Review

Dairy Product Consumption and the Risk of Breast Cancer

Peter W. Parodi, PhD

Human Nutrition and Health Research, Dairy Australia, Melbourne, AUSTRALIA

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It has been suggested in some reports that dairy product consumption may increase the risk of breast cancer. This review gives a brief overview of the etiology of breast cancer and in particular the roles of fat, bovine growth hormone, insulin-like growth factor-1 and estrogens. Evidence from animal studies and epidemiology does not support a role for fat in the etiology of breast cancer. The daily intake of insulin-like growth factor-1 and biologically active estrogens from dairy products is minute in comparison to the daily endogenous secretion of these factors in women, whereas bovine growth hormone is biologically inactive in humans. On the other hand, milk contains rumenic acid, vaccenic acid, branched chain fatty acids, butyric acid, cysteine-rich whey proteins, calcium and vitamin D; components, which have the potential to help prevent breast cancer. Evidence from more than 40 case-control studies and 12 cohort studies does not support an association between dairy product consumption and the risk of breast cancer.

Key teaching points:

- The etiology of breast cancer is still largely undetermined. A women's reproductive history provides the most consistent evidence for risk, but the relative risk for most risk factors is close to the null value of 1.
- More than 40 case-control and 12 cohort studies do not suggest that dairy product consumption is associated with the risk of breast cancer.
- It has been suggested by some researchers that dairy products may increase the risk of breast cancer due to their content of fat, insulin-like growth factor-1, estrogens or growth hormone. However, the available evidence does not support this association.
- Animal studies and epidemiology do not suggest a role for fat in the etiology of breast cancer. Bovine growth hormone is biologically inactive in humans. Daily intake of insulin-like growth factor-1 and biologically active estrogens is insignificant compared to daily endogenous secretion in women.
- Milk contains rumenic, vaccenic, butyric and branched chain fatty acids, whey protein, calcium and vitamin D, which have the potential to protect against breast cancer.

INTRODUCTION

Breast cancer is the most common - and most feared - malignancy in women living in developed countries, and is second only to lung cancer as a cause of cancer death. There is large international variation in breast cancer rates. In developed countries the age-standardized incidence rates are around 100/100,000 women with mortality rates about 25/100,000. These rates are up to 5-fold higher than those reported from Asian regions, which have the lowest incidence of breast cancer [1]. Breast cancer is rarely found before the age of 25 years. Thereafter, the incidence increases with age until menopause when the rate of increase is less pronounced. About three-quarters of diagnosed cases are in postmenopausal women [2–7].

Despite extensive research to find the cause of breast cancer the etiology is largely undetermined. It is estimated that around 75% of women who present with this malignancy have no established risk factors other than age and living in a western society [2]. When women migrate from a region of low incidence for breast cancer to one with a high incidence their risk does not immediately assume the rate in the host country. However, the risk in their descendants approaches that of their adopted country after two to three generations, which indicates that environmental factors are of greater importance than genetic factors [4,5,8,9]. Nevertheless, breast cancer is known to cluster in

Address reprint requests to: Dr Peter Parodi, 9 Hanbury St., Chermside, 4032, Queensland, AUSTRALIA. E-mail: peterparodi@uq.net.au

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families and having a first-degree relative (mother, sister, daughter) with breast cancer, especially at a young age, can double the risk of developing this cancer. Two high-penetrant genes, *BRCA1* and *BRCA2* account for the majority of inherited breast cancer, however, mutations in these and other low-penetrant susceptibility genes account for less than 5 to 10% of breast cancer cases [5,10].

From the mass of epidemiological data generated over the years, characteristics of a woman's reproductive history provide the most consistent evidence for the risk of breast cancer. Early onset of menarche, a late menopause, delayed childbirth, nulliparity and low cumulative lactation time all increase the risk of breast cancer [2,4,5]. It is believed these factors reflect a longer lifetime exposure to endogenous steroid hormones. This is supported by observations that women with bilateral oophorectomy at an early age have a decreased risk of breast cancer compared with women who had a natural menopause [4,11]. Further, there is a small increase in risk of breast cancer associated with long-term use of oral contraceptives and hormone replacement therapy (HRT) [3–5]. However, most of these risk factors are weak and the relative risk (RR) or odds ratio (OR), indices used to indicate the strength of risk, are seldom much greater than the null value of 1 [11].

A number of other important, although minor, risk factors have been noted. Women exposed to excessive levels of radiation, especially at a young age, are at increased risk of breast cancer [5,12]. Increased mammographic breast density is associated with increased risk [5,13]. Obesity is associated with a decreased risk of breast cancer in premenopausal women and an increased risk in postmenopausal women [4,11,14]. Physical activity decreases risk [4,5]. Height is a risk factor [4], and risk increases with increasing birth weight [15]. Most of this group of risk factors may influence or be influenced by steroid hormones. Although the role of diet in the etiology of breast cancer has been studied extensively there is no clear indication that any dietary item, apart from alcohol, is associated with breast cancer risk [16].

Special interest groups, media articles, books and some scientific papers have suggested that dairy product consumption can increase the risk of developing breast cancer. The rationale for this claim is that dairy products are a source of fat, including saturated fatty acids; insulin-like growth factor, a mitogen; estrogenic hormones, which are weak carcinogens and mutagens, and growth hormone [17–20]. The validity of these assertions is now examined.

DAIRY PRODUCT CONSUMPTION AND BREAST CANCER RISK: EPIDEMIOLOGY

Some 41 case-control studies together with 12 cohort and case-control studies nested within cohort studies have determined the associations between total dairy product or specific dairy item consumption and the risk of breast cancer. Knekt and Jarvinen [21] give a description and results of studies published up to 1998, and summarize in table form the strength of association for the various studies. As part of a meta-analysis on dietary fat and breast cancer risk, Boyd et al. [22] included two dairy categories, milk (16 studies) and cheese (12 studies), which showed ORs with associated 95% confidence intervals (CIs) of 1.12 (0.88–1.43) and 1.26 (0.96–1.66), respectively. Missmer et al. [23] conducted a pooled analysis of primary data from eight large prospective studies as part of the Pooling Project of Prospective Studies of Diet and Cancer. No relation was found with dairy products analyzed as total dairy fluids, total dairy solids, ten sub-groups, or seven specific dairy foods and the risk of breast cancer.

Recently, Moorman and Terry [24] summarized the results of ten cohort and 36 case-control studies that evaluated the association between dairy product consumption and breast cancer risk. They concluded that the available epidemiological evidence dose not support a strong association between the consumption of milk or other dairy products and the risk of breast cancer. Since this report [24] results have appeared for two case-control studies and two cohort studies. One case-control study found a significant negative association between high milk intake and breast cancer risk [25]. The other study [26] found a significant negative association between a high intake of total dairy and low-fat dairy intake and the risk of breast cancer, but high-fat dairy consumption was nonsignificantly associated with risk. In the Nurses' Health Study II [27] women with a high consumption of low-fat dairy products during their premenopausal years had a nonsignificant negative association with breast cancer risk. However, total dairy intake was nonsignificantly associated, and high-fat dairy intake was positively associated with risk. The other cohort study [28] assessed the risk of adolescent diet and the risk of breast cancer and will be discussed separately.

Adolescent Diet and the Risk of Breast Cancer

Exposure to initiating events during childhood, adolescence and early adulthood, when the mammary gland is attaining adult-stage morphology, may influence the risk of breast cancer in later life. Indeed, several studies show that the risk of breast cancer associated with alcohol consumption and cigarette smoking increases with decreasing age at which exposure to these practices commenced [29]. For women treated with high doses of ionising radiation for tuberculosis, acute postpartum mastitis, enlarged thymus and Hodgkin's disease, the risk of breast cancer increased with decreasing age at exposure [12]. Long-term follow-up studies of the incidence of breast cancer among atomic bomb survivors from Hiroshima and Nagasaki also show increased risk with decreasing age at exposure [12].

Three cohort and four case-control studies have examined the consumption of dairy products during adolescence and the subsequent risk of breast cancer. The results of these studies are presented in Table 1. Of the 12 associations listed, ten showed a

Table 1. Summary of Data from Cohort and Case-Control Studies Evaluating the Association between Adolescent Dairy Product Intake and The Subsequent Risk of Breast Cancer

Study	Cases	Controls or cohort size	Menopausal status at diagnosis	Product evaluated	Results OR[1] or RR[1] (95% Cl[2])
COHORT					
Frazier et al. [28]	361	47,355	94.8% premenopausal	Total dairy (less butter)	0.83 (0.56–1.24)
				High-fat dairy	1.11(0.76–1.62)
				Low-fat dairy	0.88 (0.60–1.29)
Shin et al. [139]	327	–[3]	Premenopausal	Milk	0.81 (0.51–1.28)
	1509	–[3]	Postmenopausal	Milk	1.02 (0.82–1.26)
Hjartaker et al. [140]	317	48,844	Premenopausal	Milk	0.64 (0.22–1.87)
CASE-CONTROL					
Shu et al. [141]	1459	1556	Mixed	Milk	0.76 (0.59–0.98)
Potischman et al. [142]	1647	1501	Premenopausal	Dairy products	0.98 (0.8–1.2)
Pryor et al. [143]	99	101	Premenopausal	Milk fat	0.4 (0.1–1.1)
	70	88	Postmenopausal	Milk fat	0.2 (0.0–0.8)
Hislop et al. [114]	263	306	Premenopausal	Whole milk	0.71 (0.40–1.27)
	392	435	Postmenopausal	Whole milk	0.75 (0.49–1.13)

[1]Odds ratio or relative risk for the highest category of intake vrs.the lowest. The fully adjusted models are presented.

[2]Confidence interval.

[3]Not given in text.

negative association between intake of dairy products and the risk of breast cancer, but only one achieved statistical significance.

FAT, FAT TYPE AND BREAST CANCER RISK

For many years it was considered that fat intake provided the strongest dietary link with breast cancer risk. This belief was based largely on two lines of evidence; strong correlation between *per capita* consumption of fat and breast cancer mortality in international comparison studies; and animal experiments that showed a high fat diet increased the incidence of chemically induced mammary tumors [16].

It is now realized that in cancer studies there is an interrelationship between dietary fat and calories. In studies using rodent models of carcinogenesis in which the effects of calorie intake were separated from those of the fat content, the fat content of the diet did not significantly influence tumor development. On the other hand, calorie restriction inhibited tumor development [30,31]. Because fat intake is highly correlated with energy intake it is essential to adjust for energy intake in epidemiological studies that assess associations between dietary fat intake and the risk of breast cancer.

Most international comparison (ecologic) studies show strong positive correlation between *per capita* fat consumption and mortality from breast cancer [32,33]. Ecological studies are a poor format for determining causality. Dietary information based on national food disappearance data is a poor reflection of individual consumption and tells nothing about the diets of individuals who develop cancer and those who do not. Other dietary, environmental and reproductive patterns can vary widely between countries, and are not adjusted for in this type of study [34].

Within-population epidemiological studies can avoid much of the confounding found in ecological studies. Goodwin and Boyd [35] reviewed the published results from 14 case-control studies that examined the relationship between the intake of total fat or fat containing foods and the risk of breast cancer. Eight studies examined the relationship between total fat intake and breast cancer risk. Only one study found a statistically significant positive association. Results were inconsistent in the six studies that examined the risk for various fat containing foods. Howe et al. [36] conducted a pooled analysis of the original data from 12 case-control studies of diet and breast cancer that represented 4427 cases. The RR for the highest vs. lowest quintile of total fat was 1.13 (non-significant) for premenopausal women and 1.48 (significant) for postmenopausal women. This analysis did not include the then largest study of 2024 cases [37], or a subsequent study with 2564 cases [38], both of which did not find an association between fat intake and the risk of breast cancer.

The accuracy of associations generated by case-control studies can be affected by dietary measurement error due to unreliable nutrient databases, inaccurate assessment of past diet, and dietary recall bias by subjects who have breast cancer. Inappropriate selection of control subjects can also introduce bias [34]. Prospective (cohort) studies largely overcome these biases, because diet is assessed before cancer diagnosis, and at a time closer to its initiation. In addition, control subjects belong to the same community as cases [34].

Hunter et al. [39] conducted a collaborative-pooled analysis of original data from seven large prospective studies published up to 1995 that represented 4980 cases. The analysis found no evidence of an association between the intake of cholesterol or total, saturated, monounsaturated or polyunsaturated fat and the risk of breast cancer. There was no reduction in risk among women whose energy intake from fat was less than 20% of total energy intake. What is more, for the small number of women reporting less than 15% of energy from fat, the risk of breast cancer increased more than two-fold. A follow-up pooled analysis by Smith-Warner et al. [40], with 7,329 cases, confirmed the lack of association between total fat, fat class or animal or vegetable fat intake and the risk of breast cancer. In addition, no survival advantage was found for consumption of a low fat diet or type of fat, after diagnosis of breast cancer in participants from the Nurses' Health Study [41]. High correlations between various dietary fatty acids in epidemiological studies reduce the ability to detect an independent association with cancer risk. Nevertheless, there is no convincing evidence from epidemiological studies that any individual fatty acid is associated with the risk of breast cancer [42].

Of the dietary items thought to protect against breast cancer, fruit and vegetables and fiber have received the most attention. However, a pooled analysis of cohort studies suggests that fruit and vegetable consumption, at least during adulthood, is not significantly associated with reduced breast cancer risk [43]. Likewise, evidence from well-conducted epidemiological studies does not suggest a protective effect for dietary fiber [16]. In contrast, there is consistent epidemiological evidence that alcohol consumption is positively associated with breast cancer risk [16]. Overall, there is no convincing evidence that fat intake is associated with the risk of breast cancer. The RRs and related confidence intervals associated with nearly all dietary items in the epidemiological studies are close to the null value of 1. This suggests that diet does not play an important role in the etiology of breast cancer.

INSULIN-LIKE GROWTH FACTOR AND BREAST CANCER

The Insulin-Like Growth Factor System

Insulin-like growth factors (IGFs) belong to a larger family of insulin related peptides, which include insulin, IGF-1 and IGF-2. Together with binding proteins, binding protein proteases and receptors they form the IGF system. IGFs are mitogens that play an important function in almost every organ of the body, where they regulate cell proliferation, differentiation and apoptosis [44, 45]. IGFs, particularly IGF-1, are required for normal mammary gland development, but it is also implicated in breast cancer development [46,47]. IGF-1 exerts its biological actions by interacting with a specific type 1 IGF-1 receptor (IGF-IR) associated with the cell membrane [45,46]. The bioactivity of IGF-1 depends on complex physiological regulation. Only a small portion circulates in the free form; the remainder is regulated by a series of six IGF-binding proteins (IGFBP-1 through IGFBP-6), which have a somewhat stronger affinity for IGF-1 than the receptor. More than 90% of serum IGF-1 is bound in a ternary complex with IGFBP-3 and an acid-labile subunit (ALS). This complex cannot leave the circulation and serves to both increase the half-life of IGF-1 and at the same time inhibit its mitogenic effect. The presence of IGFBP proteases in tissues can cleave the binding protein and liberate free IGF-1 [44,45,47–49]. IGFBP-3 can also modulate the IGF-1 signaling pathway independently of its IGF-1-binding ability. In mammary tissue, IGFBP-3 may interact with its own membrane receptor to inhibit growth, induce apoptosis and mediate cell growth arrest induced by other molecules [47–50].

Most IGF-1 and IGFBPs are produced in the liver under control of growth hormone, and levels can be influenced by nutritional factors. Non-hepatic tissues can also produce IGF-1 and IGFBP-3, where they exert autocrine and paracrine effects [44,45]. In the breast, IGF-1 is expressed in stromal cells adjacent to normal or malignant epithelial cells. The extent to which circulating versus endogenously produced IGF-1 is important for mammary gland development and in tumorigenesis is still to be resolved [46,51,52].

Determinants of Circulating IGF-1 and IGFBP-3 Levels

Serum IGF-1 levels are low at birth, rise during childhood and reach a peak at puberty. Thereafter, values decline with age. The age-specific distribution of IGFBP-3 and ALS is similar to the distribution for IGF-1 [44,47,53]. There is considerable heterogeneity in adult serum IGF-1 levels, with a range of 80 to 425 μg/L [53], however, an individual's circulating level of IGF-1 and IGFBP-3 is relatively constant. Thissen et al. [54] and Yu and Rohan [47] have reviewed the determinants of circulating IGF and IGFBPs. The most consistent determinant of IGF-1 levels is dietary protein. Levels are markedly lowered by severe protein and energy restriction, with essential amino acid deficiency having a severe depressive effect. Over nutrition has the opposite effect, but not to the same extent as under nutrition. There have been few studies on dietary micro- and macronutrients, and the results are conflicting. Associations between serum IGF-1 levels and other factors, such as physical activity, energy intake within normal limits, smoking, BMI and anthropometric indices have provided divergent results [47,54].

Epidemiological Studies

Many epidemiological studies have examined the association between circulating levels of IGF-1 and IGFBP-3 and the risk of breast cancer. Recently, three meta-analyses of these studies, using different exclusion criteria, were published [55–57]. Overall, there was a marginally significant association between high levels of circulating IGF-1 and increased risk of breast cancer in premenopausal women, but not in postmenopausal women. Surprisingly, there was no protective effect for

IGFBP-3, and high levels were associated with a marginally increased risk of premenopausal breast cancer.

Breast cancer cells can produce IGF-1 [46,47,58]. Also, because breast cancer cells secrete IGFBP-3 proteases, this can alter circulating levels of free IGF-1 without increasing its production [59], and breast cancer tissue exhibits higher IGF-1R levels than adjacent normal tissue [44,46,47]. An interesting sequential serum IGF-1 study was conducted in a nested case-control of prostate cancer, a hormone-related epithelial malignancy with a common pathogenic framework to breast cancer. In the prostate cancer cases serum IGF-1 levels were significantly higher at the time of diagnosis than in previous samples drawn 2 to 5 years before diagnosis [60]. Thus elevated IGF-1 levels in breast cancer patients may be a marker of, rather than a cause of the disease. Further, the positive association between serum IGFBP-3 levels and the risk of breast cancer may be a consequence of the production of IGFBP-3 by breast cancer cells [61].

IGF-1 in Milk

The IGF-1 content of bovine milk varies with the stage of lactation. A recent study showed colostrum had a level of 300ng/mL and the content dropped to 7ng/mL at 1 week postpartum. Thereafter the levels dropped further to below 2ng/mL. IGFBP-3, which inhibits the mitogenic effect of IGF-1, is by far the most abundant binding protein in milk and content varies throughout lactation in a manner similar to IGF-1 [62]. At any given stage of lactation, IGF-1 levels can vary widely between cows due to many factors including parity and farm practise [63]. The level of IGF-1 in milk is not affected by pasteurisation [64].

Milk IGF-1 and Breast Cancer

Because milk contains IGF-1, which has an identical amino acid sequence to human IGF-1 [65], it has been suggested its consumption may be linked to breast cancer [17,18]. The evidence presented to justify this connection does not stand up to serious scientific scrutiny. Firstly, the amount of IGF-1 consumed daily from milk products is minute compared to endogenous production. Based on a milk content of 4ng/mL, milk product consumption equivalent to 1.5L milk/day would contribute 6,000ng IGF-1 to the gastrointestinal tract. The gastrointestinal tract also receives considerable exogenous IGF-1 from saliva, biliary fluid, pancreatic juice and secretions from the intestinal mucosa, estimated to total 380,000ng/day [66,67]. In addition, it is estimated that in adults the liver and extra-hepatic tissues produce 10^7ng IGF-1/day [68]. Thus, milk-derived IGF-1 would contribute less than 0.06% of total daily IGF-1 production if it escaped proteolysis during intestinal passage, and was absorbed by the intestine and passed to the circulation. This is unlikely, as considerable, if not total, digestion of IGF-1 should take place in the small intestine [69].

Studies cited to justify absorption of IGF-1 from the intestine [17] used suckling rats. This is an inappropriate model, because neonates do not have a fully developed protease/peptidase system and intestinal closure has not occurred, which allows enhanced permeability of macromolecules. Even so, evidence from neonatal animal studies suggests that feeding IGF-1 results in negligible intestinal absorption [70]. Of greater significance, recent studies that fed human adults up to 60g/d of a concentrated bovine colostrum protein powder for up to 8 weeks did not find an increase in serum IGF-1 levels [71–73]. These studies provide compelling evidence that IGF-1 in dairy products is not implicated in the etiology of breast cancer.

Diet and Serum IGF-1 Levels

In an oft-cited study by Heaney et al. [74], subjects with habitual low dairy product consumption consumed their usual diet or their usual diet plus three servings of dairy per day. After 12 weeks serum IGF-1 levels increased by 12% in the milk drinkers, and decreased by 2% in the non-milk drinkers. However, the increase in IGF-1 levels in milk drinkers was accompanied by an increase in total protein intake and energy compared to non-milk drinkers. Total energy intake and protein consumption are the major determinants of circulating IGF-1 [47–54]. In a nested case-control study from the Physician's Health Study there was a modest increase in serum IGF-1 levels with increasing skim or low-fat milk consumption. Non-significant increases were found for poultry and fish consumption [75]. In a randomised double blind study, healthy men consumed 40g of soy protein (often associated with protection from breast cancer) or milk protein daily for 3 months. Serum IGF-1 levels increased from baseline with both protein supplements, but were significantly higher only for soy protein [76]. Animal studies suggest that the essential amino acid content of dietary protein may be the important determinant for IGF-1 level [77].

SEX HORMONES AND BREAST CANCER

Established risk factors for breast cancer are predominantly associated with a woman's reproductive history, which suggests they are markers for exposure to endogenous ovarian hormones, the estrogens and progestins [3,14]. Support for the concept that cumulative exposure to estrogens is a major determinant of breast cancer risk comes from several epidemiological studies and clinical observations. Women with bilateral oophorectomy have a lower risk of breast cancer than women who have a natural menopause. The younger the age of oophorectomy, the lower the risk [3,4,11]. The antiestrogenic drug tamoxifen is successful in the prevention and treatment of breast cancer, especially in women with estrogen receptor (ER) positive tumours [78]. In addition, aromatase inhibitors, which prevent the aromatase enzyme catalysing the final step in estrogen biosynthesis, are also successful in the prevention and treatment of breast cancer [79].

Use of oral contraceptives slightly increases the risk of breast cancer in young women. The risk increases with increasing duration of use, and after age 45 years. [3–5,80] Epidemiological studies show there is a modest increase in risk of breast cancer associated with hormone replacement therapy (HRT). Combined estrogen and progestogen use appears to be related to a higher risk for breast cancer than estrogen alone. Overall, the risk associated with HRT use for a year is comparable to delayed menopause for the same period of time. Risk is higher for long-term users, but risk falls when use ceases [3,81,82].

Estrogens as Carcinogens

A number of lines of evidence suggest that estradiol, the most potent estrogen, is a weak carcinogen and mutagen, although the molecular mechanisms are still incompletely understood [83–85]. Estrogens function in cells by diffusing passively through cell membranes binding to nuclear ERs and stimulating transcription of genes involved in cell proliferation. This increases the opportunity for accumulation of DNA damage that may lead to carcinogenesis. There is also accumulating evidence that estradiol can be metabolised to genotoxic compounds like 16α-hydroxy estradiol and the catechol estrogen quinones that directly damage DNA [83,85]. Estrogens act in concert and interact synergistically with elements of the IGF-1 axis. In breast cancer cells estrogens induce the expression of IGF-1 and enhance its mitogenic effect. Estrogens stimulate production of IGF-1Rs, repress synthesis of IGFBP-3 and increase the synthesis of cathepsin D, an IGFBP-3 protease. [47,86,87].

Serum Sex Hormone Level and Breast Cancer Risk

Because of the important role for sex hormones in the etiology of breast cancer, numerous studies have investigated the association between circulating sex hormone levels, particularly estradiol, and the risk of breast cancer. The physiologically significant estrogens in order of potency are estradiol (17β-estradiol), estrone and estriol in a ratio of about 100:10:4. Most circulating estradiol is bound to plasma proteins, sex hormone-binding globulin (SHBG) or albumin, which renders them biologically inactive [14].

Premenopausal Women. Key [88] lists four prospective studies that reported on estrogens and breast cancer in premenopausal women. Together, they do not suggest that a higher level of serum estradiol is associated with an increased risk of breast cancer. However, a single blood sample may not represent a woman's habitual hormone status because of large variation in hormone level during the menstrual cycle. Estradiol level varies from 6ng/100mL in the early follicular phase to 33 to 70ng/100mL in the late follicular phase, and a value around 20ng/100mL in the mid luteal phase [89].

Postmenopausal Women. About three-quarters of diagnosed breast cancer occurs in postmenopausal women. After menopause ovarian estrogen production ceases and the major circulating estrogen is estrone (30pg/mL), which is formed by aromatization of the steroid hormone androstenedione in peripheral tissues, primarily adipose tissue. Some estrone, in turn, is metabolized to estradiol (15pg/mL) [14,90].

The Endogenous Hormones and Breast Cancer Collaborative Group [91] conducted a pooled analysis of the original data from nine prospective studies. In postmenopausal women they found a statistically significant increase in the risk of breast cancer with increasing concentrations of all sex hormones examined. Interestingly, the association between the different levels of estrogens and breast cancer risk was stronger in never uses of HRT than users.

Determinants of Serum Estrogen Levels

Overweight, obese and sedentary postmenopausal women have elevated concentrations of circulating estrogens, and lower concentrations of SHBG [14,92]. Exercise can reduce serum estrogen and increase SHBG levels, but the effect is dependent on loss of body fat [92]. There is no clear association between obesity and estrogen levels in premenopausal women [14]. Many studies have investigated the role of diet on serum estrogen levels, but the results are inconclusive [14]. A relationship between dietary fat and serum estrogen levels is unclear [14,34]. Dietary fiber intake may be inversely related to concentrations of serum estrogen [14].

Estrogen Metabolism in Breast Tissue

Are high circulating levels of estrogens a cause of breast cancer, or a correlate, or a consequence of the disease? There is no simple linear relationship between serum levels and tissue concentrations of estrogens [93,94]. The levels of estradiol in normal and malignant breast tissue are similar for both premenopausal and postmenopausal women, even though serum estrogen levels are up to 50-fold lower in postmenopausal women [93,95,96]. However, estradiol levels are significantly higher in breast cancer tissue than in normal tissue for both premenopausal and postmenopausal women [93]. Levels of estrone sulphate, the major form of circulating estrogen in postmenopausal women, were significantly higher in their breast tumors than in those of premenopausal women [94].

The concentration of estrogens in breast tissue is far higher than in circulating plasma [94,97,98], which suggests that local production of estrogens in breast tissue is far more important than uptake of estrogens from the circulation [85,99]. Breast tissue contains all the enzymes necessary to synthesize the biologically active estradiol from circulating precursors. Firstly, aromatase, which converts androstenedione to estrone; secondly, estrone sulfatase that hydrolyses biologically inactive estrone sulphate to estrone; and thirdly 17β-hydroxysteroid dehydrogenase, which reduces the weakly bioactive estrone to estradiol [85]. Human breast cancer cells can adapt to a deprivation in estradiol stimulation by developing enhanced estrogen sensitivity to the residual levels of estradiol present [100] or to the precursors of estrogen by increasing the levels of estrogen synthesizing enzymes [96].

Contribution of Milk Estrogens to Circulating Levels in Women

Steroid hormones are widely distributed in the animal and vegetable products we consume [101]. Milk contains estrone and estradiol, but the concentration varies considerably during the estrous cycle and during pregnancy, especially in estrone sulfate [102,103].

As part of a German market basket survey Hartman et al. [101] purchased samples of dairy products and determined their content of estrone and estradiol. Based on previously published national nutritional data they calculated that a woman would consume about 0.05μg/day of estrogens from dairy products, with about 90% represented by the weakly bioactive estrone. These estrogens are largely conjugated and a large proportion of injested hormones are inactivated by the first-pass effect of the liver [101]. In contrast, during the late follicular phase of the menstrual cycle a woman produces up to 1mg/day of estradiol and 0.7 mg/day of estrone [89]. Postmenopausal women produce between 40 and 200μg/day of estrone from androstenedione, depending on their weight [90]. Thus, the contribution of dairy product consumption to a woman's estrogen status is infinitesimal and cannot be considered a risk for breast cancer.

GROWTH HORMONE

Growth hormone (GH) or somatotropin is secreted by the anterior pituitary gland, and regulates growth in most tissues from birth to puberty, although GH still has important metabolic effects in adults. GH levels are low in infancy, increase slightly during childhood and peak during puberty. Thereafter levels progressively decrease with age, but there is considerable inter-individual variation. There is also considerable intra- individual variation in GH levels, which are low during most of the day with bursts occurring after meals, exercise and emotional stress, but mostly during the first few hours of sleep. This pulsatile pattern of GH release by the pituitary gland is controlled by the hypothalamic factors; growth hormone-releasing hormone, which stimulates release of GH, and growth hormone-inhibitory hormone (somatostatin) that inhibits the release of GH [104].

GH is an essential factor in the development of the mammary gland. Acting through its receptor, GH induces stromal cells to synthesize IGF-1, which can stimulate proliferation and differentiation in adjacent epithelial cells in a paracrine manner [105]. Estradiol enhances the stimulatory effect of GH and IGF-1 on mammary gland development and in breast cancer cells [51,87]. The GF/IGF-1 axis also plays a role in mammary tumorigenesis. GH binds to receptors in the liver to induce IGF-1, thereby elevating circulating IGF-1 levels. On the other hand, GH also increases IGFBP-3 levels [106]. Autocrine production of GH in mammary carcinoma cells can promote cell proliferation, transcriptional activation and prevention of apoptosis. Autocrine produced GH is believed to be a more potent stimulator of mammary carcinoma cell spreading than exogenously administered GH [107].

Despite the mitogenic activity of GH, relatively few studies have addressed the role of GH in the etiology of breast cancer. Animal studies using transgenic mice that over or under express GH show that GH deficiency is associated with less tumor growth, whereas over expression of GH increases tumor development [87,108,109]. Serum GH levels in breast cancer patients were higher than in control subjects in what appears to be the only study that examined the relationship between GH level and the risk of breast cancer [110]. However, an independent role for GH in breast cancer etiology is difficult to establish because of its effect on the GH/IGF-1 axis.

Milk Derived GH

Commercial use of recombinant bovine GH (rbGH) to increase milk yield and efficiency in dairy cows commenced in the United States in 1994 [111]. This event provoked considerable debate among special interest groups, the media and in the scientific literature, as to whether milk from treated cows would cause adverse health effects [17,18,112–114].

Bovine milk naturally contains less than 1ng/mL of GH [115], whereas humans secrete 500 to 875 μg of GH per day [104]. There is no significant increase in bGH levels in milk from cows treated with rbGH [113,115]. Pasteurization of milk destroys about 90% of bGH [113]. Because bGH is a protein it is hydrolyzed in the intestinal tract during the digestion process. Should any bGH survive digestion it will have no effect on human biology, because the human GH receptor does not respond to bGH [63,116].

Administration of rbGH to cows increases the level of IGF-1 in milk, but overall the impact is minimal when considered against the large variations influenced by stage of lactation, parity, nutrition and herd environment [63,111,113]. What is more, when IGF-1 levels increase so do the levels of IGFBP-3 and ALS [111]. The unlikely survival of dietary IGF-1 in the intestinal tract to produce a biological response in humans was discussed in a previous section.

COMPONENTS OF MILK WITH THE POTENTIAL TO PREVENT BREAST CANCER

The assertion that consumption of milk and its products could increase the risk of developing breast cancer because of their content of fat, IGF-1, estrogens and GH is ill founded. On the other hand, milk contains a number of components with the potential to help prevent breast cancer.

Calcium and Vitamin D

Both calcium and vitamin D play an important role in the regulation of cell growth. In addition, vitamin D, through its active metabolite 1,25-dihydroxy vitamin $D_3(1,25(OH)_2D_3)$, is important for calcium homeostasis and absorption into cells [117,118]. Animal studies suggest that hyperproliferation and hyperplasia in mammary epithelial cells can be reduced by dietary calcium and vitamin D [117].

There are a number of possible mechanisms for the anti-proliferative action of calcium. Calcium may neutralize fatty acids and mutagenic bile acids, which can rapidly pass from the intestine to the breast where they can affect ERs and induce estrogen-regulated protein in a manner similar to estradiol [119]. Human breast cancer cells express elevated levels of fatty acid synthase [FAS], the major enzyme required for endogenous fatty acid biosynthesis, a process that has been linked to cell proliferation. Treatment of breast cancer cell lines with cerulenin, an inhibitor of FAS activity, resulted in rapid growth inhibition that was associated with apoptosis [120]. Zemel [121] recorded that high-calcium diets suppressed $1,25(OH)_2D_3$-induced calcium influx into adipocytes - the predominant cells in the breast - and inhibited FAS activity.

Increased mammographic breast density is strongly associated with the risk of breast cancer [5]. A recent study showed that an increased intake of calcium and vitamin D was associated with decreases in mammographic breast density [13]. Boyapati et al. [122] recently reported that dietary calcium intake was negatively associated with the risk of breast cancer in both premenopausal and postmenopausal women. These authors also tabulated the results of seven other case-control and two cohort studies, all of which found negative associations between calcium intake and the risk of breast cancer. In the Nurses' Health Study both calcium and dairy product intake was associated with a survival benefit for women with breast cancer [41].

Rumenic and Vaccenic Acids

Rumenic acid (RA) is the predominant natural isomer of conjugated linoleic acid (CLA), and milk fat is the richest natural source. Vaccenic acid (VA), the major *trans*-monounsaturated fatty acid in milk fat can be converted to RA in animals and humans by the enzyme Δ^9 - desaturase [123]. In normal rat mammary epithelial cells, RA inhibited cell growth and induced apoptosis [124]. At physiological concentrations RA, VA and milk fat all arrested cell growth in breast cancer cells [125,126]

When added to the diet of rats at a level of 1% or less, RA is a potent inhibitor of mammary tumor development. Tumor inhibition is independent of the amount or type (saturated or polyunsaturated) of fat in the diet, and is particularly effective when fed only during the period of mammary gland development to adult stage morphology. Feeding RA during this period resulted in a decrease in epithelial density associated with a reduced proliferation of the epithelial cells within the terminal end buds and lobular epithelium, areas where most tumors develop [124]. The anti-tumor action of RA is possibly additionally mediated by induction of apoptosis and inhibition of angiogenesis associated with decreased serum and glandular levels of vascular endothelial growth factor and its receptor Flk-1 [124,127]. RA is a potent inhibitor of FAS in human breast cancer cell lines [128,129]. As part of a CLA mixed isomer supplement, RA reduced serum IGF-1 levels in rats [130].

Epidemiological Studies. The initial case-control study found a significant inverse association between dietary intake of RA and the risk of breast cancer in Finnish postmenopausal women. Serum levels of RA and VA also showed a significant inverse relationship to breast cancer risk [131]. A study conducted in New York [132] found that there was a nonsignificant inverse association between intake of RA and incidence of breast cancer in premenopausal but not postmenopausal women. The benefit was more apparent in women with the more aggressive ER negative tumors. Three other studies did not find a relationship between RA and breast cancer risk. The methodological limitations in these, and other RA/VA studies, have been discussed [123].

Branched-Chain Fatty Acids

Branched long-chain fatty acids (BCFA) are synthesized by rumen bacteria, and iso- and anteiso-BCFAs, particularly those with a chain length of 13 to 17 carbon atoms, are found in milk fat [123]. Initially, Yang et al. [133] reported that 13-methyltetradecanoic acid (13-MTDA) induced cell death in human breast cancer cells by rapid induction of apoptosis. Recently, Wongtangtintharn et al. [129] tested the antitumour activity of a series of iso-BCFA in two human breast cancer cell lines. The highest antitumour activity was found with iso-16:0, and the activity decreased with an increase or decrease in chain-length from iso-16:0. Anteiso-BCFAs were also cytotoxic. Interestingly, cytotoxicity of 13-MTDA was comparable to RA. Both 13-MTDA and RA inhibited FAS.

Butyric Acid

Butyric acid, uniquely present in milk fat, is a potent anticancer agent, which induces differentiation and apoptosis and inhibits proliferation and angiogenesis. Although butyrate has a short half-life in the circulation this can be increased when butyrate is present as a derivative. In the case of milk fat, butyrate is esterified as a triacylglycerol, and about one-third of all milk fat triglycerides contain butyrate. Synergy with other dietary anticancer agents like vitamin A, vitamin D and resveratrol reduce the plasma concentration of butyrate required to modulate cell growth [123]. Two studies showed that dietary butyrate significantly inhibited chemically induced mammary tumor development in rats [134,135].

Milk Proteins

Evidence from animal studies and *in vitro* studies with human breast cancer cells suggest that milk proteins, especially those associated with the whey fraction, have anticarcinogenic properties [136,137]. Whey protein is a rich source of cysteine, which is essential for the synthesis of glutathione. Glutathione is a potent cellular antioxidant and also acts by itself or by its related enzymes as a detoxifying agent that facilitates the elimination of mutagens, carcinogens and other xenobiotics from the body [136]. Results from a recent nested case-control study from within the prospective Nurses' Health Study [138] show that women with higher plasma concentrations of cysteine had a significantly reduced risk of breast cancer.

CONCLUSION

The etiology of breast cancer is still largely undetermined, although a woman's reproductive history is considered an important determinant. A role for diet in breast cancer is not well established. An examination of the results from more than 40 case-control studies and 12 cohort studies does not support an association between dairy product consumption and the risk of breast cancer. The research that addresses theories about an association between dairy product consumption and breast cancer via fat, IGF-1, GH and estrogens was examined, however, the weight of evidence does not support a link. Although estrogens and the GH/IGF-1 axis play a critical role in the development of the mammary gland and in breast cancer, the mechanisms are complex and cancer is probably influenced more by autocrine/paracrine secretion than by circulating levels. Nevertheless, the daily contribution of these factors from dairy product consumption is far too small compared to daily endogenous secretion to exert a physiological effect. The presence of rumenic, vaccenic, butyric and branched chain fatty acids, cysteine-rich whey proteins, calcium and vitamin D in milk has the potential to help prevent breast cancer.

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