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REVIEW

Nutrients and nipple aspirate fluid composition: the breast microenvironment regulates protein expression and cancer aetiology

Ferdinando Mannello · Gaetana A. Tonti · Franco Canestrari

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Abstract The aetiology of breast cancer is complex and multifactorial, and may include diet and xenobiotic compounds. A change in diet affects nutrient levels in blood, but to what extent diet can affect micronutrient concentrations in the breast is not yet well established. Breast nipple aspirate fluids (NAF) can be non-invasively obtained from the breast in most women; it represents a biological tool to assess metabolic changes in the breast ductal microenvironment. A wide variation in biomolecular and hormonal composition of NAFs collected from healthy and breast cancer patient may be due to genetic and nutritional factors; however, micro- and macro-nutrients may influence the secretory status of these women, thus NAF composition and risk of breast carcinoma. The aim of this overview is to highlight the detrimental/beneficial role that diet-related compounds in nipple aspirate fluid can have in breast cancer risk.

Keywords Nutrigenomics · Diet · Benign breast diseases · Breast cancer · Nipple aspirate fluids · Nutrients

Gene–diet interactions and breast cancer risk

Breast cancer aetiology is complex, and many factors (both environmental and genetic) contribute to it. Of the environmental factors, the link between diet and BC risk has long been recognized [35]. Because benign breast disease (BBD), particularly atypical hyperplasia (AH), is a marker of increased BC risk, studies of diet and BBD have provided evidence about the effect of diet at an early stage in the process of breast carcinogenesis, reporting also some contrasting results [26, 52]. It is well known that high consumption of fruit and vegetable has been thought to provide protection against many types of cancer, including BC [15]. Their consumption could decrease BC risk through several mechanisms, mainly linked to the abundance of antioxidants contained therein, which also contain biocompounds such as isoflavones, lignans and indoles [1, 2, 64]; all of these substances have been convincingly shown to have anticarcinogenic properties and strong effects on estrogen metabolism (as reviewed in [38]), even though to date there are contrasting findings [35].

It is becoming clear that relationships between diet and breast cancer aetiology are extremely complex, and that the impact of micro- and macro-nutrients on BC risk are dependent in part on genetic factors [20, 21, 58]. Being the application of molecular epidemiology to nutrition and breast cancer in its infancy, recent studies have highlighted the importance of investigations of diet, genetic variability and breast cancer risk using a fluid non-invasively collected from breast ducts (nipple aspirate fluid, NAF), a fluid that mirrors the microenvironment of the breast tissue during physio-pathological conditions [29, 43, 44, 56].

Nipple aspirate fluid, a window on the breast tissue microenvironment

The ductal system of the breasts of non-pregnant women produces proteinaceous secretions—containing chemicals of endogenous and exogenous origin as well as epithelial cells—which can be sampled and utilized for breast cancer

F. Mannello (✉) · G. A. Tonti · F. Canestrari
Dipartimento di Scienze Biomolecolari, Sezione di Biochimica Clinica, Università Studi "Carlo Bo" di Urbino, Via O. Ubaldini 7, 61029 Urbino (PU), Italy
e-mail: ferdinando.mannello@uniurb.it

aetiology research [12, 30, 31] and breast biomonitoring [43]. These secretions are called nipple aspirate fluids (NAF) (Fig. 1). As early as the 1960s, it was hypothesized that ''locally formed toxic degradation products or carcinogenic compounds secreted into the breast ducts from the blood might be concentrated in relatively static breast secretions and be of significance in the aetiology of benign breast disease and breast cancer'' [44]. The population of women who can participate in NAF studies is more inclusive than studies involving human milk or breast tissue. Most studies indicate that NAF can be readily obtained from Caucasian and African–American women (about 75% of these women yield fluid), with Asian women less likely to be able to provide fluid (<35%) [43], even though it may be collected with higher success rate (up to 90%) [30–32, 54, 56]. The age range of women most able to produce fluid is from 30 to 55 years of age. A large case–control study of breast disease was conducted to determine factors influencing the ability to obtain NAF from non-lactating women [62]. Factors shown to increase the ability to obtain NAF are age range from 35 to 50 years, earlier age at menarche, non-Asian ethnicity, and history of lactation. Factors not associated with the ability to obtain NAF samples include exogenous estrogen use, endogenous estrogen levels, the phase of menstrual cycle, a family history of breast cancer, type of menopause (natural or surgical), and less than full-term pregnancy. In the early time of NAF analyses, investigators were able to obtain NAF from 42% of premenopausal women as compared with 17% of postmenopausal women. NAF can be obtained by aspiration (Fig. 2), a non-invasive and inexpensive method which allows to collect breast secretion and epithelial cells floating in it [29]. The most substantial drawback is the small volume of fluid which can be obtained, ranging from 1 to 500 µl [43]. Larger sample volumes allow to obtain a greater number of epithelial cells, useful for efficient optical and electronic microscopy studies [29–31]. The value of NAF has been extensively described [12, 30, 31, 43, 56], referring to breast secretions as quick, repeatable, non-invasive means for obtaining fluid and breast ductal cells. The breast is unique among secretory glands, because many chemicals in the secretions are retained and concentrated, possibly for long periods of time, and eventually metabolized by the breast epithelium or absorbed [29, 44]. This is of special interest for investigations into diseases of the breast, including breast cancer, because this peculiar secretory activity allows toxic, mutagenic, and carcinogenic substances, hormones, proteins, and growth factors, to reach breast epithelium. Once they reach the epithelium, these compounds may be taken up and metabolically activated to produce direct and/or indirect intracellular effects on the breast epithelium [30, 31]. NAF can be used to evaluate the proteinaceous secretory components that come into contact with the ductal epithelial cells, to investigate the properties of the ductal cells and their early morphological transformations, and to assess the biomolecular effect of the intake of dietary biocompounds on breast epithelial cells lining the ducts. Because BC develops from ductal and lobular epithelium, the analysis of NAF has attracted considerable interest as a non-invasive method to assess the metabolic pathways occurring within the mammary gland; this secretion represents a useful tool to clarify the biological, cellular and molecular mechanisms taking place during breast physiology and pathology, directly shedding light on the effects of exogenous and endogenous biocompounds (such as nutrients, dietary components, drugs) on the breast tissue and its modifications proning to breast cancer [54].

Apart from all the biomolecules endogenously produced, secreted and metabolized by apocrine epithelial and stromal breast cells lining the mammary ducts (such as proteins, lipids, hormones, growth factors, and antigens), recent studies have focused the attention to exogenous biocompounds (such as macro- and micro-nutrients introduced by diet intake) and their accumulation in NAF, providing the basis for their possible roles on breast tissue during different physio-pathological breast conditions.

Role of nutrients in nipple aspirate fluids: effects on breast cancer risk?

It has been long demonstrated that several micro- and macro-nutrients introduced with the diet, as well as exogenous and endogenous biocompounds, are present in nipple aspirate fluids (NAF) and may influence its biochemical composition and secretion in ductal tree; the presence in NAF of some biocompounds (e.g., proteinases) may also favour the detachment of epithelial cells lining the ducts, that can be found floating in NAF (Table 1). Actually, we cannot differentiate whether a compound derives from the diet or it is actively synthesized/secreted by breast cells, or both. Due to the difficulty to discern diet nutrients assayed in NAF from biocompounds which are determinants of NAF (e.g., lactose, which may derive both from the lactose synthase pathway in breast tissue and from the dietary intake), we present here an overview of all biocompounds detected in NAF that may be of exogenous and/or endogenous origin. Although all these components may be parameters or biomarkers of the NAF composition, we report and comment their presence considering them as characteristic determinants; in fact, two mechanisms have been suggested for their presence: on one side, passive filtration from plasma may account for the presence of nutrients in NAF at lower/equal concentrations than in plasma; on the other side, selective accumulation of

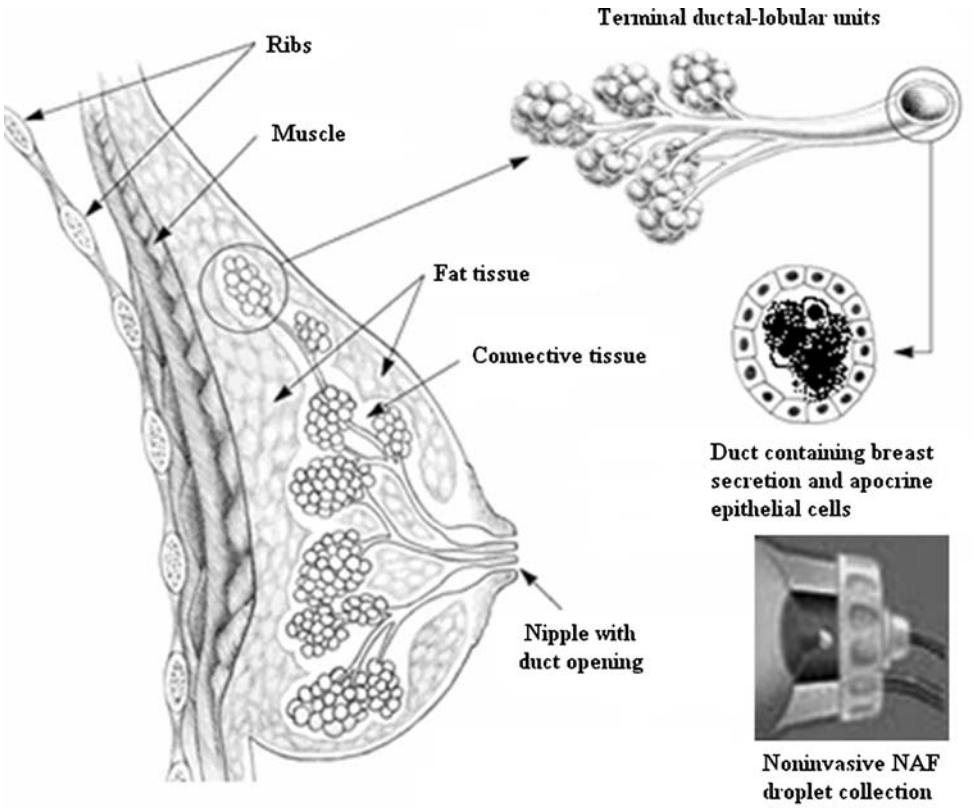

Fig. 1 Schematic representation of terminal ductal–lobular unit of human breast gland in non-lactating women. The ductal system of the breasts of non-pregnant women produces proteinaceous secretions—containing chemicals of endogenous and exogenous origin as well as epithelial cells—which can be sampled and utilized for breast cancer aetiology research and breast biomonitoring through non-invasive fluid collection technique (HALO^{TM} NAF Collection System, NeoMatrix, CA). Because BC develops from ductal and lobular epithelium, the analysis of NAF has attracted considerable interest as a non-invasive method to assess the metabolic pathways occurring within the mammary gland; this secretion represents a useful tool to clarify the biological, cellular and molecular mechanisms taking place during breast physiology and pathology, directly shedding light on the effects of exogenous and endogenous biocompounds (such as nutrients, dietary components, drugs) on the breast tissue and its modifications proning to breast cancer

nutrients may be obtained against a concentration gradient due to secretion by apocrine metabolically-active breast cells lining the ducts in conjunction with different degrees of retention or impaired reabsorption [29, 42]. Up-to-now, other than dietary intake or supplementation, no evidence of different sources for the biocompounds detected in NAF has been demonstrated. In fact, normal adult breast is a closed physiological system, and as such, the quantity and composition of secreted breast fluid must be in equilibrium with that being reabsorbed [43]. Disturbances of this equilibrium may lead to the "stagnation" of NAF fluid into breast ducts and lobules, as well as to an increased and direct exposure of breast cells lining the ducts to exogenous/endogenous mutagens and carcinogens [29, 42]; the metabolic alterations (such as the lipid and protein oxidative stress during NAF stagnation) may actively influence molecular and biochemical pathways of breast apocrine cells lining the ducts, proning them to malignant transformation [30, 31]. On the other hand, it is noteworthy that dietary intake of several antioxidants may counterbalance the detrimental effects of stagnation that play a crucial pathogenetic role for both BBD and BC development [44].

Several studies have demonstrated that modification of the diet is able to alter both the NAF composition and secretory status of some women; for example, high dietary intake of lactose [23] (a milk disaccharide not absorbed intact but metabolized in the intestine to galactose and glucose, and re-synthesized in the mammary gland by lactose synthase) may significantly increase NAF secretion due to its main osmotic property [24], but may also stimulate apocrine breast ductal/lobular cells to increase the pathways of lactose synthase through lactalbumin and galactosyl transferase activities [40, 43]. Although the enzymatic involvement of lactose synthase was found only in healthy subjects, several studies have highlighted that lactose intake and its presence in NAF were related to both the active secretory function of apocrine breast cells lining the ducts and to NAF colour [47]. In fact, virtual absence of lactose may account for the majority of NAF displaying deep yellow, brown and green colours; these NAF samples

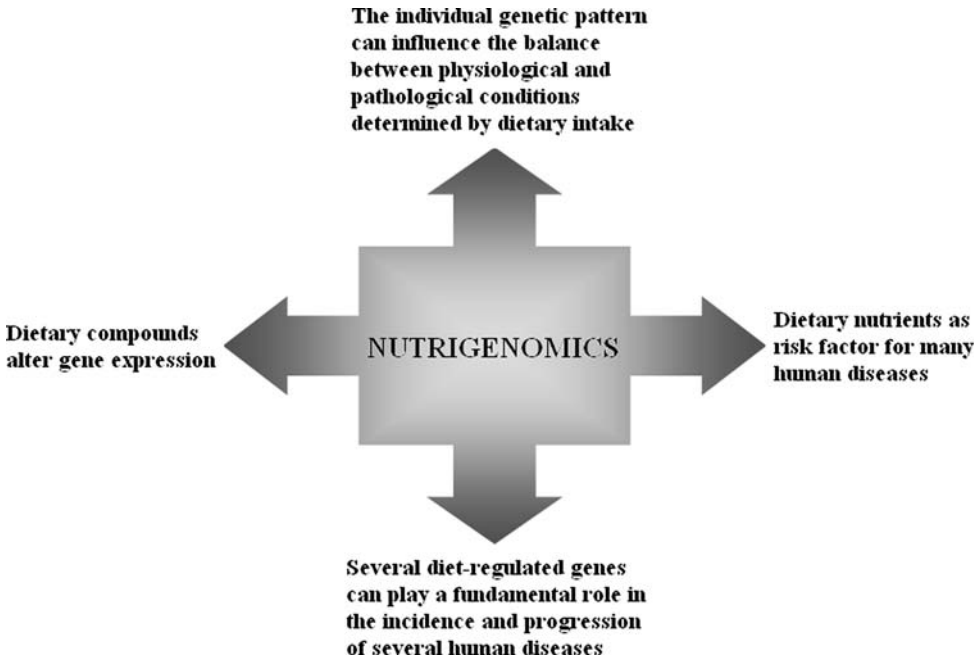

Fig. 2 The tenets of Nutrigenomics are essentially based on the concepts that diet can be an important risk factor for a wide number of diseases and that dietary compounds can directly or indirectly interact with the genome by altering gene expression. So, the individual genetic factor can influence the balance between physiological and pathological condition determined by diet and several diet-regulated genes can play a fundamental role in the incidence and progression of many chronic diseases. For these reasons, a personalized diet on the basis of nutritional status and genotype can be very helpful in preventing and curing human diseases, including cancer. The eventual implementation of these concepts in every-day clinical practice promises to revolutionize the preventive and therapeutic approach to many diseases thereby reducing the need for conventional pharmaceutical therapy

are almost collected from older age women in which prolonged stagnation occurs ([8, 19, 48, 57] and which are identified at increased BC risk [48, 57].

Among the components identified in NAF, lipids are mainly responsible for the darker colouration (mainly due to oxidized cholesterol end-products); in fact, NAF contains a wide variety of lipids, which are related to dietary intake, as well as to increased expression of binding and carrier proteins [29–31, 42]. Types and amounts of fatty acids are biochemically altered in NAF when oils of high polyunsaturated content are fed to normal women; the promptness of appearance of PUFAs in NAF following ingestion and the presence therein of fluorescent pigment indicative of lipid peroxidation have suggested that these substances may be important in the epidemiology of BC through their effects on breast epithelium [49]. Moreover, it was demonstrated that a diet habit with very-low fat consumption and enriched in fibre, significantly modulates the NAF lipid composition [4]; in fact, a progressively greater proportion of NAF yielders was found with increasing consumption of fat, indicating a positive association with dietary energy and the stimulation of prolactin gene expression and secretion [28]. In this respect, it was also reported that leptin (the product of the obesity gene which has been found to stimulate growth of breast cancer cells in vitro) significantly accumulated in NAF and was associated with body mass index in premenopausal but not in postmenopausal women [55]. Particular attention was focused to the hypothesis that chemical substances of exogenous and endogenous origin (including mutagenic and toxic substances, estrogens and other steroid hormones) are secreted and accumulated in NAF by the adult female breast, underlining that a dynamic balance between secretion, reabsorption, and turnover of these substances may be an important determinant of exposure of breast epithelia to putative initiating and carcinogenic agents [30, 31, 42]. Starting from the evidence that high fat intake is associated with increased breast secretory function [28, 63], the enhanced secretion/accumulation of lipids in NAF might increase the risk of BC through the exposure of the breast epithelium to carcinogens; in fact, oxidized cholesterol metabolites (such as cholesterol-5,6-epoxides and cholestane-3 beta, 5alpha, 6 beta-thriol) are significantly elevated in NAF of women at increased risk for BC [17, 46]. These cholesterol oxides found in breast fluids could exert genotoxic effects on the breast epithelium, and increased levels of cholesterol β-epoxide have been associated with the presence of increasing atypia in the exfoliated cells, proning women to BC progression [18, 50]. Interestingly, higher dietary intake of saturated

Table 1 Studies on the effects of dietary supplementation on NAFs collected from healthy and breast cancer women

Exogenous and endogenous compounds	References
Estrogen and steroid hormones	[18, 30, 42, 44]
Fibres	[4, 25, 28]
Fruits and vegetables	[10, 25, 53, 59]
Carotenoids	[9–11, 37, 53, 59]
α-carotene	[10, 25]
β-carotene (*trans*- and *cis*- forms)	[10, 25]
Lycopene	[7, 25]
trans- and *cis*-lycopene	[10]
2,6-cyclolycopene-1,5-diol	[7]
Lutein	[10, 25]
Zeaxanthin	[10, 25]
α-Cryptoxanthin	[10]
β-cryptoxantin	[10, 25]
Vitamins	[11, 25]
provitamin A	[9]
vitamin E	[9]
retinol	[10, 11, 25]
tocopherol	[10, 11, 25]
α– tocopherol	[10, 25]
δ-tocopherol	[10]
γ-tocopherol	[10, 25]
Lactose	[8, 19, 23, 24, 47, 48, 57]
Lipids	[4, 9, 10, 24, 25, 28–30, 42, 49, 63]
PUFA	[49]
Cholesterol, epoxides and metabolites	[10, 11, 17, 18, 25, 29, 31, 42, 44–46, 50, 62]
Isoprostanes	[10, 11, 18, 31]
Soy	[22, 33, 45]
isoflavones	[18, 45]
Genistein	[33, 45]
Daidzein	[33, 45]

fat, body mass index and body fat mass significantly affect both LDL levels and protein profile of NAF, suggesting that gene and protein expression of breast epithelial cells bathing NAF may be strongly influenced by the amounts and types of dietary and body fats [24]. A high fat-diet has been linked to elevated levels of circulating estrogens in women [62] and it was hypothesized to account for considerable variation in breast cancer risk among populations. Dietary fat may increase levels of systemic and breast tissue specific oxidative stress through lipid peroxidation pathways, that may produce and accumulate in NAF isoprostanes and prostaglandins, known effectors of breast cancer growth [30, 31]. In addition, a high-fat diet is often associated with an increased amount of adipose tissue, which is now recognized as an endocrine organ capable of producing a variety of bioactive peptides that play roles in inflammation, lipid and hormone metabolism, as well as cell growth and differentiation [6]. The human mammary gland is indeed embedded in adipose tissue and adipocyte-secreted factors have been shown to promote experimental mammary carcinogenesis; despite relatively weak associations between dietary fat and BC risk in observational and epidemiological studies, the results from intervention trials suggested that a low-fat diet may reduce the prevalence of established risk factors for BC [24], such as increased mammographic density and circulating estrogens [27]. In addition, hyperplasia, atypia and ductal carcinoma in situ are more commonly found in mammographically dense human breasts, which is consistent with a higher proportion of epithelial cells and connective tissue relative to fat [27].

Cholesterol is known to be also a carrier of carotenoids in the blood [51], and both cholesterol and protein might be responsible for the NAF viscosity [29]. Since increased blood levels of carotenoids were shown to be protective towards BC risk [53, 59–61], several studies evaluated the presence and the possible antioxidant activity of NAF carotenoids. While it was demonstrated that NAF carotenoid levels and their transport from the blood into the breast microenvironment may be enhanced by lactation [37], no association between BC risk and levels of provitamin A, carotenoid and vitamin E was found [9]. It is well known that some micronutrients can protect lipids from oxidation, and carotenoids also exhibit anticarcinogenic effects through mechanisms such as regulation of gene expression [51]. Starting from the evidence that increased levels of both carotenoids and tocopherols in plasma/serum are associated with decreased BC risk, and that low intake of carotenoids may be associated with increased BC risk [53, 59, 61], a recent study indicated that retinol, total carotenoid and total tocopherol levels were higher in NAF from women who breast-fed a child for more than 6 months versus those who never lactated, evidencing also that in women who had lactated the fat-soluble micronutrient and protein levels declined with time after weaning, while cholesterol levels significantly increased [11]. This study strengthened the hypothesis that high cholesterol levels in breast NAF are associated with increased BC risk, while carotenoids and tocopherols may be protective.

Although the role of nutritional factors in the aetiology of breast cancer has not yet been fully understood, the protective effects of vegetables and fruits have often been ascribed to antioxidant vitamins abundant in these foods, even though it is difficult to distinguish the effects of individual vitamins in observational studies. Several prospective or cohort studies have reported an inverse

association between breast cancer risk and serum/plasma carotenoids, including α- and β-carotenes and lutein [59, 60]. Despite being a relatively minor component among total carotenoids, it is intriguing that lutein showed a significant inverse association [25]. It was speculated that the chemical structure of lutein enables it to act as a more effective membrane antioxidant than β-carotene and lycopene. However, it is also possible that other micronutrients, such as isothiocyanate, lutein and folate (abundantly present in dark-green vegetables), may account for the protective effects in breast tissue [3]. In a study of Kato et al. [25], the association with antioxidant vitamin levels was only observed in plasma but not in NAF, undermining the potential effects of these vitamins on breast epithelial cells. However, it is worth noting that levels of a variety of endogenous and exogenous biochemical components in NAF, such as cholesterol, estrogens, and isoflavone, are not correlated with their blood levels [18, 45]. In fact, when correlations between plasma and NAF levels were examined for cholesterol and antioxidant vitamins at baseline, significant correlations were only observed for α- and β-carotenes, and to a lesser extent for lycopene and cryptoxanthin [25]. Carotenoids can also be oxidized in the lipid-rich environment of the breast, as well as lipid oxidation products can be at very high levels in the breast microenvironment [30, 31]. It was also found that concentrations of 5,6-cyclolycopene-1,5-diol, the oxidation product of lycopene, were much higher in NAF than in plasma, and that measurable lycopene levels in some NAF samples were all in the oxidized form [7].

Recently, it was reported that dietary fibre intake may reduce the probability of yielding NAF enriched of epithelial cells, limiting the breast ductal cell proliferation and its turnover [25]. Likewise, an inverse association between dietary fibre intake and BC risk was observed in some cohorts and case–control studies (reviewed in [34]); dietary fibre has been postulated to inhibit intestinal reabsorption of excreted biliary estrogens, limiting the increased stimulation of NAF prolactin and steroids due to increased dietary fat intake [28]. Such hormonal stimulation of the breast may also affect mammary epithelial cells, because supplementation of foods with compounds displaying weak estrogenic properties (such as the isoflavones genistein and daidzein from soy proteins) was shown to increase not only breast fluid secretion but also the appearance of hyperplastic epithelial cells in NAF, as well as plasma estradiol levels, suggesting that plant isoflavones may act directly on breast epithelial cells lining ducts [33, 45]. On the other hand, it was reported that soy supplementation increases NAF levels of genistein and daidzein, but does not modify breast epithelial cell proliferation, estrogen and progesterone receptor status, apoptosis, and mitosis [22].

An interesting study reports that the consumption of fruits, vegetables and antioxidant micronutrients may have different effects on breast tissue in pre- versus post-menopausal breast cancer patients, suggesting that the estrogen receptorial status may influence the antioxidant properties of several micronutrients [14]. In this respect, a recent comprehensive study evaluates how a change in diet might affect micronutrient levels in the NAF breast microenvironment [10]. An important indication was that carotenoids in NAF increased with a high fruit and vegetable diet regardless of whether dietary fat intake was concomitantly decreased, but surprisingly little correlation between levels of micronutrients in NAF and plasma was found [10]. Dietary carotenoids were more strongly correlated with plasma levels than with adipose tissue levels [60], suggesting that plasma levels likely reflects absorption from the diet to a relatively greater degree, with the subsequent distribution of micronutrients within the body fluids. So, the source of micronutrients in NAF may derive from both the distribution of micronutrients in breast tissue and their accumulation into the extracellular fluids. Moreover, nutrients in breast secretions may be further modified by lipid peroxidation products, since very high concentrations of several isoprostanes and cholesterol oxides were detected in NAF [18, 30, 31]. Oxidative mechanisms may negatively affect the breast cells lining the ducts, making replenishment of antioxidant micronutrients through the diet is a very important factor for maintaining breast tissue physiology and homeostasis. In the Nutrition and Breast Health Study, it was also reported that plasma levels of γ-tocopherol (but not β-tocopherol) decreased significantly in low-fat diet habit [25]. Based on the evidence that tocopherol and retinol levels found in NAF were not targeted by any intervention and did not show significant changes [10, 11, 25], it is possible that the breast tissue stores of tocopherols are less affected by diet than those of plasma. Previous studies indicate that a high fruit–vegetable diet can be useful to increase carotenoids in breast secretions (e.g., NAF and milk), which in turn may be useful for prevention of breast cancer, as increased plasma carotenoid levels have been associated with decreased BC risk [53, 59].

Role of Nutrigenomics in future perspectives

In recent years, significant advances have been made in the understanding of the complex interactions between life style and genotype, and their subsequent effects on health and disease. The increasing awareness of gene–nutrient interactions and the potential of an individual's genetic profile to alter nutrient requirements and responsiveness, as well as to modify the risk of developing diseases, will be

the key to understand the pathology and progression of metabolic and non-metabolic polygenic disorders. The study of such interactions may provide therapeutic alternatives tailored to individuals and based on their genetic background.

Although the impact that variations in NAF composition have on BC risk are not well understood [12], it is likely that an association exists since NAF bathes ductal epithelial cells [30, 31], and most breast cancer originate from such cells. While some nutrients (e.g., lipids, cholesterol, and some soy proteins) may be influenced by diet and metabolized in NAF producing mutagenic/genotoxic actions on the breast epithelium with final detrimental effects [30, 31, 46], others (like carotenoids, vegetable and fruit antioxidants) have been shown to be protective towards BC risk with final anticarcinogenic effects [10]: the balance between these effects may highlight mechanism(s) by which diet can affect breast cancer risk. All these substances, of both exogenous and endogenous origin, may be secreted and accumulated in NAF by the adult female breast. Since a dynamic balance between secretion, reabsorption, and turnover of these substances may be an important determinant of exposure of the breast epithelia to putative anticarcinogenic and carcinogenic agents [29–31, 43], dietary intake of fat, fibre, vegetables and fruits may have a crucial role in increasing or decreasing breast cancer risk through modulation of gene and protein expression, as well as hormone function. Thus, in the future, the most appropriate nutritional regimen should be tailored for each woman to maintain breast tissue health, preventing benign breast diseases and breast cancer. In this respect, Nutrigenomics (i.e., the investigation of how dietary compounds regulate gene and protein expression within the context of individual genotypes) may be of great importance to determine how dietary factors influence the occurrence of the majority of human diseases, including breast cancer [39]. During the last half of the twentieth century, most of the work focused on clarifying the functions of essential nutrients and their roles in cancer metabolic pathways, even though the mechanisms by which nutrients influence carcinogenesis still remain not clear [16, 38]. The tenets of Nutrigenomics are essentially based on the concept that individual susceptibility to nutrients is based on the environment/genotype/phenotype interplay, making this new research field extremely promising, although complex (Fig. 2). Increasing evidence indicates that dietary components are important determinants of cancer risk and tumour behaviour, even though there is considerable scientific uncertainty about the real benefit [36]. Greater attention is being focused to the potential effects of differently formulated diets on genes involved in the pathogenesis of breast cancer (BC), one of the major causes of death world-wide [5]. Thus, moderating environmental factors which we are exposed to over a lifetime, such as diet, might have a strong impact also on BC risk [13, 41].

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References

1. Ahn J, Gammon MD, Santella RM, Gaudet MM, Britton JA, Teitelbaum SL, Terry MB, Neugut AI, Josephy PD, Ambrosone CB (2004) Myeloperoxidase genotype, fruit and vegetable consumption, and breast cancer risk. Cancer Res 64:7634–7639
2. Ahn J, Gammon MD, Santella RM, Gaudet MM, Britton JA, Teitelbaum SL, Terry MB, Nowell S, Davis W, Garza C, Neugut AI, Ambrosone CB (2005) Association between breast cancer risk and the catalase genotype, fruit and vegetable consumption, and supplement use. Am J Epidemiol 162:943–952
3. Ambrosone CB, McCann SE, Freudenheim JL, Marshall JR, Zhang Y, Shields PG (2004) Breast cancer risk in premenopausal women is inversely associated with consumption of broccoli, a source of isothiocyanates, but is not modify by GST genotype. J Nutr 134:1134–1138
4. Bagga D, Ashley JM, Jeffrey S, Wang HJ, Barnard J, Elashoff R, Heber D (1994) Modulation of serum and breast ductal fluid lipids by a very-low-fat, high-fiber diet in premenopausal women. J Natl Cancer Inst 86:1419–1421
5. Bevers TB, Anderson BO, Bonaccio E, Borgen PI, Buys S, Daly MB, Dempsey PJ, Farrar WB, Fleming I, Garber JE, Harris RE, Helvie M, Hoover S, Krontiras H, Shaw S, Singletary E, Sugg Skinner C, Smith ML, Tsangaris TN, Wiley EL, Williams C, National Comprehensive Cancer Network (2006) Breast cancer screening and diagnosis. J Natl Compr Canc Netw 4:480–508
6. Chaldakov GN, Stankulov IS, Hristova M, Ghenew PI (2003) Adipobiology of disease: adipokines and adipokine-targeted pharmacology. Curr Pharm Des 9:1023–1031
7. Chen G, Djuric Z (2002) Detection of 2, 6-cyclolycopene-1, 5-diol in breast nipple aspirate fluids and plasma: a potential marker of oxidative stress. Cancer Epidemiol Biomarkers Prev 11:1592–1596
8. Chotiner HC, Adams HR, Spiekerman AM, Gilliland PF, Newmark SR (1979) Lactose and casein content of nonpuerperal breast secretion. J Reprod Med 22:267–270
9. Covington C, Mitchel-Gieleghem A, Lawson D, Eto I, Grubbs C (2001) Presence of carotenoid, an anticarcinogenic marker, in nipple aspirates postlactation. Adv Exp Med Biol 501:143–152
10. Djuric Z, Chen G, Ren J, Venkatramanamoorthy R, Covington CY, Kucuk O, Heilbrun LK (2007) Effects of high fruit–vegetable and/or low-fat intervention on breast nipple aspirate fluid micronutrient levels. Cancer Epidemiol Biomarkers Prev 16:1393–1399
11. Djuric Z, Visscher DW, Heilbrun LK, Chen G, Atkins M, Covington CY (2005) Influence of lactation history on breast nipple aspirate fluid yields and fluid composition. Breast J 11:92–99
12. Dua RS, Isacke CM, Gui JP (2006) The intraductal approach to breast cancer biomarker discovery. J Clin Oncol 24:1209–1216
13. Duncan AM (2004) The role of nutrition in the prevention of breast cancer. AACN Clin Issues 15:119–135
14. Gaudet MM, Britton JA, Kabat GC, Steck-Scott S, Eng SM, Teitelbaum SL, Terry MB, Neugut AI, Gammon MD (2004)

Fruits, vegetables, and micronutrients in relation to breast cancer modified by menopause and hormone receptor status. Cancer Epidemiol Biomarkers Prev 13:1485–1494
15. Glade MJ (1999) Food, nutrition and the prevention of cancer, a global perspective. Nutrition 15:523–526
16. Gonzales CA, Riboli E (2006) Diet and cancer prevention: where we are, where we are going. Nutr Cancer 56:225–231
17. Gruenke LD, Craig JC, Petrakis NL, Lyon MB (1987) Analysis of cholesterol, cholesterol-5, 6-epoxides and cholestane-3 beta, 5 alpha, 6 beta-thiol in nipple aspirates of human breast fluid by gas chromatography/mass spectrometry. Biomed Environ Mass Spectrom 14:335–338
18. Gruenke LD, Wrensch MR, Petrakis NL, Miike R, Ernster VL, Craig JC (1987) Breast fluid cholesterol and cholesterol epoxides: relationship to breast cancer risk factors and other characteristics. Cancer Res 47:5483–5487
19. Hagler L, RIJr Coppes, Block M, Hofeldt FD, Herman RG (1975) Clinical implications of lactose-positive breast secretions in nonpuerperal females. Obstet Gynecol 46:302–307
20. Han J, Hankinson SE, De Vivo I (2003) A prospective study of XCCR1 haplotypes and their interaction with plasma carotenoids on breast cancer risk. Cancer Res 63:8536–8541
21. Han J, Hankinson SE, Ranu H, De Vivo I, Hunter DJ (2004) Polymorphisms in DNA double-strand break repair genes and breast cancer risk in the Nurses' Health Study. Carcinogenesis 25:189–195
22. Hargreaves DF, Pottent CS, Harding C, Shaw LE, Morton MS, Roberts SA, Howell A, Bundred NJ (1999) Two-week dietary soy supplementation has an estrogenic effect on normal premenopausal breast. J Clin Endocrinol Metab 84:4017–4024
23. Huang Y, Anderson KE, Nagamani M, Grady JJ, Lu LJW (2008) Dietary intake of lactose as a strong predictor for secretory status of nipple aspirate fluid in healthy premenopausal non-lactating women. Clin Cancer Res 14:1386–1392
24. Huang Y, Nagamani M, Anderson KE, Kurosky A, Haag AM, Grady JJ, Lu LJ (2007) A strong association between body fat mass and protein profile in nipple aspirate fluids of healthy premenopausal non-lactating women. Breast Cancer Res Treat 104:57–66
25. Kato I, Ren J, Visscher DW, Djuric Z (2006) Nutritional predictors for cellular nipple aspirate fluids: nutrition and breast health study. Breast Cancer Res Treat 97:33–39
26. Ingram DM, Nottage E, Roberts T (1991) The role of diet in the development of breast cancer: a case–control study of patients with breast cancer, benign epithelial hyperplasia and fibrocystic disease of the breast. Br J Cancer 64:187–191
27. Lee MM, Petrakis NL, Wrensch MR, King EB, Miike R, Sickles E (1994) Association of abnormal nipple aspirate cytology and mammographic pattern and density. Cancer Epidemiol Biomarkers Prev 3:33–36
28. Lee MM, Wrensch MR, Miike R, Petrakis NL (1992) The association of dietary fat with ability to obtain breast fluid by nipple aspiration. Cancer Epidemiol Biomarkers Prev 1:277–280
29. Malatesta M, Mannello F, Bianchi G, Sebastiani M, Gazzanelli G (2000) Biochemical and ultrastructural features of human milk and nipple aspirate fluids. J Clin Lab Anal 14:330–335
30. Mannello F, Tonti GA, Qin W, Zhu W, Sauter ER (2007) Do nipple aspirate fluid epithelial cells and their morphology predict breast cancer development? Breast Cancer Res Treat 102:125–127
31. Mannello F, Tonti GA, Pagliarani S, Benedetti S, Canestrari F, Zhu W, Qin W, Sauter ER (2007) The 8-epimer of prostaglandin F-2a, a marker of lipid peroxidation and oxidative stress is decreased in the nipple aspirate fluid of women with breast cancer. Int J Cancer 120:1971–1976
32. Mannello F, Fabbri L, Ciandrini E, Tonti GA (2008) Increased levels of erythropoietin in nipple aspirate fluid and in ductal cells from breast cancer patients. Cell Oncol 30:51–61
33. Maskarinec G, Hebshi S, Kuster L, Franke AA (2008) The relation of soy intake and isoflavone levels in nipple aspirate fluid. Eur J Cancer Prev 17:67–70
34. Mattisson I, Wirfalt E, Johanson U, Gullberg B, Olsson H, Berglung G (2004) Intakes of plant foods, fibre and fat and risk of breast cancer—prospective study in the Malmo Diet and Cancer cohort. Br J Cancer 90:122–127
35. Michels KB, Mohllajee AP, Roset-Bahmanyar E, Beehler GP, Moysich KB (2007) Diet and breast cancer. Cancer 109:2712–2749
36. Milner JA (2006) Diet and cancer: facts and controversies. Nutr Cancer 56:216–224
37. Nantais-Smith LM, Covington CY, Nordstrom-Klee BA, Grubbs CJ, Eto I, Lawson DM, Pieper BA, Northouse LL (2001) Differences in plasma and nipple aspirate carotenoid by lactation status. Nurs Res 50:172–177
38. Nowell SA, Ahn J, Ambrosone CB (2004) Gene-nutrient interactions in cancer etiology. Nutr Rev 62:427–438
39. Ordovas JM, Mooser V (2004) Nutrigenomics and nutrigenetics. Curr Opin Lipidol 15:101–108
40. Paone JF, Waalkes TP, Baker RR, Shaper JH (1980) Serum UDP-galactosyl transferase as a potential biomarker for breast carcinoma. J Surg Oncol 15:59–66
41. Parodi PW (2005) Dairy product consumption and the risk of breast cancer. J Am Coll Nutr 24:556–568
42. Petrakis NL (1986) Physiologic, biochemical and cytologic aspects of nipple aspirate fluid. Breast Cancer Res Treat 8:7–19
43. Petrakis NL (1993) Nipple aspirate fluid in epidemiologic studies of breast diseases. Epidemiol Rev 15:188–195
44. Petrakis NL (1993) Studies on the epidemiology and natural history of benign breast disease and breast cancer using nipple aspirate fluid. Cancer Epidemiol Biomarker Prev 3:3–10
45. Petrakis NL, Barnes S, King EB, Lowenstein J, Wiencke J, Lee MM, Miike R, Kirk M, Coward L (1996) Stimulatory influence of soy protein isolate on breast secretion in pre- and post-menopausal women. Cancer Epidemiol Biomarkers Prev 5:785–794
46. Petrakis NL, Gruenke LD, Craig JC (1981) Cholesterol and cholesterol epoxides in nipple aspirates of human breast fluids. Cancer Res 41:2563–2565
47. Petrakis NL, Lee RE, Miike R, Dupuy ME, Morris M (1988) Coloration of breast fluid related to the concentrations of cholesterol, cholesterol epoxides, estrogen, and lipid peroxides. Am J Clin Pathol 89:117–120
48. Petrakis NL, Limm ML, Miike R, Lee RE, Morris M, Lee L, Mason L (1989) Nipple aspirate fluids in adult nonlactating women—lactose content, cationic Na^+, K^+, Na^+/K^+ ratio, and coloration. Breast Cancer Res Treat 13:71–78
49. Petrakis NL, Mason ML, Doherty M, Dupuy ME, Sadee G, Wilson CS (1977) Effects of altering diet fat on breast fluid lipids in women. Fed Proc 36:1167
50. Petrakis NL, Miike R, King EB, Lee L, Mason L, Chang-Lee B (1988) Association of breast fluid coloration with age, ethnicity, and cigarette smoking. Breast Cancer Res Treat 11:255–262
51. Rock CL (1997) Carotenoids: biology and treatment. Pharmacol Ther 75:185–197
52. Rohan T, Jain M, Miller AB (1998) A case-cohort study of diet and risk of benign proliferative epithelial disorders of the breast. Cancer Causes Control 9:19–27
53. Sato R, Helzlsouer KJ, Alberg AJ, Hoffman SC, Norkus EP, Comstock GW (2002) Prospective study of carotenoids, tocopherols and retinoid concentrations and the risk of breast cancer. Cancer Epidemiol Biomarker Prev 11:451–457

54. Sauter ER (2005) Analysis of nipple aspirate fluid for diagnosis of breast cancer: an alternative to invasive biopsy. Expert Rev Mol Diagn 5:873–881
55. Sauter ER, Garofalo C, Hewett J, Hewett JE, Morelli C, Surmacz E (2004) Leptin expression in breast nipple aspirate fluid (NAF) and serum is influenced by body mass index (BMI) but not by the presence of breast cancer. Horm Metab Res 36:336–340
56. Sauter ER, Ross E, Daly M (1997) Nipple aspirate fluid: a promising non-invasive method to identify cellular markers of breast cancer risk. Br J Cancer 76:494–501
57. Sauter ER, Winn JN, Dale PS, Wagner-Mann C (2006) Nipple aspirate fluid color is associated with breast cancer. Cancer Detect Prev 30:322–328
58. Tamimi RM, Hankinson SE, Spiegelman D, Colditz GA, Hunter DJ (2004) Manganese superoxide dismutase polymorphysm, plasma antioxidant, cigarette smoking, and risk of breast cancer. Cancer Epidemiol Biomarkers Prev 13:989–996
59. Tamimi RM, Hankinson SE, Campos H, Spiegelman D, Zhang S, Colditz GA, Willett WC, Hunter DJ (2005) Plasma carotenoids, retinol, and tocopherols and risk of breast cancer. Am J Epidemiol 161:153–160
60. Toniolo P, Van Kappel AL, Akhmedkhanov A, Ferrari P, Kato I, Shore RE, Riboli E (2001) Serum carotenoids and breast cancer. Am J Epidemiol 153:1142–1147
61. van der Hel OL, Peeters PH, Hein DW (2004) GSTM1 null genotype red meat consumption and breast cancer risk. Cancer Causes Control 15:295–303
62. Woods MN, Barnett JB, Spiegelman D, Trail N, Hertzmark E, Longcope C, Gorbak SL (1996) Hormone levels during dietary changes in premenopausal African–American women. J Natl Cancer Inst 88:1369–1374
63. Wrensch MR, Petrakis NL, Gruenke LD, Ernster VL, Miike R, King EB, Hauck WW (1990) Factors associated with obtaining nipple aspirate fluid: analysis of 1428 women and literature review. Breast Cancer Res Treat 15:39–51
64. Wynder EL, Hill P, Laakso K, Littner R, Kettunen K (1981) Breast secretion in Finnish women: a metabolic epidemiologic study. Cancer 47:1444–1450
65. Yuan JM, Koh WP, Sun CL, Lee HP, Yu MC (2005) Green tea intake, ACE gene polymorphism and breast cancer risk among Chinese women in Singapore. Carcinogenesis 26:1389–1394