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In utero and lactational exposure to blueberry via maternal diet promotes mammary epithelial differentiation in prepubescent female rats

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Abstract

Early developmental events influence the fine tuning of later susceptibility to adult diseases. Diet is a determinant of breast cancer risk, and our previous studies showed that diet-mediated changes in transcriptional programs promote early mammary gland differentiation. Although consumption of fruits is considered to elicit multiple health benefits, little is known on whether associated bioactive components modify the early differentiation program in developing mammary glands. Here, we evaluated the hypothesis that early exposure (in utero and lactational) to blueberry through maternal diet enhances mammary epithelial differentiation in female offspring. Pregnant Sprague-Dawley rats beginning at gestation day 4 were fed American Institute of Nutrition–based diets containing casein and whole blueberry powders added to casein at 2.5%, 5.0%, and 10% weight/weight. Female pups at weaning were evaluated for growth and mammary tissue parameters. Blueberry at 5% dose increased body and adipose fat weights, relative to the other diets. Mammary branch density and terminal end bud size were highest for the 5% blueberry group, whereas terminal end bud numbers were not affected by all diets. Mammary ductal epithelial cells of the 5% blueberry group had lower nuclear phosphorylated histone 3 and higher nuclear tumor suppressor phosphatase and tensin homolog deleted in chromosome 10 (PTEN) levels than the casein group. Although sera of both diet groups had similar antioxidant capacity, 5% blueberry sera elicited higher nuclear PTEN accumulation in human MCF-10A mammary epithelial cells. Our studies identify developing mammary glands as early targets of blueberry-associated bioactive components, possibly through systemic effects on epithelial PTEN signaling.

Keywords: Blueberry; Mammary gland; PTEN; Puberty; Rat
Abbreviations: BB, blueberry; CAS, casein; PND, postnatal day; PTEN, phosphatase and tensin homolog deleted in chromosome 10; TEB, terminal end bud.

1. Introduction

The concept that early life experiences are linked to the development of adult chronic diseases has its roots in the seminal findings of Prof David Barker and colleagues on the association between low birth weight and increased risk for adult type 2 diabetes mellitus [1]. Although emerging evidence suggests that other diseases such as breast cancer are similarly subject to considerable influence by early developmental events [2-5], the mechanisms underlying "developmental plasticity" remain poorly understood. In the case of breast cancer, a leading cause of malignancy among

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women [6], understanding the biological basis of this phenomenon is even more challenging, given the many contributing elements to its multiple histopathologies [7-10]. Among all cancer types, breast cancer is considered to have the greatest chance of detection and effective treatment; however, primary prevention of this disease by adopting healthier lifestyles has gained widespread support. In principle, the protective effects of bioactive components present in foods could result from promotion of the differentiation of the highly proliferative terminal end buds (TEBs) of the mammary gland, whose numbers are maximal at prepuberty and increasingly decrease and disappear thereafter in adult tissues [11]. Terminal end buds are presumed to contain the stem cell population [12] from which epithelial tumors originate, when these cells undergo overexpansion [13-15]. Nevertheless, other mammary compartments constitute potential targets of dietary factors as well [16].

Consumption of fruits as part of the regular diet is considered good nutritional practice, with health advocates pushing for 5 to 9 servings of fruits and vegetables a day. Although solid scientific evidence for a linkage between breast health and high intake of fruit is lacking, one case-controlled study [17] and one prospective study [18] in women provided data in support of an association of high fruit (and vegetable) intake with reduction in breast cancer risk. Among fruits, berries are valued for their oncoprotective activities, based largely on animal and human tumor cell–based studies [19-23]. Although these antitumor activities can be partly attributed to the high anthocyanin and polyphenol content of berries [24] that display high oxygen radical absorbance capacity to reduce cellular DNA damage, there are likely additional yet unknown underlying mechanisms.

The dual (protein/lipid) phosphatase, phosphatase and tensin homolog deleted in chromosome 10 (PTEN) is the second most frequently mutated tumor suppressor gene in human cancers [25-27]. PTEN dephosphorylates phosphatidylinositol 3,4,5-triphosphate to negatively regulate the PI3-kinase/Akt pathway [28]. Diminished PTEN function leads to increased proliferation and reduced apoptosis, both hallmarks of mammary tumorigenesis, and primary ductal adenocarcinomas of the breast display loss of PTEN expression [26,27]. Recent findings suggest a nuclear function for PTEN in tumor suppression and chromosomal stability [29]. The reported positive association between PTEN dosage and tumor phenotype in humans suggests that reactivation of PTEN expression may have important clinical utility [30].

The rat mammary gland constitutes a highly relevant model for studying the biological actions of dietary compounds, given its many similarities to human breast in structure, function, and development [31,32]. Ductal and lobuloalveolar development in the rat mammary gland occurs extensively at peripuberty, similar to human females. In the present study, we evaluated the hypothesis that early exposure (in utero through lactation) to blueberry via maternal diet enhances mammary epithelial differentiation in female rat offspring. To address this, we examined the effects of early exposure to dietary intake of whole blueberry (BB) by pregnant and lactating dams on mammary architecture and differentiation marker PTEN expression in prepubertal female rats at weaning. We also determined whether systemic changes due to early exposure to dietary BB underlie enhanced mammary epithelial differentiation, involving nuclear PTEN accumulation. Our findings provide the first evidence of the early influence of maternal BB diet on mammary gland development of offspring and suggest that breast health in women may be similarly enhanced by increased fruit intake by pregnant and lactating mothers.

2. Methods and materials

2.1. Rats, diets, and tissue collection

Animal care and handling followed protocols approved by the Institutional Animal Care and Use Committee of the University of Arkansas for Medical Sciences. Time impregnated Sprague-Dawley rats (Charles River Laboratories, Inc, Wilmington, Mass) were maintained in the animal facility of the Arkansas Children's Nutrition Center in a temperature- and humidity-controlled room. Pregnant females beginning at gestation day 4 were randomly assigned to the American Institute of Nutrition-93G–based pelleted diet containing casein (CON) as the major protein source to which was added freeze-dried powders of blueberry (BB) at 2.5%, 5%, and 10% by weight of feed. Freeze-dried BB powders were provided by FutureCeuticals Inc (Momence, Ill). Diet with added BB was formulated to be isoenergetic and isonitrogenous by adjusting the amounts of casein, corn starch, and cellulose fiber (Table 1). The chemical composition of the whole BB powder is presented in Table 2. All rats were provided food and water ad libitum. The amount of food

Table 1
Ingredient composition of the diets

Component (g/Kg)	CON	2.5% BB	5% BB	10% BB
Casein	200.0	200.0	198.2	196.3
L-Cystine	3.0	3.0	3.0	3.0
Corn starch	397.5	372.5	354.0	310.3
Maltodextrin	132.0	132.0	132.0	132.0
Sucrose	100.0	100.0	100.0	100.0
Corn oil	70.0	69.8	69.5	69.0
Cellulose	50.0	50.0	45.8	41.9
Mineral mix, AIN-93G-MX (TD 94046)	35.0	35.0	35.0	35.0
Vitamin mix, AIN-93G-VX (TD 94047)	10.0	10.0	10.0	10.0
Choline bitartrate	2.5	2.5	2.5	2.5
TBHQ (antioxidant)	0.014	0.014	0.014	0.014
Blueberries	–	25	50	100

AIN indicates American Institute of Nutrition.