism (60). Treatment of cells with a lipid-soluble antioxidant vitamin E blocked tamoxifen-induced caspase-3 and c-Jun NH2-terminal kinase 1 activation as well as apoptosis (60). In another study, tamoxifen modulated protein kinase C via oxidative stress in ER-negative breast cancer cells (59). Various antioxidants inhibited these cellular effects of tamoxifen. Vitamin E blocked tamoxifen-induced growth inhibition (59). These findings show that interventions to lipid peroxidation other than hormones also affect the behavior of breast cancer cells.

**Physical Activity.** Physical exercise has been consistently shown to reduce the risk of breast cancer (143-145), and it is also a potent inducer of lipid peroxidation (67-70). Multiple studies have shown markers of lipid peroxidation to be elevated in ultramarathon runners, mountain climbers, and various groups of trained and untrained humans and animals following physical activity (69, 70, 146). However, not all studies showed evidence of oxidant stress following exercise, and contradictory data do exist. Lovlin et al. (147) reported elevations in plasma malondialdehyde (a lipid peroxidation marker) in untrained males only after an exhaustive exercise bout and suggested that exercise-induced free radical generation may only occur during maximal activity. This observation has also been suggested by several human and animal studies (148-151). Lipid peroxidation induced by acute exercise has been found to increase during hypoxia in men (152), and malondialdehyde has been found to be positively correlated with maximal oxygen consumption in active older women (153). The fact that both the protective effect of exercise on breast cancer (154) and the generation of exercise-induced free radicals seem to occur after moderate to vigorous intensity exercise is consistent with the possibility that lipid peroxidation plays a role in the physical exercise-breast cancer relationship. It has been shown that the increased ROS production during exercise leads to an increase of apoptosis in cells (155, 156).

**Alcoholic Beverages.** Intake of alcoholic beverages has been consistently shown to increase breast cancer risk (157) and is...
associated with decreased lipid peroxidation (71, 75). It is possible that the decrease in lipid peroxidation caused by antioxidants in alcoholic beverages is responsible, at least in part, for the increased breast cancer risk associated with alcohol intake. Antioxidants in alcoholic beverages, especially polyphenolic compounds in red wine, have been proposed as a contributory factor to the protective effect of regular alcohol use against atherosclerotic cardiovascular disease. There is definitive in vitro evidence that extracts of red wine, white wine, and beer can inhibit lipoprotein oxidation, the degree of inhibition being directly proportional to beverage polyphenolic content and able to be abolished by prior stripping of the polyphenolics from the alcoholic beverage (73, 74, 158). In addition, recent studies have consistently shown that red wine and white wine also have beneficial effects in vitro on blood antioxidant activity (71, 72, 75, 159). However, alcohol has also been found to exhibit a pro-oxidant effect (72, 74, 160). Some studies suggest that the antioxidant or pro-oxidant activities of alcoholic beverages may depend on factors, such as dose, ethanol content, polyphenol content, ethanol metabolism, and cell type (73, 161-165), but this is still unclear. Other studies suggest that the pro-oxidant properties of alcohol in the liver may come from ethanol, which is oxidized primarily at that level, and antioxidant properties from polyphenols, but this is also unclear. Alcoholic beverages have been found to have opposite effects on lipid peroxidation (and apoptosis) depending on the cell system. For example, red wine extract and alcohol decreased lipid peroxidation and/or apoptosis in the heart (162, 166), but alcohol increased both oxidative stress and apoptosis in the liver (163, 164, 167, 168). It is unknown if the procarcinogenic effect of alcohol in breast cancer comes from its antioxidant or pro-oxidant properties, but it seems to be related to oxidative stress, as it is modified by oxidative stress genes. Zheng et al. (169) found that breast cancer risk is 6.8-fold increased for postmenopausal women with the GSTT1-null genotype who consumed a lifetime of >250 kg of spirit equivalents. An 8-fold significantly increased risk was observed among heavier drinkers who had GSTM1A- and GSTT1-null genotypes. The mechanisms underlying the alcohol-breast cancer relationship need further study.

**Obesity.** An inverse association between relative weight and breast cancer risk has been found among premenopausal women in most case-control and prospective studies (170, 171). This apparent protective effect has been reported to last for breast cancer (172) to 30 years after the index (173) among those studies reporting significant results. In a meta-analysis, Ursin et al. (174) reached the general conclusion that there was a significant trend for decreasing relative risk for premenopausal breast cancer in association with increasing body mass index. The relative risk for a body mass index difference of 8 kg/m² was 0.70 (95% confidence interval, 0.54-0.91) for 4 cohort studies and 0.88 (95% confidence interval, 0.76-1.02) for the 19 case-control studies. In light of these findings, it has been proposed that some beneficial effect coming from adiposity decreases breast cancer risk. Obesity reduces serum estradiol and progesterone levels in premenopausal women, but the mechanism underlying these observations is unknown. It is possible that obesity decreases breast cancer in premenopausal women through a specific mechanism that involves the generation of lipid peroxidation products. There exists a direct relationship between obesity and lipid peroxidation: obesity has been associated with elevated lipid peroxidation among human subjects, and the elevation seems to be removable by weight reduction (2). It has been shown that peroxidation products in low-density lipoprotein are cytotoxic to ER-negative breast tumor cells and vitamin E counteracts this effect (175).

The relation between body weight and postmenopausal breast cancer is less clear. In many case-control studies, body mass index has been positively associated with postmenopausal breast cancer. However, prospective studies have generally suggested a weaker positive association (176-178). The lack of a stronger positive association between body weight and breast cancer is perplexing because, among postmenopausal women, endogenous estrogen levels, which are believed to increase breast cancer incidence, are 50% to 100% higher among overweight women compared with lean women (170, 179, 180). This has suggested that some beneficial effect of adiposity may counterbalance an adverse effect due to higher endogenous estrogen levels (170). We suggest that increased levels of lipid peroxidation present in obese women may play a role in the postmenopausal obesity-breast cancer relationship. It is also possible that several other factors, such as the use of estrogen preparations after menopause, could obscure the adiposity-breast cancer relationship in postmenopausal women as supported by results of Huang et al. (170). In that study, the risks associated with obesity and weight gain were much stronger in postmenopausal women who never used hormones (170).

**Other Suspected Protective/Risk Factors for Breast Cancer**

There is supporting evidence that lipid peroxidation may play a role in the potential anticarcinogenic effect of other breast cancer factors, including soy (28, 181-187), marine n-3 fatty acids (46), thyroid diseases (188-202), green tea (203-207), vitamin D (208), calcium (209-212), folate (213), and isothiocyanates (214-217).

**Anticarcinogenic Mechanisms of Lipid Peroxidation—Inhibition of Cell Proliferation and Induction of Cell Differentiation and Apoptosis**

Evidence suggests a role of lipid peroxidation products in the control of cell proliferation and in the induction of differentiation, maturation, and apoptosis (218-220). It has been shown that lipid peroxidation and ROS are triggers and essential mediators of apoptosis, which eliminates precancerous and cancerous, virus-infected and otherwise damaged cells that threaten our health (1, 80, 221, 222). Although therapeutic mechanisms may not always be relevant to etiology, most chemotherapeutic agents and radiation induce mitochondrial changes and apoptosis through mechanisms associated with lipid peroxidation/ROS production (1, 223-225). Numerous in vitro studies have shown that patients treated with a wide range of chemotherapeutic agents exhibit marked increases in lipid peroxidation products (225-230). It is well known that oxidative stress provokes cell death as a result of massive cellular damage associated with lipid peroxidation and alterations of proteins and nucleic acids (224). Apoptosis occurs when, through a pathway of signaling, the mitochondrial membrane becomes permeable (231, 232). Mitochondria are the main site for ROS generation and are thought to be a major intracellular target for oxidative damage (1, 233, 234). Anticancer agents can cause mitochondrial permeabilization through enhanced generation of lipid peroxidation, and once the mitochondrial membrane barrier function is lost, several factors contribute to cell death (232). Whereas lipid peroxidation, among other factors, induces or facilitates mitochondrial permeabilization, glutathione and antioxidant enzymes inhibit it (232, 235). It has also been shown that lipid peroxidation products inhibit cell proliferation and induce cell differentiation and maturation (218-220). The most abundant aldehydes of lipid peroxidation have been identified as 4-hydroxy-2E-nonenal. This toxic product has been reported to be involved in oxidative alterations in Alzheimer’s disease and in the
formation of etheno DNA-base adducts, and because 4-hydroxy-2E-nonenal is a mutagen, it has been reported that it causes mutation in the p53 gene in human hepatocarcinoma (218). However, it has also been shown that 4-hydroxy-2E-nonenal strongly inhibits cell proliferation in a dose- and time-dependent manner in leukemic cells (219) and that it is also a strong inducer of cell differentiation and apoptosis in leukemic and osteosarcoma cell lines (219).

Potential Anticancer Effects of Lipid Peroxidation on Stages of Breast Differentiation and Development. The role of lipid peroxidation in conferring protection against breast cancer may be considered as an episodic phenomenon linked to stages in a woman’s life where lipid peroxidation—induced growth arrest or apoptosis is required for maturation, development, differentiation, and tissue turnover. The consideration of lipid peroxidation as a protective factor for the prevention of breast cancer does not contradict the conventional view that it is a cytotoxic process that is generally undesirable. This may remain the case for general biochemical processes (smoking, polycyclic aromatic hydrocarbon-DNA adducts, radiation, and other chemical exposures), as lipid peroxidation in excess disturbs normal cell processes. However, in stages in development where damage or transformed cells (not useful or functional ones) require disposal, lipid peroxidation may provide an important component for cell death and turnover.

Normal human breast epithelial cells undergo a continuous cycle of cellular proliferation, differentiation, and apoptosis (236-238), and factors that influence these processes are expected to play a role in mammary tumor development. Cell proliferation and apoptosis have been shown to undergo significant changes during the menstrual cycle, pregnancy, and lactation due to the high level of cell turnover in these states (236, 237, 239-243).

Menstrual Cycle. In the early 1980s, Ferguson and Anderson (236) provided conclusive evidence that cell turnover in the “resting” breast is influenced by the menstrual cycle with both cell proliferation and apoptosis showing cyclical changes. Since then, several investigators have confirmed these findings, showing that apoptosis occurs cyclically in normal breast tissue epithelial cells, with a peak incidence at the end of the luteal phase (238, 244). The position of the apoptotic peak at the end of the luteal phase is important because numerous rodent, mostly, and human studies have shown that lipid peroxidation/ROS significantly increases at this time and this increase is a trigger for luteal regression and luteolysis and also decreases progesterone production in luteal cells (95-108).

In addition, antioxidant and antiapoptotic Bcl-2 protein levels also decline sharply in the late luteal phase as the incidence of apoptosis increases (245). In another study, the status of the normal mammary epithelium was summarized as a balance between factors that were proapoptotic, represented by Bax, and antiapoptotic, represented by Bcl-2, Mcl-1, and Bcl-x (246). It has been shown that proapoptotic Bax functions in an oxidant way to induce apoptosis and antiapoptotic Bcl-2 functions in an antioxidant way to prevent it (80, 247, 248).

Pregnancy. In addition to the menstrual cycle, apoptosis is also influenced by pregnancy and lactation also due to the high level of cell turnover. Russo et al. (249) have shown that parity, in addition to exerting an important influence in the lobular composition of the breast, profoundly influences the proliferative activity of the mammary epithelium. Although after menopause the proliferative activity of the mammary epithelium decreases, still parous women retain a lower rate of cell proliferation than nulliparous women. Parity significantly decreases the proliferative index.

Thus, in stages of breast differentiation and maturation, lipid peroxidation may have beneficial effects for cells. The statistically significant increased levels of lipid peroxidation that have been found during the late luteal phase of the menstrual cycle, pregnancy, and menopause could decrease the risk of breast cancer by inhibition of cell proliferation and potentiation of differentiation and apoptosis.

ROS-Mediated Cell Responses

We believe that the ROS-mediated cell responses depend on several factors: (a) baseline levels of ROS, (b) cell type, and (c) duration and intensity of the cells exposing to the ROS environment.

Baseline Level of ROS. The human population is heterogeneous regarding ROS levels and lipid peroxidation generation. As ROS/lipid peroxidation are critical mediators of apoptosis, factors that increase their levels could increase apoptosis of normal, functioning cells and thus induce degenerative diseases or cancer in people with innate or acquired high levels of ROS. However, factors that increase ROS/lipid peroxidation can increase apoptosis of precancerous and cancerous cells and thus protect against cancer, particularly in people with a low innate baseline level of ROS (1).

Cell Type. Flores and McCord (250) and Aw (251) proposed a model that predicts differential cell responses to oxidative stress depending on cell type, differentiation, and ROS concentration. (a) If the cells are terminally differentiated or quiescent (such as kidney, liver, brain, and heart), they have such constraints on the ability to proliferate because of a mitotic block that, when the concentration of oxidants increases, their response will be to die by either apoptosis or necrosis. Thus, too much ROS in a slowly proliferating tissue would result in untimely death or damage of normal, functioning cells and thus possibly lead to degenerative diseases, such as Alzheimer’s, Parkinson’s, and Huntington’s, cardiovascular disease (excessive apoptosis has been found to be a key factor in the pathogenesis of these disorders; refs. 252, 253), or cancer through DNA damage and mutations. Untimely normal cell death in an organ can also lead to cancer through compensatory regeneration and consequent cell proliferation (3). In fact, several models of oxidation-induced carcinogenesis involve slowly proliferating tissue, such as kidney and liver, organs that contain stable cells that do not multiply continuously but can do when necessary (3).

(b) Low levels of ROS in mitotically competent tissue (composed of cells that maintain a genetic program that allows them to proliferate) or in a highly proliferating tissue (containing undifferentiated, transformed, precancerous, or cancerous cells) may initially provide a further stimulus to proliferation. However, higher levels of ROS in these tissues would induce apoptosis of the undifferentiated, transformed, precancerous or cancerous cells and therefore would have the potential to protect against cancer. It has been shown that nondifferentiated cells are more sensitive to ROS-induced cytotoxicity than fully differentiated, mature cells (254).

Duration and Intensity of the Cells Exposing to the ROS Environment. The intracellular concentration of ROS and lipid peroxidation products seems to be crucial for the nature of cell cycle signaling and may be a determinant for the signaling for differentiation, proliferation, transformation, or apoptosis (255). Although ROS generated at low intracellular levels can promote the proliferative stimulus provided by mitogens (256), it is well established that increased generation of ROS plays a central role in the initiation and completion of many forms of apoptosis (23, 255). Similarly, lipid peroxidation products, particularly 4-hydroxy-2E-nonenal, also affect signaling mechanisms in a concentration-dependent manner. It has been shown that, whereas low levels of 4-hydroxy-2E-nonenal...
promote proliferation, higher concentrations induce differentiation and apoptosis (255).

An example to illustrate the role of ROS concentration in cell response is radiation-induced breast cancer. ROS are involved in cellular radiation and there is a high risk of radiation-induced breast cancer in women treated for Hodgkin’s disease. The incidence of radiation-induced tumors is well known to increase in the low-dose range (which would imply subtoxic, mild concentrations of ROS), and it has been speculated that the risk declines with increasing radiation dose as radiation cell kill becomes the predominant effect (257). A recent combined analysis of eight cohorts confirmed the downturn in risk at the highest dose levels (related, in part, to the killing of cells rather than transformation; ref. 258).

**Summary**

The molecular mechanisms underlying the development of breast cancer in general and estrogen-associated breast carcinogenesis in particular are not completely understood. It is generally believed that the initiation of breast cancer results from uncontrolled cell proliferation as a consequence of cumulative genetic damages that lead to genetic alterations. However, the underlying molecular mechanism is still unclear. We raise the possibility that decreased lipid peroxidation may be a mechanism responsible, at least in part, for the increased risk associated with several hormonal and nonhormonal risk factors for breast cancer. Some of the observed inconsistencies in the epidemiology are explainable in light of the lipid peroxidation hypothesis. It is our hope that this discussion can serve as an impetus for other investigators to consider the possibility that the process of lipid peroxidation may play a role in breast cancer etiology and that studies that are able to more directly assess this possibility may be warranted.

**References**

38. Shao Y, Pardini L, Pardini RS. Dietary menhaden oils enhance mitomycin C.


Alexa ID, Jerca L, Gheorghita V. The role of lipid peroxidation and of the
123.
122.
Wang Y, Walsh SW. Placental mitochondria as a source of oxidative stress in
Cohn BA, Cirillo PM, Christianson RE, van den Berg BJ, Siiteri PK. Placental
118.
117.
Wu JJ. Lipid peroxidation in preeclamptic and eclamptic pregnancies. Eur J
116.
Walsh SW, Wang Y. Dexamethasone stimulates glutathione peroxidase activity in
preeclampsia is associated with increased placental production of thrombaxone and
115.
Walsh SW. The role of fatty acid peroxidation and antioxidant status in
114.
Uotila J, Tuimala R, Aarnio T, Pyykko K, Ahotupa M. Lipid
peroxidation products, selenium-dependent glutathione peroxidase and
42:95 – 100.
113.
1994;331:819 – 35.
112.
819 – 35.
111.
Wickens D, Wilkins MH, Lunec J, Ball G, Dormandy TL. Free radical
110.
Cohn BA, Cirillo PM, Christianson RE, van den Berg BJ, Siiteri PK. Placental
109.
Wu JJ. Lipid peroxidaion in preeclamptic and eclamptic pregnancies. Eur J
108.
Sato M, Maulik N, Das DK. Cardioprotection with alcohol: role of both
antioxidants and pro-oxidants in alcoholic beverages that might influence
the development of atherosclerotic cardiovascular disease? Neuroepidemiology
107.
Lee IM. Physical activity and cancer prevention—data from epidemiologic
106.
Thangaraju M, Vijayalakshmi T, Sachidanandan P. Effect of tamoxifen on
lipid peroxide and antioxidative system in postmenopausal women with breast
105.
Furth FW, Colditz GA, et al. Alcohol consumption induces hepatocyte
104.
103.
102.
101.
100.
Wang SW, Wang Y. Dexamethasone stimulates glutathione peroxidase activity in
preeclampsia is associated with increased placental production of thrombaxone and
99.
Walsh SW. The role of fatty acid peroxidation and antioxidant status in
98.
Uotila J, Tuimala R, Aarnio T, Pyykko K, Ahotupa M. Lipid
peroxidation products, selenium-dependent glutathione peroxidase and
42:95 – 100.
97.
1994;331:819 – 35.
96.
819 – 35.
95.
Wickens D, Wilkins MH, Lunec J, Ball G, Dormandy TL. Free radical
94.
Cohn BA, Cirillo PM, Christianson RE, van den Berg BJ, Siiteri PK. Placental
108.
Wu JJ. Lipid peroxidation in preeclamptic and eclamptic pregnancies. Eur J
93.
Cohn BA, Cirillo PM, Christianson RE, van den Berg BJ, Siiteri PK. Placental
109.
Wu JJ. Lipid peroxidaion in preeclamptic and eclamptic pregnancies. Eur J
92.
Cohn BA, Cirillo PM, Christianson RE, van den Berg BJ, Siiteri PK. Placental
108.
Wu JJ. Lipid peroxidation in preeclamptic and eclamptic pregnancies. Eur J
91.
Cohn BA, Cirillo PM, Christianson RE, van den Berg BJ, Siiteri PK. Placental
108.
Wu JJ. Lipid peroxidation in preeclamptic and eclamptic pregnancies. Eur J
90.
Cohn BA, Cirillo PM, Christianson RE, van den Berg BJ, Siiteri PK. Placental
108.
Wu JJ. Lipid peroxidation in preeclamptic and eclamptic pregnancies. Eur J
89.
Cohn BA, Cirillo PM, Christianson RE, van den Berg BJ, Siiteri PK. Placental
108.
Wu JJ. Lipid peroxidation in preeclamptic and eclamptic pregnancies. Eur J
88.
Cohn BA, Cirillo PM, Christianson RE, van den Berg BJ, Siiteri PK. Placental
108.
Wu JJ. Lipid peroxidation in preeclamptic and eclamptic pregnancies. Eur J
87.
Cohn BA, Cirillo PM, Christianson RE, van den Berg BJ, Siiteri PK. Placental
108.
Wu JJ. Lipid peroxidation in preeclamptic and eclamptic pregnancies. Eur J
86.
Cohn BA, Cirillo PM, Christianson RE, van den Berg BJ, Siiteri PK. Placental
108.
Wu JJ. Lipid peroxidation in preeclamptic and eclamptic pregnancies. Eur J
Lipid Peroxidation and Breast Cancer


180. Lipworth L, Adami HO, Trichopoulos D, Carlstrom K, Mantzoros C. Serum steroid hormone levels, sex hormone-binding globulin, and body mass index in the etiology of postmenopausal breast cancer. Epidemiology 1996; 7:96–100.


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