Endogenous hormone metabolism as an exposure marker in breast cancer chemoprevention studies

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There is overwhelming evidence that alterations in endogenous hormone metabolism — as a form of endogenous exposure—may be an important metabolic risk factor for the development of breast cancer. This chapter reviews current theories and major epidemiological findings that link endogenous hormones (sex steroids and their metabolites, but also insulin, and insulin-like growth factor-I (IGF-I)) to breast cancer risk. Knowledge about these metabolic risk factors can be used to identify women at increased risk of breast cancer, who might benefit most from chemoprevention. In addition, modification of high-risk endocrine profiles may itself become a central target of chemoprevention. Possible intervention strategies include improvement of insulin sensitivity, reducing concentrations of IGF-I in blood and breast tissue, reducing ovarian overproduction of androgens, inhibiting the activity of aromatase and other enzymes involved in estrogen formation within the breast, and modifying estrogen metabolism within the breast (e.g., decreasing 16α- and 4α-hydroxylation, and increasing O-methylation of catecholestrogens). Several of these possible strategies are illustrated with examples of chemopreventive agents currently in use or proposed for use to prevent breast cancer.

Introduction

Breast cancer is predominantly a disease of western, industrialized countries. There is overwhelming evidence from studies on migrants and from time trends in incidence rates that the high incidence rates of breast cancer in western countries are due mainly to lifestyle factors, including nutrition. Nutritional factors most strongly implicated include low levels of physical activity and an energy-dense diet rich in fats, refined carbohydrates and animal protein. These factors also appear related to an earlier average age at menarche, increasing average body height and an increasing prevalence of obesity—three well established risk factors for breast cancer that are also known to be associated with changes in hormone metabolism. An additional risk factor, which probably also affects breast cancer risk through changes in endogenous hormone metabolism, is a childbearing pattern characterized by relatively late first pregnancy and low parity. Besides endogenous hormones, the use of exogenous hormones for contraception or for treatment of menopausal symptoms has also been suggested to be related to breast cancer risk.

The identification of specific alterations in endogenous hormone metabolism as risk factors for breast cancer risk is a topic of extensive research. Knowledge of such hormonal risk factors may help understand why more traditional risk factors (e.g., obesity, lack of physical activity, late menopause, use of certain exogenous hormones) are related to breast cancer. In addition, this knowledge can be used to design and test strategies for breast cancer prevention, through changes in lifestyle (nutrition, hormone use) or by use of specific chemopreventive agents. Favourable changes in endogenous hormone profiles can then be taken as intermediate evidence for a possible preventive effect.

The first section of this chapter reviews the associations of breast cancer risk with circulating levels

of sex steroids and with modifications in sex steroid metabolism locally within the breast. In the next section, basic aspects of insulin-like growth factor-I (IGF-I) metabolism are discussed, and the associations of breast cancer risk with circulating levels of IGF-I, its binding proteins (IGFBPs) and insulin are reviewed. In the third section, a number of possible strategies for breast cancer prevention through modification of endogenous hormone metabolism are presented. The final section presents concluding remarks about the possible use of markers of endogenous hormonal exposures in prevention studies.

Sex steroids
Circulating sex steroids as determinants of breast cancer risk

Ovarian sex steroids clearly play an important role in promoting the development of breast cancer. Risk is consistently increased in women with early menarche and late menopause (Key & Pike, 1988) and hence is associated with a longer lifetime production of estradiol and progesterone by the ovaries. Furthermore, breast cancer incidence rates rise more steeply with age before menopause than after, when the ovarian synthesis of estrogens and progesterone practically ceases, and when the production of androgens gradually reduces to about half the premenopausal levels. Ovariectomy at an early age reduces breast cancer incidence and ovariectomy at the time of cancer diagnosis diminishes the risk of tumour recurrence (Secreto & Zumoff, 1994).

A popular theory is that breast cancer risk is increased in women who have increased plasma levels of bioavailable estradiol unbound to sex-hormone binding globulin, and elevated estradiol concentrations within breast tissue (estrogen excess hypothesis) (Bernstein & Ross, 1993; Key & Pike, 1988). A second theory is that breast cancer risk is increased in women with elevated levels of androgenic steroid hormones, in particular testosterone and Δ4-androstenedione (ovarian androgen excess hypothesis) (Bernstein & Ross, 1993; Kaaks, 1996; Secreto & Zumoff, 1994). These two theories are complementary, as ovarian over-production of androgens and increased plasma levels of total or bioavailable estrogens often occur together, most strikingly in patients with hyperandrogenic syndromes such as the polycystic ovary syndrome

(Ehrmann et al., 1995), but also in postmenopausal women with upper body type (android) obesity (Kaaks, 1996). Δ4-Androstenedione and testosterone are the immediate precursors for estrogen synthesis. Especially after the menopause, when the ovaries no longer produce the enzyme aromatase needed for estrogen synthesis, the levels of bioavailable androgens unbound to sex-hormone binding globulin in plasma are a major determinant of estrogens formed within adipose tissue, including the breast.

The ovarian androgen excess hypothesis and estrogen excess hypothesis are both supported by results from several prospective cohort studies, which have shown increased breast cancer risk in postmenopausal women having elevated plasma levels of testosterone and Δ4-androstenedione, reduced levels of sex-hormone binding globulin and increased levels of total and bioavailable estradiol (Thomas et al., 1997a). The increases in breast cancer risk associated with this high-androgen steroid profile may be due particularly to the rise in bioavailable plasma estrogen levels, plus increased estrogen formation within breast tissue itself. Estradiol is a strong mitogen for breast epithelial cells, and is therefore thought to have a key role in promoting tumour development (Henderson & Feigelson, 2000). However, risk may also be influenced directly by androgens, through androgen receptors within breast tissue (Adams, 1998; Birrell et al., 1998).

There is considerable evidence that the combination of estrogens and progestogens increases breast cancer risk further than exposure to excess estrogens alone (Bernstein & Ross, 1993; Key & Pike, 1988). Major observations supporting this "estrogen-plus-progestogen" hypothesis are that: (1) breast epithelial cells have the highest mitotic activity in the luteal phase of the menstrual cycle, when progesterone production peaks; (2) premenopausal women who are obese or who have severe forms of ovarian androgen excess (as in polycystic ovary syndrome) on average experience some reduction in breast cancer risk, and this may be explained by frequent anovulatory menstrual cycles and an impaired luteal-phase progesterone production; (3) in postmenopausal women, the use of combined estrogen-plus-progestogen hormone replacement therapy increases breast cancer risk to a greater extent than replacement therapy

with estrogens alone (Ross *et al.*, 2000; Schairer *et al.*, 2000). Nevertheless, several case–control studies (Secreto & Zumoff, 1994), though not one (small) prospective cohort study (Thomas *et al.*, 1997b), have shown increases in breast cancer risk in premenopausal women who have elevated androgen levels in plasma or urine. The latter suggests that only mildly hyperandrogenic women who maintain regular ovulatory cycles, but who may have somewhat elevated bioavailable estrogens, may be at increased risk, whereas the more severely hyperandrogenic women may experience a relative protection because of chronic anovulation and impaired ovarian progesterone production. Direct evaluations of associations of breast cancer risk with circulating levels of estradiol and progesterone are complicated by the wide variations of these two hormones during the menstrual cycle, and so far have not led to any definitive conclusions.

There is extensive evidence that reductions in sex-hormone binding globulin synthesis and increases in ovarian sex steroid synthesis are both related to chronic hyperinsulinaemia, which in turn is often a consequence of obesity, lack of physical activity, and hence insulin resistance (Kaaks, 1996) (Figure 1). The effects of insulin on hepatic sex-hormone binding globulin production and steroidogenesis may be mediated at least partially by an increase in IGF-I bioactivity (see below).

Estrogen synthesis and metabolism within breast tissue

Only sex steroids unbound to sex-hormone binding globulin can diffuse from the circulation into target tissues, where they may bind to estrogen and androgen receptors and exert their biological effects. Circulating levels of bioavailable sex steroids are thus a key determinant of local estrogenic or androgenic activity within the breast. Another important determinant of estrogenic activity within breast tissue, however, is the local metabolism of sex steroids (Figure 2). Indeed, studies have shown substantially higher concentrations of estrogens in breast tissue than in the circulation, especially in postmenopausal women. Furthermore, several steroid-metabolizing enzymes are present, and active, in breast tissue (Zhu & Conney, 1998). Similar enzymatic activity is found in adipose tissue elsewhere in the body.

The principal enzyme involved in producing estrogens from androgenic precursors is aromatase (CYP19), which converts Δ4-androstenedione and testosterone into estrone and estradiol, respectively. Aromatase activity is higher in adipose tissue of breast cancer patients than in tissue from women with benign breast disease (Miller & Mullen, 1993). Furthermore, in breasts containing a tumour, aromatase expression and activity are higher in quadrants bearing tumours than in the other quadrants. A second enzyme in breast tissue that may contribute to estrogen formation is 3β-hydroxysteroid dehydrogenase, which converts dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS) into Δ4-androstenedione, the principal androgenic precursor for aromatization into estrogens (estrone).

A second important regulatory mechanism for modulation of estrogenic activity within breast tissue is the interconversion of the less potent estrogen estrone into the more potent estradiol by 17β-hydroxysteroid dehydrogenase type 1, and vice versa, by 17β-hydroxysteroid dehydrogenase type 2. In normal breast tissue and breast cancer cells, the balance in activity between the two isozymes generally appears to favour the conversion of estrone into estradiol (Zhu & Conney, 1998).

A third mechanism by which hormonally active estrogens (i.e., with receptor binding activity) can be formed is the hydrolysis of sulfate or glucuronyl groups from, respectively, estrone sulfate and estrone glucuronate or estradiol glucuronate. The sulfated or glucuronated compounds are formed in the liver and other tissues by steroid sulfotransferases and glucuronosyl transferases, respectively, to form more water-soluble compounds that can be excreted in bile and urine. These compounds have little or no estrogen receptor-binding affinity, but their large amounts in the circulation form a reservoir from which active estrogens can be formed by hydrolysis (Zhu & Conney, 1998).

A fourth mechanism by which active estrogens are formed is hydrolysis of estrogen fatty acid esters. These esters are formed by estrogen acyltransferase and, because of their high lipophilicity, accumulate in relatively high concentrations in fatty tissues. While estradiol fatty acid esters have little or no estrogen receptor-binding affinity, they form an important local reservoir for estradiol formation by the action of specific esterases.
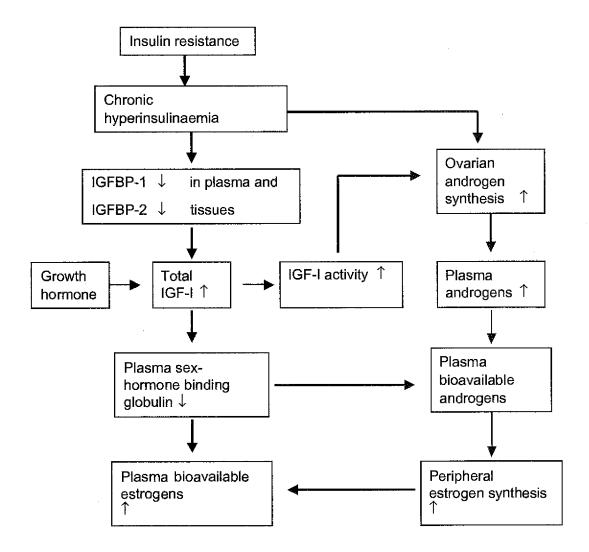
Figure 1. Role of insulin and IGF-I in regulating levels of total and bioavailable sex steroids
IGF-I, Insulin-like growth factor-I; IGFBP-1, insulin-like growth factor binding protein-1; IGFBP-2,

Besides enzymes involved in regulating levels of active versus less active or bound forms of estrogen within breast tissue, several other enzymes form estrogen metabolites that may have their own biological activities and physiological roles (Figure 2). The first major classes are cytochrome P450 enzymes (CYP1A1, CYP1A2, CYP2B1, CYP2B2, CYP2C family, CYP3A family, $P450_{C-M/F}$) that form estrogen hydroxylation products. A second type of enzyme — catechol-O-methyltransferase —further metabolizes hydroxy-estrogen metabolites to methoxy-estrogens.

The hydroxylating P450 enzymes all require NADPH, and are located in mitochondria. Estrogen
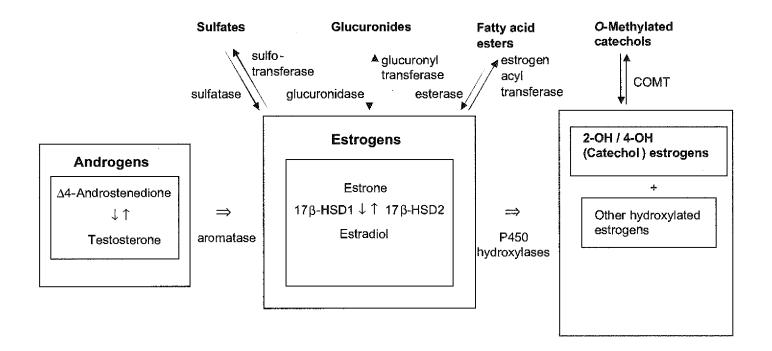
Figure 2. Synthesis and metabolism of estrogens within breast tissue
(After Zhu & Conney, 1999)

17β HSD1, 17β-hydroxysteroid dehydrogenase type 1; 17β HSD2, 17β-hydroxysteroid dehydrogenase type 2; COMT, catechol-*O*-methyl-transferase

metabolites with hydroxyl groups at positions 1, 2, 4, 6, 7, 14, 15, 16, 17 or 18 can be formed (Zhu & Conney, 1998). Some of these metabolites are formed predominantly in liver, but others (e.g., the 2-, 4- and 16-hydroxylation products) are also formed in relatively large amounts in breast tissue. 2-Hydroxyestradiol has markedly reduced estrogen receptor-binding activity, but 4-hydroxyestradiol and 16-hydroxyestradiol retain potent hormonal activity by binding estrogen receptors. Because of this and other lines of evidence, it has been hypothesized that an increase in 16-hydroxylation (relative to 2-hydroxylation) may be a determinant of breast cancer risk (Bradlow et al., 1986). This hypothesis was confirmed in some recent epidemiological studies (Kabat et al., 1997), but not by others (Ursin et al., 1999), where levels of 16- and 2-hydroxylated estrogens were measured in urine (Meilahn et al., 1998). Further recent studies do suggest, on the other hand, that increased formation of 4-hydroxyestrogens in target tissues, including human breast, may play an important role in estradiol-induced carcinogenesis. A key mechanism involved may be metabolic redox cycling of 4-hydroxyestradiol, catalysed by cytochrome P450 enzymes (Liehr, 2000). The potential role of increased 4-hydroxylation in breast cancer development awaits confirmation from epidemiological studies.

The 2- and 4-hydroxyestrogens have a catechol structure (i.e., with hydroxyl groups on two adjacent carbon atoms in the aromatic A ring), which means that these compounds are chemically reactive. Due to their reactivity, they may for instance bind to DNA, and thus cause DNA damage or mutations. A strong carcinogenic action has been observed only for 4-hydroxyestradiol, and not for the 2-hydroxy metabolite. The enzyme catechol-O-methyltransferase (COMT) further metabolizes these 'catechol estrogens' by formation of 2- and 4-methoxyestradiol compounds. O-Methylated estrogens are more lipophilic, have very long half-lives, and do not bind to the classical (alpha) estrogen receptor.

Earlier studies on the chemical reactivity and potential genotoxicity of catechol estrogens led to the suggestion that enzymatic O-methylation was primarily a detoxification pathway for these catechol intermediates. More recent results, however, suggest that the O-methylated products may have a number of unique biological activities apparently not mediated by the estrogen alpha receptor. In particular 2-methoxyestradiol inhibits the proliferation of several cancer cell lines in vitro, including human breast cancer, and in vivo also has strong anti-angiogenic effects (Zhu & Conney, 1998).

The distribution of catechol-O-methyl transferase activity appears to follow a bimodal pattern due to a polymorphism in the COMT gene. About 25% of Caucasians are homozygous for the low-activity allele. Some recent epidemiological studies (Huang et al., 1999; Lavigne et al., 1997; Thompson et al., 1998) but not all (Millikan et al., 1998) have shown an increase in breast cancer risk in women with this gene variant, although the results varied in association with either pre- or postmenopausal risk, and between obese or non-obese women. Taken together, there is some evidence that high catechol-O-methyl transferase activity may protect against breast cancer by increasing levels of 2-methoxyestradiol, which has anti-estrogenic properties, and by faster deactivation of 4-hydroxyestradiol, which is carcinogenic.

IGF-I and IGF-binding proteins

The hypothesis that risk of cancer (at various organ sites) may be related to circulating levels of total IGF-I and its binding proteins (IGFBPs), and to an increase in IGF-I bioactivity at a tissue level, has recently received much attention from epidemiologists. Reasons are that IGF-I in general inhibits apoptosis and stimulates cell proliferation (Jones & Clemmons, 1995; Werner & Le Roith, 1997) and thus stimulates tumour development. In addition, levels of IGF-I and several of its binding proteins (especially IGFBP-1, -2 and -3), as well as local IGF-I bioactivity within tissues, are intricately related to nutritional status and energy metabolism (Thissen et al., 1994). Furthermore, as mentioned above, the IGF-I/IGFBP system, in interaction with insulin, appears to be central in regulating the synthesis and plasma levels of sex-hormone binding globulin and androgens.

IGF-I, IGFBPs, and IGF-I bioactivity

IGF-I bioactivity is the overall resultant of complex interactions of endocrine, paracrine and autocrine sources of IGF-I and IGFBPs with cellular receptors. Although IGF-I bioactivity is believed to increase generally when total IGF-I concentrations rise,

IGF-I bioactivity is modulated to a large extent by the IGFBPs.

A first level at which IGFBPs modulate IGF-I bioactivity is regulation of the efflux of circulating IGF-I through the capillary barrier. At least six different IGFBPs have been identified in tissues and in the circulation (Jones & Clemmons, 1995). Although IGF-I and IGFBPs are produced in practically all human tissues, most IGF-I and IGFBPs in blood plasma are produced in the liver. Over 90% of circulating IGF-I is complexed with IGFBP-3 and with another glycoprotein called acid-labile subunit (ALS). IGFBP-3 has a very high affinity for IGF-I, and the large IGF-I/IGFBP-3/ALS complex cannot pass through the capillary barrier to target tissues. There is recent evidence that IGFBP-5, which has even higher affinity for IGF-I than IGFBP-3, may form a similar IGF-I/IGFBP-5/ALS complex. Practically all of the remaining IGF-I is bound to IGFBP-1, -2, -4 and -6, which have lower binding affinities for IGF-I (compared with IGFBP-3 and IGFBP-5), do not form complexes with ALS, and are small enough to cross the endothelial barrier. Therefore, a decrease in plasma IGFBP-3 level, with a transfer of IGF-I to smaller IGFBPs not complexed with ALS, is believed to increase IGF-I availability to its tissue receptors. Reductions in plasma concentrations of the smaller IGFBPs, and particularly IGFBP-1 and -2, are also thought to increase the bioavailability of circulating IGF-I (Jones & Clemmons, 1995).

A second level at which IGFBPs modulate IGF-I bioactivity is the target tissue itself, where IGFBPs regulate the binding of IGF-I to its receptors. Although binding proteins have been mostly proposed to inhibit receptor binding by complexing IGF-I, results from *in-vitro* studies suggest that, depending on the relative concentrations of IGF-I and IGFBPs, certain IGFBPs (e.g., IGFBP-1, -2, -3 and -5) may actually enhance IGF-I binding to its receptors (Jones & Clemmons, 1995).

Epidemiological studies on insulin, IGF-I and breast cancer risk

Three case–control studies and one prospective cohort study have shown an increase in breast cancer in women with elevated plasma IGF-I or with elevated IGF-I for a given level of IGFBP-3 (Bohlke et al., 1998; Bruning et al., 1995; Del Giudice et al., 1998; Hankinson et al., 1998; Peyrat et al., 1993).

This relationship between risk and total plasma IGF-I was found exclusively for women who developed breast cancer before the average age at menopause. Besides increased levels of IGF-I, either as total concentration or relative to IGFBP-3 levels, two case–control studies have shown that elevated plasma insulin levels also increase breast cancer risk, in both premenopausal (Bruning et al., 1995; Del Giudice et al., 1998) and postmenopausal (Bruning et al., 1992) women.

Possible strategies for chemoprevention

Different types of intervention aiming at reducing breast cancer risk by favourably changing endogenous hormone metabolism have been proposed, including energy restriction and body weight loss (in obese or overweight women), long-term changes in diet and physical activity level, and use of various chemopreventive agents. Strategies for prevention of breast cancer through alterations in hormone metabolism may target a number of mechanisms. These include reduction of circulating IGF-I levels and/or IGF-I bioactivity at a tissue level, reduction of ovarian (and/or adrenal) sex steroid production, increase of hepatic sex-hormone binding globulin production, reduction of local estrogen synthesis and modification of local estrogen metabolism. Each of these strategies in principle may lead to measurable alterations in levels of hormones or hormone metabolites in blood or urine. Chemopreventive agents may also be used to diminish the binding of hormones to their receptors.

Reducing IGF-I bioactivity

A class of compounds that lower IGF-I levels are growth hormone-releasing hormone (GHRH) antagonists, including the natural compound somatostatin, and a number of somatostatin analogues (e.g., octreotide, vaproetide, lanreotide, octastatin, somatuline (BIM 23014)). These compounds decrease IGF-I levels by inhibiting the pituitary secretion of growth hormone (Kath & Hoffken, 2000; Kineman, 2000). In addition to the suppression of the growth hormone/IGF-I axis, GHRH antagonists appear to inhibit tumour growth directly through a local mechanism, which may be related to reduced local synthesis of insulin-like growth factor-II (IGF-II). This direct effect might be mediated by blocking the

paracrine/autocrine actions of locally produced GHRH, which may normally act through receptors for the vasoactive intestinal protein or pituitary adenylate cyclase-activating polypeptide — peptides that are structurally similar to GHRH. Somatostatin analogues have been tested as part of hormonal adjuvant therapy in breast cancer patients, but have not been evaluated for chemoprevention in healthy women.

A second class of agents that decrease especially the hepatic production and circulating levels of total IGF-I are exogenous estrogens (e.g., in oral contraceptives, postmenopausal estrogen replacement therapy, or combined estrogen–progestogen replacement therapy) (Campagnoli *et al.*, 1992, 1995; Ho *et al.*, 1996) and estrogen analogues (tamoxifen, raloxifene, droloxifene) (Pollak *et al.*, 1992). Especially weak estrogen analogues have been regularly proposed as potential chemopreventive agents against breast cancer, and this was initially motivated mainly by their anti-estrogenic effects, through competitive binding to estrogen receptors (but without receptor activation). Estrogenic compounds decrease the liver synthesis of IGF-I only if taken orally but not when administered transdermally. A likely explanation is that oral administration leads to relatively high estrogen concentrations in the portal vein and hence in the liver, and that only such high concentrations can substantially decrease hepatic IGF-I production. Oral administration of androgenic compounds, including some progestagens produced from the androgenic precursor 19-nortestosterone (e.g., norethisterone, norgestrel, levonorgestrel) and present in some types of oral contraceptive and hormone replacement therapy, leads to an increase in synthesis and plasma levels of IGF-I. So far, no clear effect of phytoestrogen intake on circulating IGF-I levels has been reported.

The epidemiological evidence for associations of breast cancer risk with oral contraceptive use, use of estrogen replacement therapy or hormone replacement therapy has been reviewed (IARC, 1999), as well as that for tamoxifen (IARC, 1996). There is a small increase in risk associated with use of combined oral contraceptives, as well as with use of estrogen replacement therapy. The association of risk with oral contraceptives based on progestogens only, and with combined estrogen plus progestogen replacement therapy was difficult to evaluate because of lack of detail in the information from published studies. Nevertheless, a large case–control study and a cohort study recently showed significantly stronger increases in risk with combined estrogen plus progestogen therapy than with estrogens alone (Ross *et al.*, 2000; Schairer *et al.*, 2000).

The use of estrogen analogues for treatment and possibly chemoprevention of breast cancer was initially motivated by the concept that such compounds would diminish estrogenic effects in breast tissue by competing with natural estradiol for estrogen receptor binding, without having a receptor-activating capacity (Goldstein, 1999). A large meta-analysis of randomized intervention trials, including more than 75 000 breast cancer patients, confirmed the efficacy of such analogues, showing dramatic improvements in ten-year recurrence-free survival in the tamoxifen treatment group (Early Breast Cancer Trialists' Collaborative Group, 1992). Another large, double-blind and placebo-controlled randomized trial also showed a strong reduction in breast cancer incidence among 13 000 initially cancer-free women (Fisher *et al.*, 1998). The latter finding was not confirmed, however, by smaller trials in about 2500 women in the United Kingdom (Powles *et al.*, 1998) and in about 5400 women in Italy (Veronesi *et al.*, 1998). A recent trial among 7700 postmenopausal women with osteoporosis showed a strong protective effect of raloxifene against breast cancer occurrence (Cummings *et al.*, 1999).

A third group of agents that have been tested for potential chemopreventive activity against breast cancer, and which also appear to decrease circulating IGF-I levels, are retinoic acid analogues, such as fenretinide (4-hydroxyphenylretinamide) and all-*trans*-retinoic acid. A randomized intervention trial among about 3000 Italian women with surgically removed stage I breast cancer showed no significant effect of fenretinide on the occurrence of contralateral or ipsilateral tumors. However, an interaction was detected between fenretinide and menopausal status, with a possible beneficial effect in premenopausal women (Veronesi *et al.*, 1999).

IGF-I bioactivity may be decreased by improving insulin sensitivity. An improvement of insulin sensitivity and decreases in hepatic glucose output lead to lower endogenous insulin secretion, and

this in turn leads to higher levels of IGFBP-1 and IGFBP-2. Natural ways of improving insulin sensitivity are to lose body weight (for overweight or obese women) (Bosello *et al.*, 1997; Guzick *et al.*, 1994) or to increase physical activity. In addition, insulin-sensitizing and hypoglycaemic drugs such as metformin (Pugeat & Ducluzeau, 1999) and troglitazone (Henry, 1997; Scheen & Lefebvre, 1999), biguanides or sulfonylureas (e.g., tolbutamide and tolazamide) could be used.

Increasing plasma sex-hormone binding globulin levels

Circulating sex-hormone binding globulin is produced in the liver and, as mentioned above, is under negative control of insulin and IGF-I, which both reduce its hepatic synthesis. Approaches to increase sex-hormone binding globulin synthesis include, first of all, improvement of insulin sensitivity. Weight loss or an increase in physical activity both increase circulating sex-hormone binding globulin levels (Guzick *et al.*, 1994), as does the use of insulin-sensitizing drugs. Oral intake of exogenous estrogens and estrogen analogues (e.g., tamoxifen), which decrease the hepatic production of IGF-I, also causes a rise in plasma sex-hormone binding globulin levels (Campagnoli *et al.*, 1992). Conversely, oral intake of androgenic compounds reduces hepatic output and plasma levels of sex-hormone binding globulin (Campagnoli *et al.*, 1994). Some study results have suggested a mild increase in hepatic production (Loukovaara *et al.*, 1995) or plasma levels of sex-hormone binding globulin (Adlercreutz *et al.*, 1987) at high intakes of phytoestrogens, but this requires confirmation in larger studies.

Reducing ovarian sex steroid synthesis

Total plasma levels of testosterone are positively related to breast cancer risk. As most of the circulating testosterone comes from the ovaries, this suggests that a reduction in ovarian sex steroid output may also be an approach to prevent cancer.

A first approach to diminishing ovarian (and possibly also adrenal) androgen production is to reduce circulating insulin levels. In obese and hyperandrogenic women, energy restriction with body weight loss and use of insulin-sensitizing drugs have both been shown to decrease circulating androgen levels (Pugeat & Ducluzeau, 1999).

A second approach, which has also proven effective especially in women with polycystic ovary syndrome, is the use of combination-type oral contraceptives, which reduce ovarian hyperandrogenism by diminishing the pituitary secretion of luteinizing hormone. A third, more experimental approach is to use new types of oral contraception, which contain gonadotropin-releasing hormone agonists to inhibit secretion of pituitary luteinizing hormone, as well as small amounts of exogenous estrogens to compensate for the block of endogenous (ovarian) sex steroid production (Spicer & Pike, 1994).

Reducing estrogen formation within the breast

Since steroid sulfatases may increase the local formation within the breast of estrogenic steroids with receptor-activating capacity, steroid sulfatase inhibitors could have considerable therapeutic, and possibly preventive, potential. Several such inhibitors have now been developed. The most potent to date is estrone-3-O-sulfamate (Purohit *et al.*, 1999). Other steroidal sulfatase inhibitors are estrone-3-methylthiophosphonate (Duncan *et al.*, 1993) and 3-O-methylphosphonate derivatives of dehydroepiandrosterone (DHA-3-MTP), pregnenolone or cholesterol (Purohit *et al.*, 1994). Nonsteroidal estrone sulfatase inhibitors include 4-methylcoumarin 7-O-sulfamate and its derivatives (14, 16 and 18) (Woo *et al.*, 1998). The extent and duration of the inhibition of estrone sulfatase may be monitored by measuring the activity of this enzyme in white blood cells or by measuring the decrease in the plasma DHEA/DHEAS concentration ratio (Purohit *et al.*, 1997).

Besides steroid sulfatase inhibitors, inhibitors of the enzyme aromatase (e.g., anastrozole, letrozole, vorozole, aminoglutethimide, 4-hydroxyandrostenedione (Harvey, 1998; Singh *et al.*, 1998) and other compounds (Kelloff *et al.*, 1998)) have been tested extensively in animal models and in clinical trials for breast cancer treatment. Aromatase inhibitors are being proposed for phase III trials for breast cancer treatment. The use of aromatase inhibitors can lead to profound suppression of plasma estrogen levels (Kelloff *et al.*, 1998). Nevertheless, strategies may be developed to obtain chemopreventive effects without total suppression of aromatase and plasma estrogen levels (Kelloff *et al.*, 1998).

Several flavonoids and isoflavonoids (phyto-estrogens; e.g., coumestrol, genistein) have been found to be potent inhibitors of estrone reduction by 17β-hydroxysteroid dehydrogenase type 1 in breast cancer cells (Makela *et al.*, 1995, 1998). There is little information about compounds that may be used to regulate the balance between activities of the 17β-hydroxysteroid dehydrogenase type 1 and type 2 isozymes in favour of lower estradiol synthesis.

Modification of estrogen hydroxylation
On the basis of the theory that a high ratio of 16- to 2-hydroxyestradiol would increase risk, the use of chemicals that can shift hydroxylation towards 2-hydroxylation have been proposed as chemo-preventive agents. One such compound is indole-3-carbinol, a compound which occurs naturally in certain vegetables and which competes with estradiol for 16-hydroxylation (Michnovicz, 1998). The shift from 16-hydroxylation to 2-hydroxylation can be monitored by measuring the ratio of the two types of estrogen hydroxylation product in urine (Michnovicz *et al.*, 1997). It is possible, however, that inhibition of 16-hydroxylation leads to increased formation of 4-hydroxyestradiol, which is potentially carcinogenic.

Hormone measurements as markers of endogenous exposure in chemoprevention studies: conclusions and problem areas

As shown above, alterations in endogenous hormone metabolism — as a form of 'endogenous' exposure — may be an important metabolic risk factor for the development of breast cancer. Knowledge about such metabolic risk factors can be used to identify women at increased risk of breast cancer who might benefit most from chemoprevention. In addition, as discussed in the third section of this chapter, modification of high-risk endocrine profiles may become the central target for chemoprevention. In the latter case, it is assumed that chemopreventive modification of endogenous hormone levels (e.g., reduction in IGF-I levels in blood and other tissues; inhibition of aromatase activity; shifts of estradiol hydroxylation pathways towards less 16- or 4-hydroxylation) will lead to reductions in breast cancer risk.

This implies that either the hormonal parameter aimed at must be itself a direct cause of cancer or else it must be at least very closely associated with other metabolic factors that are on the causal pathway (Lippman *et al.*, 1990). Measurements of hormones or hormone metabolites may then be used to evaluate the efficacy of a given prevention strategy. There are number of important problems, however.

First, the evaluation of whether a given hormone is directly related to tumour development is generally not straightforward. One problem is that hormonal parameters can have quite strong physiological interrelationships. For example, in post-menopausal women, elevated plasma insulin levels lead to reductions in sex-hormone binding globulin, increases in plasma androgens and increases in bioavailable estrogens. Sorting out which of these interrelated hormonal factors (bioavailable plasma estrogens, androgens, insulin?) are more directly, or strongly, related to breast tumour development may not be possible using epidemiological methods alone, and may require complementary mechanistic evidence from experimental studies. When possible effects on breast tissue of physiologically interrelated hormones cannot be disentangled, a reasonable approach to prevention may be to take modification of a global endocrine profile as the target for prevention. For example, by improving insulin sensitivity, one may at the same time obtain an increase in plasma sex-hormone binding globulin levels and reductions in plasma levels of total and bioavailable androgens and estrogens.

Second, it is not always clear whether circulating levels of hormones are a main determinant, or at least a good indicator, of levels within breast tissue. For example, in postmenopausal women, who have very low plasma concentrations of total and non-sex-hormone binding globulin-bound estradiol, the main determinant of estradiol concentrations within the breast may be its local synthesis from androgen precursors or from estrone. Therefore, if estradiol is a key hormone in promoting breast tumorigenesis, increased circulating bioavailable androgens, or increased breast tissue activities of aromatase or 17β-hydroxylase, may be more important determinants of risk than plasma concentrations of bioavailable estradiol. Another example is the control of IGF-I bioactivity

within breast tissue, which depends not only on circulating levels of IGF-I and IGFBPs, but also on local synthesis. Thus, it is not entirely clear, for instance, whether factors such as estrogen replacement therapy or tamoxifen that may decrease the hepatic synthesis and output of IGF-I, therefore reducing its plasma concentrations, will also substantially reduce IGF-I bioactivity within the breast. A third example is formation of hydroxy- and methoxy-metabolites of estrogens within the breast. Blood concentrations of these metabolites may not reflect very accurately their levels in breast tissue, because they can also be formed in adipose tissue elsewhere in the body.

Besides the problem that circulating levels of a given hormone may not always reflect breast tissue concentrations, there can be major technical or logistic problems in the measurement of hormones or their metabolites in blood, urine or saliva. Such problems are the lack of sensitivity and specificity of methods for the detection of specific sex steroids and their metabolites (e.g., hydroxylation products) and problems due to cyclic or pulsatile variations in hormone secretion over time (Michaud *et al.*, 1999; Xu *et al.*, 1999).

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