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REVIEW/HYPOTHESIS

Nutrition, hormones, and breast cancer: Is insulin the missing link?

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Breast cancer incidence rates are high in societies with a Western lifestyle characterized by low levels of physical activity, and by an energy-dense diet rich in total and saturated fat and refined carbohydrates. Epidemiologic studies, so far mostly on postmenopausal women, have shown that breast cancer risk is increased in hyperandrogenic women, with decreased levels of plasma sex-hormone binding globulin, and with increased levels of testosterone and of free estrogens. This paper describes the role of hyperinsulinemia as a physiologic link between nutritional lifestyle factors, obesity, and the development of a hyperandrogenic endocrine profile, and reviews evidence that may or may not support the theory that chronic hyperinsulinemia is an underlying cause of breast cancer. An hypothesis is presented, stipulating that breast cancer risk is increased not only in hyperandrogenic postmenopausal women, but also in premenopausal women with mild hyperandrogenism and normal (ovulatory) menstrual cycles. The author suggests further investigation as to whether there is a positive association between risk of breast cancer before menopause and subclinical forms of the polycystic ovary syndrome (PCOS), and to what extent diet and physical activity during childhood, by modulating the degree of insulin resistance during adolescence, may or may not be determinants of a PCO-like hyperandrogenic endocrine profile persisting into adulthood. *Cancer Causes and Control* 1996, 7, 605-625

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1. Introduction

Breast cancer incidence rates are up to five, or even 10 times higher in western Europe and North America than in rural Africa or Asia.[1] Studies on changing cancer incidence patterns in migrant populations,[2,3] and on time trends in breast cancer incidence rates over time in Western or 'Westernizing' countries[4-6] suggest that these differences in breast cancer incidence are determined largely by environmental factors, probably diet and nutrition-related lifestyle. In countries with high breast cancer incidence rates, dietary intake patterns generally are characterized by high intake levels of total and saturated fat and of refined carbohydrates.[7,8] In these high-risk countries, the average age at menarche tends to be lower,[9] and women reach a higher adult body stature,[10,11] which suggests that factors determining breast cancer risk act early in life. Additional suggestions that environmental influences may act at an early age come from studies on changes over time in age-specific breast cancer incidence rates, which appear to occur cohort-wise (*i.e.*, by birth year).[5,6,12] The cohort-wise changes in incidence rates also suggest that similar environmental factors determine the risk of developing breast cancer before or after menopause. Effects of nutrition may be related particularly to energy balance. A high level of physical activity during

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adolescence has been found to be protective against breast cancer.[13-15] In animal studies, caloric restriction has been shown to reduce considerably the development of mammary tumors.[16]

Case-control studies and prospective cohort studies have confirmed the existence of associations of the risk of developing breast cancer – either before or after menopause – with age at menarche[17] and body stature.[18] In addition, these studies show a relation between overweight, as body mass index (wt/ht^2) (BMI), and breast cancer risk, but only for breast cancers occurring after menopause. Besides the BMI, a high ratio of waist circumference divided by hip circumference (waist-to-hip ratio, or WHR) is associated with increased risk of postmenopausal breast cancer, suggesting that intra-abdominal fat stores in particular are a risk factor.[19-23] Interestingly, however, there is no strong evidence for a positive association between a high BMI and breast cancer before menopause; on the contrary, results from a recent meta-analysis indicate that this association may even be weakly negative.[24]

Results from case-control and cohort studies on the relation between breast cancer risk and dietary composition are less consistent. Reviews and meta-analyses of case-control studies suggest that the risk of developing breast cancer may be increased by high intake levels of either total or, more specifically, saturated fat, whereas risk may be decreased by high consumption levels of vegetables, fruits, or dietary fiber.[18, 25-27] These results, however, have not been confirmed clearly by prospective cohort studies thus far.[18,27] One hypothesis, that has been put forward to explain the lack of clear associations between breast cancer risk and dietary intake patterns in mid-life, is that nutritional factors may have their strongest impact before or around puberty.[18,25]

In terms of physiologic mechanisms, nutritional factors are thought to modify breast cancer risk by influencing levels of endogenous hormones. Nutrition-induced differences in hormonal metabolism also would explain associations between breast cancer risk and age at menarche, adult body stature, and obesity. Until recently, however, the understanding of the link between nutrition and endocrine profiles predisposing to the development of breast cancer has been rather limited.

Section 2 of this review briefly describes two main hypotheses on endogenous steroid hormones in relation to breast cancer, for which evidence has accumulated from epidemiologic studies. Sections 3 and 4 discuss the relations between hyperinsulinemia and steroid hormonal profiles that seem to be associated with increased breast cancer risk, and how plasma insulin levels may be co-determined by dietary composition and physical activity. Sections 5 and 6 cover the transient insulin resistance generally observed during puberty, and its possible role in the development of the polycystic ovary syndrome (PCOS) as a form of 'functional ovarian hyperandrogenism' (FOH). Section 7 ('Discussion and synthesis') is an overall discussion of observations that do, or do not, support the hypothesis that breast cancer risk generally is increased in women with a hyperandrogenic endocrine profile, which may develop mainly as a consequence of chronic hyperinsulinemia.

2. Endogenous steroid hormones and breast cancer: free estrogens and ovarian androgen excess

Early hypotheses on associations between endogenous hormones and development of breast cancer focused primarily on the effects of ovarian hormones, and especially estrogens, because it had been recognized very early that:

i. estrogens can induce mammary tumors in rodents;[28]

ii. the risk of developing breast cancer risk is increased among women with early menarche[29] or late menopause,[30] and age-specific incidence rates rise less steeply after the age of natural menopause when ovarian estrogen production has ceased;[31] and

iii. breast cancer risk decreases after artificial menopause (ovariectomy).[32]

These observations initially led to a search for evidence of an excess of endogenous estrogen production in women with breast cancer. The 'estrogen excess hypothesis' evolved into the 'free estrogen hypothesis' (reviewed by Zumoff[33]) when, in the early 1980s, Siiteri *et al*[34] reported that only plasma levels of free estradiol, unbound to sex-hormone binding globulin (SHBG), were elevated in women with breast cancer, even though total estradiol concentrations did not differ. It is now a well-established concept that only the fraction of estrogens unbound to SHBG is biologically available to receptors in target cells.[35,36] Since the 1960s, a large number of case-control studies on endogenous steroid hormone levels have been conducted, the results of which have been reviewed by Key and Pike,[37] and by Bernstein and Ross.[38] As discussed in these reviews, there is substantial evidence that high plasma levels of available estrogen are associated with increased risk of breast cancer, at least among postmenopausal women (on whom most studies have focused thus far). This conclusion receives further support from the New York Women's Health Study[39] on 130 cases of breast cancer detected during an average of five and one-half years of follow-up of a cohort of about 7,000 postmenopausal women, with a relative risk (RR) of about 4.0 between the extreme quartiles of the plasma concentrations of free

estradiol. A similar RR of about 4.0 for high *cf* low levels of free estradiol was observed recently in a prospective study of 71 breast cases in a cohort of 3,375 postmenopausal women (the 'Breast Cancer Serum Bank' study).[40]

In parallel with the earliest studies leading to the estrogen-excess hypothesis, Grattarola[41,42] observed that a substantial subset of breast cancer patients had chronic anovulation, if premenopausal, and excessive urinary excretion of testosterone irrespective of menopausal status. Histologically, resected ovaries of these patients showed hyperplasia of interstitial cells, as already reported by Sommers.[43,44] These histologic changes in the ovary are similar to those seen in women with polycystic ovary syndrome (PCOS), a condition characterized by enlarged ovaries containing an increased number of follicles, and by clinical symptoms related to hyperandrogenism (irregularity of menstrual cycles, anovulation, hirsutism).[45-47] Subsequent studies by Secreto *et al* confirmed Grattarola's findings, and showed an elevated urinary excretion of androgens,[48,49] as well as elevated plasma concentrations of testosterone in both premenopausal[50,51] and postmenopausal[52,53] patients with breast cancer. These observations eventually led to the formulation of the 'ovarian androgen excess' hypothesis.[33] Besides the studies by Secreto *et al*, increased plasma levels of testosterone have been observed in several other studies comparing breast cancer cases with controls.[54-57] Although in two early prospective studies, breast cancer risk was not associated significantly with total plasma testosterone levels, either before[58] or after menopause,[58,59] significantly increased risks were observed in the Italian 'ORDET' study[60] (24 women with breast cancer compared with 88 selected controls within a cohort of 4,053 postmenopausal women; RR = 7.0 for the highest *cf* lowest tertile), and in the Breast Cancer Serum Bank Study[40] (RR = 6.2 for the highest *cf* lowest quartile). In the New York Women's Health Study, breast cancer risk also was increased significantly in postmenopausal women with high plasma testosterone levels (Toniolo, New York University, NY, USA; personal communication, June 1996).

3. Free estrogens and ovarian androgen excess: relation to obesity and insulin resistance

Studies on both pre- and postmenopausal women show that obesity is associated with increased plasma concentrations of testosterone,[61-66] and decreased concentrations of sex-hormone binding globulin (SHBG).[61,64-70] Due to the rise in testosterone production and the drop in SHBG levels, and because testosterone has a higher affinity than estrogens for SHBG,[71,72] free estrogen concentrations increase. In addition, obesity often is associated with insulin resistance, a nutritional-metabolic state characterized by less than normal sensitivity of target tissues (liver, muscle, adipose tissue) to the physiologic effects of insulin (stimulation of glucose uptake, inhibition of lipolysis). To compensate for the loss of insulin sensitivity, larger amounts of insulin are secreted by the pancreatic β-cells to maintain normal plasma concentrations of glucose. Thus, even though there may be no clear signs of glucose intolerance (*i.e.*, the pancreas may still secrete sufficient amounts of insulin to maintain plasma glucose concentrations within the normal homeostatic range),[73] fasting plasma concentrations of insulin and postprandrial secretions of insulin by the pancreas are increased.[61-65,67-70,73,74] These hormonal imbalances (androgen excess, low SHBG levels, and insulin resistance) are related in particular to a high WHR as a measure of 'central' or 'intra-abdominal' obesity[66,75-77] and, importantly, this relation between hormonal imbalances and WHR also is observed in normal-weight women,[62,63] or in overweight women after adjustment for BMI.[66,78]

Until the late 1980s, the predominant view was that insulin resistance arises subsequent to an overproduction of ovarian androgens, in particular testosterone,[77,79] or to an imbalance between androgens from either ovarian, or adrenal origin.[80,81] Direct mechanisms by which androgens may affect insulin sensitivity are the subject of much debate (see discussions in Buffington *et al*[81] and Poretsky[82]). Nevertheless, it seems clear that androgens may favor a more centripetal body fat distribution pattern,[75,83-85] or increase the mobilization of free fatty acids from adipose tissue,[86,87] which relates to development of insulin resistance after long-term overeating (see section 4). On the other hand, there is substantial evidence for an inverse causal relation, whereby high insulin levels potentiate the luteinizing hormone (LH)-stimulated production of ovarian steroids, and particularly of testosterone.[88-92]

The decrease in plasma levels of SHBG in obese women originally was thought also to be the result of high plasma concentrations of testosterone, or by a high ratio of testosterone to estradiol.[71,72] Again, however, there is now at least equally compelling evidence that insulin is the central regulating factor.[93,94] Insulin has been shown to inhibit the production of SHBG in liver cells,[93,95-97] and relatively strong negative correlations have been found between plasma concentrations of insulin and SHBG in obese women, or in women with PCOS.[98-102]

Insulin, and insulin-like growth factors

Insulin-like growth factor I (IGF-I) is a structural homologue of insulin,[103] and has long been known to be a mediator of growth hormone signal, stimulating anabolic processes required for physical growth during puberty and adolescence, as well as during adult life. Many tissue types have been shown to have receptors for IGF-I.[104-107]

These receptors also show considerable homology with those for insulin,[108] and insulin and IGF-I both have some capacity to cross-bind to their mutual receptors.[109]

A major part of the IGF-I circulating in blood plasma is released by the liver, where its production is stimulated primarily by growth hormone (GH). In addition, IGF-I is known to be produced within many of its target tissues,[105,107,110] where it is involved in the paracrine control of cell proliferation.[104,107] Like insulin, IGF-I has a gonadotropic action, potentiating the production of steroid hormones – in particular androgens – in the ovaries[90,111] and, like insulin, it inhibits the hepatic production of SHBG.[96,97]

Plasma concentrations of IGF-I are several hundred-fold higher than those of insulin, but most of the circulating IGF-I (±95 percent) is bound to a number of different IGF-binding proteins (IGFBP) and therefore is not directly available to target tissues.[112,113] The plasma concentrations of IGF-I, and of its most abundant binding protein, IGFBP-3, are regulated primarily by growth hormone (GH) activity,[113] as a function of nutritional status, in particular an adequate dietary supply of energy and protein.[107,113-115] Fasting,[116] weight loss,[117] or an increase in energy expenditure due to physical activity[118] can induce a drop in plasma IGF-I levels within days, while these levels can be restored quickly by nutritional repletion.[107,113,115]

A smaller fraction of IGF-I is bound to a smaller binding protein, IGFBP-1. This binding protein shows marked circadian variation that parallels the natural secretory pattern of insulin during the day, and plasma levels of the binding protein were shown to decrease immediately after infusion of either glucose or insulin.[119-123] Moreover, in women with obesity,[65] or with PCOS,[100,124] and in diabetic adolescents,[125] fasting insulin concentrations have been found to be correlated negatively with IGFBP-1 levels. More definite evidence that plasma levels of IGFBP-1 are down-regulated by insulin, rather than by glucose, has come from a study by Suikkari *et al*[126] where human volunteers were given an infusion of insulin while blood glucose was clamped at euglycemic levels. In addition, insulin has been shown to inhibit IGFBP-1 production in liver (hepatoma) cells.[97,127-129]

The decrease in IGFBP-1 concentrations is believed to correspond to an increased biological activity of IGF-I at the level of target organs which, among a wide variety of body tissues, includes the ovaries and the breast. Thus, through a down-regulation of IGFBP-1, insulin may amplify its effects on steroid hormone metabolism by increasing the availability of IGF-I within the ovaries, and the effects of insulin on steroid hormone metabolism even may be mediated entirely by an increased physiologic activity of IGF-I[90] (see Figure 1). The mechanisms underlying this increased availability, however, are not yet fully understood. Possibly, a decrease in IGFBP-1 levels results in a higher concentration of unbound IGF-I, which then can diffuse freely towards target cells. There also is some evidence, however, that insulin stimulates the active transport of the IGFBP-1-IGF-1 across the endothelial surface of blood vessels.[130]

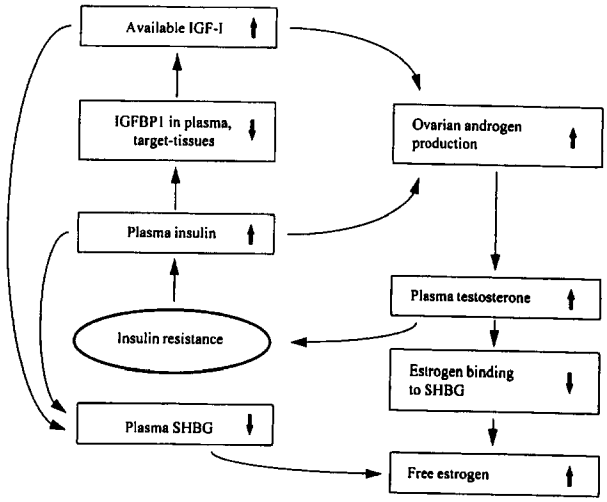

Figure 1. The role of insulin resistance in the development of a hyperandrogenic endocrine profile.

Like IGF-I itself, IGF-binding proteins are produced locally within most of its target tissues,[104,107,131] and IGFBP-1 (as well as other IGF-binding proteins) is synthesized by ovarian granulosa cells,[89,131,132] and it has been detected in follicular fluid.[124] Thus, another postulated mechanism by which high-plasma insulin levels may produce hyperandrogenism is a decrease in the local production of IGFBP-1 within the ovarian tissues, thereby increasing the local biological activity of IGF-I.[90,133]

4. Insulin resistance and hyperinsulinemia: relations with diet and physical activity

Some severe, relatively infrequent forms of insulin resistance are known to be caused by a genetic defect in the insulin receptor ('type A' syndrome), or by the production of auto-antibodies against the insulin receptor ('type B' syndrome).[134] The more common forms of insulin resistance usually are related to obesity, or to diseases associated with obesity such as non-insulin-dependent diabetes mellitus (NIDDM) or PCOS.[134,135] Nevertheless, subgroups of young adults with reduced insulin sensitivity in the absence of overweight also have been identified,[136-139] and, in a survey in the United States, it has been estimated that about 20 percent of the normal-weight men and women, with normal glucose tolerance, had decreased insulin sensitivity comparable to that of individuals with obesity or non-insulin-dependent

diabetes mellitus (NIDDM).[140] As in overweight individuals, however, reduced insulin sensitivity in normal-weight individuals is associated with an increased WHR.[62,63]

A pathogenic model of insulin resistance

Mechanistic models to explain the nutrition-related pathogenesis of insulin resistance emphasize the role of excess oxidation of free (non-esterified) fatty acids (FFA).[73,86,87,141-144] Excessive oxidation of FFA by liver or muscle cells leads to a biochemical inhibition of enzymes involved in the intracellular glucolytic cascade (Krebs cycle), and in intracellular glucose storage in the form of glycogen.[141,145-149] In response to these lowered enzyme activities, the rate of glucose transport into the cell decreases. Variability in the rate of insulin-stimulated glucose storage,[150] and in the glycogen synthesizing capacity of muscle cells,[151] has been shown to account for a large proportion of between-person variations in insulin sensitivity impairment in normal subjects, and in NIDDM patients, respectively. In skeletal muscle, insulin action also has been shown to be related inversely to the size of intracellular stores of triglycerides, which can be increased by a high-fat diet.[152,153]

The starting point in the development of insulin resistance induced by overnutrition is thought to be an increase in the amount of intra-abdominal fat.[86,87,154] Intra-abdominal fat stores are mobilized more easily than fat stored in femoral-gluteal region, because of their sensitivity to lipolytic stimuli (*e.g.*, by adrenaline, through ß3-adrenergic receptors[155]), and because of a relative insensitivity to the antilipolytic effects of insulin.[156] The release of FFA from visceral fat into the portal blood may inhibit glucose uptake and metabolism by the liver and impair hepatic glycogen storage.[141] The mobilization of fat from body fat deposits also will increase FFA levels in peripheral blood, with similar effects on the insulin sensitivity of skeletal muscles. In addition, increased oxidation of FFA by the liver is associated with a stimulation of gluconeogenesis.[157-162] The reduced uptake of glucose by the liver and skeletal muscle, combined with an increased hepatic gluconeogenesis, results in raised fasting plasma-glucose levels which, in turn, lead to chronically elevated (fasting) plasma-insulin concentrations. There is experimental evidence that insulin can down-regulate the concentration of its own receptors on the membranes of target cells,[161,162] and the degree of hyper-insulinemia in insulin-resistant individuals correlates with the reduction in the number of insulin receptors in liver, muscle, or adipose tissue cells.[163,164] The resulting reduction of insulin sensitivity may be aggravated by additional, post-receptoral defects in the response to insulin binding.[134] As discussed in the previous sections, an increase in insulin levels reduces plasma concentrations of SHBG, and may increase concentrations of testosterone (Figure 1). The resulting increase in androgenicity, combined with high plasma insulin levels, favors a centripetal type of body fat distribution[75,83-85,165] and, in interaction with GH, at the same time may stimulate the mobilization of FFA from body fat deposits.[86,87] Thus, a cycle is established in which a decrease in insulin sensitivity, initially associated with relatively mild intra-abdominal adiposity, through an increase in free androgen levels causes a more androgenic body fat distribution which, in turn, will exacerbate the insulin resistance (Figure 2).

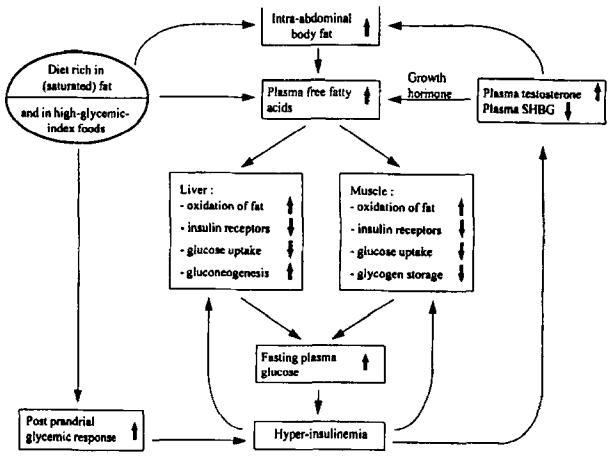

Figure 2. Mechanisms of nutritionally-induced insulin resistance.

It is well-established that genetic factors play an important role in determining the predisposition to overweight,[85,166] and in determining body fat distribution and insulin resistance.[85,167] Nevertheless, the development of central obesity and related insulin resistance in susceptible individuals generally requires interaction with an environmental precipitant in the form of overnutrition. There is much evidence to suggest that overnutrition may be caused by a Western type of diet, rich in total and saturated fat and rapidly digestible carbohydrates, and by a lack of physical activity. The requirement of an environmental precipitant, even in the presence of a very strong genetic predisposition to develop obesity, insulin resistance, and NIDDM, is well-illustrated by studies on Australian aboriginals, and on Pima Indians, who develop these metabolic disorders at much higher rates after adopting a Western type of lifestyle.[144,168]

Dietary fat, obesity, and insulin resistance

According to a well-accepted theoretical model by Flatt,[169] supported by detailed metabolic and biochemical studies, energy balance under unrestricted food intake is achieved by a separate regulation of carbohydrate, fat, and protein balances.[166,169-172] To cover minimal carbohydrate requirements for energy metabolism, and because body stores of carbohydrates are very limited, a higher energy intake

is required on a high-fat diet than on a low-fat diet. It has been shown that a positive fat balance does not enhance fat oxidation acutely[173,174] but rather through a slower, progressive increase in fat stores. Fat oxidation then is raised due to the increasing availability of free fatty acids in blood plasma, the increase in intracellular fat stores, and the development of insulin resistance. The development of insulin resistance thus may be regarded as a compensatory mechanism by which fat oxidation is increased to an amount commensurate with dietary fat intake, and by which the gain in body weight eventually will flatten off to reach a stable body mass.[169,175,176] Thus, a primary dietary factor leading to obesity and insulin resistance would be a high percentage of energy from fat. The latter is confirmed by many,[177-182] though not all,[183,184] clinical studies in which experimental high-fat diets had adverse effects on glucose and insulin metabolism. Cross-sectional epidemiologic studies on free-living populations[176,185-191] also indicate that a diet with a high percentage of energy from fat favors the development of overweight. Similar cross-sectional studies have shown an association between a high intake of total or saturated fat and reduced insulin sensitivity[192-195] or glucose intolerance.[196-198]

While high total fat intake generally has been found to be associated with a reduction in insulin sensitivity, it appears that this effect may be modified by the type of fat consumed. In rats fed high-fat diets, the development of insulin resistance was prevented by increasing the ratio of poly-unsaturated to saturated fatty acids (P/S ratio),[199] or by increasing the amount of long-chain n-3 fatty acids from fish oil.[200,201] Similarly, insulin sensitivity correlated positively with the P/S ratio of phospholipids in serum,[202] muscle cells,[203] and red blood-cell membranes[204] of human subjects. In a study of obese pubertal children,[205] peak insulinemia and the insulinemic area resulting from a standard oral glucose-tolerance test were associated positively with concentrations of saturated, as well as mono-unsaturated fatty acids, and negatively with concentrations of poly-unsaturated 20:5 n-3 fatty acids, measured in total plasma, and in circulating and erythrocyte phospholipids. In the Zutphen Elderly Study,[206] insulin levels during a glucose tolerance test correlated positively with the estimated intakes of saturated, and negatively with the intakes of polyunsaturated fats. In an Italian population of almost 5,000 men and women, a negative correlation was found between consumption levels of olive oil (particularly rich in mono-unsaturated fatty acids) and fasting blood glucose concentrations.[207] One suggested explanation for the effects due to fat composition is that high intake of polyunsaturated fatty acids tends to lower plasma levels of (VLDL-) triglycerides,[208,209] thereby reducing fat available for storage in muscle cells. Another possible explanation, supported by evidence from *in vitro* experiments,[209-211] is that poly-unsaturated fatty acids may improve insulin action by modifying the fluidity of cellular membranes.

Postprandrial glycemic and insulinemic responses to foods rich in carbohydrates

The consumption of carbohydrates induces an immediate, postprandrial rise in plasma glucose concentrations, which, in turn, stimulates the release of insulin from the pancreas. The glycemic and insulinemic responses to food intake (both measured as the integrated areas under the curves of concentrations against time) are correlated highly for a given individual. The glycemic response depends on the physical form of a food, as well as on the specific types of carbohydrate it contains.[212,213] For directly available sugars (mono- and disaccharides), the effect on the rise of blood glucose levels is a function primarily of the proportions of fructose (with low glycemic response) and of glucose (with high response). For starches, the glycemic response is determined by the morphology of the starch granules, and by the degree of branching of the polysaccharide chains. Of the two types of starch that are hydrolyzed enzymatically in the human gut – amylopectin and amylose – the former has more branched polysaccharide chains that are more accessible to the enzyme amylase and hence are digested more easily. Industrial processing (*e.g.*, gelatinization) often aims at increasing the degree of branching of starch polysaccharide chains to make foods more palatable, and thereby also enhance glycemic and insulinemic responses. Similarly, cooking and processing with heat also may produce a greater digestibility of starches, and increase the glycemic response to foods.[214,215]

Water-soluble dietary fibers from fruits, vegetables, or legumes – consisting of pectines and hemicelluloses – can form a gel-like matrix in the small intestine which retains nutrients and attenuates the glycemic and insulinemic responses.[216,217] The addition of water-insoluble types of fiber from cereals to flour products such as bread or spaghetti does not have such attenuating effects on the glycemic response.[218] Nevertheless, the disruption of the physical structure of grains and cereals by grinding and milling (breaking cell walls) has been shown to increase the digestibility of cereal starches.[219,220]

Besides determining plasma insulin responses immediately after a meal, there is some evidence that high consumption of sugars or foods with high glycemic response, in the long term, may lead to a decrease in insulin sensitivity of target tissues. In several epidemiologic studies, reduced insulin sensitivity, or the presence or development of glucose intolerance, was correlated positively with high intakes of sugar,[206,221,222] and negatively with high intakes of dietary fiber.[296,206,222,223] One suggested mechanism to explain these results is that, in the long

term, high postprandrial insulinemic responses may down-regulate the levels of insulin receptors in target cells. Other epidemiologic studies,[224] however, have provided conflicting results on the relation between insulin resistance and sugar intake. Also, from a recent review of epidemiologic and metabolic studies, there appears to be little evidence for a positive association between high-sugar diets and obesity;[225] on the contrary, epidemiologic data quite consistently show an inverse correlation between obesity and percent energy derived from both simple and complex carbohydrates (both correlated with a lower percent of energy from fat) but a positive association with fat intake.

Physical activity and insulin resistance

It is well-recognized that a high level of physical activity helps to prevent the development of overweight. Even independently of influencing body mass, however, physical activity is associated positively with measures of insulin sensitivity, and negatively with the risk of developing glucose intolerance.[206,226-231]

5. Insulin resistance, and steroid hormone profiles during puberty and adolescence

The metabolic-hormonal pattern observed in obese adult women, or in women with PCOS, shows interesting parallels with that during puberty, a period of physical development which also is marked by reduced plasma levels of SHBG,[232-237] and IGFBP-1,[232,238,239] as well as by insulin resistance.[232,238,240-246] During early adulthood, insulin sensitivity normally returns to pre-pubertal levels. Plasma concentrations of IGF-I also show a steady increase from birth to puberty and, under the influence of increased growth hormone secretion, peak at about 13 years of age.[239,244,247-250] After reaching their peak, IGF-I concentrations progressively decrease more than 2.5-fold towards the third decade of life, and even further in old age.[251] In studies comparing children and adults in different age groups, Holly and co-workers[232,238] found that the pubertal decreases in both SHBG and IGFBP-1 levels were correlated with the rise in plasma insulin concentrations. Thus, as in obese adult women, the drop in plasma levels of SHBG and IGFBP-1 seems to be due to direct downregulation by insulin, which thus appears to be implicated in the physiologic mechanisms of sexual maturation and growth.[232,238]

Like insulin, plasma concentrations of growth hormone reach peak levels during puberty (resulting in an increase also of IGF-I and IGFBP-3), and the reduction in insulin sensitivity correlates with the onset of pulsatile growth hormone secretion.[252] The transient decrease in insulin sensitivity during adolescence appears to be due largely to the lipolytic actions of growth hormone, as opposed to those of insulin, leading to increased oxidation of fats instead of carbohydrates.[253-259] Nevertheless, various observations suggest that the degree of insulin resistance in adolescent children is modulated also by type of diet, and by the level of physical activity. First, plasma levels of insulin in pubertal and adolescent children show associations similar to those among adults with BMI or WHR[260,261] and, in obese children, insulin sensitivity improves after weight loss induced by calorie restriction.[262-264] Second, the increase over time in the BMI of adolescent children was related inversely to level of physical activity during leisure time, and was reduced by a decrease in daily fat intake.[265,266] Finally, as mentioned earlier,[205] indices of insulin sensitivity were found to be associated with the fatty acid composition of total plasma lipids of obese pubertal children.

6. Insulin resistance, hyperinsulinemia, and the polycystic ovary syndrome

As mentioned in the introduction, Grattarola *et al*[41] found that breast cancer patients often had ovaries with similar characteristics to those of women with PCOS: an overall increase in size, an increased amount of stromal tissue and hyperplasia of the thecal-interstitial cells where androgens are produced, and an increased number of follicles.[46,47,133] Usually, PCOS is diagnosed only when women seek medical advice for problems of hirsutism, oligomenorrhea, or infertility. These women generally also have increased plasma concentrations of androgens, particularly testosterone and Δ4-androstenedione. Further, women with PCOS generally are insulin resistant, and often have increased plasma levels of LH, or a high ratio of LH relative to follicle stimulating hormone (FSH).

About 30 to 50 percent of women with clinical symptoms of PCOS are overweight, often even severely so, but PCOS also is observed in normal-weight women.[46,47] In studies where body fat distribution has been measured, PCOS was associated with an increased WHR.[267-269] Normal-weight PCOS patients are insulin-resistant compared with normal-weight women without clinical signs of ovarian disease.[270-276] The combined presence of PCOS and obesity, however, is associated with a much stronger degree of insulin resistance than is observed, on average, in women with obesity alone, or in normal-weight women with PCOS.[99,272,276] In contrast, plasma concentrations of testosterone and LH can be elevated equally in obese, or non-obese women with PCOS.[45-47,99,270,271] Since plasma levels of SHBG and IGFBP-1 correlate negatively with fasting insulin concentrations, the levels of SHBG and IGFBP-1 generally are decreased more in obese, than in non-obese PCOS patients.[47,98,99,101,124,273,277,278] Probably because of their

lower plasma levels of SHBG levels, and hence a further increase in free testosterone concentrations, oligomenorrhea – a major clinical manifestation of PCOS – is more frequent in the more insulin resistant women.[100,102,273,275,277,279] In some studies,[275,276] where PCOS was defined by the combined presence of hyperandrogenism and chronic anovulation, insulin resistance was found to be of equal magnitude in obese and non-obese patients.

Depending on the diagnostic criteria used, PCOS has been estimated to be present in about five to eight percent of premenopausal women.[46,47] Using ultrasound (echographic) detection methods, however, the prevalence of an ovarian morphology typical of PCOS (increased ovarian volume, increased number of follicles) was shown to be as high as 20 percent among pubertal girls,[280] as well as among adult women.[46,47,281-283] In a considerable proportion of these relatively asymptomatic women with PCO-like ovarian morphology, plasma levels of testosterone and LH were elevated,[47,281,283] but on average their BMI was similar to that of women with normal ovarian morphology and function.[46,283]

PCOS has been described as the result of an 'exaggerated puberty,'[284] as it resembles physiological adolescent anovulation in a number of ways:

i. both are characterized by a similar 'polycystic' ovarian morphology;

ii. the pattern of gonadotropin secretion – hyperresponse of LH to exogenous luteinizing hormone-releasing hormone (LHRH), and elevated plasma levels of LH – resembles that seen in puberty; and

iii. the 17α-hydroxylase and 17-20-desmolase activities of the enzyme cytochrome P450c17 – an enzyme which is involved in ovarian and adrenal androgen production, and of whose susceptibility to stimulation by adreno- corticotrope hormone (ACTH) increases sharply at adrenarche, an early event in sexual maturation – are excessively accentuated.[285]

The origin of PCOS is called 'functional,' as it has no single identifiable organic cause, but appears to result from a complex dysregulation of hormonal metabolism.[286] PCOS also is being increasingly referred to as a form of 'functional ovarian hyperandrogenism' (FOH). Most authors now concur that chronic overstimulation of the ovarian steroidogenesis by insulin and/or IGF-I is a central feature of the hormonal dysregulations leading to the syndrome (Figure 3).[90,133,284,286-288] The hyperandrogenism associated with PCOS may be due particularly to a hyperfunction of the enzyme P450c17 in the ovarian thecal cells.[289] The expression of this enzyme has been shown to be under the direct influence of insulin and IGF-I, in interaction with luteinizing hormone.[290,291] The

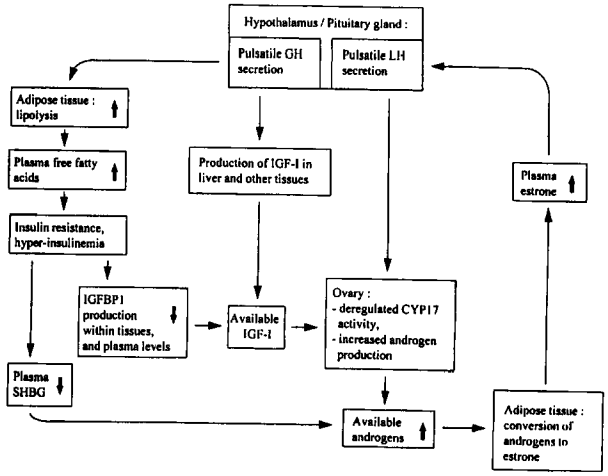

Figure 3. Endocrine mechanisms related to the development of polycystic ovary disease (PCOD).

intra-ovarian androgen excess, possibly accentuated by a down-regulation of plasma SHBG levels due to hyperinsulinemia, is believed to be the direct cause of follicular atresia, and of hyperplasia of thecal-interstitial cells. The combination of hyperplasia of thecal-interstitial cells (which produce testosterone), and atresia of follicular cells (which convert testosterone into estrogens), may explain why overstimulation of the ovarian steroid production gradually leads to an excess of androgens, but not of estradiol. The peripheral aromatization of androgens in adipose tissue leads to increased levels of estrone, which may inhibit slightly the secretion of follicle stimulating hormone (FSH), but which may increase the secretion of LH by sensitizing the pituitary to the effects of LHRH.[133,284,286] Further, an increase in bioactive IGF-I may augment the release of LHRH.[292] The rise in plasma concentrations of LH, combined with the lower levels of FSH, further aggravates the ovarian androgen excess and interstitial hyperplasia, and leads to the formation of multiple follicles arrested in their course of maturation.

The onset of clinical symptoms of PCOS is usually at about the time of menarche, seldom in adulthood. Even in women developing type II diabetes with insulin resistance and hyperinsulinemia, the onset of PCOS at adult age has not been reported.[133] This contrast, plus the fact that PCOS seems a continuation of a physiologic state of anovulation often seen during puberty (but which normally evolves after adolescence), have led several authors to postulate that the critical period for the development of PCOS may be during the peripubertal years[90,133,284] (although some believe that development of the syndrome may have its earliest origins *in utero*[293]). The endocrine-metabolic profile during puberty – characterized by increased plasma levels of GH and total IGF-I, as well as insulin resistance and reduced levels of SHBG and IGFBP-1 – would particularly enhance the

gonadotropic action (of LH and insulin/IGF-I) on the ovary. In many girls, the PCO-like symptoms and changes in ovarian morphology disappear after adolescence, when plasma levels of insulin and of available IGF-I decrease progressively.[294] In girls susceptible to becoming hyperandrogenic, however, a cycle of insulin resistance leading to ovarian androgen excess, thecal-interstitial hyperplasia and follicular atresia, and of androgen excess contributing to the maintenance of the insulin resistant state may persist into adulthood.[90,133,284] Such persistence of a hyperandrogenic endocrine profile was demonstrated by a longitudinal study in Finland,[295] showing positive correlations between serum androgen concentrations during adolescence, and 12 years later.

Studies of familial clustering of PCOS show that genetic predisposition may contribute to the development of the syndrome.[296] In a family study by Carey *et al*,[297] polycystic ovaries and male baldness were demonstrated to be the female and male phenotypes of a condition that seemed to be due to a single gene defect. Further work[298] showed that the expression of PCOS/male baldness was associated significantly (odds ratio of 3.57) with a single base change in the 5' promotor region of P450c17, which would create an additional promotor site for the gene, and thereby lead to its increased expression. The single base change was excluded, however, as a primary defect responsible for the familial clustering. Dunaif[299] proposed that two types of genetic predisposition may be involved in the pathogenesis of PCOS: a 'PCO gene' conferring a specific susceptibility to developing the reproductive abnormalities of PCOS (*e.g.*, by hypersensitivity of ovarian androgen production to stimulation by insulin/IGF-I and LH), and a genetic predisposition to developing a minimum level of insulin resistance, needed for the PCO syndrome to be expressed.

It is still unknown why some women with PCOS become obese and, on average, more insulin resistant, whereas others stay comparatively lean. One can speculate that, due to variations in genetic susceptibility (*e.g.*, the 'PCO-gene'; see above), different magnitudes of insulin resistance, augmented or nonaugmented by obesity, may be required for the ovarian androgen production to be overexpressed. Further, some forms of insulin resistance observed frequently in women with PCOS may be determined genetically and be relatively independent of body weight.[276] Alternatively, on the basis of observations that plasma levels of GH were significantly lower in obese than in non-obese women with PCOS,[300,301] Insler[302,303] has postulated that, in obese girls or women, hyperinsulinemia may be the more predominant derangement causing hyperfunction of androgen producing enzymes in the ovary, whereas in normal-weight women the more predominant derangement would be an increased activity of GH (particularly during puberty). Besides raising plasma and tissue concentrations of IGF-I, which directly stimulates ovarian androgen production, an increased GH activity contributes to the development of insulin resistance because of its lipolytic action. In addition, especially in interaction with androgens, GH favors the growth of lean body mass but limits the size of body fat deposits.

Given the postulated central role for hyperinsulinemia in the etiology of PCOS, it seems reasonable to suppose that lifestyle factors (diet, physical activity) that modulate plasma insulin levels also may influence the development or persistence of the syndrome. In studies with adult PCOS patients,[92,304-307] and with obese adolescent girls,[308] weight loss induced by a calorie-restricted diet indeed resulted in a significant fall of plasma levels of insulin, total and free testosterone, free estradiol and the ratio of LH/FSH, and in a significant rise in SHBG levels. This suggests that obesity, by adding to the relative degree of hyperinsulinemia, at least may contribute to the maintenance of the PCO syndrome. Longitudinal studies are needed, however, to investigate whether diet and physical activity, especially during peripubertal years and adolescence, may or may not have long-lasting effects on the development of a hyperandrogenic endocrine profile persisting into adulthood, and whether such effects are similar in girls who do, or who do not, develop obesity.

7. Discussion and synthesis

This paper reviews the possible role of insulin in the relation between nutrition-related lifestyle factors and the development of a hormonal-metabolic profile associated with an increased risk of breast cancer in women.

The current view of carcinogenesis is that it results from an imbalance between proliferation, differentiation, and programmed death (apoptosis) of cells, with clonal selection and expansion of colonies of preneoplastic and, ultimately, neoplastic cells that have become progressively independent of normal growth control.[309-313] A high rate of cell proliferation is believed to be a key factor in increasing the risk of cancer development, as cell division is necessary for the accumulation of genetic mutations related to neoplastic transformation and clonal selection of transformed cells, as well as for the growth of established tumors. A hormonal environment that inhibits full differentiation of cells may increase breast cancer risk by augmenting the pool of cells remaining responsive to proliferative stimuli.

Steroid hormones, and peptide growth factors have been shown to be crucial in the paracrine and endocrine intercellular communication processes controlling the differentiation[314] and proliferation of human breast cells. Regarding the effect of steroid hormones on the proliferation of epithelial cells, evidence suggests that

progestogens may be the predominant mitogen for normal epithelium, and that estrogens (especially 17-β-estradiol) may potentiate this effect via induction of increased progesterone receptor numbers. On the other hand, in neoplastic epithelium, estrogens appear to be the more predominant mitogen.[311,312] Based on clinical observations that proliferation rates of normal breast epithelium are highest during the luteal phase of the menstrual cycle, Henderson *et al* [313,315] have postulated that tumor development is promoted most strongly at a simultaneous exposure to estrogens plus progesterone. Pike *et al* [37,316,317] have argued that this 'estrogen-plus-progestogen' hypothesis also might explain the finding that, in premenopausal women, extreme obesity seems to be associated with a reduction in breast cancer risk, even though obesity increases the risk of endometrial cancer (which is believed to be due to an exposure to estrogens 'unopposed' by progestogens).[318]

As for peptide hormones and growth factors, numerous studies during the last five to 10 years (reviewed in Macaulay[319] and Yee *et al* [320]) have shown that IGF-I, especially in interaction with estrogens, is a potent inducer of mitosis in breast cancer cells. During the 1970s, experiments with animals and breast cancer cell lines, reviewed by Hilf [321] had demonstrated already that insulin also might promote the growth of breast tumors. The high degree of homology between insulin and IGF-I (at that time known as somatomedin C) and between their receptors, however, had not yet been established, and effects on tumor growth originally attributed to the binding of insulin to its specific receptor actually may have been mediated by an increased availability of IGF-I (in animal experiments), or by the cross-binding of insulin (often used at quite high concentrations in studies with cell cultures) to the IGF-I receptor.

In line with the experimental and clinical evidence relating estrogens – probably in interaction with progesterone and IGF-I – to increased growth rates of breast tumors, epidemiologic observations (reviewed in section 2) show that women developing breast cancer tend to have a hyperandrogenic hormonal profile characterized by higher plasma levels of testosterone, lower plasma levels of SHBG and, hence, higher levels of free estradiol. As described in sections 3 and 6, hyperinsulinemia appears to play a key role in the development of this endocrine profile, either in association with simple obesity, or as a form of functional ovarian hyperandrogenism. One therefore may hypothesize that chronic elevation of plasma insulin levels increases the risk of developing breast cancer. This 'insulin hypothesis' fits with the positive association between obesity and breast cancer observed in postmenopausal women, as recently discussed also by Stoll[322] and with results from ecologic studies showing that breast cancer incidence rates are high in countries with a more sedentary lifestyle, and with a diet rich in saturated (animal) fats and refined carbohydrates. The hypothesis has received its first direct support from a case-control study by Bruning *et al*,[323] in which both pre- and postmenopausal breast cancer patients were shown to have increased serum levels of C-peptide, a marker of pancreatic insulin secretion.

Although the above considerations lend considerable support to the insulin hypothesis, there are also several complicating observations.

First, in apparent contrast with the insulin hypothesis, case-control and cohort studies have not provided consistent evidence for an association between breast cancer risk and diabetes.[324-330] The interpretation of these studies, however, is problematic. In some women with non-insulin dependent (Type 2) diabetes, residual pancreatic β-cell activity can be sufficient for them to have fasting plasma insulin levels as high as insulin-resistant, but glucose-tolerant, women; and, it has been shown that these women may have a similar steroid hormonal profile, marked by low plasma SHBG levels and high free steroid concentrations, to non-diabetic women with similar BMI.[331] Women with insulin treated (Type 1 or Type 2) diabetes, however, in whom the endogenous production of insulin is reduced substantially or even practically nonexistent, can have a steroid hormone profile either very similar to, or different from, that of insulin resistant but non-diabetic women. For instance, in a study of postmenopausal women with insulin-treated Type 2 diabetes, total levels of estrogens and androgens were increased, but a simultaneous rise in SHBG kept the free fractions of these sex hormones within normal limits.[332] In another study of adult women with IDDM, by Djursing *et al*,[333] diabetics with regular menstrual cycles had significantly higher serum concentrations of androgens than controls with a normal cycle, whereas amenorrheic patients had lower serum concentrations of SHBG, LH, total androgens, and free and total estrogens. Prelevic *et al*[334] further distinguished between women with IDDM who did (C-peptide positives), or did not (C-peptide negatives) have some residual pancreatic insulin secretion. All C-peptide negatives with amenorrhea had decreased LH levels, a low LH/FSH ratio, and low levels of testosterone, as observed in the study by Djursin *et al*.[333] On the contrary, C-peptide positives with amenorrhea had the classic hormonal profile of PCOS (increased serum testosterone and LH/FSH ratio, decreased SHBG), and was associated with a history of oligomenorrhea and excess weight before the onset of diabetes. In view of this heterogeneity of steroid hormonal profiles in diabetic women, and in the absence of further information about the type and severity of diabetes, it seems difficult to predict whether diabetes will generally augment or decrease breast cancer risk.

In several studies, acromegaly – a pathologic condition characterized not only by severe insulin resistance but also by high plasma levels of IGF-I – was shown to be associated with increased occurrence of cancer.[335-339] Although most of these studies were too small to estimate the relative incidence rates of cancer at specific sites, one study showed an excess of breast cancer among females. A number of additional studies provided evidence for an increased occurrence of colon cancer, and more particularly of colonic polyps, a relatively frequent preneoplastic lesion.[340-346]

Second, in contrast to international correlation studies, individual-level studies, especially those of a prospective cohort design, have failed to show a clear association between high intake levels of (saturated) fat and breast cancer risk. This seems surprising, since physiologic studies of energy metabolism and cross-sectional epidemiologic analyses indicate that a high intake of (especially saturated) fat is one of the dietary factors associated most clearly with development of obesity and insulin resistance (see section 4). The lack of association between level of fat intake and breast cancer risk in individual-level studies has been attributed to methodologic problems, such as the difficulty of measuring quite small differences in dietary intake patterns of individuals all living in the same, geographically limited area.[347] A complimentary explanation could be that, in populations with a predominantly Western lifestyle and generalized conditions of overnutrition, in most individuals the development of chronic hyperinsulinemia and related endocrine dysregulations may depend more on genetic factors determining their physiologic response to overnutrition, than on comparatively small differences in their habitual diets. In addition, the development of breast cancer through nutritionally induced endocrine dysregulations may be determined predominantly during pre- or peripubertal years, while dietary habits during middle life may no longer reflect those of childhood.[18,25] In this respect, breast cancer seems to differ from colon cancer – although international variations in incidence rates very much parallel those of breast cancer and the development of colon cancer has also recently been postulated to be related to chronic hyper-insulinemia[348,349] – as colon cancer risk in prospective studies appears associated more clearly to aspects of habitual diet (*i.e.*, measured relatively shortly before the diagnosis of the disease).

A third confusing observation is the lack of positive association between obesity and the risk of breast cancer before menopause. This is particularly puzzling, as several case-control studies have shown that not only post-, but also premenopausal women who develop breast cancer have a more hyperandrogenic endocrine profile[49-51] and higher plasma insulin levels[323] than disease-free controls. Further, cross-sectional epidemiologic studies of disease-free, premenopausal women do show a positive association between BMI and insulin resistance.[61,63,74,78] These puzzling observations lead to the following questions:

i. How can obesity be unassociated, or possibly even associated negatively, with risk of breast cancer in premenopausal women? and

ii. How can a hyperandrogenic endocrine profile with increased plasma levels of free estrogens, and with some degree of insulin resistance, develop in the absence of obesity?

An answer to the first question may be that, especially in hyperandrogenic women, overweight is associated with more severe degrees of insulin resistance and, before menopause, with oligomenorrhea. Henderson[313,315] and Pike *et al*[37,316,317] have postulated that women with menstrual irregularities spend a smaller cumulative lifetime in the luteal phase of their cycles, and therefore have a lower lifetime exposure to the combination of estradiol (produced during both the follicular and luteal phases of the cycle) and progesterone (produced during the luteal phase only). Moreover, during anovulatory cycles, the maturation of the dominant follicle is incomplete and the ovarian production of progesterone is subnormal. Thus, assuming that the development of breast tumors is promoted most strongly by a combined exposure to estradiol and progesterone, hyperandrogenic women who are also obese may have a relative protection against breast cancer before menopause. On the other hand, following the same estrogen-plus-progestogen hypothesis, hyperandrogenic women with regular menstrual cycles (in general less obese, and less severely insulin resistant) would be expected to have a relative increase in breast cancer risk, due to greater cumulative exposure to (free) estrogens plus normal levels of progesterone.

As for the second question, an answer may be found by further investigating the mechanisms of development of FOH (see section 6). About 30 to 50 percent of women seeking medical advice for symptoms typical of PCOS are obese, the others having a normal body weight. Moreover, studies based on echographic screening have shown that the prevalence of milder, subclinical forms of PCOS may be considerably higher than that of the clinically manifest syndrome,[45,281-283] and that, on average, women with milder forms of PCOS have a BMI similar to that of women with normal ovarian appearance and function.[282,283] It may be that particularly the latter category of relatively asymptomatic women has increased risk of breast cancer before menopause.

In summary, I surmise that the risk of developing breast cancer generally is increased in hyperandrogenemic women, because of a higher exposure to estrogens unbound to SHBG. Chronic hyperinsulinemia appears

to be a central physiologic link between dietary composition, nutritional status (size of triglyceride stores in adipose tissue and skeletal muscle; relative contributions of the oxidation of fats and carbohydrates to energy metabolism), and development of the hyperandrogenic endocrine profile. In young women, hyperandrogenism may develop more often as a form of FOH, due to a genetically determined sensitivity of the ovaries to the stimulating effects of insulin/IGF-I and LH on androgen production, or because of a genetic predisposition to develop a higher-than-average degree of insulin resistance (with or without obesity) at a young age. In these women, hyperandrogenism may frequently also come to expression in the form of an ovarian morphology typical of PCOS. With age, high plasma androgen levels are probably associated increasingly with a high BMI, due to the rising prevalence of obesity in women less strongly predisposed to develop obesity and hyperandrogenism earlier in life.

Following the estrogen-plus-progestogen hypothesis, the increase in breast cancer risk due to a rise in free estrogens is amplified by the presence of progesterone. Therefore, premenopausal women with more severe forms of insulin resistance and hyperandrogenism, and with chronic anovulation, may turn out to have a relative protection against breast cancer compared with the general population (at least before menopause). The risk of breast cancer is expected to be increased, however, in hyperandrogenic women who maintain regular menstrual cycles. After menopause, when the ovarian production of progesterone and estradiol has ceased, and when breast cancer risk in all women is determined more predominantly by available levels of estrogens, formed in adipose tissue by the aromatization of androgens,[350,351] breast cancer risk would be expected to be increased in all hyperandrogenic women, especially when they are obese, and even when they are more severely insulin resistant.

Further prospective cohort studies are needed to confirm further the presence of the hypothesized relations between breast cancer risk and plasma hormone levels (testosterone, SHBG, total and free estradiol, insulin), not only after, but also before menopause. Additionally, a case-control study should be undertaken to investigate whether the incidence of breast cancer before menopause is increased in women with normal cycles and subclinical forms of PCOS (*e.g.*, diagnosed by echographic examination of the ovaries, combined with measurements of plasma hormone levels).

Thus far, only two epidemiologic studies appear to have been conducted to investigate the possible relation between PCOS and increased breast cancer risk. In a cohort study on 1,270 women with previously diagnosed PCOS,[352] there were three, four and five cases with pre-, peri-, and postmenopausal breast cancer, respectively. The five postmenopausal cases were in significant excess compared with the number of (1.4) cases expected, as computed from average breast cancer rates in the corresponding general population. In the second, a multi-center study of 4,730 cases and 4,688 geographically matched controls,[353] self-reports of PCOS (diagnosed previously by a physician) were against all expectations associated negatively with premenopausal breast cancer risk. Both studies focused on women with rather severe manifestations of the PCO syndrome, however, who sought medical advice because of complaints of hirsutism, menstrual irregularity, or infertility. The negative association between PCOS and breast cancer risk observed in the second study therefore may be explained by reduced exposure to progesterone, because of chronic anovulation, and thus would not contradict the hypothesis of an excess breast cancer risk in hyperandrogenic, but normally cycling premenopausal women.

The observation of an increased prevalence of milder forms of PCOS in premenopausal breast cancer patients may give additional support to the idea that breast cancer risk is determined largely by metabolic dysregulation occurring early in life. Further studies then might evaluate whether the development of PCOS is related to early sexual maturation (as has been suggested recently by some studies on sexually precocious girls[354,355]) a well-established risk factor for breast cancer. Finally, it would then be of interest to investigate whether puberty is indeed the critical period for development of the syndrome (also its subclinical forms), and to evaluate to what extent diet and physical activity during peripubertal years and adolescence, by influencing the degree of insulin resistance, may or may not be determinants for the development of a PCO-like hyperandrogenic profile persisting into adulthood.

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