Adipose tissue as a source of hormones[1-3]

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Introduction

The deleterious effects of obesity on human health, ranging from the increased workload on the heart to abnormalities in carbohydrate metabolism related to insulin receptor dysfunction, are well recognized. Obesity also has been associated with an increased risk of cancer, particularly of the endometrium, in both young (1) and postmenopausal women (2–4). Furthermore, many studies of cancer in experimental animal models have demonstrated that both the frequency and growth rate of various tumors can be modified by dietary manipulations.

The mechanism(s) by which dietary changes influence cancer development remain unclear, however. The cancer-causing effects of diet and/or obesity have been ascribed to perturbations of the endocrine system, the prostaglandins, the immune system, hepatic mixed-function oxidases, intestinal flora, and cellular-membrane lipid composition (5–7). Our attention has been directed toward the effects of obesity on endocrine function as it relates to reproductive hormones because of the strong evidence linking obesity to cancer of the reproductive tract, which accounts for half of all cancers in women.

This brief review considers the well-defined changes in the estrogenic milieu of obese women that promotes cancer development in estrogen-target tissues. Human studies are emphasized, inasmuch as the information obtained from rodent and other animal models has not led to unifying hypotheses that are easily transferable to human cancer. The reason for this disparity may reflect the fact that reproductive processes and their regulation by hormones vary enormously among species when compared to the regulation of metabolic functions by hormones such as insulin, thyroxine, or glucocorticoids. Important temporal differences in development and differentiation of target organs as well as the enormous differences in production of hormones during pregnancy make interspecies comparisons of hormonal effects on tumorigenesis extremely tenuous.

Estrogens and breast cancer

Many observations implicate estrogens as having a role in cancer of reproductive tissues, including the breast, endometrium, cervix, and ovary. They can be divided into two categories: those that suggest that estrogens play a role in the tumorigenic process and those that demonstrate estrogen responsiveness of established tumors. Included in the former category are *1*) the nearly total absence of breast or endometrial cancer in prepubertal girls and women with either congenital absence or surgical removal of the ovaries at an early age; *2*) the large (100:1) sex difference in incidence of breast cancer between women and men, which diminishes if men are exposed to either endogenous or exogenous estrogens; and *3*) the association of estrogen consumption with increased risk for development of endometrial cancer (8–12).

That established tumors may be responsive to estrogens is clearly evident from the therapeutic effects of surgical removal of organs involved in production of estrogen (including the ovaries, adrenals, and pituitary); inhibition of aromatase, the estrogen-forming enzyme (13); and administration of synthetic antiestrogens to breast cancer patients (14) or progestins to patients with endometrial cancer. Furthermore, the presence or absence of estrogen receptors appears to distinguish be-

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tween most estrogen-dependent and -independent tumors. When carefully controlled studies are examined, however, no clear evidence for increased plasma or urinary estrogen levels in women with or at risk for breast or endometrial cancer can be found.

Nevertheless, this finding does not mean that estrogens are unimportant in causing cancer. Although the question of direct estrogen carcinogenicity is not settled, much evidence suggests that they can act as co-carcinogens or promoters. Therefore, differences in exposure to environmental carcinogens may determine who will develop cancer. In other words, a certain level of estrogen-induced target cell proliferation may be necessary for transformation to occur. According to this view, the levels of antagonists like progesterone must also be considered when evaluating the role of estrogens (*see* below).

Some workers have suggested that differences in metabolism of the primary estrogens, estradiol and estrone, also may be important in this regard (15). This hypothesis suggests that breast cancer patients have increased metabolism through 16 α-hydroxylation to yield 16 α-hydroxy estrone and estriol, compounds with weak but demonstrable uterotrophic activity, as compared to the inert catechol metabolites formed by 2-hydroxylation. While this hypothesis is attractive, no compelling evidence for accumulation or action of estrogen metabolites other than estrone and estradiol in human tissues is presently available.

The potential importance of extraglandular estrogen in relation to endometrial and breast cancer became apparent many years ago (16–18). This process, in which adrenal and/or gonadal androgens are converted to estrogens in peripheral tissues, is known to be accelerated in obese individuals (19, 20). The percentage conversion of androstenedione to estrone rises from 1–2% in normal weight subjects to 12–15% in women who weigh 300–400 lb (135–181 kg).

The aromatase enzyme that carries out this transformation is present in many human nonendocrine tissues, including adipose tissue (21, 22) and cells (23, 24), which can explain the enhanced conversion of androstenedione to estrone found in obese women. That the estrogen derived by this process is physiologically active has been demonstrated in several studies of postmenopausal women with uterine bleeding in whom this is the only source of estrogen. These findings, coupled with observations that exogenous estrogen consumption increases risk (8–12), clearly can explain the increased risk for endometrial cancer associated with obesity in postmenopausal women.

The dramatic increase in the incidence of this cancer following menopause most probably reflects the continuing production of estrogens in the absence of antagonism by progesterone. In addition to increased conversion of androgens to estrogens, adrenal secretion of cortisol and androgens is increased in obese subjects, which also may enhance peripheral estrogen production.

Several studies have shown that human stromal cells are more active than adipocytes and that the level of aromatase activity can be increased by as much as 50-fold by Dexamethasone® treatment (24). The reduced aromatase activity of adipocytes in culture appears to be due to sequestration of substrate within lipid droplets. Because enforced weight reduction of up to $\leq$ 100 lb (45 kg) does not decrease the efficiency of conversion of A to E_1 in vivo (2), it is possible that the increased conversion in obese women reflects the level of aromatase activity in stromal cells that is determined by endogenous glucocorticoids rather than the total mass of adipose tissue. If this explanation is correct, then hypercortisolism associated with other conditions, such as stress, may indirectly increase exposure of target tissues to estrogens.

Peripheral estrogen synthesis in other species

Whether or not adipose tissue from nonprimate species also can synthesize estrogen is not clear, although some negative results have been obtained in several common laboratory animals (unpublished observations). Although many environmental and dietary differences exist, the virtual absence of breast and endometrial cancer in nonhuman primates may reflect the absence of obesity and low peripheral estrogen production.

Low conversion of androstenedione to estrone and its inhibition have been demonstrated in the rhesus monkey (25). Recent

studies of New World primates that exhibit extraordinarily high plasma levels of steroid hormones also are of interest in this regard. Several reports have shown that the plasma levels of free cortisol are 50- to 100-fold higher than those found in humans and Old World primates (26–28). Plasma levels of testosterone are also markedly elevated, particularly in male squirrel monkeys during the breeding season (29, 30). While both sexes gain weight during this period, males are unique in that their body weights increase as much as 20–40% as a result of the accumulation of upper-body fat (fatting response), which results in a seasonal cushingoid appearance.

Plasma estrogens are also markedly elevated in male squirrel monkeys during the breeding season. For this reason we measured the extent of peripheral conversion of androstenedione to estrone in squirrel monkeys; it was 1000-fold greater than that found in humans when differences in body weight were taken into account (unpublished observations).

The combined effects of high plasma levels of glucocorticoids, which induce the aromatase enzyme, together with high testosterone substrate levels can account for the high plasma estrogens in the breeding male squirrel monkey. Although the relevance of these observations to breast cancer may seem obscure, similar mechanisms operating in the human breast could have a major impact on the local estrogen milieu and the potential for development of cancer.

Although many studies of aromatase activity in human breast tumors have been reported (reviewed in ref 31), relatively little is known about estrogen synthesis in normal breast tissue. Aromatase activity has been demonstrated in breast adipose tissue, however (32), and local differences in activity have been observed (33).

Recent studies have shown that the hormonal milieu of the breasts is much different from that found in the blood circulation. For example, extremely high levels of androgen sulfates (34, 35) and estriol sulfate (36) have been found in fluids aspirated from breast cysts. Others have shown that the concentrations of steroids, including estrogens and prolactin (37), in fluid obtained from nipple aspirates are much higher than those found in blood.

We have also measured the levels of estrone and estradiol in breast fluids from more than 400 women as part of an ongoing endocrine-epidemiologic study of breast cancer (38). In a preliminary analysis of the results, the levels of both estrogens are extremely variable, ranging from undetectable amounts to > 10 ng/ml in nonpregnant, nonlactating women. No significant differences have been found between the mean levels in fluid from normal women and those with either benign or malignant breast disease, as yet. Interestingly, the ratio of breast fluid to serum estrogen levels is extremely high (up to 100:1) and also variable, except in pregnant or lactating women in whom no gradient is observed.

These results indicate that estrogens and other endogenous or exogenous substances like nicotine (39) and unidentified mutagens (40) accumulate in the nonfunctioning breast and support the old idea that *stagnation* may be a contributing factor to the development of breast disease. The observation that estrogens do not accumulate during pregnancy or lactation clearly suggests that the functioning breasts rid themselves of hormones and other noxious agents. This factor may be important in explaining the protective effect of pregnancy against the development of breast cancer.

Contrary to earlier reports, a recent study (41) has shown clearly that lengthy periods of breast-feeding significantly reduce the risk for breast cancer. Continual turnover of breast constituents despite prolonged hormonal stimulation may also explain the low breast cancer rates found in populations, such as the Hutterites, in which the number of offspring is very high. Taken together, these observations suggest that the breast cancer problem, which is most severe in affluent societies, may represent a conflict between evolutionary drive for maximal reproductive performance and the social, economic, and other factors that have led to the practice of birth control.

Estrogen transport

As indicated earlier, the mechanisms leading to accumulation of estrogens within the breast are not completely understood. In addition to accumulation by local synthesis in fat cells, uptake from the circulation is also likely. Sev-

eral studies have indicated that the risk for breast cancer in young women is increased in proportion to the age at menarche and the duration of normal ovulatory cycles. If, indeed, circulating estrogens are *trapped* in the nonfunctioning breast, then one would predict that high blood levels of estrogens during normal menstrual cycles will lead to higher local tissue levels in the breast. Many factors, however, including dietary considerations, may influence levels of plasma estrogen.

For example, some studies have suggested that women who consume vegetarian diets have lower levels of estrogens in plasma or urine compared with women who consume normal diets containing meat. A study by Goldin et al (42) suggested that increased fecal excretion of estrogens associated with a vegetarian diet may reduce levels of plasma estrogen, but the study did not address body weight and the importance of obesity vs high- or low-fat diets could therefore not be determined. In addition to urinary estrogens, Armstrong et al (43) found lower levels of plasma prolactin in vegetarians; these differences could not be explained by body weight or obesity. Interestingly, this study demonstrated that the plasma levels of sex-hormone-binding globulin (SHBG) were highly correlated with plasma high-density lipoprotein cholesterol levels, both of which were higher in vegetarians than in nonvegetarians. Although the difference in SHBG levels [1.92 vs 1.66 μg dihydrotestosterone (DHT) bound/100 mL plasma] was small, these results suggest that dietary factors may alter the transport of estrogens independently of their production rates.

Alterations in estrogen binding to plasma proteins may be of considerable importance in relation to increased risk for breast and endometrial cancer. It is generally held that only the free or unbound fraction of steroid hormones in plasma is biologically active. Estradiol (E_2) binds to SHBG, albeit with a lower affinity than the androgens testosterone or DHT. Because of this weaker binding, the potential influence of fluctuations in serum SHBG levels on the availability of serum E_2 has received little attention as compared with androgens.

Anderson (44) suggested that the binding interaction was important inasmuch as he found that the percentage of free E_2 in serum is increased at low levels of SHBG. His observations that the percentage of free E_2 is increased when levels of SHBG are depressed have been confirmed. Furthermore, virtually all women who weigh > 50 lb (23 kg) above their ideal weight have serum SHBG-binding capacity that is only 20–30% of that found in normal-weight postmenopausal women. As a consequence, the free (nonprotein-bound) and albumin-bound fractions of E_2 are increased 2- to 3-fold.

Although no significant differences in either SHBG-binding capacity or percentage of free E_2 were found in women with endometrial carcinoma compared with appropriate weight-matched controls (45, 46), long-term prospective studies are needed to evaluate fully the relationships among obesity, reduced levels of SHBG, and endometrial cancer.

Only a few studies have examined levels of SHBG or percentage of free E_2 in breast cancer patients. Total- and unbound-E_2 levels are significantly higher in breast cancer patients than in appropriate controls (47). Low levels of SHBG associated with obesity could explain the results in postmenopausal but not premenopausal women. Another report (48) has confirmed the effect of obesity but did not find differences when breast cancer patients and controls were compared.

The reason for low serum SHBG-binding capacity in serum of obese subjects is not known. Conditions known to be associated with reduced plasma SHBG-binding capacity include hyperandrogenism and hypothyroidism (44). The depressed SHBG-binding capacity found in obesity may therefore be the result of elevated secretion of adrenal androgens and/or clinically undetectable hypothyroidism. Obesity has long been known to be associated with increased adrenal secretion of cortisol (49), but few studies of adrenal androgen secretion have been made in obese subjects. It has been demonstrated, however, that puberty occurs earlier in obese girls and that they have elevated serum levels of adrenal androgens (50).

Traditionally, endocrine-related changes in plasma SHBG have been interpreted to reflect effects on the liver that change the rate of SHBG synthesis. Changes in the clearance rate of SHBG may also be important in determin-

ing blood levels (27). For example, if SHBG-bound testosterone is taken up by androgen-target cells, the plasma SHBG levels would be reduced without a change in SHBG synthetic rate and the free hormone fraction would be increased in accord with the *free hormone* concept of hormone action. Recent studies of plasma-binding proteins are in accord with this possibility (26, 51).

Whatever the mechanisms are that result in accumulation of estrogen in the breast, the high levels found in breast fluids in the absence of overt disease suggest that their estrogenic effects are somehow neutralized. Although progesterone is important in this regard because it is clearly an estrogen antagonist in the uterus, whether or not this relationship is true in the breast is not certain. Clearly, additional studies are needed to evaluate not only the estrogen-progesterone relationship but other potential estrogen antagonists, such as androgens in the breast.

Summary

Obesity is known to increase the risk for cancer of the reproductive tract in women. The mechanism underlying this association can be explained by increased estrogenic stimulus to estrogen-target tissues as the result of three factors. First, increased adrenal secretory activity makes more androgen precursors available for conversion to estrogen in peripheral tissues. Second, the efficiency of conversion of androstenedione (A) to estrone (E_1) is elevated in obese subjects because adipose tissue is the major tissue site of conversion. Third, plasma levels of SHBG, which binds estradiol (E_2), are depressed in obese subjects and greater than normal amounts of serum estradiol are therefore available to target tissues from the circulation.

Recent studies have shown that the levels of estrogens and other steroid hormones in breast fluids are much higher than in serum, which may be the result of local synthesis or increased uptake from the circulation. No differences in estrogen levels of breast fluid have been found between normal women and those with breast disease. A possible explanation may be differences in the levels of estrogen antagonists, such as progesterone.

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