Journal of Molecular Pathophysiology
www.scopemed.org
DOI: 10.5455/jmp.20140526044640

Hypothesis

GESDAV

A potential pathogenic mechanism linking obesity, breast cancer and estrogen receptor expression

David Pisani[1], Etienne Mark Paris[1], Gordon Caruana Dingli[1,2]

[1]Department of Surgery (Breast Clinic), Mater Dei Hospital, Triq Dun Karm, Msida, Malta, [2]Department of Medicine University of Malta Medical School, Malta

Address for correspondence:
Dr. David Pisani,
E-mail: pisanidavid@gmail.com

Received: March 17, 2014
Accepted: May 26, 2014
Published: September 07, 2014

ABSTRACT

Obesity is a growing problem in the Western world and has been strongly linked to post-menopausal breast cancer. After menopause, adipose tissue assumes a major role in estrogen production, primarily because it expresses aromatase, which is critical in estrogen synthesis. Obese women have higher levels of circulating estrogen and lower levels of sex hormone-binding globulin. Free estrogen can promote fat deposition in the breast, which further increases local breast estrogen production. Estrogen promotes ductal proliferation by interacting with intracellular receptors, especially estrogen receptor-α (ER-α), but also through a host of membrane receptors like G protein-coupled receptor 30, which terminate on cyclin D1 activation, amongst other signals. Supplemental estrogen for this process can be provided by local macrophages and stromal cells. Excessive ductal proliferation is associated with accumulation of mutations in various genes, including *HER2/neu*, *DIRAS3*, *TP53* and *ESR1*, which promote aberrant cell proliferation. This process is aided by genotoxic estrogen metabolites and by increased enterohepatic circulation of estrogen byproducts. In addition, hyperestrogenemia inhibits ubiquitin-mediated receptor degradation, hence promoting ER expression in tumor cell lines. Other adipocytokines, like leptin, interleukin-6, adiponectin and insulin, have also been linked to breast cancer, with several molecular cross-interactions with ER signaling pathways.

KEY WORDS: Adipocytokine, adipose breast cancer, estrogen, estrogen receptor

INTRODUCTION

Obesity is an ever-growing problem owing to a lifestyle poor in physical activity and rich in carbohydrate and lipid-laden food. Obesity has been linked to numerous malignancies, including colon cancer, endometrial cancer, renal cell carcinoma, esophageal adenocarcinoma and post-menopausal breast cancer, to such an extent that current data suggests that in non-smokers, obesity is the most important avoidable cause of cancer [1,2]. In the context of breast malignancy, adipose tissue provides a wealth of hormones that help in sustaining tumor growth.

ESTROGEN SYNTHESIS AND ACTION

In premenopausal women, the production of estrogen is largely the function of the ovaries, which are under the control of pituitary-derived gonadotropin-releasing hormone, follicle-stimulating hormone and luteinizing hormone. These in turn stimulate oögenesis in the ovary, with the granulosa cell acting as the main center for estrogen production, synthesizing this from cholesterol-derived androstendione produced by the theca interna [3,4]. This C-19 to C-18 steroid conversion is mediated by aromatase; a p450 heme-protein derived from transcription of the *CYP19* gene [4,5]. Aromatase converts androstendione to estrone (E1), which can be subsequently converted to 17 β-estradiol (E2) through the enzyme 17 β-hydroxysteroid dehydrogenase. The third main estrogen, estriol (E3), is synthesized largely during pregnancy by the placenta, through a dehydroepiandrosterone sulfate (DHEA-S) intermediate produced in the fetal adrenal gland. Aromatase is, once again, involved in the conversion of DHEA-S to E3. In the non-pregnant female, E1 levels are the lowest of the three main estrogens, while those of E2, which is the main active hormone, are the highest [3].

In postmenopausal women, the ovaries cease production of estrogen and adipose tissue, together with other tissues, assumes a major role in estrogen secretion. Adipose tissue is well adapted for such roles, as it is rich in aromatase [4,5]. Estrogen levels have been shown to increase proportionally with increasing adipose body mass [4,6], with high-body mass index patients having significantly higher levels of free estrone and free estradiol when compared to non-obese patients [7]. Furthermore, obesity is associated with reduced levels of sex hormone-binding globulin, which results in higher levels of free, bioactive, circulating estrogen level [8].

Both estrogens and androgens promote the deposition of adipose tissue in the hypodermis. However, while androgens promote selective deposition in the abdominal region

(i.e. central obesity), estrogens promote selective deposition in breast tissue and thighs [9]. Hence, one can propose a positive feedback system, which propagates further local breast estrogen synthesis in obese patients, as more adipose tissue deposits with further rising estrogen levels.

Estrogen acts through a host of cellular mechanisms. The estrogen receptors (ERs) are a functionally diverse class of proteins, which belong to the nuclear receptor superfamily [10]. The receptors function as ligand-dependent nuclear transcription factors. Two forms of the receptor have been identified; referred to as the alpha and beta variants (ER-α and ER-β respectively). Both receptors have similar structures, being composed of six domains, labeled A through F. Domains A and B are able to weakly activate gene transcription in the absence of bound ligand, allowing ligand-independent basal transcription rates. The C domain is a deoxyribonucleic acid (DNA)-binding domain that binds to estrogen-response elements in the genome. Domains E and F comprise the ligand-binding domain, which binds to estradiol, together with coactivators and corepressors. The D domain is a hinge region [3]. Once the receptor is bound to ligand, the receptor dimerizes to form homodimers or heterodimers (ER-α/α, ER-β/β or ER-α/β) [11]. The classically described physiological pathway for estrogen cellular activity is that the estrogen receptor dimerizes when bound to ligand and migrates to the nucleus to promote or repress transcription of specific estrogen-response elements. The transcription process takes hours to days to completely fulfill [12].

MOLECULAR LINKS BETWEEN ESTROGEN AND BREAST CANCER

Breast cancer cells often exhibit hormone-dependent growth, with ERs being expressed in over 50% of tumors [13]. The ER-α variant seems to be very important in the pathogenesis of breast cancer, however, the function of the ER-β form is poorly understood, although many cancers express both receptor variants [14]. One experiment demonstrated that activating expression the ER-β variant in an ER-α-producing cell line actually suppresses of tumor growth [15].

The mechanisms behind the ER and breast cancer proliferation must lie in an aberration of the normal physiology behind hormone-mediated duct proliferation. Estrogen acts in concordance with other hormones to mediate its effect on breast tissue, with growth hormone and insulin-like growth factor-1 (IGF-1) being the main concordant hormone axis involved. ER-α stimulates growth through a host of mechanisms which terminate on the epidermal growth factor receptor (EGFR). The main postulated mechanism is that activated ER-α stimulates A disintegrin and metalloproteinase (ADAM)-17 on the membrane of mammary epithelial cells, which promotes cleavage of membrane-bound pro-amphiregulin to amphiregulin. The latter stimulates EGFR on nearby stromal cells. Poorly characterized downstream EGFR signals, including fibroblast growth factor (FGF) and matrix metalloproteinase Type II (MMP2) are thus activated [16-18]. FGF, together with other factors, can promote activation of cyclin D1 promoting G_1-to-S-phase transition during cell division [19]. Moreover, MMP2, which is a gelatinase degrading Type IV collagen, is important in stromal loosening, allowing tubular epithelium to branch and invade surrounding stroma [20].

The initiating insult for breast cancer, as for many other malignancies, remains poorly characterized. However, increased estrogen concentrations in obesity may work indirectly by promoting accelerated cellular division, which may, in itself, compromise the integrity of cellular DNA, resulting in an accumulation of mutations, hence promoting carcinogenesis. Other unclear mechanisms have linked a hyperestrogenemic state with decreased rates of ubiquitin-mediated receptor degradation [21], hence allowing sustained estrogen receptivity in tumors without the expected cellular receptor desensitization. Various somatic mutations have been described in breast cancer, including mutations in the *HER2/neu* gene, which in itself encodes an EGFR and which is overexpressed in up to 30% of breast cancers [22], *DIRAS3*, which encodes a G-protein-coupled receptor (GPR) normally involved in down-regulating cellular growth signals [23], and *TP53*, which encodes p53, an important tumor suppressor gene which controls of cell growth and DNA integrity [24]. Other mutations have also been described in the ER itself. Mutations in the *ESR1* gene (6q25.1), which codes for ER-α, can greatly increase the affinity of the receptor for estrogen and can, in rare cases, promote estrogen-independent signaling. Amongst these mutations are the *Pvu*II and *Xba*I restriction site polymorphisms, which involve mutations in the first intron of the gene. Mutations in the promoter region can also result in gene amplification [25]. In one study, analysis of the *ESR1* gene in 118 receptor-positive cases revealed several nucleotide polymorphisms, which implies that mutations in the ER seems to play a key role in the pathogenesis of some forms of breast cancer [26].

The importance of estrogen in the pathogenesis of breast cancer seems so great that several breast cancer cells have been found to secrete preproopiomelanocortin (POMC). POMC can be broken down to adrenocorticotropic hormone, which can subsequently influence the adrenocortical zona fasciculata and reticularis to release sex steroids [27]. Moreover, it has been shown that the macrophages and lymphocytes that infiltrate breast tissue also express high levels of aromatase [4,28-30]. These macrophages can produce interleukin-1 (IL-1), which further promotes expression of the enzyme [31]. This local estrogen is probably even more important than circulating estrogen in pathogenesis, as it stimulates cancer cells in a paracrine fashion [29].

The classical ER is not, however, the only receptor involved in estrogen-mediated breast cancer pathogenesis. Various other receptors have been implicated, including the protein G protein-coupled receptor 30 (GPR30), which is a G-protein-coupled, membrane-bound ER, which stimulates the phosphatidylinositol (PI3K) cascade when triggered, part of which involves activation of cyclin D1 [32,33], Increased PI3K expression is a feature of the pathogenesis in many forms of human breast cancer [34]. The role of this receptor seems to be particularly high in cases of triple-negative breast cancer, where GPR30 is often overexpressed, and mediates estrogen-dependent growth [35]. This receptor has thus provided a novel target in the treatment of triple-negative breast cancer.

The effects of estrogen on breast cancer are also present by virtue of its unique metabolism. Both E1 and E2 are metabolized in the liver to 2- and 4-hydroxyestradiol through a host of cytochrome complexes. These compounds are subsequently eliminated by virtue of sulfation and glucuronidation, either directly or indirectly via conversion to 2- and 4-hydroxyestradiol intermediates. Sulfated and glucuronidated estrogens are subsequently eliminated in bile [3]. However, 4-methoxyestradiol in particular, can also be metabolized to a host of quinone derivatives, particularly 3,4-quinones. These metabolic byproducts can integrate with DNA, namely adenine and guanine nucleotide residues. These adducts are either depurinated or result in inappropriate base pairing during DNA synthesis [36,37]. Novel mechanisms have also implicated the importance of gut microflora in the pathogenesis of breast cancer. Certain gut microorganisms interact with the aforementioned metabolic pathway by deconjugating and desulfating estrogen metabolic products, hence releasing free lipophilic estrogen derivatives that can be reabsorbed as part of the enterohepatic circulation [38]. Moreover, it has been shown that certain gut microbes can synthesize estrogens from bile steroids, the bowel concentration of which is directly proportional to oral intake of lipids [39].

OTHER ADIPOCYTOKINES AND BREAST CANCER

It is highly unlikely that estrogen is the only link between obesity and breast cancer. Adipose tissue produces a host of other substances, referred to as adipocytokines, each of which can play a role in carcinogenesis.

Leptin, which is coded for by the *ob* gene, is important in the control of satiety. Studies have shown that both leptin and the leptin receptor are overexpressed in breast cancer tissue. Binding of leptin with its receptor activates both mitogen-activated protein kinase (MAPK) and PI3K signaling pathways, which are highly mitogenic in breast cancer tumour cell lines [40]. Leptin has been shown to increase the expression of ER-α, hence potentiating the effects of estrogen on breast cancer [41]. It also promotes the expression of local aromatase, which increases estrogen concentration [42]. Moreover, crosstalk has been demonstrated between the leptin receptor and the HER2/neu receptor, with the leptin receptor being able to transactivate the HER2/neu receptor, promoting epidermal growth factor-independent signaling [41].

IL-6 is another adipocytokine that is produced in high concentrations in obese individuals. IL-6 works in a similar fashion to IL-1, promoting the synthesis of aromatase in macrophages and other white cells in breast cancer tissue [30]. IL-6 also increases the transcription rate of ER-α in breast cancer cells [43], acting similar to leptin in this respect. In addition, IL-6 reduces the expression of the cell adhesion molecules E-cadherin, Twist and Snail in ER positive breast cancer cells, which seems to be important in the pathogenesis of breast cancer metastasis [44]. This may, in part, explain the poorer prognosis in obese women [7].

Interestingly, adipose tissue also produces an anti-neoplastic hormone, referred to as adiponectin. Breast cancer cells express both AdipoR1 and AdipoR2 receptors and binding of adiponectin to these receptors is associated with decreased levels of cyclin A2 and activation of BAX-induced apoptosis. However, estradiol has been found to suppress expression of both receptors, hence increasing the malignant potential of breast tumour cell lines [45].

The importance of insulin in breast cancer is less clearly understood. Many studies have associated obesity with increased insulin resistance and thus, increased basal levels of insulin [46]. It has been found that insulin has potent effects on the proliferation of estrogen-dependent breast cancer cells. Crosstalk between estrogen and insulin signaling pathways has been demonstrated [47]. Interactions between the insulin-insulin receptor complex and unliganded ER-α are known to occur. These interactions are associated with ligand-independent activation of the ER-α receptor [48]. Furthermore, insulin has been shown to act as a mitogen, binding avidly to tumor cells and promoting the phosphorylation of MAPK and potently activating the Sonic Hedgehog (Shc) pathway. The latter pathway stimulates the enzymes farnesyl transferase and geranylgeranyl transferase. These enzymes are involved in transferring the appropriate lipid residues (i.e. a farnesyl or a geranylgeranyl residue) to proteins like p21. Prenylation of p21 is associated with increased cellular oncogenic activity [49]. Insulin can also act by increasing the local production of vascular endothelial growth factor in breast cancer tissue, which promotes neoangiogenesis and vascularization of the tumor. This can, in turn, increase the chance of hematogenous metastasis. Moreover, insulin can promote the production of hepatic insulin-like growth factor 1 [50], which binds to overexpressed IGF-1R receptors on breast cancer cells. These are receptor tyrosine kinases which autophosphorylate upon binding to IGF-1, subsequently activating downstream signaling events that are involved in cell division. Moreover, crosstalk has also been demonstrated between IGF-1R and the ER [51].

CONCLUSION

Overall, it can thus be seen that estrogen plays important roles in carcinogenesis. So critical is estrogen in the pathogenesis that this has manifested itself as a difference in therapy between obese and normal weight individuals, with one study demonstrating that 26% of obese individuals receive endocrine chemotherapy, as opposed to only 13% of normal weight patients [52]. Clinically, a loss in body weight by 10% has been shown to reduce the serum concentrations of estrogen, leptin, insulin and pro-inflammatory cytokines and to increase the concentration of sex hormone-binding globulin and adiponectin [53,54], which may be of significant benefit in those with ER positive malignancies.

REFERENCES

1. Calle EE, Thun MJ. Obesity and cancer. Oncogene 2004;23:6365-78.
2. Abu-Abid S, Szold A, Klausner J. Obesity and cancer. J Med 2002;33:73-86.
3. Nilsson S, Mäkelä S, Treuter E, Tujague M, Thomsen J,

Andersson G, *et al.* Mechanisms of estrogen action. Physiol Rev 2001;81:1535-65.
4. Nelson LR, Bulun SE. Estrogen production and action. J Am Acad Dermatol 2001;45:S116-24.
5. Ronti T, Lupattelli G, Mannarino E. The endocrine function of adipose tissue: An update. Clin Endocrinol (Oxf) 2006;64:355-65.
6. Sue L, Fuhrman B, Xu X, Gail MH, Falk RT, Wu AH, *et al.* Relationships between body mass index, endogenous estrogen levels, and patterns of estrogen metabolism in Asian-American women. FASEB J 2009;23:551.33.
7. McTiernan A, Rajan KB, Tworoger SS, Irwin M, Bernstein L, Baumgartner R, *et al.* Adiposity and sex hormones in postmenopausal breast cancer survivors. J Clin Oncol 2003;21:1961-6.
8. Hautanen A. Synthesis and regulation of sex hormone-binding globulin in obesity. Int J Obes Relat Metab Disord 2000;24 Suppl 2:S64-70.
9. Björntorp P. The regulation of adipose tissue distribution in humans. Int J Obes Relat Metab Disord 1996;20:291-302.
10. Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schütz G, Umesono K, *et al.* The nuclear receptor superfamily: The second decade. Cell 1995;83:835-9.
11. Nilsson S, Mäkelä S, Treuter E, Tujague M, Thomsen J, Andersson G, *et al.* Mechanisms of estrogen action. Physiol Rev 2001;81:1535-65.
12. Micevych P, Kuo J, Christensen A. Physiology of membrane oestrogen receptor signalling in reproduction. J Neuroendocrinol 2009;21:249-56.
13. Ali S, Coombes RC. Estrogen receptor alpha in human breast cancer: Occurrence and significance. J Mammary Gland Biol Neoplasia 2000;5:271-81.
14. Gross JM, Yee D. How does the estrogen receptor work? Breast Cancer Res 2002;4:62-4.
15. Paruthiyil S, Parmar H, Kerekatte V, Cunha GR, Firestone GL, Leitman DC. Estrogen receptor beta inhibits human breast cancer cell proliferation and tumor formation by causing a G2 cell cycle arrest. Cancer Res 2004;64:423-8.
16. Lubarsky B, Krasnow MA. Tube morphogenesis: Making and shaping biological tubes. Cell 2003;112:19-28.
17. Sternlicht MD. Key stages in mammary gland development: The cues that regulate ductal branching morphogenesis. Breast Cancer Res 2006;8:201.
18. LaMarca HL, Rosen JM. Estrogen regulation of mammary gland development and breast cancer: Amphiregulin takes center stage. Breast Cancer Res 2007;9:304.
19. Yang C, Trent S, Ionescu-Tiba V, Lan L, Shioda T, Sgroi D, *et al.* Identification of cyclin D1- and estrogen-regulated genes contributing to breast carcinogenesis and progression. Cancer Res 2006;66:11649-58.
20. Nakopoulou L, Tsirmpa I, Alexandrou P, Louvrou A, Ampela C, Markaki S, *et al.* MMP-2 protein in invasive breast cancer and the impact of MMP-2/TIMP-2 phenotype on overall survival. Breast Cancer Res Treat 2003;77:145-55.
21. Miyoshi Y, Murase K, Saito M, Imamura M, Oh K. Mechanisms of estrogen receptor-a upregulation in breast cancers. Med Mol Morphol 2010;43:193-6.
22. Mitri Z, Constantine T, O'Regan R. The HER2 Receptor in Breast Cancer: Pathophysiology, Clinical Use, and New Advances in Therapy. Chemother Res Pract 2012;2012:743193.
23. Zou CF, Jia L, Jin H, Yao M, Zhao N, Huan J, *et al.* Re-expression of ARHI (DIRAS3) induces autophagy in breast cancer cells and enhances the inhibitory effect of paclitaxel. BMC Cancer 2011;11:22.
24. Gasco M, Shami S, Crook T. The p53 pathway in breast cancer. Breast Cancer Res 2002;4:70-6.
25. Jakimiuk A, Nowicka M, Bogusiewicz M, Adamiak A, Skorupski P, Miotla P, *et al.* Prevalence of estrogen receptor alpha PvuII and XbaI polymorphism in population of Polish postmenopausal women. Folia Histochem Cytobiol 2007;45:331-8.
26. Sellers TA. Genetic factors in the pathogenesis of breast cancer: Their role and relative importance. J Nutr 1997;127 5 Suppl:929S-32.
27. Ray A, Ratnakar N, Murthy NS, Sharma BK. Adrenocorticotropic hormone and growth factor receptors in breast cancer. Indian J Exp Biol 2000;38:663-8.
28. Suzuki T, Moriya T, Ishida T, Kimura M, Ohuchi N, Sasano H. In situ production of estrogens in human breast carcinoma. Breast Cancer 2002;9:296-302.
29. Chen S. Aromatase and breast cancer. Front Biosci 1998;3:d922-33.
30. Schmidt M, Kreutz M, Löffler G, Schölmerich J, Straub RH. Conversion of dehydroepiandrosterone to downstream steroid hormones in macrophages. J Endocrinol 2000;164:161-9.
31. Honma S, Shimodaira K, Shimizu Y, Tsuchiya N, Saito H, Yanaihara T, *et al.* The influence of inflammatory cytokines on estrogen production and cell proliferation in human breast cancer cells. Endocr J 2002;49:371-7.
32. O'Dowd BF, Nguyen T, Marchese A, Cheng R, Lynch KR, Heng HH, *et al.* Discovery of three novel G-protein-coupled receptor genes. Genomics 1998;47:310-3.
33. Alvarez B, Garrido E, Garcia-Sanz JA, Carrera AC. Phosphoinositide 3-kinase activation regulates cell division time by coordinated control of cell mass and cell cycle progression rate. J Biol Chem 2003;278:26466-73.
34. Gershtein ES, Scherbakov AM, Shatskaya VA, Kushlinsky NE, Krasil'nikov MA. Phosphatidylinositol 3-kinase/AKT signalling pathway components in human breast cancer: Clinicopathological correlations. Anticancer Res 2007;27:1777-82.
35. Steiman J, Peralta EA, Louis S, Kamel O. Biology of the estrogen receptor, GPR30, in triple negative breast cancer. Am J Surg 2013;206:698-703.
36. Yue W, Wang JP, Li Y, Bocchinfuso WP, Korach KS, Devanesan PD, *et al.* Tamoxifen versus aromatase inhibitors for breast cancer prevention. Clin Cancer Res 2005;11:925S-30.
37. Zahid M, Kohli E, Saeed M, Rogan E, Cavalieri E. The greater reactivity of estradiol-3,4-quinone vs estradiol-2,3-quinone with DNA in the formation of depurinating adducts: Implications for tumor-initiating activity. Chem Res Toxicol 2006;19:164-72.
38. Gorbach SL. Estrogens, breast cancer, and intestinal flora. Rev Infect Dis 1984;6 Suppl 1:S85-90.
39. Hill MJ, Goddard P, Williams RE. Gut bacteria and aetiology of cancer of the breast. Lancet 1971;2:472-3.
40. Frankenberry KA, Skinner H, Somasundar P, McFadden DW, Vona-Davis LC. Leptin receptor expression and cell signaling in breast cancer. Int J Oncol 2006;28:985-93.
41. Fiorio E, Mercanti A, Terrasi M, Micciolo R, Remo A, Auriemma A, *et al.* Leptin/HER2 crosstalk in breast cancer: In vitro study and preliminary in vivo analysis. BMC Cancer 2008;8:305.
42. Sinicrope FA, Dannenberg AJ. Obesity and breast cancer prognosis: Weight of the evidence. J Clin Oncol 2011;29:4-7.
43. Speirs V, Kerin MJ, Walton DS, Newton CJ, Desai SB, Atkin SL. Direct activation of oestrogen receptor-alpha by interleukin-6 in primary cultures of breast cancer epithelial cells. Br J Cancer 2000;82:1312-6.
44. Sullivan NJ. Interleukin-6 as a Potential Mediator of Breast Cancer Progression and Non-Melanoma Skin Carcinogenesis. Thesis, (PhD). Ohio State University; 2009.
45. Pfeiler GH, Buechler C, Neumeier M, Schäffler A, Schmitz G, Ortmann O, *et al.* Adiponectin effects on human breast cancer cells are dependent on 17-beta estradiol. Oncol Rep 2008;19:787-93.
46. Casazza K, Phadke RP, Fernandez JR, Watanabe RM, Goran MI, Gower BA. Obesity attenuates the contribution of African admixture to the insulin secretory profile in peripubertal children: A longitudinal analysis. Obesity (Silver Spring) 2009;17:1318-25.
47. Lanzino M, Morelli C, Garofalo C, Panno ML, Mauro L, Andò S, *et al.* Interaction between estrogen receptor alpha and insulin/IGF signaling in breast cancer. Curr Cancer Drug Targets 2008;8:597-610.
48. Patrone C, Ma ZQ, Pollio G, Agrati P, Parker MG, Maggi A. Cross-coupling between insulin and estrogen receptor in human neuroblastoma cells. Mol Endocrinol 1996;10:499-507.
49. Finlayson CA, Chappell J, Leitner JW, Goalstone ML, Garrity M, Nawaz S, *et al.* Enhanced insulin signaling via Shc in human breast cancer. Metabolism 2003;52:1606-11.
50. Brismar K, Fernqvist-Forbes E, Wahren J, Hall K. Effect of insulin on the hepatic production of insulin-like growth factor-binding protein-1 (IGFBP-1), IGFBP-3, and IGF-I in insulin-dependent diabetes. J Clin Endocrinol Metab 1994;79:872-8.
51. Zhang X, Yee D. Tyrosine kinase signalling in breast cancer: Insulin-like growth factors and their receptors in breast cancer. Breast Cancer Res 2000;2:170-5.
52. Ewertz M, Jensen MB, Gunnarsdóttir KÁ, Højris I, Jakobsen EH, Nielsen D, *et al.* Effect of obesity on prognosis after early-stage

breast cancer. J Clin Oncol 2011;29:25-31.
53. Jen KL, Djuric Z, DiLaura NM, Buison A, Redd JN, Maranci V, *et al.* Improvement of metabolism among obese breast cancer survivors in differing weight loss regimens. Obes Res 2004;12:306-12.
54. Bastard JP, Jardel C, Bruckert E, Blondy P, Capeau J, Laville M, *et al.* Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. J Clin Endocrinol Metab 2000;85:3338-42.

Source of Support: Nil, Conflict of Interest: None declared.