Fatty Acids Influence Susceptibility to DMBA-Induced Carcinogenesis

JoAnne Schneider, Holly Hendrix, Meena Mistry, Mario Medvedovic, Robin Gear, Deborah Clegg
Department of Psychiatry, University of Cincinnati, Cincinnati OH

During recent decades, industrialized countries around the world have seen a parallel increase in the incidence of obesity and breast cancer. While the specific etiology of breast cancer remains unknown, the incidence appears to be correlated with obesity and specifically consumption of high fat diets. For example, rates of breast cancer are higher in the U.S., where 30-40% of calories are consumed as fat. By contrast, many East Asian countries consume significantly less fat and have lower rates of breast cancer. Importantly, the type of fat that may be responsible for increased breast cancer susceptibility in women or their offspring remain unknown. Therefore, we are testing whether early life exposure to specific fatty acids causes obesity and predisposes rats to DMBA-induced breast cancer. To accomplish this, female rats are exposed to diets high in saturated fats from animals (i.e., butter), monounsaturated fats (i.e. olive oil), omega-3 fatty acids (fish oils), or polyunsaturated fats (PUFA) which are abundant in vegetable oils. Females are then impregnated, carry pups to term, and then lactate on these specific diets. Pups from each dietary condition are then subjected to a tumor-generating dose of DMBA. The objective of the study is to determine the rates of tumorogenesis in each dietary condition. Additionally, mammary gland morphology, onset of puberty, hormonal changes, and tumor formation are compared across each dietary condition. Finally, using microarray gene analysis of mammary gland epithelial cells, we are assessing gene expression changes associated with increased susceptibility to carcinogenesis based on dietary fat treatment.

Mouse Models of Adolescent Obesity and Breast Development

Karl Olson, PhD, Michigan State University, East Lansing, MI 48823

Increased body weight and adiposity caused by poor diets and sedentary lifestyles have been associated with early onset of menarche in girls - a known risk for breast cancer. Elevated adiposity during puberty may alter breast cancer risk by perturbing breast development, which may have long-term consequences on function and hormonal responsiveness of the adult gland. To investigate the impact of pubertal obesity on breast development, obesity-susceptible C57BL/6 mice and obesity-insusceptible Balb/c mice were fed a high fat diet from weaning (3 wks old) to 7 weeks of age. A set of C57BL/6 mice was also ovariectomized (OVX) to study the effect of obesity on estrogen (E) and/or progesterone (P) regulation of end bud formation and epithelial cell proliferation. The high fat (HF) diet consisted of 60% kcal fat, 20% kcal carbohydrate and 20% kcal protein, whereas the control (C) diet was 10% kcal fat, 70% kcal carbohydrate and 20% kcal protein. C57BL/6 mice fed a HF diet for 4 wks were significantly heavier than C fed mice. Consistent with increased adiposity and mild insulin resistance, C57BL/6 mice fed a HF diet had elevated leptin and insulin levels and diminished glucose tolerance. HF fed C57BL/6 mice had increased breast fat pad and epithelial cell area. The epithelium also appeared have more completely infiltrated the stroma and had less developed end buds compared to controls. Even though the epithelial cells had greater outgrowth, there were fewer progesterone receptor A (PRA) positive duct epithelial cells in HF fed C57/BL6 mice. Ovariectomy led to increased weight gain and leptin levels in both HF and C diet fed C57BL/6 mice. Breast fat pad and epithelial cell area was increased in OVX C57BL/6 mice and this was substantially augmented by HF diet. OVX C57BL/6 mice were refractory for E- and/or P-induced end bud formation. Duct epithelial cells of OVX C57BL/6 mice had markedly reduced numbers of PRA positive cells. Treatment of OVX C57BL/6 mice with E and P, independent of diet, led to a partial recovery of PRA positive cells and duct cell proliferation. Overall this data show that increased body weight during adolescence can result in increased levels of insulin and leptin, breast fat pad and epithelial cell area, and alterations in end bud morphology and hormone responsiveness.

The Crucial Role of Intestinal Metabolism in the Bioavailability and Actions of Dietary Phytoestrogens in Soy Foods

Kenneth D. R. Setchell, Department of Pathology, Cincinnati Children's Hospital Medical Center and the Department of Pediatrics, University of Cincinnati College of Medicine

Interest in the nutritional value of phytoestrogens, a class of polyphenols, has grown at an unprecedented rate following the wealth of scientific data showing that these bioactive non-nutrients possess potent and wide-ranging biological activity. While phytoestrogens are ubiquitous to the plant kingdom, several plants and plant-based foods are notorious for their relatively high contents of phytoestrogens. These include foods made from, or incorporating soybeans and the nutritional values and properties of such products has been rapidly exploited by the food industry. Soy-based foods, which contain the isoflavones, daidzein and genistein and their β-glycoside conjugates, are widely consumed in Asian countries and the relatively low incidence of many hormone-dependent diseases, such as breast and prostate cancers, cardiovascular disease, and osteoporosis compared with Western countries led to the hypothesis that these phytoestrogens play a role in disease prevention.

The bioavailability and therefore clinical effectiveness of soy isoflavones is crucially dependent on intestinal metabolism. Intestinal hydrolysis of the conjugated sugar moieties is a prerequisite for their absorption. After hydrolysis, the aglycones, daidzein and genistein are released and become available for absorption. Typically, plasma isoflavone concentrations attain maximal levels 6-8 h after ingestion and the plasma elimination half-life of isoflavones is 6-8 hours. The volume of distribution (Vd) of isoflavones is high and consistent with extensive tissue distribution following their absorption. Consumption of typical dietary intakes of isoflavones (15-50 mg/day) leads to circulating concentrations of isoflavones that exceed plasma estrogens by several orders of magnitude and sufficient to expect hormonal effects. Intestinal bacterial conversion of daidzein to equol [7-hydroxy-3-(4'-hydroxyphenyl)-chroman] appears to be a highly important phenomenon that may enhance the biological effects of soy-based diets.

Interestingly, equol has a chiral center and therefore can occur as 2 distinct enantiomers, S-, and R-equol. We have shown that human intestinal bacteria produce exclusively S-equol. Most animal species metabolism soy isoflavones extensively to S-equol, and this is especially the case for rodents. However, humans differ because S-equol is only found in a small proportion of adults after consuming soy foods. The frequency of equol-producers is only 20-30% for adult Westerners, but Asian adults and vegetarians show a much greater propensity to produce equol from soy foods. This intestinal metabolism may be a key factor in determining the extent of hormonal effects of diets containing soy isoflavones. S-equol possesses significant estrogenic activity having specific affinity for ERβ and is therefore, by definition is a SERM. It is also unique in possessing antiandrogen effects in vivo in antagonizing the actions of the potent androgen dihydrotestosterone (DHT). Furthermore, equol is superior to all other isoflavones in its antioxidant activity. Several recent dietary intervention studies examining the health effects of soy isoflavones highlight the potential importance of equol by establishing that maximal clinical responses to soy protein diets are observed in people who are good 'equol-producers'.

In view of the therapeutic value of SERM's in breast cancer treatment and prevention, we are currently examining the potential role of S-equol in breast cancer prevention and thus the potential importance of being able to convert soy isoflavones to equol. Prepubertal exposure to S-equol significantly increased mammary gland size whether given by injection, or by feeding and the effects were greater than for genistein. Using the classical animal model of breast cancer, early exposure to S-equol in the diet increased early latency and caused a reduction in the numbers of mammary tumors chemically-induced by the potent carcinogen DMBA. Given the relatively high frequency of equol-producers in Asians consuming soy foods, and the epidemiological evidence supporting an inverse relationship between breast cancer risk and early soy food intake in Asians, these findings suggest that intestinal formation of equol may be an important factor in chemopreventive and in part explain the reduced incidence of breast cancer in Asian women. Exposure to dietary equol in adult life did not increase the numbers of tumors formed providing some assurance that equol is unlikely to increase risk of breast cancer in women at risk especially if they are equol-producers.

Non-absorbable Fat Affects the Absorption, Storage and Excretion of PCBs, PFOA and Hexachlorobenzene

RJ Jandacek and P Tso, Department of Pathology and Laboratory Medicine, University of Cincinnati

Oral ingestion is the primary route of entry into humans for organochlorine compounds and other halogenated hydrocarbons. Because of their lipophilicity, organochlorine compounds accompany dietary fat into the gastrointestinal tract where they are absorbed via both the lymphatic and the portal routes. After initial absorption, organochlorines and their metabolites are distributed into adipose tissue and into organs and other tissues. The parent compounds and their lipophilic metabolites are involved in enterohepatic circulation with movement from tissue into the intestine. Entry into the intestine occurs both in bile and via a non-biliary process that may include direct secretion from the enterocyte and/or normal enterocyte turnover. Reabsorption of part of the organochlorines that enter the intestine and distribution of the absorbed material to tissues completes the enterohepatic cycle. Longitudinal studies are consistent with absorption being more rapid than the rate of excretion in humans. This imbalance suggests the intestine as a site for intervention to reduce the body burden of organochlorines. Hindrance of absorption of an organochlorine from the intestine can both hasten the rate of excretion of stored material and reduce its input from the diet. We have studied the effects of unabsorbed fat on the processes of absorption and reabsorption in enterohepatic circulation. Both in animal models and in humans, unabsorbed fat can favorably reduce absorption in a clinically significant manner. The results and implications of these studies will be addressed.