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# Persistent organic pollutants & obesity: potential mechanisms for breast cancer promotion?

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## Abstract

Dietary ingestion of persistent organic pollutants (POPs) correlates with developing obesity. Obesity alters metabolism, induces an inflammatory tissue microenvironment, and is also linked with diabetes and breast cancer risk/promotion of the disease. However, no direct evidence exists exploring the correlation among all three of these factors (POPs, obesity, and breast cancer). Herein, we present current correlative studies suggesting a causal link between POPs exposure through diet and their bioaccumulation in adipose that promotes the development of obesity and ultimately influences breast cancer development and/or progression. Furthermore, as endocrine disruptors, POPs can potentially interfere with hormonally responsive tissue functions causing dysregulation in hormone signaling and cell function. This review highlights the critical need for advanced *in vitro* and *in vivo* model systems to understand the complex relationship between obesity, POPs, breast cancer, and, more importantly, to delineate their multifaceted molecular, cellular, and biochemical mechanisms. Comprehensive *in vitro* and *in vivo* studies directly testing the observed correlations as well as detailing their molecular mechanisms are vital to cancer research and, ultimately, public health.

### Keywords

Breast Cancer; Persistent Organic Pollutants; Obesity; Metabolism

## Introduction

Persistent Organic Pollutants (POPs), a group of chemicals such as organochlorine pesticides (OCP) and polychlorinated biphenyls (PCBs), are ubiquitous pollutants found globally. These chemicals have common properties of persistence including lipophilicity, toxicity, and bioaccumulation (Lee, et al. 2010; Woodwell 1967). Due to their high

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**Conflict of Interest**

The authors declare that they have no conflict of interest.

lipophilic properties, POPs (organo-hydrocarbons with chlorine, fluoride and bromine groups) are most notably resistant to degradation in both humans and the environment, thus proving to be a bigger threat to global health (Yu, et al. 2011). With their half-lives varying from months to decades due to their high lipid solubility that resist biological, photolytic and chemical degradation, POPs are also semi-volatile allowing them to evaporate into the atmosphere and deposit back to the earth in precipitates (Thundiyil, et al. 2007). The Stockholm Convention on POPs was adopted on May 22nd, 2001 and entered into force on May 17th 2004 with the goal of managing, cleaning and eliminating the production of POPs in the environment. In the US, production and distribution of many POPs have been banned under the Safe Chemicals Act of 2011. However, according to the Environmental Protection Agency and other regulatory sources, chronic POPs exposure continues due to contamination in the food chain, both in artificial and natural environments, and their continued use in under-developed countries with little to no regulation (La Merrill, et al. 2013; Lee et al. 2010; Ljunggren, et al. 2014; Noyes, et al. 2009; Ritter, et al. 2002; Weber, et al. 2008). POPs accumulation in adipose tissues has been proposed as the likely stimuli for their pathological manifestations. While non-lipophilic POPs are not the focus of this review, it is important to note that compounds such as perfluorooctane sulfonate and perfluorooctanoic acid, commonly used in industry as flame-retardants and fabric protectors, do not accumulate in fatty tissue but bioaccumulate due to their extremely stable carbon–fluorine bonds. These POPs have been shown to bind to proteins ultimately interfering with their function (Corsini, et al. 2014; DeWitt, et al. 2012; Domingo 2012; Jones, et al. 2003). Exposure to any type of POPs has the potential for long-term disruption of metabolic, immune, and endocrine system functions. Consequently, POPs are gaining research and public health attention due to their strong link to type 2 diabetes, metabolic syndrome, and cancer, each of which are intimately linked with obesity (Lee et al. 2010; Park, et al. 2014b; Prieto-Hontoria, et al. 2011). Human exposure to POPs occurs through consumption of seafood and livestock and because POPs are water insoluble and not easily metabolized, their levels accumulate in adipose tissue (Lee et al. 2010; Woodwell 1967).

As endocrine disruptors, POPs interfere with hormonally responsive tissue functions via dysregulation in hormone signaling and cell function (Lara, et al. 2012; Meeker 2012; Pombo and Castro-Feijoo 2005; Rylander, et al. 2014; Solomon and Schettler 2000). Specific to this review, dysregulation of estrogen, progesterone, and prolactin, important breast development hormones, can cause a multitude of growth dysfunctions including fibrocystic breast disease and/or breast cancer (Hovey, et al. 2002; Vonderhaar 1988). Breast development is unique compared to other major organs as the majority of development occurs after birth. Rudimentary epithelial and stromal growth is isometric until the onset of puberty, whereupon allometric growth of the epithelial ducts and supportive stromal tissues occur (Hassiotou and Geddes 2013). Post-puberty, breast tissue continues to proliferate and regress in response to hormonal-stimuli during each menstruation cycle. Depending on the woman's life choices, subsequent key developmental stages may include pregnancy- and lactation- and post-lactation associated remodeling. Ultimately, every woman experiences post-menopausal involution, initiated via declining ovarian function and subsequent decreased circulating estrogen and progesterone. Atrophy and regression of glandular epithelium with a concurrent increase in adipose and a reduced elasticity of the supporting

connective tissue are observed (Hassiotou and Geddes 2013). In sum, breast tissue is highly dynamic throughout a woman's life, and during any of these unique stages, a disruption in the delicate balance of growth factor and hormonal signaling between the stroma and epithelium has the potential to promote disease.

The global obesity epidemic compounds any predisposition to breast disease due to changes in hormone and growth factor signaling. Obesity and metabolic syndrome promotes an inflammatory microenvironment, thereby disrupting tissue homeostasis (Park et al. 2014b). Inflammatory microenvironment is known to promote aggressive breast cancer cell behaviors and affect tumor subtype, ultimately leading to poor prognosis and decreased survival (Casbas-Hernandez, et al. 2014; Ham and Moon 2013; Nguyen, et al. 2013; Rose and Vona-Davis 2014; Sun, et al. 2012). Consumption and bioaccumulation of POPs in adipose enhances the obesity-driven dysregulation of cell function, as POPs accumulation is hypothesized to promote obesity (Lee et al. 2010; Woodwell 1967). Thus, the potential exists for POPs and obesity-related factors to work together in a self-reinforcing mode to magnify the effects of endocrine disruption and altered cell signaling, thereby increasing the risk of developing breast cancer or existing tumors progressing into more aggressive subtypes. Herein, we highlight the potential interaction between POPs accumulation, development of obesity, and breast cancer.

## Adipose Tissue and POPs Localization

Each adipose tissue depot (serum, subcutaneous, visceral, and inter-muscular) has differing endocrine, metabolic, and cell signaling functions (Bloor and Symonds 2014; Gilsanz, et al. 2013; La Merrill et al. 2013; Rosenwald and Wolfrum 2014). Women are unique in that they possess an additional, highly dynamic and functional source of adipose in their breast tissue. Within each of these adipose depots resides a multitude of different cell subtypes. White adipocyte tissue (WAT), found mainly around the waist and thighs, have a single lipid droplet and function as energy stores that secret various hormones with endocrine and immune functions. Residing in WAT are brown adipocytes derived from precursor cells differing from classical brown adipocytes that are closer to the white adipocyte cell lineage termed "brite adipocytes." These brite cells appear at anatomical sites corresponding to WAT after thermogenic stimuli (Giralt and Villarroya 2013). Conversely, brown adipocyte tissue (BAT) is primarily involved in body temperature maintenance (non-shivering thermogenesis) and body adiposity. In adult humans, BAT is mainly found around the cervical-supraclavicular area, contains many small lipid droplets, a high number of mitochondria, higher oxygen consumption, high capillary perfusion and sympathetic nervous system innervation (Bloor and Symonds 2014; Gilsanz et al. 2013; Rosenwald and Wolfrum 2014; Seale, et al. 2009; Seale and Lazar 2009). Lipid droplet proteins have been shown to regulate BAT metabolism in adult mice while the role of human BAT metabolism, as well as their overall function, needs further analyses to fully appreciate their physiological roles (Giralt and Villarroya 2013; Seale et al. 2009; Seale and Lazar 2009). Of note, poor BAT activity in humans correlates with ageing, BMI and measures of metabolic disease, and the study of adipocyte transdifferentiation mechanisms may lead to therapeutic strategies against metabolic syndrome and cellular metabolic deregulation observed in breast

cancer (Giordano, et al. 2014; Giralt and Villarroya 2013; Seale et al. 2009; Seale and Lazar 2009).

In women, a large source of WAT can be found in breast tissue. Adipose tissue comprises the majority of breast volume and is altered due to puberty, pregnancy, lactation, and menopause (Hassiotou and Geddes 2013). During puberty, periductal connective tissue is generated in breast adipose tissue with thickening and elongation of the ductal system. In pregnancy and lactation, active glandular tissue doubles in relation to adipose tissue but reverts once lactation ceases. Starting around age 40, ductal and lobular breast tissue begins to atrophy with involution of glandular tissue that is replaced by fat and connective tissue. Both systemic and local growth factors direct this continual remodeling of the gland. Breast adipocyte secretion of adipokines, cytokines, and hormones such as estrogen, has been linked to numerous biological processes including cell proliferation, communication, immune response, apoptosis, and cell metabolism (Park et al. 2014b). Estrogens also regulate adipose deposition, adipogenesis and adipocyte differentiation. Any deregulation in the production or distribution of estrogens can potentially lead to disease or breast cancer (Park, et al. 2014a; Planey, et al. 2014). Moreover, it has been shown that adipocytes can be manipulated by cancer cells to promote tumor invasion, characterized by adipocyte hypertrophy and the accumulation of neutrophils and macrophages in adipose tissue, creating an imbalance and poor prognosis for cancer patients (Park et al. 2014b; Prieto-Hontoria et al. 2011).

Adipose tissue contains high levels of aromatase, an enzyme that converts androgens to estrogen (Simpson 2003). In obesity, estrogen levels have been shown to be increased in postmenopausal women but decreased in premenopausal women (De Pergola and Silvestris 2013; Suba 2013). These obesity driven changes in estrogen regulation are suggested to play a role in the complex relationship to premenopausal and postmenopausal breast cancer, and the clinical behavior of the disease. A meta-analysis from seven cohort studies reported a direct link between obesity and an elevated risk of breast cancer in postmenopausal women, with the inverse being true in premenopausal women (van den Brandt, et al. 2000). However, obesity is correlated with advanced disease at diagnosis and poor prognosis at any age (Stephenson and Rose 2003; van den Brandt et al. 2000). Furthermore, excessive weight gain during pregnancy, fetal exposure to high fat diet, high birth weight, and postnatal exposure to high fat diets during lactation have each been shown to increase breast cancer susceptibility and development in adults in carcinogen-induced rat model studies (De Assis and Hilakivi-Clarke 2006; de Oliveira Andrade, et al. 2014; Hilakivi-Clarke and de Assis 2006; Hilakivi-Clarke, et al. 2005; Hilakivi-Clarke, et al. 2004). Collectively these data suggest that in addition to increased risk of cancer later in life, childhood obesity and obesity-related intrauterine factors may ultimately increase breast cancer risk in women.

A recent study focused on understanding the effects on POPs exposure on adipose tissue demonstrated exposure of human preadipocytes to PCB126 affected their ability to differentiate into mature adipocytes. PCB126 stimulated intrinsic changes in cell function by decreasing transcript levels of a key regulator of adipogenesis, PPARγ (Gadupudi, et al. 2014). The ability of preadipocytes to mature is integral to development and homeostasis. POP exposure compromising the ability of preadipocytes to differentiate in children and

adolescents has the potential to alter their ability to form normal adipose tissue, ultimately leading to dysfunction and disease. In adults, chronic POPs exposure may alter their ability to replace mature adipocytes caused by cell death, again potentially leading to dysfunctional adipose tissue (Gadupudi et al. 2014).

Two well-studied sources of adipose tissue are visceral and subcutaneous, each having unique physiological and clinical features. In a study investigating POPs content of these sources, Korean patients ($n$ = 50) undergoing surgery for gallbladder or liver lesions had samples isolated and tested for a set of POPs congeners (Kim, et al. 2014). POPs accumulation were correlated with both sources of adipose. However, researchers found five to 10 times higher absolute concentration of PCB congeners in visceral (VAT) versus subcutaneous adipose tissue (SAT). A pattern also emerged in patients with diabetes, showing a set of OCPs and PCB congeners significantly correlated with VAT (Kim et al. 2014). The authors propose that these correlations may be due to biological properties of the VAT adipocytes as these cells have enhanced sensitivity to lipolysis, are more metabolically active, and have increased insulin resistance compared to SAT. Given the emerging complex biological roles of adipose, it is important to ascertain whether POPs distribute equally throughout all adipose sources in the body or are preferentially localized.

In a parallel study, Yu and colleagues analyzed ten PCB congeners and OCPs in serum levels of both lean and obese subjects. Serum samples, visceral and subcutaneous adipose biopsies were taken from subjects during laparotomy and evaluated. Overall, higher levels of OCPs were found in VAT while PCBs accumulated more readily in SAT (Yu et al. 2011). Variations were contributed to exposure level, BMI, and genetic differences of the individual highlighting the fact that POPs-containing food sources vary from each geographical region, and within ethnicities. This study is very limited (n=7) with only one woman included in this report, and despite gender-specific adipose distribution, limited studies have directly observed gender differences with respect to POPs accumulation. A recent, more comprehensive study evaluated the accumulation of 13 types of POPs and in VAT and SAT from Portuguese obese (>35 BMI) bariatric surgery patients (n=189) of which 166 were females (Pestana, et al. 2014). While gender and breast adipose was also not specifically studied in this report, the data confirm those found in the Kim and Yu *et al.* studies. Pestana and colleagues show POPs were prevalent in this obese population (96.3% of detection on both tissues), their abundance increased with age and duration of obesity. An increase in POPs deposition in VAT was observed, a positive correlation between POP levels and the presence of metabolic syndrome, and a relation of higher POP levels with lower weight loss in older patients (Pestana et al. 2014).

While none of these studies focus on gender differences, women tend to have overall higher adipose levels, with the majority localized to the hips and thighs (Karastergiou, et al. 2012) and a significant amount in breast tissue, while men tend to exhibit a preferential accumulation of abdominal adipose. Limited information exists on breast adipose tissue and POPs accumulation. Three methods papers have directly demonstrated POPs accumulation in breast adipose tissue; however, no analysis on health or etiology of disease was performed. The first report validated the use of chromatography-time-of-flight mass spectrometry (GC-TOF MS) for screening anthropogenic organic contaminants in 40 human

breast adipose tissues show that both target and non-target approaches detected pollutants including *p,p′*-DDE, hexachlorobenzene (HCB), and several PCBs, polyaromatic hydrocarbons (PAHs), 5-di-*tert*-butyl-4-hydroxy-toluene (BHT) and its metabolite 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde (BHT-CHO), and dibenzylamine, *N*-butyl benzenesulfonamide (*N*-BBSA) (Hernandez, et al. 2009). The second report by the same authors used a multiresidue method for the quantification and confirmation of 30 organohalogenated compounds in human breast tissue samples (Medina, et al. 2009). Lastly, the presence of prevalent dioxins, furans, PCBs, OCPs, and brominated diphenyl ethers in both breast and abdominal adipose tissues of 21 women were reported. These results show that, with some notable exceptions, measurements in breast and abdominal adipose tissues were correlated and that concentrations of POPs in one tissue could be derived from measurements in the other tissue (Petreas, et al. 2004). Again, no functional studies on the effects of these POPs on cell behavior were performed, however these data highlight the accumulation of the potentially disruptive POPs in the breast microenvironment.

In support of lipid-specific accumulation, the levels of PCBs uptake were directly correlated with triglyceride levels in adipocyte lipid droplets (Bourez, et al. 2012; Bourez, et al. 2013). Lipid droplets are organelles composed of a phospholipid monolayer and a lipid ester core that occupy a large portion of cell volume in many cells including mammalian, plant, and microorganisms. Obesity, characterized by excessive adipocytes, is essentially a disease of lipid droplet excess (Walther and Farese 2009). To identify rate of absorption and localization of PCBs into cells, Bourez *et al.* assayed the well-characterized 3T3-L1 murine cell model system for adipogenesis, as well as primary murine embryonic adipocytes by exposing them to a cocktail of PCB congeners. After 90 minutes of incubation, the majority of all congeners were recovered inside the differentiated adipocytes, and not the control pre-differentiated fibroblasts. After 24 hours, the intracellular PCB accumulation was almost exclusively recovered in the high triglyceride-containing lipid droplet fraction of the adipocytes compared to the membrane fraction (Bourez et al. 2012), indicating that PCB accumulation in adipocytes is dependent on the triglyceride content of the adipose, and highlighting the potential for greater risk in accumulation of POPs in obese individuals. A limitation of this study is the use of murine cells and cell lines. It is unknown if human pre-adipocytes would have a comparable cellular differentiation process in the presence of the PCB congeners, or if human adipocytes would display similar uptake patterns of the congeners used in the study.

Complement to these studies, Gutleb and colleagues developed a method to visualize cellular localization and semi-quantitatively calculate concentrations of halogenated POPs via secondary ion mass spectrometry (Gutleb, et al. 2012). Using a human adrenocortical carcinoma and a rat pituitary tumor cell line, bromine-containing POP tetrabromobisphenol A (TBBPA; commonly used flame retardant) and the fluorine POP perfluorooctane sulfonate (PFOS; a fabric protector) were localized to the lipid bilayer of cell membranes (Gutleb et al. 2012). Again, a limitation to this study is the lack of normal/non-transformed human cells and tissues to observe POPs incorporation and localization. It is unknown if the effects of POPs localization in tumor cells would be applicable to normal human epithelial cells, given the well-documented altered cellular metabolism and membrane lipid content in

cancer cells (Baumann, et al. 2013; Ibarguren, et al. 2014; Siddiqui, et al. 2007; Zaidi, et al. 2013). To date, no additional studies have investigated such detailed molecular localization of POPs in human tissues and/or cell culture model systems.

This would be of particular interest in breast tissue, as the specific mechanisms of fatty acid uptake in breast epithelium during various human developmental stages (puberty, pregnancy, lactation, involution) have not been as well identified compared to rodents and livestock (Barber, et al. 1997; McManaman 2014). Lipophilic POPs would be hypothesized to be potentially endocytosed by cells via LPL and CD36, two proposed mediators of breast lipid endocytosis (Kuemmerle, et al. 2011; McManaman 2014). The significant increase in epithelial cell lipid uptake during lactation to produce milk proteins would suggest a potential route of transmission of POPs from mother to the developing child.

In a developmental study on prenatal exposure to POPs, Valvi *et al.* (2014) collected information on pregnant women and their infants (n=1285) in a population-based birth cohort study. POP concentrations (DDT, DDE, HCB, β-hexachlorocyclohexane, and the PCB congeners 28, 52, 101, 118, 138, 153, and 180) were measured in serum collected from the pregnant women in the $7^{th}$ and $26^{th}$ week of pregnancy (Valvi, et al. 2014). The infant model included, age, sex, gestational age, breast feeding duration, and several maternal characteristics. The researchers found that prenatal exposure to DDE and HCB was associated with rapid growth in the first 6 months and overweight infants at 14 months. This suggests a direct link of childhood obesity and prenatal exposure to POPs. Interestingly, prenatal growth was not influenced by PCB exposure (Valvi et al. 2014) suggesting different mechanisms for various POPs. Human exposure to POPs is unavoidable, however to understand tissue-specific differences in POPs metabolism, as well as cell-type specific reactions and localization, further studies are needed.

Studies investigating the mechanisms of POPs cellular uptake and localization in breast cells (epithelium vs. adipose) would further advance our understanding of breast and lactation physiology as well as cellular lipid metabolism. Moreover, detailing the alterations in POPs uptake and localization in cancer cells and their tumor microenvironment, compared to healthy breast epithelium and supportive stroma, may provide further insight into the transition of cellular lipid metabolism and deregulation of cellular respiration during tumorigenesis.

## Obesity and POPs

Adipose tissue is classified as a metabolically active endocrine organ and obesity-induced dysregulation stimulates an enhanced inflammatory tissue microenvironment, including altered secretion of chemokines/cytokines (monocyte chemotactic protein 1, interleukin-6 and TNF-alpha) and adipokines (haptogolin, PAI, leptin, resistan, visfatin, adiponectin and VEGF) (Myre and Imbeault 2014; Prieto-Hontoria et al.). Obesity-stimulated production of these factors is directly correlated to conditions such as chronic inflammation, cardiovascular disease, atherosclerosis, cancer (including breast cancer), and lipotoxicity (Prieto-Hontoria et al. 2011). Moreover, emerging research suggests a strong relationship between POPs, obesity, and the development and/or progression of disease (Bourez et al.

2012; Bourez et al. 2013; La Merrill et al. 2013; Ljunggren et al. 2014; Magliano, et al. 2014; Myre and Imbeault 2014; Pereira-Fernandes, et al. 2014; Taylor, et al. 2013). The "obesogen hypothesis" postulates that chemical pollutants (obesogens) promote obesity by disrupting appetite controls and promoting adipocyte hypertrophy and hyperplasia, thereby causing weight gain (Baillie-Hamilton 2002; Decherf and Demeneix 2011; Grun and Blumberg 2006; Pereira-Fernandes et al. 2014). The link between dysregulated adipose tissue in lipid metabolism, cancer, cardiovascular disease and diabetes has been extensively researched (Matsuda and Shimomura 2013; Prieto-Hontoria et al. 2011; Schwab, et al. 2014). The molecular mechanisms of POPs signaling in driving the development or progression of these diseases remain to be fully understood.

Dirinck and colleagues assessed the association between serum POPs levels and the prevalence of obesity in a cross-sectional study of 98 obese and 47 lean adults (Dirinck, et al. 2011). BMI, glucose tolerance, adipose storage deposits, and measurement of waist and hip circumference were recorded, and serum levels were analyzed for lipophilic PCB congeners 153, 138, 180, and 170, and for the hydrophilic OCPs: dichloro-diphenyl-dichloroethylene (pp-DDE) and β-hexachlorocyclohexane (βHCH). Results show as BMI increased, the serum levels of highly lipophilic congeners decreased. The converse was true for the less lipophilic congeners (Dirinck et al. 2011). The authors suggest that the lipophilic POPs are preferably stored in adipose tissue and a higher percentage of body fat will lead to fast and efficient storage, with lower serum levels as a consequence. While not discussed here, this inverse relationship has been observed by others (Agudo, et al. 2009; Wolff, et al. 2005). In the Dirinck study, serum βHCH had a significant positive correlation with BMI in both genders. In females, fat mass percentage and increased waist circumference had a significant negative correlation with all PCBs, while only PCB 180 and 170 had a negative correlation with waist and fat mass percentage in males. When analyzing the abdominal fat distribution in more detail, a significant negative correlation between the PCBs and abdominal fat was almost solely due to subcutaneous fat mass. In contrast, the positive correlation of βHCH in serum was observed regardless of adipose distribution and localization (Dirinck et al. 2011). While no tissue samples were taken to directly measure POPs levels in adipose tissue depots, these data show a correlation of obesity and serum POPs levels and, highlight gender differences with adipose localization and serum POPs levels.

Herein it should be stated that BMI is the most commonly used method to characterize obesity and is routinely applied in estimating body fat (adiposity), yet this method is inaccurate when based solely on weight and height, excluding total body fat. In addition, BMI provides little to no information on overall health status or disease severity, cannot be generalized among different ethnic groups or gender, and is especially inaccurate in individuals with lean body mass (Green and Duffull 2004; WHO 2000; Whyte, et al. 2014). The limitations of BMI as a risk assessment tool are recognized, and there is continuing interest in identifying substitute or complementary indicators linking adiposity and disease risk. For example, visceral adiposity plays a key role in the metabolic disorders associated with obesity, yet the lack of a practical method to assess visceral fat in routine examinations precludes its use as a screening tool for the general population. Developing simple and

reliable methods to assess body fat compartments should be an important priority of obesity research (Caballero 2007).

A common source of lipophilic POPs exposure in humans is the consumption of fish, especially fatty fish such as salmon. In an effort to directly test the accumulation of POPs from dietary consumption and its downstream effects on insulin resistance and metabolism, Ruzzin and colleagues investigated various high fat diets in rats (Ruzzin, et al. 2010). Male rats were fed a high fat diet of crude salmon oil (HFC) containing high levels of POPs or refined salmon oil (HFR) with significantly lower POPs levels. After 28 days, rats fed the HFC had significantly higher concentrations of VAT or abdominal obesity, and elevated levels of diacylglycerol, triacylglycerol and total cholesterol compared to the HFR rats. Moreover, HFC-fed rats developed insulin resistance, impaired lipid and glucose metabolism, and hepatosteatosis, confirming that chronic exposure to POPs severely impairs insulin sensitivity and contributes to abdominal obesity in male rats (Ruzzin et al. 2010). Parallel studies in female rats were not performed, thus the effects of estrogens and potential gender-specific consequences of this study are unknown. In this same study, *in vitro* exposure of adipocytes to a cocktail of POPs showed a significant inhibition of insulin-dependent glucose uptake, providing further evidence of the risk of insulin resistance and metabolic disorders associated with the ingestion of POPs.

POPs have been shown to cause metabolic disruption. The Pestana study focused on assessing the association between POPs and this imbalance. VAT and SAT adipose tissue were collected from obese (>35 BMI) bariatric surgery patients (n=189) of whom 166 were females, and tested them for 13 POPs (Pestana et al. 2014). Higher concentrations of POPs were found in VAT; however, detectable quantities of all compounds were found in virtually all patients. VAT acts as a highly metabolic active tissue with susceptibility to lipolysis and thereby causes POP release (Roos, et al. 2013). Metabolic syndrome was found in 65.8% of the cohorts and in this subset, total level of POPs in both VAT and SAT were higher. Notably the researchers found that the metabolic abnormalities had a correlation with dysglycaemia, high blood pressure, and cardiovascular risk (Pestana et al. 2014). This study highlights the link between obesity and dysregulated adipose tissue due to POPs exposure underlining the need for more research into the mechanisms behind the dysregulation.

## POPs, Obesity, and Breast Cancer

Obesity-driven changes in the normal and cancerous breast microenvironment, alterations in metabolism, and release of signaling molecules such as endocrine, growth, and inflammatory mediators are known to increase breast cancer risk as well as promote breast cancer progression (Harvey, et al. 2011; Nalabolu, et al. 2014; Sundaram, et al. 2013). However, studies investigating a direct mechanistic link between breast cancer, POPs accumulation, and obesity are remarkably lacking despite the strong correlation between POPs, obesity, and disease (Prieto-Hontoria et al. 2011; Taylor et al. 2013; Zeliger 2013a, b).

While a correlation between high levels of POPs and breast cancer suggests that POPs may be a risk factor for breast cancer (Bonefeld-Jorgensen, et al. 2011; Cabaravdic 2006;

Lemaire, et al. 2006; Warner, et al. 2002), a direct relationship and corresponding biochemical mechanisms remain relatively undefined. The majority of associations between POPs and breast cancer risk have been attributed to the estrogenic effects of particular POPs on breast lesions. Basal-like tumors are characterized by lack of ER expression, an expression signature similar to the basal/myoepithelium, and are reported to have characteristics similar to those tumors arising from *BRCA1* germline mutations carriers. They are unresponsive to endocrine-targeted therapies such as tamoxifin and aromatase inhibitors, and are associated with almost twice the risk of death when compared to ER positive tumors (Dunnwald, et al. 2007). Whether POPs affect both ER positive and ER negative breast cancer subtypes needs further investigation. In an attempt to address POPs effects on breast cancer cell proliferation, Aube *et al.* (2011) exposed four breast cancer cell lines to a cocktail of 15 different organochlorine compounds frequently found in the serum of women all around the world. ER positive MCF-7, T47D, and CAMA-1, and the ER negative MDA-MB231 were tested. Results show the non-hormone dependent MDA-MB231 cell line had decreased proliferation in the presence of the OC cocktail, and ER positive T47D cells had no response. Low levels of exposure to the OC cocktail induced significant proliferation in the MCF7 cells, while high levels were inhibitory (Aube, et al. 2011). The OC cocktail also had the capacity to stimulate the proliferation of CAMA-1 cells, however this was only in the presence of exogenous 17β-estradiol and dihydrotestosterone. Clearly more research is needed to understand these results and potential clinical implications. Defining the effects of POPs on the various breast cancer subtypes have never been directly addressed, but are critical to fully understanding whether POPs differentially influence the behavior or promotion of various breast cancer subtypes.

In a comprehensive report focused on estrogen receptor tested 49 POPs for ER alpha and beta (ERα/ERβ) activation or inhibition via transfection of a luciferase-based reporter construct in HeLa cells (Lemaire et al. 2006). Fifteen of the POPs tested stimulated ERα-mediated transcription in a dose-dependent manner, and five were capable of activating ERβ. Antagonistic activities toward ERα and ERβ were also shown in select POPs. Most interestingly, chlordecone and methoxychlor, the most effective antagonist compounds for ERβ, were strong agonists for ERα. These data underscore the complexity of POPs activation of ER signaling. Although ER activation potential of the POPs was documented in reporter assays, the results were not confirmed in breast cancer cells and the effects of these POPs on cell behavior were not investigated (Lemaire et al. 2006). In another study, the effects of the POP DDT and its metabolites on ERα expressing MCF7 and ERα-negative MDA-MB231 cell lines were investigated. Notably, the POPs tested had strong opposing results on the two cell lines, with the invasive potential of the MCF7 cells enhanced while the invasion of MDA-MB-231 cells significantly reduced (Lemaire et al. 2006). While the estrogenic effects of POPs on breast cancer cells are apparent, the POPs induced inflammatory microenvironment and their paracrine effects on ER negative or any breast cancer subtype remain to be defined.

A study released in 2011 focused on the observation that California has some of the highest breast cancer rates in the world, and some of the highest body burden levels of polybrominated diphenyl ethers (PBDE; flame retardants common in plastics, electronics

and building materials), with levels ranging tenfold higher than those reported among European and Asian populations. The authors postulated that the estrogenic effects of PBDE might influence breast cancer risk (Petreas, et al. 2011). Breast biopsies of women from the San Francisco Bay area were analyzed for five major PBDE congeners. Results suggested that elevated BDE-154 correlated with breast cancer risk, but sample size was limited. Overall the data provided no significant evidence of an association between breast tissue concentrations of PBDE and breast cancer risk, though the breast tissue levels of PBDE were the highest ever reported. Study limitations were a significant factor, including that the levels of PBDEs were measured at/near the time of diagnosis and therefore may not be representative of lifetime exposures or exposures during potentially critical windows of susceptibility during earlier life. Second, the control group included women with proliferative benign breast lesions and evidence suggests these women are at increased risk for breast cancer (Aroner, et al. 2010; Collins, et al. 2006; Schnitt 2001). Third, PBDE levels are inversely correlated with age and recruitment resulted in a skewed age distribution such that controls were significantly younger than cases. Moreover, it is of note that out of the 78 cases and 56 controls only a total of seven women had a BMI over 30. As presented herein, high BMI combined with POPs is a factor worthy of investigation, but not adequately represent by this cohort (Petreas et al. 2011).

Until the 1970s, the Inuit population of Greenland and Canada held one of the lowest breast cancer rates in the world. No cases were reported before 1967, however a surge of 193 cases was reported between 1969 and 1997 (Bonefeld-Jorgensen 2010; Bonefeld-Jorgensen et al. 2011; Nielsen and Hansen 1980). Congruently, the Inuit population exhibited some the highest serum concentrations of POPs globally. This bioaccumulation has been proposed to be a direct result from the high dietary consumption of fats from fish, seal, whale, polar bear and seabirds leading researchers to theorize the high concentration of POPs found within tissues was related to the recent rise in breast cancer cases (Bonefeld-Jorgensen 2010). To further investigate, serum levels of breast cancer patients were analyzed for POPs between 2000 and 2003. Results showed significantly higher levels of perfluorinated compounds (PFCs), as well as double the median level of perfluorooctane sulfonate (PFOs) and sum of perfluorsulfonated acids (sumPFSA) in breast cancer patients compared to controls. No difference was observed in the serum levels of OCPs and PCBs. However, when PCBs were subdivided, significantly higher concentrations were seen in the highest quartiles. Collectively, these data indicate high levels of POPs are directly related increased breast cancer rates in the Inuit population. As studies continue to show increasing concentrations of select POPs in artic mammals and fish, it is critical to understand the molecular mechanism contributing to cancer risk or progression to provide better guidelines for targeted therapies and preventative measures (Butt, et al. 2010; Letcher, et al. 2010; Sonne 2010).

In the past decade, the role of the cancer microenvironment has come to the forefront of research. It is now well documented that the surrounding stromal cells, including adipocytes, fibroblasts, immune, and endothelial cells, have a significant effect on breast cancer progression (Casbas-Hernandez, et al. 2011; Sundaram et al. 2013). As the majority of breast tissue is adipose, and numerous reports have shown dramatic effects of POPs on the adipose, coculture studies observing the direct effects of POPs on adipocytes and subsequent

paracrine effect on breast cancer cell behavior are necessary. *Ex vivo* studies using breast cancer cell cocultures with adipocytes are an excellent starting point for comprehensive paracrine studies on POPs influence on cancer cell behavior. Salamah and colleagues (Salameh, et al. 2013) isolated rat mammary fatpads, devoid of epithelium, and cultured these glands *in vitro.* The cultured tissues were injected with the CRL1743 rat mammary tumor epithelial cells and their migration and tumorigenesis documented. Results using coherent anti-Stokes Raman scattering (CARS) microscopy showed the morphological outgrowths mimicked cancer cell morphology *in vivo* and highlighted the potential for future studies. This model also provides a platform to develop paracrine cell interaction studies investigating the effects of POPs incorporation during various metabolic states (i.e. high fat/ western vs. lean diet). A second 3D model system, similar to a skin culture system, demonstrated the interactions between pre-adipocytes, adipocytes, fibroblasts, and breast cancer cells (Delort, et al. 2013). Contact with breast cancer cells enhanced the differentiation of pre-adipocytes into adipocytes, and completely modified their transcriptional programs by increasing the expression of genes involved in cell proliferation (cyclinD1, MAPK), angiogenesis (MMP9, VEGF) and hormonal signaling (ESR1, IL6). Moreover, another 3D model of differentiated T3T-L1 adipocytes with normal murine mammary epithelial cells demonstrated that adipocyte-rich stroma induces branching through paracrine signals, including hepatocyte growth factor secretion, and may serve as a good model for observations during pubertal development (Pavlovich, et al. 2010). The addition of POPs could show potential alterations in branching morphogenesis or initiation of pre-cancerous lesions during puberty. This issue is imperative as it directly relates to contemporary issues involving childhood obesity and cancer risk later in life.

Serum levels of leptin, a key modulator in regulating energy intake and expenditure, is increased in breast cancer patients and has been implicated in the association between high BMI and breast cancer risk (Romero-Figueroa Mdel, et al. 2013). Leptin also transactivates key breast cancer promoting targets, including HER2, ERα, EGFR, and IGF-I receptors. Notably, correlations between POPs and leptin deregulation are strong (Pereira-Fernandes et al. 2014; Saxena, et al. 2008). Perfluorooctane sulfonate (PFOS), which belongs to the degradation product of many perfluorinated POPs, was fed to maternal rats and the effects of ingestion were investigated on the offspring in adulthood (Lv, et al. 2013). During gestation and lactation, offspring were indirectly exposed to 0.5 mg/kg/day or 1.5 mg/kg/day PFOS, respectively, from gestation day 0 to postnatal day 21. The offspring exhibited low body weight until weaning and displayed signs of a pre-diabetic state including elevated fasting serum leptin levels, elevated insulin and impaired glucose tolerance, regardless of exposure levels. These results suggested that developmental exposure to PFOS may contribute to glucose and lipid metabolic disorder in adulthood.

Further highlighting the potential differential gender impact, a clinical-based analysis of adipose depots with POPs accumulation found a significant correlation between high leptin serum concentrations and several PCBs in women, whereas no correlations were found in men (Pereira-Fernandes et al. 2014). Though breast tissue was not analyzed, in visceral fat a significant correlation was found between leptin, POPs and obesity in women. Leptin gene expression was also positively correlated with key POPs, such as CB180 and BDE153.

These data indicate a potential interaction between POPs and leptin signaling, however, definitive studies testing whether circulating POPs disrupts leptin signaling remain to be performed. Further extending these observations on changes in leptin levels in response to POPs to whether or not they influence breast cancer tumorigenesis also remains unexamined.

POPs levels are exponentially higher in adipose tissue among obese people than normal weight individuals (Airaksinen, et al. 2011), and obesity causes adipose tissue to become inflamed thus promoting dysfunction (Prieto-Hontoria et al. 2011). POPs exposure and bioaccumulation in tissue also promotes deregulated adipokines, including adiponectin (Kim, et al. 2010; Pereira-Fernandes et al. 2014). For example, adiponectin expression in VAT correlated negatively with the PCB congener CB138, indicating that POPs could have an influence on obesity-related disorders by suppressing the expression of adiponectin, a protective adipokine (Pereira-Fernandes et al. 2014). Another study demonstrated *in vitro* the effects of another PCB congener, CB77, on the suppression of adiponectin expression of mature adipocytes (Arsenescu, et al. 2008). Furthermore, in overweight and obese individuals, adiponectin is downregulated and inversely associated with sex steroids (Duggan, et al. 2011), all of which are markers for increased risk of breast cancer risk in pre- and post-menopausal women. Whether POPs stimulate a decrease in adiponectin specifically in breast tissue is unknown, and the direct relationship between POPs exposure driving a reduction in adiponectin and the subsequent effects on breast cancer cell behavior has not been investigated. However, adiponectin inhibits important metastatic properties such as adhesion, invasion, and migration of breast cancer cells. Consequently, breast tumors arising in a low adiponectin environment are associated with a more aggressive phenotype (Hwang, et al. 2013; Saxena and Sharma 2010). Thus, further investigation as to whether POPs would stimulate more aggressive breast cancer subtypes via suppression of adiponectin is warranted.

Conversely, a recent study by Salisbury *et al*. (2013) implicated an inverse correlation between POPs and breast cancer incidence in the binding and activation of the aryl hydrocarbon receptor (AhR) (Salisbury, et al. 2013). AhR is a diverse transcription factor involved in regulating xenobiotic metabolism as well as genes relevant to obesity and inflammation (Beischlag, et al. 2008; Bourdon, et al. 2010; Kerley-Hamilton, et al. 2012; La Merrill et al. 2013; Myre and Imbeault 2014). The AhR affects signaling pathways including the retinoic acid receptor, ER and the NFkB (Beischlag et al. 2008). In their study, AhR signaling activation-enhanced proliferation of ER positive breast cancer cell lines (MCF7 and T47D) via IGF-II-containing adipocyte conditioned media. Conversely TCDD, a highly specific AhR agonist and POP, blocked IGF-II signaling and cell proliferation (Salisbury et al. 2013) indicating that select POPs in the local microenvironment may inhibit cancer cell growth via activation of AhR. However, the paracrine effects of POPs in the tissue microenvironment must be investigated in combination with tumor cell growth. A study using human multipotent adipose-derived stem cells found that exposure to the AhR ligands TCDD and PCB congener 126 significantly regulated genes involved in inflammatory/ immune response, cancer, and metabolism pathways (Kim, et al. 2012). This was observed in both precursor and adipocytes, and demonstrated that AhR activation was the major

pathway inducing these changes via inhibition of AhR using the AhR antagonist α-naphthoflavone. Regulation of the inflammatory pathway via TCDD and PCB-126 was also observed in wildtype but not *AhR*-knockout mice. While this study did not directly observe the paracrine effect of the adipose on tumor cells, the pathways activated in the precursor and adipose suggest potential augmentation of tumorigenesis.

Another potential mechanism of action for POPs and obesity on breast tumorigenesis is through the action of fatty acid synthase (FASN), an enzyme responsible for producing endogenous fatty acids. This enzyme has been shown to be necessary for proliferation and survival effects of numerous malignant cancers promoting tumor aggression (Puig, et al. 2009). Specific to breast cancer, studies have shown significantly elevated levels of FASN *in vitro* and *in vivo*, and have directly correlated this enhanced expression with enhance aggressive breast cancer cell behaviors, enhanced tumor progression and poor prognosis (Hilvo, et al. 2011; Hopperton, et al. 2014; Hunt, et al. 2007; Khan, et al. 2014). FASN is also linked to the accumulation of visceral fat, the development of type 2 diabetes and obesity (Berndt, et al. 2007). Obesity generates free fatty acids that in turn elevates tissue levels FASN and gives cancer cells heightened *de novo* lipogenesis (Hunt et al. 2007). This cyclic observation highlights a molecular mechanism for obesity driving breast cancer. To tie in POPs with this mechanism, we highlight a study focused on determining whether the antioxidant glutathione was important in protecting liver tissue from TCDD-mediated toxicity and development of nonalcoholic fatty liver disease with associated steatosis (Chen, et al. 2012). Results show TCDD stimulates liver toxicity and altered lipid metabolism via several lipid metabolism genes, including FASN. Gclm(−/−) knockout mice, a model of liver glutathione deficiency, were protected from liver steatosis and correspondingly had significantly decreased FASN expression levels. This data suggests that altered lipid metabolism, in part by FASN function, is induced via the POPs TCDD. It would be of interested to determine whether POPs similarly function in part through FASN to alter lipid metabolism in breast tissue, thereby potentially promoting breast tumorigenesis.

Lifetime exposure to POPs through dietary intake would, over time, result in continual increases of bioaccumulation in adipose tissue due to POPs lipophilic nature. It is also of note that age is the most significant risk factor in developing breast cancer (Carriot, et al. 2014). It could be hypothesized that POPs bioaccumulation with age could additively/synergistically promote breast cancer progression. The collective interaction of inflammation is with altered local hormone production and altered immune function as seen with age (Chung, et al. 2008; Priyanka, et al. 2013). In post-menopausal women, a significant amount of estrogen is supplied by adipose tissue (Huang, et al. 1997). By adding a lifetime exposure to POPs, bioaccumulation in adipose could have severe consequences, potentially further compounded by obesity. It is important to ward off this increased risk as 70% of breast cancers are estrogen receptor positive and higher levels of estrogen increases risk (Carriot et al. 2014; Santen, et al. 2014).

## Socioeconomic Impact of POPs and Breast Cancer

Multiple epidemiological studies indicate a worse prognosis for women who are obese at the time of breast cancer diagnosis compared with non-obese patients (Protani, et al. 2010). A

meta-analysis of 43 studies of obesity and breast cancer revealed that obese patients were 33% more likely than non-obese patients to die of breast cancer (Protani et al. 2010). Socioeconomic status has been classically known to impact on diet, with low level of education and moderate physical activity level, cost per calorie as important mediators identified in the socioeconomic status-BMI relationship (Dunneram and Jeewon 2013; Fokeena and Jeewon 2012). Previous trends have shown an increase in BMI and metabolic syndrome in the populations of higher socioeconomic strata among developing countries (Caballero 2007). But, the impact of socioeconomic status on obesity has been shifting, affecting a broader range of societies, and is suggested to be due to increased consumption of sweetened beverages, and increased availability of low-cost, energy-dense foods in urban areas of developing countries (Caballero 2007). Recent studies show obesity is rising among middle-aged (Caballero 2007; Dunneram and Jeewon 2013) and postmenopausal women (Bhurosy and Jeewon 2013; Caballero 2007) and adolescents of low socioeconomic status in middle-income countries in Africa, as well as urban and rural areas in the poorest countries of sub-Saharan Africa and South Asia to populations in countries with higher income levels (Bhurosy and Jeewon 2014; Popkin, et al. 2012). Similarly, another socioeconomic study across 22 western European countries reported that obesity (BMI>30) was more common among women of lower education level (Mackenbach, et al. 2008).

With respect to breast cancer mortality, African-American women suffer disproportionately compared to other racial/ethnic groups (DeSantis, et al. 2014; Jemal, et al. 2007; Newman, et al. 2002) and this has been related to their increased prevalence of high fat/poor diet, obesity and metabolic syndrome. Both social and biological mechanisms are contributory, including a higher prevalence of aggressive basal-like, “triple-negative” breast cancers in young, premenopausal African-American women (Boyle 2012). This subtype does not express detectable levels of ER, PR and does not over-express the growth factor Her2. Therefore, this subtype is deemed to be unresponsive to anti-estrogenic compounds, currently no effective targeted therapies exist, and patient survival is poor (Sorlie, et al. 2001). Even when African-American women (of any age) are diagnosed with ‘good prognosis’ Luminal A breast cancers, they fare worse than Caucasian women with the same subtype (Murray, et al. 2010). Correspondingly, the prevalence of metabolic syndrome is higher among Luminal breast cancers in postmenopausal women [OR 1.37 (95% CI 1.07–2.80) $P$=0.03] and most notably, BMI alone was associated to Luminal A subtype breast cancer risk [OR 1.12 (95% CI 0.96–2.196 p = 0.2] (Capasso, et al. 2014). Unfortunately race was not addressed in this study.

Social disparities research show the percentage of POPs to be at its highest in women without a formal education and at its lowest in affluent women (Arrebola, et al. 2013; Freire, et al. 2011; Gasull, et al. 2013; Wilcox, et al. 2013). In low socioeconomic ethnic-based communities, the availability of healthy food is inconsistent at best. Fresh fruits and vegetables are somewhat non-existent or too expensive to feed a family. A poor diet of processed/fast foods and fatty meats all contribute to the obesity epidemic prevalent in the African-American community increasing their susceptibility to hypertension, cardiovascular disease, diabetes, and obesity (Caprio, et al. 2008a, b; Hebert, et al. 2013; Wilcox et al. 2013). In a study by Chandran *et al* (2012), African-American women were shown to be less

physically active, overweight or obese, and have a higher intake of total cholesterol and fat than other ethnicities (Chandran, et al. 2012). In addition to dietary choices and food availability, a multitude of factors influence the observed disparities in obesity. For example, a study involving the Jackson Heart Study participants (N = 2,881) reported that travel for healthier food options was inhibited due to safety reasons and perceived neighborhood safety (Pham do, et al. 2014). African American women who strongly disagreed their neighborhood was safe from crime had a higher BMI compared to women who felt safe, and premenopausal African American women who felt most unsafe had significantly higher BMI, waist circumference, and volumes of visceral and total adipose tissue than those who felt safe (Pham do, et al. 2014). These data highlight that in addition to dietary factors, emotional stress may play a role in the promotion of obesity in these communities. It has also been shown that African-American women have a higher burden of PCDD/PCDFs compared to other ethnicities (Reynolds, et al. 2005). These women do not have a higher incidence of breast cancer but, more importantly, they have the worst prognosis and survival rates (Chandran et al. 2012). If it is true that lower socioeconomic classes have higher levels of POPs and POPs accumulates in adipose tissue, then it can be hypothesized that the poor prognosis of African-American women and breast cancer could be attributed to the level of POPs in their adipose tissue.

## Model Systems

To study breast development, deregulation and cancer, mouse and rat animal models are frequently used. Numerous murine models have been developed to study normal human breast development and cancer, including the most widely used human xenograft models. In these models, human breast epithelium, stromal and/or tumor cells are transplanted orthotopically into immunocompromised mice that do not reject human cells (Proia and Kuperwasser 2006; Richmond and Su 2008). The three-dimensional tissue organization, whole body metabolism, hormone and growth factor interactions are a highlighted benefit to understanding post-pubertal breast development and function compared to limited *in vitro* co-culture studies. However, significant limitations include cross-species complications in signaling molecules and the lack of immune cell interactions, a known critical component of tumorigenesis and progression. Knockout mouse models are useful in studying the effects of a single gene during disease. Two knockout mouse models have been used to study the effects of POPs *in vivo*, though we were unable to find any studies involving mammary function and POPs interactions. The *AhR*-knockout mouse model was used to test the effects of TCDD on epididymal adipose tissue compared to wild type controls. Male mice were treated with a non-acute toxic dose of TCDD and results demonstrated that pre-adipocytes and adipocytes are targets of POPs and that one of the main pathways activated was inflammation. Activation of the inflammatory pathways was not observed in *AhR*-knockout, thus this model demonstrated that a major pathway of TCDD was via AhR activation (Kim et al. 2012). The Apo E−/− mouse model has also been used to study POPs *in vivo* confirming, or highlighting, the synergistic effects of POPs on atherosclerosis (Conklin, et al. 2010; Shan, et al. 2014). Another model for studying human breast cancer are genetically engineered mouse (GEM) models, in which mice carry the same mutation within the endogenous locus, the gene is expressed within the specific cell types that occur in human

tumors, and is silent during embryogenesis (Shoushtari, et al. 2007). Although knockout and GEM models are highly useful for evaluating the effects of specific mutation, deletion or gene amplification of one or two genes during breast or tumor progression, they usually cannot fully reproduce the genetic complexity of human tumors. However, it cannot be argued that many advances have been gained from these models and they will continue to provide valuable data to the field.

Potentially one of the most significant limitations to using mouse models is the morphological and structural differences among the epithelium and stroma in murine fat pad compared to human breast. The mouse mammary gland contains large depots of adipose laced with small amounts of interspersed connective tissue (Parmar and Cunha 2004). The functional lobular units of the mouse gland are embedded within the fat pad, and have a considerable amount of space between the minimally branched ducts. In contrast, the lobular units of the human breast are surrounded by loose intralobular connective tissue, consisting primarily of fibroblasts. This intralobular stroma is subsequently surrounded by a more compact interlobular stroma, which detaches the lobules and intralobular stroma from any substantial direct contact with the adipose tissue (Fleming, et al. 2008). Thus, alterations in breast cellular communication in response to obesity in humans compared to mice may substantially vary, given the differences in amount and proximity of the adipose to the epithelium. Stemming from the observations that these stromal subtypes differ in their physical location in relation to epithelial lobules, and that epithelial/stromal interactions can promote or inhibit tumorigenesis, animal models that better resemble human breast structure are critically needed to relate experimental studies investigating collective metabolic, developmental and environmental interactions.

Rats have been highlighted as a more appropriate model due to the similarities in ductal and lobular mammary structures, spatial proximity to fibroblasts and adipose, total adipose content, as well as hormone and paracrine cell interactions (Shepel and Gould 1999; Vargo-Gogola and Rosen 2007). One slight developmental variation in rats is a constant growth of lobular and ductal structures into the adipose; human lobular structures do not extend into fat tissue and ductal structures grow along connective tissue septa (Russo 1983; Russo, et al. 1990). However, a significant advantage of using rat models is the close association of rat and human breast cancer genes and risk alleles (Sanders and Samuelson 2014). Moreover, in contrast to mouse models, chemically induced rat mammary tumors are generally hormone-dependent adenocarcinomas. For this reason, the rat mammary carcinogenesis models have been utilized extensively to investigate hormone-dependent breast cancer and the protective role of pregnancy in breast cancer (Vargo-Gogola and Rosen 2007). Rat models are also popular for metabolic and nutritional studies, and historically chemically induced tumors have been evaluated in correlation with diet-induced obesity. Collectively, these studies have shown that 7, 12, dimethylbenz [a]anthracene (DMBA) and N-nitrosomethylurea (NMU) induced mammary tumors had enhanced proliferation in obese rats compared to normal weight controls (Chan, et al. 1977; Hakkak, et al. 2005; Hakkak, et al. 2007; Lautenbach, et al. 2009). It is of note that the use of more relevant studies to human health and breast cancer, such as dietary POPs accumulation, their contribution to obesity, mammary adipose/stromal function together with mammary cancers have not been studied in the rat model.

## Conclusions

In conclusion, numerous correlative studies suggest a strong association between POPs exposure through diet and their bioaccumulation in adipose promotes the development of obesity and ultimately influences breast cancer development and/or progression (Figure 1). There is a critical need for advanced model systems and pioneering *in vitro* and *in vivo* studies to understand the complex relationship between these causative factors and, more importantly, to delineate their multifaceted molecular, cellular, and biochemical mechanisms.

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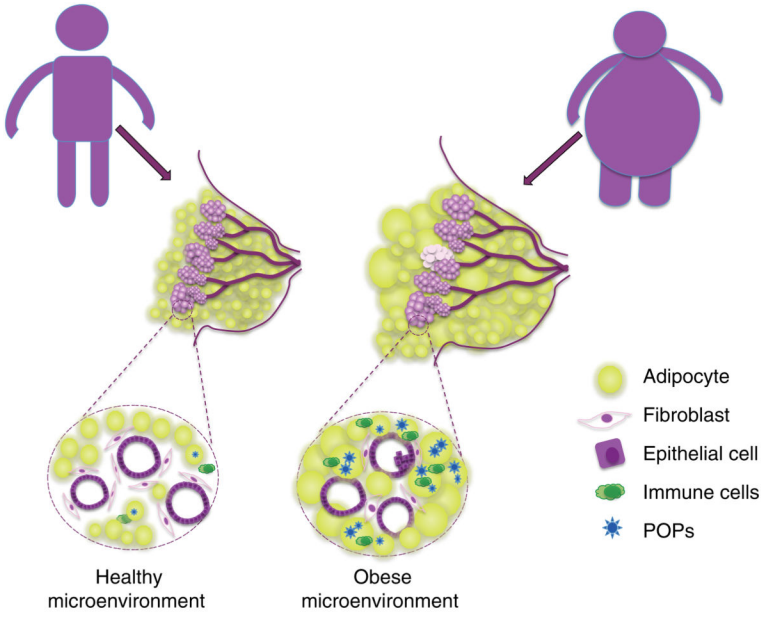


**Figure 1. Potential mechanisms for POPs in the promotion of breast cancer**

Obesity-associated endocrine and metabolic mediators are suspected to play a role in oncogenesis by modifying both systemic nutrient metabolism and the nutrients available locally in the supportive stromal tissue. Excessive adipose in obese women also serve as storage depots for POPs obtained from dietary sources. The accumulated POPs stimulate additional adipocyte proliferation and recruitment of immune cells, leading to an inflammatory microenvironment. Collectively, these factors may promote breast cancer development and/or direct cancer cell phenotypes.