Adverse effects of obesity on breast cancer prognosis, and the biological actions of leptin (Review)

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Abstract. Leptin is a hormone with multiple biological actions which is produced predominantly by adipose tissue; in humans, plasma levels correlate with total body fat, and particularly high concentrations occur in obese women. Several actions of leptin, including the stimulation of normal and tumor cell growth, migration and invasion, and enhancement of angiogenesis, suggest that this hormone can promote an aggressive breast cancer phenotype which can be estrogen-independent. This effect may involve activation of the transcription factor NFκB. Leptin can also induce aromatase activity, with the potential for the promotion of estrogen production from androstenedione in adipose tissue, and hence the stimulation of estrogen-dependent breast cancer progression. On this basis, we hypothesize that leptin, perhaps in association with insulin, the plasma concentrations of which correlate with those of leptin, has an important role in the known adverse effect of obesity on breast cancer.

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1. Introduction

An association between breast cancer and obesity has been recognized for at least 40 years (1), although the relationship is a complex one, and is still not completely understood. Most epidemiological studies found that obesity is a risk factor for postmenopausal breast cancer (2), whereas it has been associated with a reduced risk for breast cancer in premenopausal women (3). Furthermore, obese breast cancer patients are more likely to have tumors that have already spread beyond the primary site at the time of diagnosis, but this local and systemic metastatic disease, and the related reduced breast cancer survival, occur in both premenopausal and postmenopausal overweight women (4).

The mechanisms by which obesity affects breast cancer risk and prognosis continue to be a vigorous area of clinical and basic research. Although the involvement of the estrogens is well described (5), an additional candidate, for which we propose a major role in obesity-mediated breast cancer progression, is leptin. This hormone, which was first identified in 1995 (6,7), has a broad spectrum of biological activities, several of which parallel those known to be involved in cancer cell invasion and metastasis. Moreover, leptin is capable of activating nuclear factor-kappa B (8), a transcription factor which has also been associated with the metastatic phenotype (9,10).

2. Obesity and breast cancer prognosis

The extensive literature which describes the adverse influence of obesity on breast cancer prognosis has been reviewed by Chlebowski et al (4). The extent to which obesity reduces breast cancer survival because it impedes early detection, as opposed to it having a causal association with an aggressive, potentially highly metastatic, tumor phenotype, remains an unresolved issue. However, Boyd and his colleagues (11) and Verreault et al (12) both concluded from their epidemiological studies that the deleterious effect of obesity on prognosis was unlikely to be due to the confounding influences of delayed diagnosis. Moreover, the diminished efficacy of adjuvant chemotherapy in overweight premenopausal patients, which was observed in an Eastern Cooperative Oncology Group trial (13), also suggests that impaired tumor detection does not provide the entire explanation.

In fact, the mechanisms by which body weight affects breast cancer outcome probably involve a complex relationship between obesity, breast physiology and tumor biology. While there is convincing evidence that this includes an obesity-induced elevation in biologically available estrogen, due to both elevated aromatase activity with an increase in estrogen production from the C19 steroid androstenedione, and reduced sex hormone-binding globulin levels (5), an alternative explanation is necessary in the case of estrogen receptor...
dependent on a variety of growth factors, fatty acid-derived eicosanoids, and protein kinase C (PKC) activity (29) (Table I).

There are a number of recognized angiogenic factors, among which vascular endothelial growth factor (VEGF) has been shown to be a potent, and specific, mitogen for endothelial cells (30). The recognition that leptin also performs as an angiogenic factor was first reported independently by Sierra-Honigmann and coworkers (31), and Bouloumié et al (32) in 1998. Both groups of investigators demonstrated that leptin stimulated angiogenesis in the same in vitro assay and in vivo model, and Bouloumié et al (32) also showed that it enhanced vascular endothelial cell proliferation in vitro with a potency similar to that of VEGF. Subsequent studies confirmed the stimulatory effect of leptin on endothelial cell growth and migration (33,34), demonstrated a complementary anti-apoptotic effect on vascular endothelial cells which required increased expression of the apoptosis inhibitor Bcl-2 (35), and found leptin also acted synergistically with VEGF and fibroblast growth factor-2 (FGF-2) to promote angiogenesis (36), and that it was partially dependent on FGF-2 for its angiogenic activity (37). Moreover, leptin induced an elevation in the expression of the 72 kDa (MMP-2) and 92 kDa (MMP-9) gene products (33,34), MMPs that are key components of the process of angiogenesis as well as tumor cell invasion.

The regulation of angiogenesis by prostaglandins and other eicosanoid products of fatty acid metabolism was reviewed by Rose and Connolly (29), but there is also an emerging relationship between leptin and the prostaglandins. Prostaglandin E2 (PGE2), which is synthesized from arachidonic acid, a polyunsaturated ω-6 fatty acid, under the influence of cyclooxygenase (COX), promotes angiogenesis by its stimulatory effects on vascular endothelial cell migration, and the organization of these cells into pre-capillary tubes. Also, PGE2 has the potential for stimulating endothelial cell growth by way of VEGF induction.

The relationship between PGE2 and leptin is a bi-directional one. Thus, in the hypothalamus both leptin and PGs act to modulate food intake, and the hormone was found to stimulate PGE2 production by rat hypothalamic cells (38). Conversely, Fain et al (39) showed that PGE2 stimulated leptin release from cultured mouse adipose tissue. In a second study (40), leptin secretion by explants of human adipose tissue obtained from obese individuals was enhanced by both PGE2 and its metabolic precursor, arachidonic acid. This increased leptin production in the presence of arachidonic acid was inhibited by a selective pharmacological inhibitor of the COX-2 isozyme.

Arachidonic acid exerts some of its biological effects not only by way of PG synthesis, but also via PGE2-mediated activation of PKC. Therefore, it is of interest for the present discussion that PKC, and specifically the activation of PKC-α, is required for vascular endothelial cell growth and migration, and tube formation (41). Furthermore, leptin was found to induce increases in PKC-α, and two other Ca2+-dependent PKC isoenzymes in adrenal cells (42).

4. Breast cancer progression and leptin

Regional and distant metastases are the principal causes of treatment failure in breast cancer patients. The process of
metastasis involves cell proliferation, angiogenesis, and a series of critical steps in the invasive/metastatic process whereby tumor cells penetrate the epithelial basement membrane, and enter the underlying interstitial stroma (Table I). This is followed by the tumor cells gaining access to lymphatic and blood vessels, their transportation and extravasation into distant anatomical sites, and further cellular proliferation to establish metastatic foci (43). Only a small minority of the cells comprising the primary tumor mass acquire the biological and biochemical features of the phenotype necessary to perform all these steps in the metastatic cascade. The underlying basis for the hypothesis presented in this report is that increased leptin bioactivity, a consequence of obesity and, perhaps, high intake of specific dietary fatty acids, is a major factor in this acquisition process.

Both the levels and types of dietary fat influence the development of chemically-induced rat colon (44) and mammary (45,46) carcinomas, and the growth and metastasis of human breast cancer cell solid tumors in athymic nude mice (47). In each of these models, polyunsaturated ω-6 fatty acids exert stimulatory, and ω-3 fatty acids exert inhibitory, effects on carcinogenesis and tumor progression. High-fat, ω-6 fatty acid-rich diets also elevated rodent adipose tissue leptin mRNA levels (48), whereas ω-3 fatty acids had an inhibitory effect on both adipose leptin mRNA (49), and plasma leptin concentrations (50,51).

Leptin acts as a mitogen on a variety of cell types (Table I), including not only vascular endothelial cells (32,33) and vascular smooth muscle cells (52), but also normal and neoplastic colon cancer cells (53,54), and breast cancer cells (55,56). Liu et al (54) showed that leptin enhanced human colon cancer cell growth in vitro, and also that in the 1,2-dimethylhydrazine-induced rat colon cancer model a high-fat, ω-6 fatty acid-rich, diet produced an elevation in serum leptin levels which correlated with colon cell proliferation and the formation of aberrant crypt foci. It seems logical to postulate that a similar relationship between dietary fat, specific fatty acids, and leptin may be present in rat mammary carcinogenesis models.

Expression of the leptin gene occurs in normal mammary tissue (57), and in breast cancer cell lines and solid tumors (58). The presence of leptin receptors was reported in breast cancer cells (55), and leptin stimulated growth of ER-positive human breast cancer cell lines by a mechanism which involved activation of the MAP kinase pathway (55,56).

Breast cancer proliferation is stimulated by estrogens, and various other hormones and growth factors. Among these are several which are candidates as intermediaries in, or biological proximate effectors for, leptin mitogenic activity. Estrogens produced in postmenopausal women under the influence of aromatase activity are one example, because, as noted earlier, this enzyme can be induced by leptin (23). Insulin is a mitogen for breast cancer cells (14), and its capacity to stimulate leptin release, and elevate circulating leptin levels (20) provides a potential interaction between insulin and components of the metastatic cascade that are targets for leptin bioactivity. We may hypothesize further
that the relationship between hyperinsulinism and a poor breast cancer prognosis reported by Goodwin et al (14) is, at least in part, mediated by way of enhanced leptin production by adipose tissue, and elevated levels of the hormone in obese patients.

Angiogenesis is now established as a biomarker of a poor prognosis in invasive breast cancer (59-62), as is the expression of VEGF (60,63,64). The nuclear phosphoprotein p53, which normally is a regulator of the cell cycle and apoptosis, undergoes mutations that are among the most common genetic defects in cancer cells, and are involved in both tumor development and progression. A number of studies have associated alterations in p53 with a poor breast cancer prognosis, but such abnormalities were also correlated with increased VEGF expression (64), Gasparini et al (59), in a comparison of a number of prognostic indicators, found that the relationship between p53 and relapse-free survival of breast cancer patients was dependent on the level of angiogenic activity.

The emerging relationship between p53, its mutated forms, and angiogenesis is of interest in the present context because in experimental studies leptin was implicated in the development of mammary carcinomas in wild-type p53-deficient mice (65). In this model, caloric restriction increased the time before the appearance of tumors, and this was associated with a reduction in serum leptin and IGF-I concentrations. It should be noted that this relationship between delayed tumor development and a reduced serum leptin level does not establish causality, and further studies will be necessary to determine the true significance of this result.

Cancer cell invasion requires the secretion of a complex system of proteolytic enzymes and their inhibitors, the net activity of which is responsible for the digestion of matrix proteins as a preliminary to cell migration (66). Among these enzymes are the MMPs which degrade type IV collagen; both MMP-2 and MMP-9 are secreted by malignant cells, and expressed in breast carcinoma tissues (67,68). Although leptin has not been reported to be a regulator of MMPs in cancer cells, it does stimulate the secretion of both MMP-2 and MMP-9 by invasive cytotoxophoblastic cells (69). Furthermore, this hormone was found to enhance the invasive capacity of premalignant adenomatous colonic cells (70). Consequently, we anticipate that when a search is made, MMP expression and secretion by human breast cancer tissue will be found to be promoted by leptin.

Prostaglandins and other eicosanoids derived from the ω-6 fatty acid arachidonic acid are involved in breast cancer invasion and metastasis (71), as well as in the process of angiogenesis (29). Thus, the reports that arachidonic acid and PGE2 stimulated the release of leptin from adipose tissue (39,40) are of particular interest. Furthermore, it may be relevant that this leptin secretion in response to arachidonic acid was blocked by a selective inhibitor of COX-2 (40), the isoform of the PGE2-synthesizing enzyme which has been associated with metastatic potential in breast cancer (72,73).

Protein kinase C, and more importantly the relative expression of its isoenzymes, exerts a powerful influence on breast cancer biology (74). In addition to tumor angiogenesis, PKC-α contributes to defining the metastatic phenotype (74,75) and is associated with aggressive ER-negative breast cancer cell lines (74). While these effects involve a number of signal transduction pathways, the reports that leptin activates PKC (42), and that PKC mediates leptin secretion from adipocytes (76) merit further investigation.

The intravascular stage of metastasis requires a complex interaction between tumor cells and platelets to form platelet aggregates and mixed platelet-tumor cell microemboli, which involves the participation of eicosanoid products of arachidonic acid metabolism (77,78), and PKC (79). It has also been reported that leptin promoted platelet aggregation in vitro at concentrations which were equivalent to those occurring in human obesity (80).

5. NFκB

Obesity can produce a situation of oxidative stress (81) with increased formation of reactive oxygen species (ROS), which activates transcription factors such as NFκB (82,83). The activation of NFκB by leptin was described by Bouloumié et al (8) in vascular endothelial cells, but similar effects in other tissues may prove to be involved in the relationship between obesity and breast cancer progression, and prognosis.

The stimulatory actions of leptin on tumor cell proliferation and invasion, MMP expression and angiogenesis described earlier are paralleled by similar effects of NFκB, which regulates a large number of genes, including those involved in the suppression of tumor cell apoptosis (84), and tumorigenesis (9,85). Yang et al (86) reported the upregulation of MMP-9 in rat kidney epithelial cells which overexpressed v-Ha-Ras with an associated enhancement of NFκB activity; in a human prostate cancer cell line, inhibition of NFκB activity suppressed expression of MMP-9 and VEGF in vitro and in vivo, and led to impaired invasive capacity in vitro (10). Moreover, ROS and NFκB have been shown to promote the initial steps in neovascularization in an in vitro angiogenesis model (87). Thus, it is plausible to propose that the transcription factor can act as a second messenger in the biological actions of leptin, and that it is intimately involved in obesity-related effects of leptin on breast cancer growth and metastasis.

6. Clinical studies of leptin in breast cancer

At the time of preparing this report, there have been two studies published which describe blood leptin levels in patients with invasive breast cancer, although neither addressed directly the potential association with obesity and prognosis.

A small study from Italy found elevated plasma leptin levels in 23 breast cancer patients, notably those with metastatic disease, and there were corresponding increases in adipose tissue leptin mRNA levels (88). There was no correlation with the BMI, but the plasma insulin levels were elevated in the breast cancer group, and these were positively related to the leptin concentrations. It is worth noting that the mean age of the control group of women was 20 years younger than that of the breast cancer patients, but that the mean BMI value did not show the expected increase with age. A second study, from Greece, was described by Petridou et al (89). There were 75 patients with mammographically-detected
breast cancers, and these were compared with 75 age-matched women with normal mammograms. In the 14 premenopausal patients the serum leptin levels were significantly lower compared with those of the premenopausal control women; there was no difference between the two postmenopausal groups. However, there is cause for concern in that the serum IGF-I concentrations were normal in the premenopausal breast cancer patients, which is contrary to other reports from the USA (90,91) and the Netherlands (92) that circulating IGF-I concentrations are elevated in these women.

7. Commentary

We conclude from our present understanding of leptin biology that this hormone, perhaps by NFκB activation, is a strong candidate for a role as a proximate effector in mediating the adverse influence of obesity on breast cancer prognosis (Fig. 1). One area worthy of future study is the potential relationship between obesity and breast cancer angiogenesis. Mukherjee et al (93) examined the relationship between host caloric intake, cell proliferation, angiogenesis, and VEGF expression in transplanted rat and human prostate cancers. Feeding an energy-restricted diet retarded tumor growth, and reduced tumor microvessel density and VEGF levels in both models. There was also a concomitant decrease in circulating IGF-I concentrations. Plasma leptin levels were not determined in this study, but, as we remarked earlier, energy restriction in p53-deficient mice was associated with reductions in both blood IGF-1 and leptin concentrations (65). It may also be relevant that the neovascularization which is an integral part of proliferative diabetic retinopathy (27) was reported to be associated with plasma leptin levels which were higher than those present in patients with non-proliferative retinopathy (94).

The relationships between leptin and estrogen production in postmenopausal breast cancer also merit attention. We have seen how leptin can act as an inducer of aromatase activity, and how this suggests a part in the causal associations between obesity, enhanced estrogen bioactivity, and postmenopausal breast cancer risk and disease progression. Although the hormones are commonly thought of as exerting their biological effects by their secretion from specific production sites, and blood-borne transportation to the target sites of action, these same compounds may also act in an autocrine and paracrine manner. A paracrine relationship occurs between breast adipose stromal cells in the immediate vicinity of a carcinoma, and the breast cancer cells. Under the influence of the tumor, these stromal cells possess particularly high levels of aromatase activity (95), which, we hypothesize, is a consequence of enzyme induction by leptin (23) produced locally by the breast cancer cells. While both antiestrogen and aromatase inhibitor therapies could serve to suppress these aromatase-mediated adverse estrogenic effects, it may be significant that tamoxifen and toremifene, the two antiestrogens in general clinical use, were found to behave like estrogens in elevating the blood leptin levels of postmenopausal breast cancer patients (96). In this context, it is of interest that an initial analysis of data from a comparative clinical trial found an aromatase inhibitor, anastrozole, to be more effective than tamoxifen as breast cancer adjuvant therapy for these women (97).

The fat content of the diet, because of its relatively high caloric density, may be a disproportionate contributor to the development of obesity, but in addition it provides fatty acids of diverse, and to some extent opposing, biological actions. In this context, further investigations to pursue the relationship between dietary fatty acids, leptin and experimental breast cancer progression should take account of the likely role for α-6 fatty acid-derived eicosanoids. We have noted already that arachidonic acid and its cyclooxygenase-mediated product PGE2 enhanced leptin production in adipose tissues (39,40), and that both α-6 fatty acid-induced colon carcino-
References

1. De Waard F, Baanders-van Halewijn EA and Huizinga J: The
bilateral age distribution of patients with mammary carcinoma.

2. Fatty Acids Mg, Panz VR, Crowther NJ, et al: Free fatty acids
and plasma biomarkers of obesity and cardiovascular disease

human leptin in vivo: effects of hydrocortisone and insulin. Int J


5. Caprio M, Fabbrini E, Iosidori AM, Aversa A and Fabbri A:
Leptin in reproduction. Trends Endocrinol Metab 12: 65-72,

6. Magoffin DA, Weitsman SR, Aagarwal SK and Jakimiuk AJ:
Leptin regulation of aromatase activity in adipose stromal cells

7. Ruohola JK, Valve EM, Karkkainen MJ, Joukov V, Alitalo K
and Harkonen PL: Vascular endothelial growth factors are
differentially regulated by steroid hormones and antiestrogens in

8. Liens S, de Clercq E and Neyts J: Angiogenesis: regulators and

9. Smit SK: Regulation of angiogenesis in the endometrium.

10. Spranger J and Pfeiffer AF: New concepts in pathogenesis and
treatment of diabetic retinopathy. Exp Clin Endocrinol Diabetes

11. Folkman J: What is the evidence that tumors are angiogenesis

12. Rose DP and Connolly JM: Regulation of tumor angiogenesis
by dietary fatty acids and eicosanoids. Nutr Cancer 37: 119-127,
2000.

13. Thomas KA: Vascular endothelial growth factor, a potent and

action of leptin as an angiogenic factor. Science 281:

15. Bouloumée A, Drexler HC, Lafontan M and Busse R: Leptin
induces oxidative stress in human endothelial cells. FASEB J

16. Biswas DK, Cruz AP, Gansberger E and Pardee AB: Epidermal
growth factor-induced nuclear factor kappa B activation:
egenesis (98), and human breast cancer cell progression in
nude mice (99) are suppressed by pharmacological cyclo-
ovoxygenase inhibitors. Moreover, cyclooxygenase-2 expression
in human breast cancer cell lines (100) correlated with their
capacity for metastasis in nude mice (reviewed in ref. 47),
and with the metastatic potential of mouse mammary
acinoma cells (73).

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