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Alcohol consumption promotes mammary tumor growth and insulin sensitivity

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Abstract

Epidemiological data show that in women, alcohol has a beneficial effect by increasing insulin sensitivity but also a deleterious effect by increasing breast cancer risk. These effects have not been shown concurrently in an animal model of breast cancer. Our objective is to identify a mouse model of breast cancer whereby alcohol increases insulin sensitivity and promotes mammary tumorigenesis. Our results from the glucose tolerance test and the homeostasis model assessment show that alcohol consumption improved insulin sensitivity. However, alcohol-consuming mice developed larger mammary tumors and developed them earlier than water-consuming mice. *In vitro* results showed that alcohol exposure increased the invasiveness of breast cancer cells in a dose-dependent manner. Thus, this animal model, an *in vitro* model of breast cancer, may be used to elucidate the mechanism(s) by which alcohol affects breast cancer.

Keywords

alcohol; breast cancer; insulin sensitivity; estrogen; leptin

1. Introduction

Breast cancer is the most common malignancy affecting women and the second leading cause of death among women in the United States [1]. The American Cancer Society estimates that in 2009 there will be approximately 180,000 new cases of invasive breast cancer and about 40,000 women will die from the disease [1]. Epidemiological studies reveal that alcohol consumption increases breast cancer risk in a dose-dependent manner [1,2]. In women, breast cancer accounts for approximately 60% of alcohol-related cancers [3]. Studies indicate that breast cancer risk increases by approximately 10% per drink consumed per day [1,2]. A drink is defined as 12 ounces of beer or 5 ounces of wine. Women who consume 4-5 drinks per day have about a 50% higher probability of developing breast cancer than those who abstain [4]. Others have suggested that alcohol is a tumor promoter [2]. Additionally, epidemiological studies suggest that alcohol intake may increase the aggressiveness of breast cancer cells to invade and metastasize [5,6]. Alcohol consumption may also promote the growth of existing

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breast tumors [7]. Studies by others support the notion that alcohol increases the invasiveness of breast cancer cells through a direct mechanism [8,9]. Specifically, alcohol may increase the invasiveness of breast cancer cells via the human epidermal growth factor Receptor 2 also known as ErbB2 [8]. However, it is not clear if the *in vitro* effects of alcohol on cancer cells are similar to or different from those observed in an animal model of breast cancer.

The role of alcohol consumption in disease is contradictory. It has both a deleterious effect, in that it promotes breast cancer development, but also a beneficial effect, in that it increases insulin sensitivity. A meta-analysis study reported that daily consumption of 6-48g of alcohol (compared to non-consumption) decreased the risk of type 2 diabetes by approximately 15-30% [10]. A randomized control trial study showed that women who drank 30g of alcohol per day for 8 weeks had an improved response to exogenous insulin, thus showing that alcohol consumption promotes insulin sensitivity [11]. Alcohol may increase insulin sensitivity and breast cancer by a similar mechanism. Alcohol consumption may increase insulin sensitivity by enhancing systemic hormones, such as insulin, estrogen, and leptin [12-14]. By increasing the levels of these hormones alcohol may prevent insulin resistance; however, high levels of these hormones may also promote breast cancer development [15-19]. Our notion is that by understanding both the beneficial and the deleterious effects of alcohol simultaneously in the same animal, we will be able to determine how alcohol affects breast cancer progression. Thus, our objective is to identify a mouse model of breast cancer whereby alcohol increases insulin sensitivity and promotes mammary tumorigenesis.

Our results show that alcohol consumption sensitizes mice to insulin. Thus, it is feasible that alcohol decreases the risk of type 2 diabetes by ameliorating insulin resistance in tissues regulating glucose metabolism such as skeletal muscle, adipose tissue, or the liver. However, if alcohol sensitizes mammary cancer cells to insulin or other growth factors such as estrogen, then the tumors of alcohol-consuming mice may grow at a faster rate, or breast cancer cells may obtain a more aggressive metastatic phenotype when they encounter alcohol. In fact, our data show that mammary tumor growth rate is accelerated in alcohol-consuming mice, and in cell culture studies, alcohol increased the invasive ability of breast cancer cells in a dose-dependent manner. Furthermore, the combination of estrogen and alcohol magnified the invasive ability of breast cancer at a higher level than either one alone. Also, mice consuming alcohol have higher levels of estrogen and leptin; thus, it is conceivable that alcohol consumption increases the production of these growth factors by adipose tissue and simultaneously increases the sensitivity of breast cancer cells to these hormones. Thus, our studies suggest that by understanding both the beneficial and the deleterious effects of alcohol simultaneously in an animal model of breast cancer, the mechanism by which alcohol affects breast cancer may be elucidated.

2. Materials and Methods

2.1. Animals, alcohol, and diet

A total of 65 specific pathogen-free female mice were obtained from the Jackson Laboratory (Bar Harbor, ME) at 6 weeks of age and housed in the Animal Resources Center at the University of Texas at Austin (UT). Animal care was provided in accordance with the procedures outlined in "Guide for the Care and Use of Laboratory Animals" (NIH Publication No. 86-23, 1985). All animal procedures were approved by UT's Institutional Animal Care and Use Committee. Mice were singly housed in a 22-24°C room and maintained on a 12-hr light/dark cycle. Following two weeks of acclimation, mice were randomized into one of two treatment groups defined by the liquid provided: 30 mice on water and 35 mice on alcohol. Mice were fed a chow diet as described previously [20]. Mice consumed either water or 20% w/v alcohol in the drinking water *ad libitum* throughout the study. Insulin sensitivity was measured after 16 weeks, and mice were injected with mammary cancer cells after 18 weeks.

Body weights, food consumption, and liquid consumption were measured weekly. Serum was collected at the end of the study to determine systemic levels of insulin, leptin, IGF-1, estradiol, and blood alcohol levels.

2.2. Glucose clearance and insulin sensitivity

To determine the effects of alcohol consumption on glucose regulation and insulin sensitivity, we performed a glucose tolerance test (GTT) as previously described [20]. As an indicator of insulin sensitivity, we used the homeostasis model assessment (HOMA) [21]. For this purpose, we measured fasting insulin and glucose levels, which were used to calculate HOMA values using the formula below:

$$\text{HOMAIR} = \frac{\text{fasting insulin } (\mu\text{U/ml}) \times \text{fasting glucose (mmol/l)}}{22.5}$$

Mouse serum was collected from blood obtained through the retro-orbital vein. Insulin levels were measured using an insulin ELISA kit (Mercodia; Winston-Salem, NC), and glucose levels were measured with a Glucometer Elite (Bayer; Elkhart, IN).

2.3. Cancer cell line and tumor growth

Mice were injected with Met-1 mouse mammary cancer cells [22]. These breast cancer cells were originally derived from mammary tumors dissected from polyomavirus middle T antigen transgenic mice (PyMT) [22]. They were kindly provided by Jeffrey P. Gregg (Department of Pathology, School of Medicine, University of California-Davis, Sacramento, California, USA). Syngeneic female FVB/N mice were injected subcutaneously in the lower back with 25,000 Met-1 cells. Once tumors were detected, tumor volume was measured three times a week with calipers.

2.4. Body composition

Final body composition was calculated using Dual energy X-ray Absorptiometry (DXA) with a GE Lunar Piximus II densitometer (Madison, WI). Using this method, we determined percent body fat, lean mass, and bone mineral density (BMD).

2.5. Immunoassay and blood alcohol concentration

Serum leptin and insulin levels were measured using a LINCOplex kit (LINCO Research; St. Charles, MO). Serum estrogen (E2) and IGF-1 levels were measured using a 17 β -estradiol ELISA (IBL-America; Minneapolis, MN) and a mouse IGF-1 immunoassay kit (R&D Systems; Minneapolis, MN). Blood alcohol levels were measured using an NADH kit (Sigma-Aldrich; St. Louis, MO). Manufacturer instructions were followed for all kits.

2.6. Invasion assay

T47D human breast cancer cells were grown in 75-cm³ tissue culture flasks (Falcon Labware, BD; Franklin Lakes, NJ) to 80% confluence. Cancer cells were serum-starved overnight in DMEM and harvested with 0.25% Trypsin (Sigma-Aldrich; St. Louis, MO). Cells (5×10^4) were seeded on the upper chamber of a Boyden chamber in DMEM treated or untreated with 0, 0.1, 0.2, or 0.5% alcohol and/or 20nM estrogen (Sigma-Aldrich; St. Louis, MO) and 0.01% bovine serum albumin. The lower chamber contained DMEM and 10% FBS as a chemoattractant, and 0, 0.1, 0.2, or 0.5% alcohol and/or 20nM estrogen. After 24-hour incubation at 37°C and 5% CO₂, cells remaining on the upper membrane surface were removed with a cotton swab. Cells on the lower surface of the filter were fixed and stained with Diff-

Quik (Dade-Behring; Newark, DE). Five fields of adherent cells were randomly counted in each well with a Nikon Diaphot-TMD inverted microscope at 20x magnification.

2.7. Statistical analysis

Body composition (body weight, body fat, and BMD), calorie consumption, serum analysis (estrogen, IGF-1, insulin, and leptin), GTT, and HOMA were analyzed by an independent t-test. Mann-Whitney U test and log transformation were used to analyze the tumor data due to the skewed nature of the data. Invasion assays were examined by one-way analyses of variance (ANOVA) with post-hoc comparison of the means using Tukey's Honestly Significant Difference. SPSS version 15.0 for Windows (SPSS Inc., Chicago, IL) was used for all statistical comparisons. To detect statistical significance, p value was set at 0.05, and data are presented as the mean $\pm$ standard error of the mean (SEM).

3. Results

3.1. Body weight and body composition

Mean baseline body weight values were similar between water-consuming (17.3 ± 0.2 g) and alcohol-consuming (17.4 ± 0.2 g) mice ($p > 0.05$). Furthermore, body weights between water- and alcohol-consuming mice were similar throughout the study (Table 1). At the end of the study, there was no significant difference in percent body fat and lean mass between the two groups. However, alcohol-consuming mice had significantly lower bone mineral density values compared to water-consuming mice (Table 1, $p < 0.05$).

3.2. Calorie consumption and blood alcohol levels

Alcohol-consuming mice tended to eat fewer calories from food, yet the mean values were not statistically different compared to water-consuming control mice (Table 1). Total calorie consumption (calories from food and alcohol) were higher in alcohol-consuming mice, which consumed an average of 5.4 ± 0.22 kcal per day from alcohol. Alcohol-consuming mice had blood alcohol levels of approximately 80 mg/dL (Table 2).

3.3 Alcohol consumption and insulin sensitivity

To determine the effect of alcohol consumption on insulin sensitivity, we evaluated GTT and HOMA on mice. The GTT results show that mice consuming alcohol cleared injected glucose more rapidly than mice consuming water (Figure 1A). We fasted water- and alcohol-consuming mice overnight and measured serum glucose and insulin levels, which were used to determine HOMA values. As shown in Figure 1B, alcohol-consuming mice exhibited lower HOMA values compared to mice drinking water, indicating that alcohol increased insulin sensitivity. Collectively, the GTT and HOMA values showed that alcohol consumption promoted insulin sensitivity.

3.4. Alcohol consumption and mammary tumor growth

A greater number of alcohol-consuming mice developed palpable tumors earlier than water-consuming mice (Figure 2A). Alcohol ingestion, in contrast to water ingestion, also resulted in the development of larger tumors (Figure 2B, $p < 0.05$). At most time points, the average tumor volume was significantly higher in the alcohol-consuming mice ($p < 0.05$).

3.5. Alcohol intake and hormones

To determine the relationship between blood hormone levels and improved insulin sensitivity, tumor progression, and alcohol consumption, we measured insulin, leptin, estrogen, and IGF-1 in our mice (Table 2). Results show that serum leptin and estrogen levels were significantly

elevated in alcohol-consuming mice compared to water-consuming mice ($p<0.05$). IGF-1 and insulin levels were not statistically different between the two groups ($p>0.05$).

3.6. Effect of alcohol and estrogen on cancer cell invasiveness

To determine if alcohol affects the metastatic ability of breast cancer cells, we measured the effects of alcohol on human T47D breast cancer cell invasiveness. Results show that alcohol exposure increased the invasiveness of T47D cancer cells in a dose-dependent manner (Figure 3A, $p<0.01$). Using the invasion assay, we also show that estrogen at 20nM increased the invasiveness of T47D cancer cells ($p<0.05$); however, the combination of alcohol and estrogen intensified the ability of the cancer cells to invade significantly more than either treatment alone ($p<0.01$) (Figure 3B).

4. Discussion

Alcohol consumption has a negative effect by increasing breast cancer risk [2] but also a positive effect by increasing insulin sensitivity [21]. The mechanism by which alcohol increases both breast cancer risk and insulin sensitivity is unclear; thus, we devised a mouse model to simultaneously show the effects of alcohol on breast cancer and insulin sensitivity. We show that alcohol consumption increased insulin sensitivity (Figure 1) and promoted tumor growth (Figure 2) in the same animals.

The effects of alcohol on insulin sensitivity and breast cancer are not due to negative or positive effects of alcohol on body weight and body fat levels. Body weight and percent body fat levels were similar in alcohol- and water-consuming mice (Table 1). Some epidemiological studies show that alcohol consumption leads to weight loss in women [23-25] while others, conversely, report weight gain [26,27]. On the other hand, work by Shah *et al.* showed that alcohol consumption did not affect either body weight or body fat in women [28]. These conflicting results may be due to the lack of control for confounding factors that can affect body weight, such as caloric consumption and initial body weight at the beginning of the study. An advantage of our study is that we measured calorie consumption and body composition throughout the study. In fact, total calorie consumption, body weight, and percent body fat levels between water- and alcohol-consuming mice were similar at the beginning and the end of the study. The only factor affected by alcohol was bone mineral density (BMD); alcohol-consuming mice had lower BMD than water-consuming mice (Table 1).

Epidemiological data show that alcohol clearly increases breast cancer risk in women when 3-4 drinks or 30-40 grams of alcohol are consumed per day [1]. If a 140-pound woman consumes 3-4 drinks daily, it would lead to about a 0.08% blood alcohol level (www.dot.wisconsin.gov/safety/docs/08law.pdf). In women, 3-4 drinks of alcohol increases breast cancer risk by about 40% [4]. Our results show that mice consuming 20% alcohol had 80 mg/dL alcohol levels in their blood (Table 1). This translates to about a 0.08% blood alcohol level in humans, a level that would classify an adult woman as legally intoxicated. Thus, feeding 20% alcohol to mice allowed us to determine the effects of alcohol on insulin sensitivity and mammary cancer at high levels yet physiological levels found in women.

Previously, we showed that alcohol consumption promoted insulin sensitivity in male mice without affecting body weight and body fat levels [20]. Our GTT and HOMA results showed that alcohol also increased insulin sensitivity in female mice without affecting body weight or body fat levels (Figure 1A). Our current results showed that the injected glucose was cleared faster in female mice consuming alcohol than those consuming water. Also, the lower HOMA values in alcohol-consuming mice indicated that alcohol increased insulin sensitivity (Figure 1B). Insulin sensitivity is oftentimes proportional to body fat levels; however, in our studies, alcohol consumption enhanced insulin sensitivity without affecting body fat levels (Table 1).

Moreover, alcohol-consuming mice had higher serum levels of estrogen and leptin (Table 2). Studies by others show that estrogen and leptin, which are involved in energy homeostasis, are capable of affecting insulin sensitivity [29,30]. Estrogen and leptin are positively associated with PI3K/Akt signaling pathway [29,30]. It is possible that alcohol increases estrogen and/or leptin levels which activate the insulin signaling pathway, specifically PI3K/Akt, to initiate glucose uptake. Activation of this pathway leads to the translocation of glucose transporter-4 (GLUT-4) to the cell membrane, where it acts as a channel allowing glucose to enter the cell from the blood [31]. Feng et al. reported that ethanol-consuming rats had enhanced GLUT-4 expression in adipose tissue [32]. Regarding breast cancer, activation of the PI3K/Akt signaling pathway is associated with increased breast cancer development [33]. Activation of PI3K/Akt in breast cancer cells leads to increased cell growth, increased survival, increased metastasis, and a more aggressive breast cancer phenotype [34]. Thus, it is possible that alcohol affects breast cancer by increasing the sensitivity of the cancer cells to hormones such as insulin or estrogen.

It is unclear how alcohol consumption promotes breast cancer progression, although it has been suggested that alcohol may affect breast cancer risk by: 1) inducing DNA damage through reactive oxygen species (ROS) and acetaldehyde, a toxic ethanol metabolite [35], or 2) increasing the production of hormones such as estrogen, which can promote tumor growth and metastases [2,36,37]. Our hypothesis is that alcohol affects breast cancer progression by increasing systemic hormone levels (e.g., estrogen, leptin) and increasing the sensitivity of breast cancer cells to the effects of these hormones. Our results support the hypothesis in that alcohol consumption sensitizes mice to insulin; this is reflected by faster glucose clearance in alcohol-consuming mice after insulin injection [20]. Sensitizing tissues such as adipose tissue to the effects of insulin by alcohol is beneficial to prevent insulin resistance and eventual type-2 diabetes. However, if alcohol sensitizes mammary cancer cells to growth factors (e.g., estrogen, insulin), then the tumors of alcohol-consuming mice will grow at a faster rate. In fact, our results showed that alcohol-consuming mice developed larger mammary tumors earlier than water-consuming mice.

Alcohol may influence breast cancer progression by way of estrogen, leptin, insulin, or IGF-1, which are known growth factors of breast cancer [38]. Hormones such as estrogen and leptin have been found to influence the growth of breast cancer cells during *in vitro* studies [39]. Moreover, alcohol consumption has been shown to increase the systemic levels of estrogen and leptin, further implicating them in the modulation of breast cancer risk [4,38]. In our studies, alcohol consumption significantly increased systemic estrogen and leptin levels. Elevated estrogen levels are considered to be a risk factor for breast cancer [40]. Therefore, it is possible that alcohol increases breast cancer risk by increasing the production of estrogen. Also, others have shown that leptin increases the growth and invasiveness of breast cancer cells [19,41]. Leptin can also affect breast cancer cells through an indirect mechanism by up-regulating aromatase activity and promoting breast cancer development through increased estrogen levels in the mammary gland [13]. It is feasible that alcohol increases the production of estrogen and leptin and, at the same time, sensitizes breast cancer cells to the effects of these hormones. Our *in vitro* results support this notion. Alcohol increased the aggressiveness of breast cancer cells to invade in a dose-dependent manner, and estrogen treatment slightly increased the invasiveness of the cancer cells; however, the combination of alcohol and estrogen intensified the ability of the cancer cells to invade more than either treatment alone. In future experiments, we will test the hypothesis that alcohol promotes breast cancer development by sensitizing breast cancer cells to hormones such as estrogen and leptin.

In summary, we propose that by investigating the beneficial and deleterious effects of alcohol simultaneously, the mechanism by which alcohol affects breast cancer may be elucidated. We showed that alcohol has a beneficial effect, in that it increased insulin sensitivity, and a

deleterious effect, in that it promoted tumor growth. We also showed that alcohol consumption increased serum levels of leptin and estrogen. *In vitro* experiments showed alcohol increased the invasive ability of breast cancer cells in a dose-dependent manner. Moreover, the combination of alcohol and estrogen intensified the ability of the cancer cells to invade more than either treatment alone.

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References

1. ACS. Breast Cancer Facts & Figures 2009-2010. American Cancer Society; Atlanta: 2009. p. 1-36.
2. Singletary KW, Gapstur SM. Alcohol and breast cancer: review of epidemiologic and experimental evidence and potential mechanisms. *JAMA* 2001;286:2143-51. [PubMed: 11694156]
3. Boffetta P, Hashibe M, La Vecchia C, Zatonski W, Rehm J. The burden of cancer attributable to alcohol drinking. *Int J Cancer* 2006;119:884-7. [PubMed: 16557583]
4. Smith-Warner SA, Spiegelman D, Yaun SS, van den Brandt PA, Folsom AR, Goldbohm RA, Graham S, Holmberg L, Howe GR, Marshall JR, Miller AB, Potter JD, Speizer FE, Willett WC, Wolk A, Hunter DJ. Alcohol and breast cancer in women: a pooled analysis of cohort studies. *JAMA* 1998;279:535-40. [PubMed: 9480365]
5. Weiss HA, Brinton LA, Brogan D, Coates RJ, Gammon MD, Malone KE, Schoenberg JB, Swanson CA. Epidemiology of in situ and invasive breast cancer in women aged under 45. *Br J Cancer* 1996;73:1298-305. [PubMed: 8630296]
6. Stoll BA. Alcohol intake and late-stage promotion of breast cancer. *Eur J Cancer* 1999;35:1653-8. [PubMed: 10674009]
7. Vaeth PA, Satariano WA. Alcohol consumption and breast cancer stage at diagnosis. *Alcohol Clin Exp Res* 1998;22:928-34. [PubMed: 9660324]
8. Ke Z, Lin H, Fan Z, Cai TQ, Kaplan RA, Ma C, Bower KA, Shi X, Luo J. MMP-2 mediates ethanol-induced invasion of mammary epithelial cells over-expressing ErbB2. *Int J Cancer* 2006;119:8-16. [PubMed: 16450376]
9. Luo J. Role of matrix metalloproteinase-2 in ethanol-induced invasion by breast cancer cells. *J Gastroenterol Hepatol* 2006;21(Suppl 3):S65-8. [PubMed: 16958676]
10. Koppes LL, Dekker JM, Hendriks HF, Bouter LM, Heine RJ. Moderate alcohol consumption lowers the risk of type 2 diabetes: a meta-analysis of prospective observational studies. *Diabetes Care* 2005;28:719-25. [PubMed: 15735217]
11. Davies MJ, Baer DJ, Judd JT, Brown ED, Campbell WS, Taylor PR. Effects of moderate alcohol intake on fasting insulin and glucose concentrations and insulin sensitivity in postmenopausal women: a randomized controlled trial. *JAMA* 2002;287:2559-62. [PubMed: 12020337]
12. Greenfield JR, Samaras K, Jenkins AB, Kelly PJ, Spector TD, Campbell LV. Moderate alcohol consumption, estrogen replacement therapy, and physical activity are associated with increased insulin sensitivity: is abdominal adiposity the mediator? *Diabetes Care* 2003;26:2734-40. [PubMed: 14514572]
13. Roth MJ, Baer DJ, Albert PS, Castonguay TW, Dorgan JF, Dawsey SM, Brown ED, Hartman TJ, Campbell WS, Giffen CA, Judd JT, Taylor PR. Relationship between serum leptin levels and alcohol consumption in a controlled feeding and alcohol ingestion study. *J Natl Cancer Inst* 2003;95:1722-5. [PubMed: 14625264]
14. Huang Z, Sjöholm A. Ethanol acutely stimulates islet blood flow, amplifies insulin secretion, and induces hypoglycemia via nitric oxide and vagally mediated mechanisms. *Endocrinology* 2008;149:232-6. [PubMed: 17916634]
15. Nunez NP, Carter PA, Meadows GG. Alcohol consumption promotes body weight loss in melanoma-bearing mice. *Alcohol Clin Exp Res* 2002;26:617-26. [PubMed: 12045469]

16. Dorgan JF, Baer DJ, Albert PS, Judd JT, Brown ED, Corle DK, Campbell WS, Hartman TJ, Tejpar AA, Clevidence BA, Giffen CA, Chandler DW, Stanczyk FZ, Taylor PR. Serum hormones and the alcohol-breast cancer association in postmenopausal women. *J Natl Cancer Inst* 2001;93:710–5. [PubMed: 11333294]
17. Gavalier JS, Rosenblum E. Exposure-dependent effects of ethanol on serum estradiol and uterus mass in sexually mature oophorectomized rats: a model for bilaterally ovariectomized-postmenopausal women. *J Stud Alcohol* 1987;48:295–303. [PubMed: 3613579]
18. Ginsburg ES, Walsh BW, Shea BF, Gao X, Gleason RE, Barbieri RL. The effects of ethanol on the clearance of estradiol in postmenopausal women. *Fertil Steril* 1995;63:1227–30. [PubMed: 7750592]
19. Sulkowska M, Golaszewska J, Wincewicz A, Koda M, Baltaziak M, Sulkowski S. Leptin--from regulation of fat metabolism to stimulation of breast cancer growth. *Pathol Oncol Res* 2006;12:69–72. [PubMed: 16799705]
20. Hong J, Smith RR, Harvey AE, Nunez NP. Alcohol consumption promotes insulin sensitivity without affecting body fat levels. *Int J Obes (Lond)* 2009;33:197–203. [PubMed: 19125162]
21. Zilkens RR, Burke V, Watts G, Beilin LJ, Puddey IB. The effect of alcohol intake on insulin sensitivity in men: a randomized controlled trial. *Diabetes Care* 2003;26:608–12. [PubMed: 12610009]
22. Borowsky AD, Namba R, Young LJ, Hunter KW, Hodgson JG, Tepper CG, McGoldrick ET, Muller WJ, Cardiff RD, Gregg JP. Syngeneic mouse mammary carcinoma cell lines: two closely related cell lines with divergent metastatic behavior. *Clin Exp Metastasis* 2005;22:47–59. [PubMed: 16132578]
23. Williamson DF, Forman MR, Binkin NJ, Gentry EM, Remington PL, Trowbridge FL. Alcohol and body weight in United States adults. *Am J Public Health* 1987;77:1324–30. [PubMed: 3498373]
24. Liu S, Serdula MK, Williamson DF, Mokdad AH, Byers T. A prospective study of alcohol intake and change in body weight among US adults. *Am J Epidemiol* 1994;140:912–20. [PubMed: 7977278]
25. Greenfield JR, Samaras K, Jenkins AB, Kelly PJ, Spector TD, Campbell LV. Moderate alcohol consumption, dietary fat composition, and abdominal obesity in women: evidence for gene-environment interaction. *J Clin Endocrinol Metab* 2003;88:5381–6. [PubMed: 14602777]
26. Wannamethee SG, Shaper AG, Whincup PH. Alcohol and adiposity: effects of quantity and type of drink and time relation with meals. *Int J Obes (Lond)* 2005;29:1436–44. [PubMed: 1607718]
27. Wannamethee SG, Field AE, Colditz GA, Rimm EB. Alcohol intake and 8-year weight gain in women: a prospective study. *Obes Res* 2004;12:1386–96. [PubMed: 15483203]
28. Shah M, Jeffery RW, Hannan PJ, Onstad L. Relationship between socio-demographic and behaviour variables, and body mass index in a population with high-normal blood pressure: Hypertension Prevention Trial. *Eur J Clin Nutr* 1989;43:583–96. [PubMed: 2606089]
29. Cirillo D, Rachiglio AM, la Montagna R, Giordano A, Normanno N. Leptin signaling in breast cancer: an overview. *J Cell Biochem* 2008;105:956–64. [PubMed: 18821585]
30. Riant E, Waget A, Cogo H, Arnal JF, Burcelin R, Gourdy P. Estrogens protect against high-fat diet-induced insulin resistance and glucose intolerance in mice. *Endocrinology* 2009;150:2109–17. [PubMed: 19164473]
31. Saltiel AR, Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* 2001;414:799–806. [PubMed: 11742412]
32. Feng L, Gao L, Guan Q, Hou X, Wan Q, Wang X, Zhao J. Long-term moderate ethanol consumption restores insulin sensitivity in high-fat-fed rats by increasing SLC2A4 (GLUT4) in the adipose tissue by AMP-activated protein kinase activation. *J Endocrinol* 2008;199:95–104. [PubMed: 18719073]
33. Perez-Tenorio G, Stal O. Activation of AKT/PKB in breast cancer predicts a worse outcome among endocrine treated patients. *Br J Cancer* 2002;86:540–5. [PubMed: 11870534]
34. Stemke-Hale K, Gonzalez-Angulo AM, Lluch A, Neve RM, Kuo WL, Davies M, Carey M, Hu Z, Guan Y, Sahin A, Symmans WF, Pusztai L, Nolden LK, Horlings H, Berns K, Hung MC, van de Vijver MJ, Valero V, Gray JW, Bernards R, Mills GB, Hennessy BT. An integrative genomic and proteomic analysis of PIK3CA, PTEN, and AKT mutations in breast cancer. *Cancer Res* 2008;68:6084–91. [PubMed: 18676830]
35. Dumitrescu RG, Shields PG. The etiology of alcohol-induced breast cancer. *Alcohol* 2005;35:213–25. [PubMed: 16054983]

36. Aye MM, Ma C, Lin H, Bower KA, Wiggins RC, Luo J. Ethanol-induced in vitro invasion of breast cancer cells: the contribution of MMP-2 by fibroblasts. *Int J Cancer* 2004;112:738–46. [PubMed: 15386367]
37. Lew JQ, Freedman ND, Leitzmann MF, Brinton LA, Hoover RN, Hollenbeck AR, Schatzkin A, Park Y. Alcohol and Risk of Breast Cancer by Histologic Type and Hormone Receptor Status in Postmenopausal Women: The NIH-AARP Diet and Health Study. *Am J Epidemiol.* 2009
38. Wayne S, Neuhouser ML, Ulrich CM, Koprowski C, Wiggins C, Baumgartner KB, Bernstein L, Baumgartner RN, Gilliland F, McTiernan A, Ballard-Barbash R. Association between alcohol intake and serum sex hormones and peptides differs by tamoxifen use in breast cancer survivors. *Cancer Epidemiol Biomarkers Prev* 2008;17:3224–32. [PubMed: 18957523]
39. O’Neil JS, Burow ME, Green AE, McLachlan JA, Henson MC. Effects of estrogen on leptin gene promoter activation in MCF-7 breast cancer and JEG-3 choriocarcinoma cells: selective regulation via estrogen receptors alpha and beta. *Mol Cell Endocrinol* 2001;176:67–75. [PubMed: 11369444]
40. Suzuki R, Ye W, Rylander-Rudqvist T, Saji S, Colditz GA, Wolk A. Alcohol and postmenopausal breast cancer risk defined by estrogen and progesterone receptor status: a prospective cohort study. *J Natl Cancer Inst* 2005;97:1601–8. [PubMed: 16264180]
41. Caldefie-Chezet F, Damez M, de Latour M, Konska G, Mishellani F, Fusillier C, Guerry M, Penault-Llorca F, Guillot J, Vasson MP. Leptin: a proliferative factor for breast cancer? Study on human ductal carcinoma. *Biochem Biophys Res Commun* 2005;334:737–41. [PubMed: 16009333]

Figure 1. Insulin sensitivity in water- and alcohol-consuming mice

(A) Glucose tolerance test (GTT) of mice consuming either water or 20% alcohol at week 16 (10 mice/group). The graph portrays blood glucose levels at baseline, 15, 30, 60, and 120 minutes after glucose injection. Water-consuming mice are represented by closed diamonds; alcohol-consuming mice by open squares. (B) HOMA values. *Indicates a significant difference between water- and alcohol-consuming mice ($p < 0.05$).

Figure 2. Tumor development in water-consuming and alcohol-consuming mice

Mice were injected with Met-1 mammary cancer cells subcutaneously. (A) Percent palpable tumors were calculated every 2 or 4 days after cancer cell injection. (B) Tumor volume was determined every 2 or 4 days beginning 2 weeks after cancer cell injection. *Significant difference between water- and alcohol-consuming mice ($p < 0.05$).

Figure 3. Ethanol treatment and invasion assay in vitro

(A) In vitro, alcohol consumption increases the invasive phenotype of T47D human breast cancer cells in a dose-dependent manner (0%, 0.1%, 0.2%, or 0.5% ethanol). *Significant difference from control ($p < 0.05$) and **significant difference from other groups ($p < 0.05$). (B) Estrogen increases the invasiveness of T47D cells; however, the alcohol-estrogen combination increases the invasive ability more than either treatment alone. *Significant difference from control ($p < 0.05$) and **significant difference from control and from 20nM E2 treatment ($p < 0.05$). The images shown are pictures of cells that invaded during the 24-hr assay; images were captured at 20x magnification after staining.

Table I

Final body composition and total calorie consumption in water or alcohol consuming mice

	Group		<i>p value</i>
	Water	Alcohol	
Body weight (g)	22 ± 0.6	23 ± 0.5	<i>P</i> >0.05
Body fat (%)	24 ± 0.5	24 ± 0.4	<i>P</i> >0.05
Lean mass (%)	76 ± 0.5	76 ± 0.5	<i>P</i> >0.05
BMD (g/cm ²)	0.0725 ± 0.00196	0.0513 ± 0.00046	<i>P</i> <0.05
Food (kcal)	12.8 ± 0.46	11.5 ± 0.42	<i>P</i> >0.05
Alcohol (kcal)		5.4 ± 0.22	
Total energy intake (kcal)	12.8 ± 0.46	16.9 ± 0.43	<i>P</i> <0.05

Values represent the mean of 30 mice in the water and 35 mice in the alcohol group (± SEM).

Values determined at the end of the study.

Table II

Hormone levels and blood alcohol concentration in water or alcohol consuming mice

	Group		<i>p value</i>
	Water	Alcohol	
HOMA	26.4 ± 2.69	13.8 ± 1.15	<i>P</i> <0.05
Glucose (mg/dl)	202 ± 13.5	135 ± 9.5	<i>P</i> <0.05
Insulin (ng/ml)	19.7 ± 1.25	16.0 ± 1.03	<i>P</i> >0.05
Leptin (pg/ml)	1521 ± 210	2326 ± 256	<i>P</i> <0.05
IGF-1 (pg/ml)	760 ± 29.8	685 ± 35.2	<i>P</i> >0.05
Estradiol (pg/ml)	14.1 ± 0.50	34.6 ± 6.48	<i>P</i> <0.05
Blood alcohol (mg/dl)	0.0 ± 0.00	79.5 ± 7.81	<i>P</i> <0.01

Values represent the mean of 15 mice in each group (± SEM).

Values determined at the end of the study.