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Cytochrome P450 1A2 (CYP1A2) activity and risk factors for breast cancer: a cross-sectional study

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Abstract

Introduction Breast cancer risk may be determined by various genetic, metabolic, and lifestyle factors that alter sex hormone metabolism. Cytochrome P450 1A2 (CYP1A2) is responsible for the metabolism of estrogens and many exogenous compounds, including caffeine.

Methods In a cross-sectional study of 146 premenopausal and 149 postmenopausal women, we examined the relationships between CYP1A2 activity and known or suspected risk factors for breast cancer. Blood levels of sex hormones, lipids, and growth factors were measured. *In vivo* CYP1A2 activity was assessed by measuring caffeine metabolites in urine. Stepwise and maximum R regression analyses were used to identify covariates related to CYP1A2 activity after adjustment for ethnicity.

Results In both menopausal groups CYP1A2 activity was positively related to smoking and levels of sex hormone binding globulin. In premenopausal women, CYP1A2 activity was also positively related to insulin levels, caffeine intake, age, and plasma triglyceride levels, and negatively related with total cholesterol levels and body mass index. In postmenopausal women CYP1A2 activity was positively associated with insulin-like growth factor-1, and negatively associated with plasma triglyceride, high-density lipoprotein cholesterol, and age at menarche.

Conclusion These results suggest that CYP1A2 activity is correlated with hormones, blood lipids, and lifestyle factors associated with breast cancer risk, although some of the observed associations were contrary to hypothesized directions and suggest that increased CYP1A2 function may be associated with increased risk for breast cancer.

Keywords: anthropometric measurements, CYP1A2, insulin-like growth factor-1, mammographic density, sex hormones

Introduction

Epidemiologic evidence suggests that endocrine factors alter breast cancer risk. In both premenopausal and postmenopausal women cancer risk is associated with events that alter hormonal balance, such as age at menarche, parity, body weight, body fat distribution, and use of exogenous hormones. Prospective data from postmenopausal women have also shown that modest increases (+15%) in circulating sex hormone levels are associated with increased future risk for developing breast cancer [1].

Exposure to sex hormones may be affected by various genetic, metabolic, and lifestyle factors that can alter the biosynthesis and metabolism of sex hormones. Function of the cytochrome P450 1A2 (CYP1A2) enzyme might play a role in determining estrogen exposure because it is principally responsible for the metabolism of 17β-estradiol after initial conversion to estrone. CYP1A2 is a major phase I enzyme, and accounts for about 15% of total liver P450 content [2]. The enzyme is most active in catalyzing 2-hydroxylations [3], and in humans between 40% and 50%

AAMU = 5-acetylamino-6-amino-3-methyluracil; AFMU = 5-acetylamino-6-formylamino-3-methyluracil; BMI = body mass index; CMR = caffeine metabolic ratio; CYP1A2 = cytochrome P450 1A2; GH = growth hormone; HDL = high-density lipoprotein; IGF = insulin-like growth factor; IGFBP = insulin-like growth factor binding protein; LDL = low-density lipoprotein; SHBG = sex hormone binding globulin; 1U = 1-methylurate; 1, 3U = 1, 3-dimethylurate; 1, 7U = 1, 7-dimethylurate; 1X = 1-methylxanthine; WHR = waist–hip ratio.

of estrogens are hydroxylated at the C-2 position [4]. Increased formation of 2-hydroxylated estrogens would be consistent with a reduced risk for breast cancer under a 'receptor-mediated' hypothesis because 2-hydroxyestrogens can bind only weakly to the estrogen receptor [5]. Higher CYP1A2 activity is therefore postulated to be associated with reduced risk for breast cancer.

Previous research has shown that CYP1A2 activity can be influenced by a number of factors including use of exogenous hormones, body size, tobacco smoke., and intake of coffee and cruciferous vegetables [6-8]. The rate of estrogen 2-hydroxylation can also be influenced by dietary factors [9]. The goal of the present study, conducted in a cross-sectional sample of 295 women, was to identify the determinants and covariates of CYP1A2 activity, particularly those related to breast cancer risk. These include potential associations with reproductive events, sex hormone levels, the growth hormone (GH)/insulin-like growth factor (IGF)-1 axis, body size, plasma lipids, smoking status, and diet.

In vivo CYP1A2 function was examined by assessing caffeine clearance. Caffeine is a good probe for CYP1A2 function because 90% of caffeine clearance is mediated by CYP1A2 [10]. Urinary caffeine metabolites have been used to calculate a number of ratios that indicate CYP1A2 function. We chose to use the caffeine metabolic ratio (CMR), which is calculated as follows: CMR = (5-acetylamino-6-formylamino-3-methyluracil [AFMU] + 1-methylurate [1U] + 1 methylxanthine [1X])/1, 7-dimethylurate (1,7U). This is because this parameter is not dependent on renal flow [11] and is based only on metabolic end products of caffeine metabolism, thus making the amount and timing of urine collection relatively unimportant [10]. Measurement of the CMR in 24-hour urine samples has been shown to be a viable method of assessing CYP1A2 intake in populations with widespread caffeine use [12]. In the present study 93% of women consumed sufficient levels of caffeine to estimate CMR. Because daily caffeine intake is relatively constant, urinary recovery of caffeine metabolites over a 24-hour period reflects CMRs observed with standardized dosing [12].

Methods

The methods employed in the present study are published in detail elsewhere [13] and are only briefly described here. Ethical approval for the study protocol was given by the Human Subjects Review Committee at the University of Toronto, Canada.

Source of study subjects

Between 1994 and 1997, potential participants were identified from the mammographic units of Mount Sinai, Women's College, and St. Michael's Hospital in Toronto. The extent of mammographic density for all patients was visually estimated by a radiologist and expressed as a percentage of breast area on a five-point scale. The purpose of our approach to recruitment was to assemble a group of women without breast cancer and with a wide range of density levels by recruiting approximately equal numbers of women in each of five categories of density, with over-representation of the extreme categories. The number of patients recruited into each of the five categories of radiological density were as follows: <10%, $n = 101$; 10% to <25%, $n = 62$; 25% to <50%, $n = 60$; 50% to <75%, $n = 60$; and = 75%, $n = 99$.

Recruitment

Potential participants were sent a letter and subsequently telephoned about the study. Premenopausal women were eligible if they were menstruating regularly, not pregnant or breast-feeding, and had not had a hysterectomy or oophorectomy. Postmenopausal women were eligible if they had had spontaneous amenorrhea for at least 12 months, or had had a hysterectomy and were 50 years of age or older, or had had a bilateral oophorectomy at any age. A woman was excluded if she was taking any type of exogenous hormone preparation, had breast augmentation or reduction or a personal history of breast cancer, or was being investigated for breast cancer. In total, 382 women agreed to participate in the study, representing 88% of those who were contacted and found to be eligible.

Measurements

Data and blood samples were collected after a 12-hour overnight fast, and during the luteal phase of the menstrual cycle (days 20–24) for premenopausal women. The mammogram closest to the time of the blood draw was used (mean difference 32 weeks).

Obtaining consent

Because examination of CYP1A2 activity was not a goal of the original study, patients were subsequently mailed a letter describing the goals of this component of the study, and written consent was obtained to measure CYP1A2 activity. Information on ethnicity was also obtained at this time. Of 382 eligible patients (193 premenopausal and 189 postmenopausal women), 357 (93%) gave consent. Eight women could not be contacted because they had moved and could not be traced through either telephone directories or their physicians. Sixteen women were nonresponders after a minimum of four telephone reminders, and one did not provide consent.

Ethnicity

By questionnaire (see below), each patient was asked their country of birth, as well as the countries of birth for each of their parents and grandparents. They were also asked the question, 'What is your ethnic or cultural background?' and

given instructions to mark all appropriate categories. Subjects were classified as follows: black; white (e.g. British, French, European, Latin/South American of European background); native/aboriginal people of North America (North American Indian, Inuit, Métis); East Asian (e.g. Chinese, Japanese, Korean, Vietnamese); South Asian (e.g. Indian from India, Pakistani, Punjabi, Tamil); other, with specification; and 'don't know'. Because of low numbers in groups other than Caucasians, the categories were collapsed and described as Caucasian (white), East Asians, Jewish, and other.

Epidemiologic and anthropometric data

Information about epidemiologic risk factors for breast density and breast cancer was collected by questionnaire, and dietary information was obtained using a list-based food frequency questionnaire developed by Block and coworkers [14]. Each woman was weighed and measured for height, and waist and hip circumference.

CYP1A2 activity

Caffeine (1, 3, 7-trimethylxanthine) is metabolized by CYP1A2 and has been used to evaluate CYP1A2 activity *in vivo* [15]. The best urinary metabolic ratio appears to be (AFMU + 1X + 1U)/1,7U (i.e. the CMR) [16].

Urinary caffeine metabolites were measured by high-performance liquid chromatography as previously described [15], except for a modification of the composition of the mobile phase. The mobile phase was composed of 1.3% isopropanol, 0.2% isonitrile, and 0.1% phosphoric acid. The caffeine metabolites were eluted at 1 ml/min and detected by ultraviolet absorbance (0.05) at 280 nm. The retention times of 1U, 1X and 1,7U and the internal standard (N-acetyl-*p*-aminophenol) were 9.9, 11.9, 29.8, and 14.2 min, respectively.

Urinary AFMU was first deformylated to stable 5-acetylamino-6-amino-3-methyluracil (AAMU) and then measured using the high-performance liquid chromatography method reported by Tang and coworkers [15]. The mobile phase consisted of 0.075% acetic acid and 0.075% phosphoric acid. AAMU and the internal standard (hydrobenzyl alcohol) were eluted at a flow of 0.9 ml/min and monitored by ultraviolet absorbance (0.04) at 263 nm. The retention times of AAMU and the internal standard were 13 and 36 min, respectively.

A standard urine sample with known caffeine metabolite concentrations was analyzed across all days of sample analyses with an interassay coefficient of variation of 9%. Accuracy of the CYP1A2 measurement did not vary with caffeine intake. After adjustment for smoking status, age, body mass index (BMI), waist–hip ratio (WHR), and ethnicity, coefficient of variations associated with mean CYP1A2 function across quartiles of caffeine intake were 56.8%, 55.3%, 57.0%, and 46.9% for premenopausal women, and 39.8%, 44.1%, 43.5%, and 42.4% for postmenopausal women. Kashuba and coworkers [17] investigated the intraindividual variability in CYP1A2 activity over a 3-month period and the median coefficient of variation was 16.8% (range 4.5–49.3%).

Caffeine intake

Dietary caffeine intake was assessed by summing the molar concentrations of AAMU, 1, 7U, 1U, and 1, 3-dimethylurate (1,3U) in urine and multiplying them by the 24-hour urine volume to obtain total amount of caffeine metabolites excreted in moles. This amount was multiplied by the molecular weight of caffeine (194.19 g/mol) and divided by 1000 to obtain caffeine intake in milligrams. A correction factor of 1.49 (1/0.67) was applied because the above metabolites, on average, account for 67% of all excreted caffeine metabolites [18].

Measurement of blood samples

Serum estradiol in premenopausal women and sex hormone binding globulin (SHBG) levels were measured at the London Regional Cancer Center in Ontario, Canada in the laboratory of Geoffrey Hammond [19]. Percentage free estradiol was estimated from a nomogram describing the relationship between serum SHBG levels and percentages of free estradiol in a reference population of premenopausal and postmenopausal women of normal weight [20]. These values were used to calculate the free estradiol concentrations from the total estradiol measurements. Serum estradiol levels in postmenopausal women, and IGF-1, IGF binding protein (IGFBP)-3, and GH levels were measured by Esoterix Center for Clinical Trials (Calabasas Hills, CA, USA) [13]. Insulin and progesterone levels were measured by the Clinical Biochemistry Laboratory at the Wellesley Hospital in Toronto, Canada. Measurements of lipids and lipoproteins were performed at the J Alick Little Lipid Research Laboratory (St. Michael's Hospital, Toronto), using the standardized Lipid Research Clinic method [21]. For hormone measurements, the percentage coefficient of variation within hormone assays was less than 7% for all (except for progesterone, which was 8.7%), and between assays it was less than 10% for all (except progesterone, which was 11.9%). The interassay coefficient of variation was less than 4% for total cholesterol, triglycerides, and high-density lipoprotein (HDL)-cholesterol.

Statistical methods

Data were analyzed using the SAS statistical software package (version 6.12; SAS Institute Inc., Cary, NC, USA) [22]. Data were inspected for normality and, when necessary, transformed to approximate a normal distribution. Details of the transformations used are given in the footnotes of tables. Differences in results were found for

Table 1

Selected characteristics of study subjects by ethnicity: premenopausal women

	With CYP1A2 results				No CYP1A2 results[5] ($n = 36$)
	Caucasian[1] ($n = 125$)	East Asian[2] ($n = 4$)	Jewish[3] ($n = 10$)	Other[4] ($n = 7$)	
Risk factors					
Age (years)	44.8 (4.8)	46.8 (4.5)	45.4 (3.1)	44.7 (5.1)	45.1 (4.4)
Weight (kg)	68.1 (16.6)	54.8 (3.1)	59.4 (10.7)	64.9 (17.9)	69.1 (13.3)
BMI (kg/m^2)	25.3 (6.1)	23.2 (0.9)	22.6 (3.5)	25.9 (6.5)	25.8 (5.1)
WHR	0.75 (0.06)	0.75 (0.04)	0.73 (0.07)	0.77 (0.05)	0.74 (0.06)
Age at menarche (years)	12.8 (1.5)	11.5 (1.3)	12.2 (0.6)	13.7 (1.6)	12.6 (1.5)
Age at first birth (years)	27.8 (5.7)	29.5 (0.7)	29.5 (2.7)	25.6 (9.7)	29.0 (6.4)
Number of live births	1.4 (1.2)	1.0 (1.2)	2.1 (1.3)	1.6 (1.3)	1.5 (1.2)
Mammographic density (%)[6]	27.4 (23.2)	47.6 (17.0)	40.2 (22.7)	25.9 (20.0)	28.4 (20.5)
CYP1A2 activity (CMR)[7]	6.3 (3.0)	6.5 (1.0)	5.0 (1.0)	6.1 (3.5)	-
Hormones					
Estradiol (pmol/l)	326.2 (217.0)	188.0 (127.6)	241.6 (173.3)	337.9 (188.4)	295.9 (221.5)
Free estradiol (%)	2.1 (0.7)	1.7 (0.5)	1.8 (0.7)	2.3 (0.7)	2.3 (0.5)
SHBG (nmol/l)	57.3 (27.1)	70.8 (17.2)	69.1 (26.6)	48.4 (24.6)	48.3 (19.0)
IGF-1 (ng/ml)	158.5 (35.6)	154.5 (36.2)	139.7 (36.6)	144.4 (24.8)	143.4 (34.0)
IGFBP-3 (mg/l)	2.7 (0.5)	2.6 (0.4)	2.7 (0.6)	2.7 (0.4)	2.6 (0.5)
Insulin (pmol/l)	74.0 (43.6)	47.4 (20.2)	53.8 (33.6)	86.0 (40.8)	77.0 (36.8)
Nutritional variables					
Caffeine intake (mg/day)	161.0 (100.8)	138.8 (90.9)	132.0 (109.1)	212.9 (148.6)	58.0 (58.8)
Total energy intake (kcal/day)	1707.9 (467.1)	1640.0 (624.9)	1568.2 (346.4)	1995.4 (877.1)	1749.3 (485.2)
Blood lipids					
Total cholesterol (mmol/l)	4.7 (0.9)	4.8 (0.4)	4.8 (0.8)	5.1 (0.9)	5.0 (1.0)
Triglycerides (mmol/l)	1.3 (0.9)	1.1 (0.1)	1.1 (0.5)	1.0 (0.6)	1.5 (1.1)

Values are expressed as mean (standard deviation). [1]$n = 86$ for age at first birth; $n = 124$ for free estradiol, insulin-like growth factor (IGF-1), and IGF binding protein (IGFBP)-3; $n = 121$ for caffeine intake; and $n = 123$ for total energy intake. [2]$n = 2$ for age at first birth. [3]$n = 8$ for age at first birth. [4]$n = 5$ for age at first birth. [5]$n = 25$ for age at first birth and $n = 7$ for caffeine. [6]Proportion of breast area occupied by dense tissue. [7]Estimated by caffeine metabolic ratio (CMR; see text for details). BMI, body mass index; WHR, waist–hip ratio.

premenopausal and postmenopausal women; thus, all data are presented stratified by menopausal status.

Relationships between CYP1A2 activity and hormone levels, anthropometric measurements, blood lipids, and diet were explored using multiple regression analysis. Ethnicity, age, body size (BMI and WHR), and smoking status as determinants of CYP1A2 activity [7] were included in all models as potential confounders. Further adjustments were made to control for potential confounding variables, and details are given in the tables and figures. Because the data analysis was exploratory in nature, the goal of the study was to provide results for all relationships that might potentially be associated with CYP1A2 activity. Subsequent research will be needed to confirm any relationships identified here. Differences were thus considered signifi-

Table 2

Selected characteristics of study subjects by ethnicity: postmenopausal women

	With CYP1A2 results				No CYP1A2 results[5] ($n = 26$)
	Caucasian[1] ($n = 126$)	East Asian[2] ($n = 6$)	Jewish[3] ($n = 5$)	Other[4] ($n = 12$)	
Risk factors					
Age (years)	56.3 (4.6)	54.8 (3.8)	53.4 (2.5)	55.9 (4.2)	56.0 (4.5)
Weight (kg)	72.5 (16.6)	55.8 (9.8)	70.5 (18.1)	66.6 (15.7)	63.7 (17.0)
BMI (kg/m^2)	26.8 (6.2)	23.2 (4.7)	25.9 (6.8)	25.6 (5.4)	23.3 (5.2)
WHR	0.77 (0.07)	0.77 (0.08)	0.80 (0.09)	0.80 (0.06)	0.75 (0.11)
Age at menarche (years)	13.1 (1.6)	13.0 (1.7)	12.6 (2.6)	13.0 (2.1)	12.8 (1.7)
Age at menopause (years)	49.1 (4.8)	50.8 (3.3)	49.1 (3.7)	48.8 (2.5)	48.2 (4.3)
Age at first birth (years)	26.0 (5.2)	34.6 (6.8)	22.8 (3.8)	31.0 (8.7)	25.4 (4.1)
Number of live births	1.8 (1.5)	1.8 (1.5)	2.2 (1.6)	1.0 (1.0)	1.3 (1.4)
Mammographic density (%)[6]	19.8 (18.5)	48.2 (8.9)	22.3 (24.6)	30.1 (23.8)	36.0 (22.1)
CYP1A2 activity (CMR)[7]	5.7 (2.2)	8.9 (1.8)	3.5 (1.7)	6.6 (2.8)	-
Hormones					
Estradiol (pmol/l)	44.6 (91.1)	31.8 (14.3)	27.9 (10.6)	51.1 (33.4)	72.6 (117.5)
Free estradiol (%)	2.6 (0.6)	2.4 (0.5)	2.4 (0.6)	2.7 (0.4)	2.2 (0.7)
SHBG (nmol/l)	41.0 (22.2)	47.8 (16.8)	47.6 (22.3)	36.9 (14.8)	54.2 (26.5)
IGF-1 (ng/ml)	127.8 (33.0)	126.7 (42.6)	137.0 (34.7)	137.3 (36.6)	139.3 (37.5)
IGFBP-3 (mg/l)	2.8 (0.5)	2.6 (0.4)	3.0 (0.5)	2.8 (0.4)	2.8 (0.6)
Insulin (pmol/l)	77.1 (46.4)	78.6 (46.7)	91.3 (40.1)	103.7 (53.9)	75.9 (52.9)
Nutritional variables					
Caffeine intake (mg/day)	193.0 (117.3)	179.0 (147.6)	93.0 (59.4)	118.5 (73.2)	9.0 (9.7)
Total energy intake (kcal/day)	1779.7 (491.1)	1884.7 (646.4)	1234.3 (215.3)	1579.8 (274.3)	1648.0 (523.3)
Blood lipids					
Total cholesterol (mmol/l)	5.6 (0.8)	5.7 (0.7)	5.4 (1.0)	5.6 (0.9)	5.2 (1.1)
Triglycerides (mmol/l)	1.5 (0.8)	1.4 (0.8)	1.8 (0.9)	1.4 (0.6)	1.2 (0.5)

Values are expressed as mean (standard deviation). [1]$n = 117$ for age at menopause; $n = 97$ for age at first birth; $n = 105$ for total estradiol; and $n = 125$ for free estradiol, insulin-like growth factor (IGF-1), and IGF binding protein (IGFBP)-3. [2]$n = 5$ for age at first birth, free estradiol, and sex hormone binding globulin (SHBG). [3]$n = 4$ for age at first birth. [4]$n = 11$ for menopausal age and total estradiol and $n = 8$ for age at first birth. [5]$n = 23$ for menopausal age, $n = 14$ for age at first birth, $n = 22$ for total estradiol, and $n = 15$ for caffeine. [6]Proportion of breast area occupied by dense tissue. [7]Estimated by caffeine metabolic ratio (CMR; see text for details). BMI, body mass index; WHR, waist–hip ratio.

cant at $P \leq 0.05$, but for descriptive purposes quartile least square means were determined for variables associated with CYP1A2 activity at $P \leq 0.20$, and tests for trends were performed as an additional method of assessing the data for these variables. Although these results are based on analyses of transformed data, they are presented in their original units with 95% confidence intervals.

Table 3

CYP1A2 activity and risk factors for breast cancer: premenopausal women

Variable	β (SE)	F	*P*	Quartiles (mean CMR [95% confidence interval])				*P* for trend
				Q1	Q2	Q3	Q4	
Age (years)	0.001 (0.007)	0.01	0.93					
Age at first birth (years)[2]	0.16 (0.21)	0.56	0.46					
Age at menarche (years)	0.03 (0.03)	1.80	0.18	6.11 (5.12–7.29)	6.55 (5.49–7.81)	7.39 (5.84–9.35)	6.62 (5.03–8.71)	0.16
BMI (kg/m²)[2]	-0.43 (0.19)	5.05	0.03	7.76 (6.32–9.53)	6.11 (5.09–7.33)	6.17 (5.02–7.57)	6.16 (4.89–7.76)	0.04
WHR	1.26 (0.70)	3.23	0.07	5.89 (4.80–7.24)	6.01 (4.90–7.37)	6.46 (5.31–7.86)	7.20 (5.87–8.83)	0.06
Caffeine (mg/day)[1]	0.020 (0.007)	6.32	0.01	5.42 (4.49–6.54)	6.37 (5.29–7.68)	6.28 (5.17–7.64)	6.94 (5.84–8.24)	0.02
Alcohol (kcal/day)[2]	0.03 (0.02)	2.13	0.15	5.17 (4.33–6.16)	5.17 (4.29–6.23)	5.88 (4.85–7.12)	6.12 (5.03–7.45)	0.05
Cholesterol (mg/day)[2]	0.09 (0.08)	1.29	0.26					
Total energy (kcal/day)[1]	0.01 (0.01)	6.05	0.02	5.73 (4.70–6.98)	6.18 (5.00–7.64)	6.58 (5.46–7.93)	6.81 (5.56–8.33)	0.06
Carbohydrate (g/day)[1]	0.02 (0.02)	2.26	0.14	5.87 (4.84–7.13)	6.75 (5.51–8.26)	6.30 (5.13–7.74)	6.63 (5.45–8.07)	0.34
Fat intake (g/day)[1]	0.05 (0.02)	4.33	0.04	6.04 (5.20–7.01)	6.94 (5.98–8.05)	6.38 (5.46–7.45)	7.01 (6.03–8.15)	0.25
Protein intake (g/day)[2]	0.29 (0.11)	7.81	0.006	6.01 (4.97–7.27)	6.74 (5.45–8.33)	6.20 (5.08–7.55)	6.93 (5.65–8.50)	0.28
Total estradiol (pmol/l)[3]	-0.001 (0.006)	0.01	0.92					
Free estradiol (%)[2]	-0.05 (0.01)	11.02	0.001	6.62 (5.51–7.96)	7.46 (6.16–9.04)	5.99 (4.90–7.31)	5.16 (4.19–6.35)	0.007
SHBG (nmol/l)[4]	0.07 (0.02)	10.89	0.001	5.13 (4.16–6.35)	6.09 (4.96–7.46)	7.57 (6.17–9.28)	6.73 (5.64–8.03)	0.004
Progesterone (nmol/l)[1]	-0.01 (0.02)	0.40	0.53					
FSH (IU/l)[2]	0.01 (0.04)	0.01	0.91					
GH (μg/l)[5]	0.01 (0.03)	0.14	0.71					
IGF-1 (ng/ml)[6]	0.30 (0.22)	1.73	0.19	5.59 (4.59–6.81)	6.59 (5.38–8.06)	7.63 (6.19–9.41)	6.75 (5.41–8.43)	0.07
IGFBP-3 (mg/l)[7]	-0.14 (0.31)	0.20	0.66					
Insulin (pmol/l)[2]	0.24 (0.09)	6.91	0.01	5.44 (4.51–6.57)	5.52 (4.49–6.78)	7.16 (5.91–8.68)	7.61 (6.19–9.37)	0.0009
Total cholesterol (mmol/l)[2]	-0.74 (0.20)	13.84	0.0003	7.37 (5.93–9.17)	6.88 (5.71–8.28)	6.24 (5.14–7.58)	5.64 (4.62–6.89)	0.006
LDL-cholesterol (mmol/l)[2]	-0.44 (0.15)	8.82	0.004	7.28 (5.91–8.96)	6.77 (5.60–8.19)	6.67 (5.54–8.04)	5.24 (4.30–6.39)	0.004
HDL-cholesterol (mmol/l)[2]	-0.27 (0.15)	3.35	0.07	7.05 (5.76–8.63)	6.17 (5.06–7.52)	6.83 (5.60–8.32)	5.83 (4.79–7.09)	0.11
Triglycerides (mmol/l)[2]	-0.02 (0.07)	0.04	0.83					

A total of 146 premenopausal women were studied ($n = 101$ for age at first birth; $n = 144$ total estradiol, sex hormone binding globulin [SHBG], alcohol intake, dietary cholesterol, energy intake, carbohydrate intake, total fat intake, and protein intake; $n = 145$ for free estradiol; $n = 143$ for growth hormone [GH], insulin-like growth factor [IGF]-1, and IGF binding protein [IGFBP]-3; and $n = 142$ for caffeine intake). Cytochrome P450 1A2 (CYP1A2) activity is log transformed and modeled as the dependent variable for all models. Models are adjusted for age, smoking status, body mass index (BMI), waist–hip ratio (WHR), and ethnicity. BMI models do not adjust for BMI and WHR models do not adjust for WHR. Quartile means (95% confidence interval) are provided for variables associated with CYP1A2 activity ($P \leq 0.20$). [1]Square root transformed. [2]Log transformed. [3]Square root transformed; models adjusted for SHBG. [4]Square root transformed; models adjusted for total estradiol levels. [5]Log transformed; analyses adjusted for IGF-1 and IGFBP-3. [6]Square root transformed; analyses adjusted for growth hormone and IGFBP-3. [7]Square root transformed; analyses adjusted for GH and IGF-1. CMR, caffeine metabolic ratio; FSH, follicle-stimulating hormone; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SE, standard error.

Table 4

CYP1A2 activity and risk factors for breast cancer: postmenopausal women

Variable	β (SE)	F	*P*	Quartiles (mean CMR [95% confidence interval])				*P* for trend
				Q1	Q2	Q3	Q4	
Age (years)	-0.012 (0.006)	4.51	0.04	7.17 (6.18–8.32)	6.42 (5.60–7.37)	6.69 (5.72–7.82)	5.93 (5.07–6.94)	0.02
Age at first birth (years)[2]	0.02 (0.15)	0.03	0.87					
Age at menarche (years)	-0.03 (0.02)	3.97	0.05	6.75 (5.91–7.72)	6.96 (5.90–8.20)	6.69 (5.60–7.98)	5.75 (4.92–6.73)	0.04
Age at menopause (years)	-0.004 (0.006)	0.33	0.57					
BMI (kg/m^2)[2]	-0.45 (0.14)	0.10	0.75					
WHR	-0.96 (0.42)	5.19	0.02	6.69 (5.67–7.91)	6.83 (5.79–8.05)	6.39 (5.49–7.43)	6.04 (5.17–7.05)	0.17
Caffeine (mg/day)[1]	-0.003 (0.006)	0.23	0.64					
Alcohol (kcal/day)[2]	-0.02 (0.02)	1.26	0.26					
Cholesterol (mg/day)[2]	0.02 (0.06)	0.08	0.78					
Total energy (kcal/day)[1]	0.003 (0.005)	0.42	0.52					
Carbohydrate (g/day)[1]	0.01 (0.01)	0.32	0.57					
Fat intake (g/day)[1]	0.02 (0.02)	1.03	0.31					
Protein intake (g/day)[2]	0.04 (0.09)	0.20	0.66					
Total estradiol (pmol/l)[3]	-0.26 (0.42)	0.40	0.53					
Free estradiol (%)[2]	-0.03 (0.01)	5.11	0.03	7.04 (6.03–8.22)	7.07 (6.04–8.27)	5.86 (5.05–6.80)	5.96 (4.97–7.15)	0.01
SHBG (nmol/l)[4]	0.03 (0.02)	1.80	0.18	5.96 (4.98–7.14)	5.69 (4.88–6.63)	6.98 (5.91–8.24)	6.55 (5.55–7.72)	0.08
Progesterone, (nmol/l)[2]	-0.02 (0.05)	0.23	0.63					
FSH (IU/l)[2]	0.06 (0.07)	0.60	0.44					
GH (μg/l)[5]	0.02 (0.02)	0.57	0.45					
IGF-1 (ng/ml)[6]	0.85 (0.33)	6.68	0.01	6.30 (5.30–7.48)	6.49 (5.50–7.65)	6.42 (5.43–7.60)	7.46 (6.27–8.89)	0.13
IGFBP-3 (mg/l)[7]	-0.66 (0.23)	8.32	0.005	8.04 (6.82–9.48)	6.27 (5.29–7.44)	6.40 (5.46–7.51)	6.01 (5.11–7.07)	0.003
Insulin (pmol/l)[2]	-0.01 (0.08)	0.01	0.92					
Total cholesterol (mmol/l)[2]	-0.35 (0.18)	3.70	0.06	6.98 (5.97–8.16)	6.88 (5.90–8.04)	6.40 (5.46–7.51)	6.13 (5.28–7.11)	0.05
LDL-cholesterol (mmol/l)[2]	-0.13 (0.13)	1.00	0.32					
HDL-cholesterol (mmol/l)[2]	-0.16 (0.12)	1.82	0.18	5.77 (5.02–6.63)	5.53 (4.83–6.34)	5.63 (4.89–6.48)	4.85 (4.17–5.66)	0.08
Triglycerides (mmol/l)[2]	-0.12 (0.06)	3.37	0.07	6.94 (5.90–8.16)	6.68 (5.75–7.75)	6.52 (5.52–7.70)	6.15 (5.24–7.20)	0.15

A total of 149 postmenopausal women were studied ($n = 114$ for age at first birth; $n = 139$ for age at menopause; $n = 126$ for total estradiol and sex hormone binding globulin [SHBG]; $n = 147$ for free estradiol; and $n = 134$ for growth hormone [GH], insulin-like growth factor [IGF]-1, and IGF binding protein [IGFBP]-3). Cytochrome P450 1A2 (CYP1A2) activity is log transformed and modeled as the dependent variable for all models. Models are adjusted for age, smoking status, body mass index (BMI), waist–hip ratio (WHR), and ethnicity. BMI models do not adjust for BMI and WHR models do not adjust for WHR. Quartile means (95% confidence interval) are provided for variables associated with CYP1A2 activity ($P \leq 0.20$). [1]Square root transformed. [2]Log transformed. [3]Power transformation used, in which y = (1 - 1/sqrt [estradiol + 0.5]) × 2; models adjusted for SHBG. [4]Square root transformed; models adjusted for total estradiol levels. [5]Log transformed; analyses adjusted for IGF-1 and IGFBP-3. [6]Square root transformed; analyses adjusted for growth hormone and IGFBP-3. [7]Square root transformed; analyses adjusted for GH and IGF-1. CMR, caffeine metabolic ratio; FSH, follicle-stimulating hormone; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SE, standard error.

Stepwise and maximum R regression analyses were used to identify covariates related to CYP1A2 activity after controlling for potential confounders. If variables were highly correlated ($R \geq 0.75$), then the variable most strongly associated with CYP1A2 activity (from the analyses presented in Tables 3 and 4) was included in the model. In general, variables correlated at less than 0.8 will not pose problems in regression analysis [23]. Percentage free estradiol was not included in the models as a potential covariate because values were calculated from total serum estradiol and SHBG levels [24]. Low-density lipoprotein (LDL)-cholesterol levels were not included as a potential covariate because of high correlations with total cholesterol level ($R > 0.84$). Total energy intake was not included in the models because of high correlations with protein, fat, and carbohydrate intake ($R > 0.79$). Variables identified from the stepwise and maximum R regressions with $P \leq 0.15$ were reanalyzed with linear regression, and only variables that continued to be significant at the $P \leq 0.15$ level were reported. For descriptive purposes, each variable included in the final model was also categorized, in turn, into quartiles so that least square (adjusted) means for CYP1A2 activity could be determined. *P* values for trend across quartiles of each variable were determined.

Values for GH were missing in 19 (premenopausal, $n = 3$; postmenopausal, $n = 16$) and GH was undetectable in 120 (premenopausal, $n = 53$; postmenopausal, $n = 67$) of 357 (34%) women in the study. A missing value occurred when the volume of serum available for a participant was insufficient for both a GH and IGF-1 analysis; in such instances, IGF-1 values were determined and GH assays were not performed. Nondeterminate values were assumed to be due to the episodic and pulsatile nature of GH release, which results in considerable variability in basal hormone levels. A value of 0.2 ng/l was assigned to the 120 undetectable measurements and represents the lower limit of sensitivity for the assay. To determine whether we were introducing a bias into the results, we looked for a relationship between GH detectability and *CYP1A2* activity, and found that none existed ($P = 0.71$).

Results

Participation rate

Of 357 (182 premenopausal and 175 postmenopausal) women who consented to participate in the study, 40 (11.2%; 29 premenopausal and 11 postmenopausal) did not have urine samples and 22 (6.2%; seven premenopausal and 15 postmenopausal) had insufficient excretion of caffeine metabolites for estimating CYP1A2 activity. This left 146 postmenopausal women and 149 postmenopausal women in whom CYP1A2 activity was measurable, representing 93% of those with urine samples and 83% of eligible women. The overall participation rate for this portion of the study was 77% (295/382).

Subject characteristics by ethnicity

Selected characteristics of the 146 premenopausal and 149 postmenopausal study participants with CYP1A2 phenotype data are shown according to ethnicity in Tables 1 and 2, respectively. Of the women studied, 85% were Caucasian, 3.4% were East Asian, 5.1% were Jewish, and 6.4% were from other ethnic groups. The mean age was 45 years in premenopausal and 56 years in postmenopausal women. The two groups were similar in age at menarche, age at first birth, total number of live births, insulin levels, and daily caffeine and total energy intake. Postmenopausal women had higher average BMI and WHR, and lower circulating levels of estradiol, SHBG and IGF-1, lower levels of percentage mammographic density, and higher levels of free estradiol, IGFBP-3, and total plasma cholesterol and triglycerides.

Compared with Caucasians, premenopausal and postmenopausal East Asian women and premenopausal Jewish women had lower mean BMIs, lower levels of total and free estradiol, higher levels of SHBG, and greater percentage breast density. Among premenopausal women, both East Asians and Jewish women had lower levels of circulating insulin than did Caucasians, and Jewish women also had lower IGF-1 levels. In postmenopausal women, compared with Caucasians, East Asians were older at age of first birth and had higher levels of CYP1A2 activity, and Jewish women had lower CYP1A2 activity, higher levels of SHBG, IGF-1, and insulin, and lower levels of daily energy intake.

In premenopausal women, those without CYP1A2 data had slightly lower SHBG and IGF-1 levels. In postmenopausal women, those without CYP1A2 results weighed less, and had lower BMIs and WHRs, and lower levels of total blood cholesterol and triglycerides. Mammographic density, estradiol, SHBG, and IGF-1 levels were also higher in postmenopausal women without CYP1A2 results.

CYP1A2 activity

In premenopausal women the CMR ranged from 0.77 to 23.5, with a mean ± standard deviation of 6.21 ± 2.86 and a median of 5.84. In postmenopausal women the ratio ranged from 1.84 to 17.06 with a mean ± standard deviation of 5.85 ± 2.32, and median of 5.40. This magnitude of interindividual variation is similar to that in previous reports showing up to a 30-fold variation in enzyme activity [25,26]. The population distribution of CMR in the present study was log-normal, and similar to that in other studies using the same urinary ratio [7,12,26].

When CYP1A2 activity was compared between different ethnic groups (Tables 1 and 2), there was some indication, based on small numbers, that Jewish women may have lower CMRs than Caucasians (premenopausal 5.0 ± 1.0, postmenopausal 3.5 ± 1.7). Postmenopausal East Asian

women (n = 6) were observed to have a higher mean CMR than Caucasian women (8.9 ± 1.8). This, however, was likely to have occurred by chance due to low numbers because previous studies generally showed CYP1A2 function to be lower [27] or not different [7] between Orientals and Caucasians.

CYP1A2 activity and risk factors for breast cancer

Tables 3 and 4 show relationships between CYP1A2 activity and known or suspected risk factors for breast cancer. Ethnicity, age, body size (BMI and WHR), and smoking status as determinants of CYP1A2 activity [7] were included in all models as potential confounders. For variables associated with CYP1A2 activity at $P \leq 0.20$, quartile least square means were determined for descriptive purposes.

Age and reproductive variables

CYP1A2 activity was not related to menopausal status ($P = 0.51$; data not shown) or to age at menopause in postmenopausal women ($P = 0.57$). CYP1A2 activity was not related to parity ($P > 0.61$; data not shown) or age at first full-term pregnancy ($P > 0.46$) in either menopausal groups, but in postmenopausal women was negatively associated with age ($P = 0.04$) and age at menarche ($P = 0.05$). Adjusted mean CYP1A2 activities were 17.3% and 14.8% lower for postmenopausal women in the fourth quartile of age (P for trend = 0.02) and age at menarche (P for trend = 0.04), respectively, compared with those in the first quartile.

Body weight

BMI was inversely associated with CYP1A2 activity in premenopausal ($P = 0.03$) but not in postmenopausal women ($P = 0.75$). In premenopausal women, despite a significant P value for trend (P for trend 0.04), mean CMR was higher only for women in the lowest quartile of BMI (7.76) as compared with the other quartiles (6.11–6.17). There was some evidence that WHR was positively associated with CYP1A2 activity in premenopausal women ($P = 0.07$), and inversely related to CYP1A2 activity in postmenopausal women ($P = 0.02$). Mean CMRs were respectively 22.2% higher and 9.7% lower in premenopausal and postmenopuasal women for those in the highest quartile of WHR as compared with those in the lowest quartile.

Lifestyle factors

Lifestyle factors examined were smoking and diet. Smoking is known to induce CYP1A2 activity [7], and as expected smokers had higher CYP1A2 activity than did nonsmokers. Compared with nonsmokers, mean CMR was 55.6% higher in premenopausal smokers and 54.3% higher in postmenopausal smokers (data not shown).

Of the nutritional variables examined, caffeine ($P = 0.01$), total energy intake ($P = 0.02$), fat intake ($P = 0.04$), and protein intake ($P = 0.006$) were positively associated with CYP1A2 activity in premenopausal women. Of these, only caffeine (P for trend = 0.02) and total energy intake (P for trend 0.06) exhibited significant or borderline significant associations based on tests for trend across quartiles of intake. Compared with women in the lowest quartiles, mean CMRs were 21.2% and 18.8% higher, respectively, for women in the highest quartile of caffeine and total energy intake. In postmenopausal women, CYP1A2 activity did not vary with caffeine intake or any of the other dietary components tested.

Sex hormones

CYP1A2 activity was not associated with total estradiol ($P > 0.53$), progesterone ($P > 0.53$), or follicle-stimulating hormone ($P > 0.44$) levels in either menopausal group. CYP1A2 activity was, however, negatively associated with percentage free estradiol in both premenopausal ($P = 0.001$) and postmenopausal ($P = 0.03$) women. Mean CMRs were respectively 22% and 15% lower for premenopausal (P for trend = 0.007) and postmenopausal (P for trend = 0.01) women in the highest quartile of circulating estradiol levels compared with women in the lowest quartile. A positive association with SHBG was observed for premenopausal women ($P = 0.001$), in which women in the highest quartile of SHBG levels had a 31.2% higher mean CMR than did women in the lowest quartile (P for trend 0.004).

Insulin and growth hormone/insulin-like growth factor-1 axis

GH levels were not associated with CYP1A2 activity in either menopausal groups ($P = 0.45$). For premenopausal women, a positive relationship was observed between endogenous insulin levels and CYP1A2 activity ($P = 0.01$). Those in the highest quartile had a 39.9% higher mean CMR than did those in the lowest quartile. The test for trend was also significant ($P = 0.0009$). No other associations were observed. In postmenopausal women, CYP1A2 activity was positively related to IGF-1 levels ($P = 0.01$), negatively associated with its main binding protein IGFBP-3 ($P = 0.005$), and not associated with endogenous insulin levels ($P = 0.92$). Mean CMRs were only raised for women in the highest quartile of IGF-1, at 7.5 (+15 to 19%), as compared with mean CMRs of 6.3–6.5 in the first three quartiles; the test for trend was not statistically significant ($P = 0.13$). Women in the highest quartile for IGFBP-3 had a 25.2% lower mean CMR than did those in the lowest quartile.

Blood lipids

In premenopausal women, CYP1A2 activity was inversely associated with total plasma cholesterol ($P = 0.0003$), LDL-cholesterol ($P = 0.004$) and HDL-cholesterol ($P = 0.07$) levels, but not with triglyceride levels ($P = 0.83$). Tests for trends, however, were only significant for total (P

Table 5

Covariates of CYP1A2 activity based on maximum R and stepwise regression: premenopausal women

Variable	β (SE)	F	*P*	Quartiles (mean [95% confidence interval])				*P* for trend
	Model $R^2 = 0.42$			Q1	Q2	Q3	Q4	
Current smoker?								
No ($n = 121$)	5.35 (4.76–6.03)[1]	19.48	<0.0001					
Yes ($n = 21$)	7.69 (6.41–9.23)							
SHBG[2]	0.07 (0.02)	14.21	0.0002	5.23 (4.39–6.22)	6.33 (5.34–7.50)	7.25 (6.11–8.60)	7.06 (6.08–8.21)	0.0004
Total cholesterol (mmol/day)[3]	-0.77 (0.21)	14.02	0.0003	7.02 (5.75–8.58)	6.39 (5.42–7.54)	6.40 (5.42–7.55)	5.55 (4.66–6.60)	0.04
Insulin (pmol/l)[3]	0.21 (0.08)	7.24	0.008	5.66 (4.82–6.65)	5.69 (4.75–6.82)	6.63 (5.64–7.78)	7.61 (6.41–9.05)	0.001
Caffeine intake (mg/day)[2]	0.019 (0.007)	7.59	0.007	5.70 (4.82–6.75)	6.28 (5.31–7.42)	6.59 (5.51–7.87)	7.17 (6.15–8.35)	0.006
Age (years)	0.014 (0.006)	5.00	0.03	5.84 (4.93–6.93)	6.11 (5.11–7.30)	6.63 (5.71–7.70)	6.98 (5.84–8.34)	0.02
Triglycerides (mmol/day)[3]	0.14 (0.07)	4.18	0.04	6.12 (5.14–7.28)	6.27 (5.35–7.36)	6.34 (5.38–7.48)	7.13 (5.81–8.74)	0.24
BMI	-0.37 (0.18)	4.51	0.04	7.65 (6.49–9.02)	6.25 (5.38–7.27)	5.91 (5.00–6.98)	6.53 (5.41–7.89)	0.01
HDL (mmol/day)	-0.20 (0.14)	2.20	0.14	7.23 (6.11–8.56)	6.20 (5.29–7.27)	6.82 (5.80–8.03)	5.63 (4.77–6.65)	0.05

A total of 142 premenopausal women were studied. Model has been adjusted for ethnicity. Potential predictors of cytochrome P450 1A2 (CYP1A2) activity included in the stepwise and maximum R regression models were: age, number of livebirths, age at menarche, smoking status, body mass index (BMI), waist–hip ratio (WHR), caffeine intake, alcohol intake, dietary cholesterol, total carbohydrate intake, total fat intake, protein intake, total blood cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, dehydroepiandrosterone sulphate (DHEAS), total estradiol, sex hormone binding globulin (SHBG), progesterone, follicle stimulating hormone (FSH), growth hormone, insulin-like growth factor (IGF)-1, and IGF binding protein (IGFBP)-3. [1]Least square means (95% confidence interval) reported for smokers and nonsmokers. Quartile least square means (95% confidence interval) provided for variables associated with CYP1A2 activity ($P \leq 0.15$). [2]Square root transformed. [3]Natural log transformed. SE, standard error.

= 0.006) and LDL ($P = 0.004$) cholesterol. Mean CMRs were 23.5% and 28.0% lower for women in the highest quartile of total cholesterol and LDL-cholesterol, respectively, as compared with those in the lowest quartile. In contrast, no statistically significant relationships were detected between CYP1A2 activity in postmenopausal women and plasma LDL-cholesterol, although borderline negative relationships were observed for total cholesterol ($P = 0.06$) and triglyceride levels ($P = 0.07$). Mean CMRs for women in the highest quartile of total cholesterol (P for trend = 0.05) and blood triglyceride levels (P for trend = 0.15) were 12.2% and 11.4% lower, respectively, than for women in the lowest quartile.

Family history of breast cancer

Family history of breast cancer was not significantly related to CYP1A2 activity in either premenopausal or postmenopausal women (data not shown; $P > 0.10$).

Covariates of CYP1A2 activity

Stepwise and maximum R regression analyses were used to identify covariates related to CYP1A2 activity after controlling for any mutual confounding. All variables shown in Tables 3 and 4, as well as smoking status, were included in the models except those that were highly correlated (see Statistical methods section, above, for details). Findings are shown in Tables 5 and 6.

In both menopausal groups CYP1A2 activity was positively related to smoking ($P < 0.0001$) and SHBG ($P \leq 0.004$). In premenopausal women CYP1A2 activity was also positively related to endogenous insulin levels ($P = 0.008$), caffeine intake ($P = 0.007$), age ($P = 0.03$) and plasma triglyceride levels ($P = 0.04$), and negatively related to total cholesterol levels ($P = 0.0003$) and BMI ($P = 0.04$). All tests for trend across quartiles for each variable were significant except for blood triglycerides (P for trend = 0.24). In postmenopausal women, CYP1A2 activity was positively associated with IGF-1 levels ($P = 0.03$), and negatively associated with plasma triglyceride ($P = 0.0001$) and HDL-cholesterol levels ($P = 0.002$), and age at menarche ($P = 0.02$). All tests for trend across quartiles of each variable were significant except for IGF-1 (P for trend = 0.22). Of the variance in CYP1A2 activity, 42% and 41% was

Table 6

Covariates of CYP1A2 activity based on maximum R and stepwise regression: postmenopausal women

Variable	β (SE)	F	*P*	Quartiles (mean [95% confidence interval])				*P* for trend
	Model $R^2 = 0.41$			Q1	Q2	Q3	Q4	
Current smoker?								
No ($n = 133$)	5.05 (4.58, 5.57)[1]	24.52	<0.0001					
Yes ($n = 14$)	7.69 (6.45–9.17)							
Triglycerides (mmol/day)[3]	-0.28 (0.07)	15.49	0.0001	7.22 (6.15–8.42)	6.40 (5.53–7.42)	6.28 (5.34–7.39)	5.41 (4.59–6.38)	0.003
HDL (mmol/day)[3]	-0.42 (0.13)	10.57	0.001	7.39 (6.48–8.43)	6.49 (5.66–7.44)	6.62 (5.83–7.52)	5.58 (4.80–6.49)	0.008
SHBG[2]	0.05 (0.02)	8.49	0.004	5.95 (5.19–6.82)	6.30 (5.58–7.11)	7.39 (6.48–8.43)	7.03 (6.19–7.98)	0.01
Age at menarche (years)	-0.03 (0.02)	5.17	0.02	6.73 (6.01–7.54)	7.08 (6.27–7.99)	6.69 (5.76–7.78)	5.60 (4.89–6.41)	0.03
IGF-1 (ng/ml)[2]	0.04 (0.02)	4.74	0.03	6.49 (5.71–7.39)	6.55 (5.77–7.44)	6.28 (5.59–7.05)	7.34 (6.40–8.41)	0.22

A total of 147 postmenopausal women were studied. Model has been adjusted for ethnicity. Potential predictors of cytochrome P450 1A2 (CYP1A2) activity included in the stepwise and maximum R regression models were: age, number of livebirths, age at menarche, age at menopause, smoking status, body mass index (BMI), waist–hip ratio (WHR), caffeine intake, alcohol intake, dietary cholesterol, total carbohydrate intake, total fat intake, protein intake, total blood cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, dehydroepiandrosterone sulphate (DHEAS), total estradiol, sex hormone binding globulin (SHBG), progesterone, follicle stimulating hormone (FSH), growth hormone, insulin-like growth factor (IGF)-1, and IGF binding protein (IGFBP)-3. [1]Least square means (95% confidence interval) reported for smokers and nonsmokers. Quartile means (95% confidence interval) provided for variables associated with CYP1A2 activity ($P \leq 0.15$). [2]Square root transformed. [3]Natural log transformed. SE, standard error.

explained in premenopausal and postmenopausal women, respectively.

Discussion

In the present study we found that CYP1A2 activity, as measured by the CMR, varied 30-fold in premenopausal women and ninefold in postmenopausal women, but as in previous studies mean values did not vary with menopausal status [6]. CYP1A2 activity was found in both menopausal groups, through stepwise and maximum R regression, to be independently associated with a number of covariates, including smoking status, serum levels of SHBG, circulating levels of blood cholesterol, and triglycerides. CYP1A2 activity was also associated with age, BMI, endogenous insulin levels, and caffeine intake in premenopausal women, and with IGF-1 levels and age at menarche in postmenopausal women. These results suggest that CYP1A2 activity is associated with a number of risk factors associated with breast cancer.

In both premenopausal and postmenopausal women, approximately 40% of the variability associated with CYP1A2 activity was explained by the lifestyle, hormonal, and host factors examined in the study. The remaining 60% of variability not explained by our models point to unexamined factors, which may in part be genetic. In a twin study of Caucasians conducted in Denmark [28], CYP1A2 activity was determined for 49 monozygotic twin pairs and 34 same-sex dizygotic twin pairs concordant for nonsmoking and nonuse of oral contraceptives. A biometrical model for the caffeine ratio including only additive genetic factors and unique environmental factors was the best fitting model and gave a heritability estimate of 0.725 (95% confidence interval 0.577–0.822), suggesting that CYP1A2 activity is mainly under genetic control. Several genetic polymorphisms have been identified to date in the *CYP1A2* gene, including one associated with greater inducibility [29] and one associated with decreased inducibility [8]. The extent, however, to which these polymorphisms explain the interindividual variation associated with CYP1A2 activity is unclear, particularly in nonsmokers [29]. Results from this study add to the evidence that CYP1A2 function is related to traits that are in part under genetic control, such as blood levels of IGF-1 and body size [30,31].

In the present study, CYP1A2 function was positively related to insulin levels in premenopausal women and positively, but weakly, related to IGF-1 levels in postmenopausal women. Because both insulin [32,33]. and IGF-1 [34] levels have been linked to increased risk for breast cancer and/or recurrence, our findings suggest that CYP1A2 function is associated with increased rather than decreased breast cancer risk as hypothesized.

Given the cross-sectional nature of the study, it is unclear whether CYP1A2 activity is affected by insulin and IGF-1

levels, or *vice versa*, although there is evidence to suggest that these variables may mutually affect each other. In a recent randomized study of healthy elderly (≥ 70 years) men on the effects of administered recombinant human GH [35], those given GH had increased IGF-1 levels and increased CMR, as a measure of CYP1A2 activity, compared with those given placebo. There is also some evidence from animal studies that insulin can induce CYP1A2 activity [36], and obese Zucker rats, which exhibit hyperinsulinemia, hyperglycemia, and hypercholesterolemia compared with lean Zucker rats, also exhibit higher CYP1A2 levels [37].

In the opposite direction, microarray analysis of CYP1A2$^{-/-}$ knockout mice suggests a role for CYP1A2 in the expression of insulin and IGF-1 genes. CYP1A2$^{-/-}$ mice exhibited downregulation of genes involved in insulin action, including IGFBP-1, glucokinase, and phosphenolpyruvate carboxykinase-1, which catalyzes the rate-limiting step in glucogenesis [38]. These changes in gene expression may be linked to an overall downregulation of cell proliferation, changes in sex steroid and SHBG production, and/or alterations in lipid metabolism [38].

Increased levels of insulin and/or IGF-1 can potentially increase breast cancer risk through a number of mechanisms. Insulin can decrease SHBG levels [39], and inverse associations were observed in the present study between SHBG and insulin levels (premenopausal: $\beta = -0.74 \pm 0.37$, $F = 4.07$, $P = 0.05$; postmenopausal: $\beta = -0.91 \pm 0.32$, $F = 7.92$, $P = 0.006$) as well as IGF-1 (in postmenopausal women only: $\beta = -0.22 \pm 0.08$, $F = 8.04$, $P = 0.005$) after adjustment for age, BMI, ethnicity, and WHR. Insulin can also stimulate adrenocortical aromatase P450 mRNA levels [40]; upregulate steroidogenic acute regulatory protein, which increases the rate of cholesterol transport for steroidogenesis [41]; and upregulate transcription of the gene encoding sterol response-binding protein-1c, which is involved in the expression of lipogenic genes [38].

In both menopausal groups, CYP1A2 phenotype was not associated with total estradiol levels, but it was negatively associated with percentage free estradiol, which in turn is strongly related to SHBG levels. The positive association between CYP1A2 activity and SHBG levels (and inverse associations between CYP1A2 and percentage free estradiol) was independent of the relationship of CYP1A2 with insulin and IGF-1 levels. Because estrogens increase serum SHBG levels presumably through enhanced hepatic synthesis [42], lower levels of CYP1A2 activity in the liver might have a direct effect on enhancing SHBG production due to an increased level of estrogen activity in the liver, and this would account for a lowering of plasma free estradiol levels.

Inverse associations between CYP1A2 activity and percentage free estradiol are consistent with previous reports that female sex hormones have an inhibitory effect on CYP1A2 activity. CYP1A2 activity is lower in women [6], reduced by 35–50% during pregnancy [7], inhibited by oral contraceptives [6] and hormone replacement therapy [43], and may be lower during the late luteal phase of the menstrual cycle, when estradiol concentrations peak [44].

CYP1A2 activity varied negatively with total cholesterol levels in premenopausal women and HDL-cholesterol levels in postmenopausal women. Relationships between estrogens and plasma lipids are well documented, and exogenous estrogens are known to reduce serum lipids and lipoproteins [45]. Furthermore, 2-hydroxyestrone, a product of CYP1A2 activity, has been shown in rats to have a serum triglyceride reducing effect as strong as that of 17β-estradiol [46]. Elevated triglyceride levels have been associated with increased breast cancer risk in both premenopausal and postmenopausal women [47,48]. In this study, however, circulating triglyceride levels were positively associated with CYP1A2 activity in premenopausal women, and negatively associated in postmenopausal women. The reason for this is unclear, although findings were independent of BMI and WHR, which were included in the analyses as potential predictors of CYP1A2 function. Findings were not altered when BMI and WHR were forced into the regression models as potential confounders (data not shown).

Recent research shows that several genes involved in fatty acid and cholesterol biosynthetic pathways are downregulated in the CYP1A2 knockout mouse [38]. This may affect breast cancer risk since steroidogenesis is influenced by the provision of cholesterol as a substrate [49]. These findings, however, support a positive association between CYP1A2 activity and lipid and cholesterol biosynthesis, whereas our findings generally showed inverse associations between CYP1A2 activity and circulating cholesterol and triglyceride levels (in postmenopausal women).

As in previous studies, CYP1A2 function was strongly induced by tobacco smoke [6,7]. The effect of smoking on CYP1A2 function may explain why smoking is not an important risk factor for breast cancer and may actually be protective. Cigarette smoking has been observed to increase 2-hydroxylation [50] and reduce the conversion rate of androgens to estrogens [51]. Brunet and coworkers [52] recently reported that smoking reduces risk for breast cancer by 44% in carriers of *BRCA1* or *BRCA2* gene mutations.

CYP1A2 activity was found to vary inversely with BMI in premenopausal women after adjusting for other covariates. These findings were similar to that observed in a study of

786 Caucasian women [6], which was the first to find an inverse association between CYP1A2 activity and BMI. Because increased BMI is associated with reduced risk before the menopause [53], our findings would suggest that lower CYP1A2 activity (associated with greater BMI) in these women would be associated with lower breast cancer risk. Low 2-hydroxyestrone levels, however, have been associated with higher risk for breast cancer in premenopausal women [54]. Further study is needed to elucidate the relationship between CYP1A2 activity, body size, and breast cancer risk.

CYP1A2 activity was associated with a number of dietary factors in premenopausal women but not in postmenopausal women. After adjusting for body size, age, ethnicity, and smoking status, positive associations were observed with intake of caffeine, total energy, fat, and protein. After adjusting for other covariates through stepwise and maximum R regression, however, only caffeine intake remained significant. The positive association between caffeine intake and CYP1A2 activity in premenopausal women is consistent with results from other studies that suggest that caffeine can induce CYP1A2 activity [6,7,55]. This has been observed in population [12] as well as rat studies [56].

Limitations of the present study include the small number of participants, particularly after stratification by menopausal status. The research was exploratory in nature, and so novel findings, such as associations observed with insulin and IGF-1 levels, as well as with blood lipids and cholesterol, are more likely due to chance and need replication in future studies.

Conclusion

Results from the present cross-sectional study suggests that several factors associated with breast cancer risk are associated with CYP1A2 activity, including SHBG and free estradiol levels, insulin and IGF-1, blood lipids and cholesterol, body size, and smoking status. Some of the observed associations, however, were contrary to hypothesized directions and suggest that increased CYP1A2 function may be associated with increased risk for breast cancer. These preliminary findings need confirmation in future studies. The association of risk factors with the activity of metabolic enzymes, particularly those under substantial genetic control, may suggest important pathways in the development of breast cancer. Further research is required to elucidate the relationships between CYP1A2 genotype, CYP1A2 phenotype, their influence on risk factors associated with breast cancer, and their impact on breast cancer risk.

Competing interests

None declared.

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