Low Urinary Estrogen Glucuronides in Women at Risk for Familial Breast Cancer

Abstract. *Daily (12-hour) urine collections taken throughout the menstrual cycle were obtained from 30 young women who by genetic analysis were at risk for familial breast cancer, and from 30 control women carefully matched for age, height, and reproductive history. Steroids in the urine were extracted by glucuronidase hydrolysis, and the primary glucocorticoid, androgen, and estrogen hormones and their metabolites were measured by radioimmunoassay. Highly significant differences were observed only in the case of estrone and estradiol, with the high-risk subjects exhibiting lower values than the controls. This endocrine abnormality in young women at risk for breast cancer may be a potential discriminant for identifying women at risk for the disease in the population at large.*

Extensive epidemiological studies have established that a family history of breast cancer increases an individual's risk for the disease (*1*). The increased risk factor varies with the number and degree of affected relatives and reaches a ninefold value for premenopausal women who have one or more first-degree relatives (mothers and sisters) with premenopausal bilateral breast cancer (*2*). The factor responsible for the increased risk is unknown. The evidence of hormonal participation in the etiology of breast cancer (*3*) suggests, however, that the inherited risk for the disease might be mediated through a genetically transmitted endocrine factor. If such a factor were identified, it would provide an insight into endocrine relationship with the disease.

Previous studies directed to isolating an endocrine change in patients with familial breast cancer have failed to show any significant differences between subjects at risk and matched controls, except for some transient increases in plasma estradiol and prolactin in the high-risk individuals (*4-6*), which reached significance only when the increases for both hormones were considered together. These studies were limited, however, in their frequency of sampling throughout the menstrual cycle, during which large fluctuations in hormone levels occur. We now report that young women considered at risk for breast cancer because of a positive family history of the disease exhibit a urinary estrogen profile which is significantly different from that of carefully matched controls. This constitutes evidence that an endocrine difference exists between subjects at risk for breast cancer and those who are not, and that this difference may be responsible for the increased risk of the disease.

Thirty young postpubertal and premenopausal women were selected from pedigrees comprising the Familial Breast Cancer Resource at the Creighton University College of Medicine, Omaha. These women were, by genetic analysis, at clearly defined high-risk for the disease. Eight of them had at least two first-degree relatives with the disease, 12 had one first-degree relative and at least one grandmother with the disease, and 10 had only one first-degree relative with breast cancer. All of the subjects were selected through a detailed history, physical examination, and clinical laboratory tests to eliminate those with significant menstrual irregularities and with serious gynecological or other health problems. Liver and kidney functions were required to be normal, and the subjects must not have used oral contraceptives, thyroid medication, or tranquilizers for at least 6 months before beginning the study. The control women, none of whom had any first-degree relatives with breast cancer, were selected to match as closely as possible each individual high-risk case in specific variables such as weight, height, age, age at first pregnancy, age at menarche, and parity. Some of these variables have been implicated as possible risk factors for breast cancer (*7*) and have also been associated with endocrine perturbations (*8*). A correspondence in these variables between the high-risk cases and the controls was essential to preclude a misinterpretation of the results in the present study. The fidelity of matching subjects and controls is illustrated in Table 1. The only statistically significant difference encountered was in weight, the high-risk cases being an average of 8.6 pounds heavier. This difference is unlikely to be significant for this study, since it is too small to result in endocrine changes (*9*), and since height rather than weight has been reported to be the more important risk factor for breast cancer (*10*). The high-risk and control women were also selected to be of similar ethnic origins.

A concerted effort was made to obtain samples from each matched subject-control pair at the same time of the year to preclude possible differences due to seasonal endocrine fluctuation. The details of the selection and matching process have already been described (*11*). The experiment was specifically designed to obtain hormonal values throughout the menstrual cycle in order to incorporate the large hormonal fluctuations associated with ovulation. Urine samples (7 p.m. to 7 a.m., 12-hour collections) were obtained daily from the cessation of menstrual flow until the onset of the next menstruation. Blood samples were obtained on alternate days throughout the same collection period. The samples were shipped frozen by air to the Institute for Steroid Research, Montefiore Hospital and Medical Center, New York, where all of the analyses were carried out. The creatinine content of each urine sample was determined to ensure the completeness of the collection. All hormone measurements on plasma and urine were done without knowledge as to the category (high-risk or control) of the

Table 1. Matching of high- and low-risk populations. Thirty women who were at high risk for developing breast cancer were matched with 30 control women as described in the text. When analyzed by Student's *t*-test, the groups did not significantly differ from each other except that the high-risk subjects weighed more than the controls; however, this parameter has not been correlated with risk for breast cancer. Abbreviations: N.S., not significant; LH, luteinizing hormone.

Parameter	Number of pairs	High-risk subjects	Control	High-risk − control (mean ± standard error)	*t*	*P*
Year of birth	30	1943.97	1943.73	0.24 ± 0.44	0.53	N.S.
Age at menarche (years)	30	12.33	12.73	−0.40 ± 0.36	−1.01	N.S.
Parity	30	1.77	2.37	−0.60 ± 0.32	−1.87	N.S.
Age at first pregnancy	21	21.67	21.67	0.00 ± 0.29	0.00	N.S.
Menstrual cycle length (days)	30	27.33	26.73	0.60 ± 0.85	0.70	N.S.
LH peak (days)	30	13.73	13.20	0.53 ± 0.96	0.55	N.S.
Weight (pounds)	30	137.57	128.97	8.60 ± 3.49	2.46	<.05
Height (inches)	30	65.60	64.70	0.90 ± 0.53	1.57	N.S.

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sample. The plasma samples were analyzed (*11*) for estrone, estradiol, estriol, progesterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and prolactin. No significant differences were found between the two groups in the mean plasma concentrations of any of the above hormones in either the follicular or luteal stages of the cycle.

Individual urine collections were hydrolyzed with β-glucuronidase, and the released steroids were extracted with ether (*12*). Separate portions of the urinary extracts, suitably diluted, were assayed for their content of estrone, estradiol, estriol, cortisol, tetrahydrocortisol, allotetrahydrocortisol, testosterone, dihydrotestosterone, androsterone, etiocholanolone, and dehydroisoandrosterone. All of the determinations, with the exception of cortisol, were carried out by radioimmunoassay procedures, using antiserums highly specific for each steroid so that no elaborate separation procedures (with the attendant losses) were required. In the case of the estrogens, it was found desirable to partition the urine extract first between water and benzene and then ethyl acetate. Passage of the benzene through a short deactivated alumina column gave a fraction containing estrone plus estradiol, while the ethyl acetate extract processed similarly gave estriol. These fractions were then assayed for estrone and estradiol, and estriol, using specific antiserums that exhibited essentially no cross-reactivity between the three estrogens. Suitably labeled ligands were added to the urine extracts to monitor recovery, with labeled estrone or estradiol being added to alternate samples.

All of the urinary steroid concentrations were expressed per gram of creatinine, to correct for any variability in collection schedules. For the purpose of the initial computation, the mean steroid content for the entire cycle and that for the follicular and the luteal phases separately was calculated. The follicular phase was defined as those samples up to and including the day of the plasma LH peak, while the luteal phase contained all urine samples subsequent to the LH peak until the onset of menstruation. On the basis of the plasma estradiol, LH, FSH, and progesterone determinations, ovulation occurred in each cycle studied. The follicular and luteal mean concentrations as well as the full cycle mean concentrations of the urinary steroids were compared in each of the 30 matched pairs. The statistical significance of the differences was calculated by Student's two-tailed t test. No significant differences were found between the high-risk subjects and the controls in the urinary concentrations of any of the corticosteroids and androgens measured. However, highly significant differences were observed in two of the urinary estrogens, estrone and estradiol (Table 2). The controls had a higher mean full-cycle concentration of estrone and estradiol than the high-risk subjects ($P < .01$ and $P < .005$, respectively). The follicular stage mean concentrations were only significantly different for estradiol ($P < .25$) and not for estrone, while the luteal stage mean concentrations were significantly different for both estrone and estradiol ($P < .1$ and $P < .025$, respectively), the controls having the higher values in each instance. In contrast, urinary estriol concentrations were higher in the high-risk subjects, but the difference from the controls was not statistically significant.

These significant differences in urinary content of estrone and estradiol but not estriol should be considered with the fact that none of the other hormones measured in the urine, including dehydroisoandrosterone, showed any significant difference or trend between the controls and high-risk subjects. Also, none of the hormones measured in plasma, including the estrogens, exhibited any significant differences between the two populations (*11*). Thus the high-risk individuals showed only two endocrine differences from controls, namely lower urinary excretion of estrone and estradiol, and these are the hormones most closely linked to the etiology of breast cancer.

The finding of differences in urinary but not in plasma estrogen concentrations can be rationalized by the cumulative nature of the urinary concentrations, which could reveal differences not detectable in isolated plasma samples. An alternative explanation is that in this study, only the estrogen glucuronides in urine have been measured, and the estrogens excreted as sulfate conjugates have not. We preferred to limit our analysis to the glucuronides because they generally represent 90 percent or more of the urinary estrogens and they can be released by a mild, nondestructive enzymic hydrolysis. In this study, therefore, a change in conjugative pattern of estrone and estradiol in the high-risk subjects with decreased glucuronidation and increased sulfation would result in lower urinary estrone and estradiol values without any differences in the plasma concentrations. The reported absence of any abnormalities in urinary estrogen excretion in the daughters of breast cancer–affected mothers (*13*) is, therefore, not necessarily in conflict with our results, since in that study, in addition to other methodological differences, both

Table 2. Comparison of urinary estrogens in women at risk for familial cancer and controls. Individual urine samples were incubated with β-glucuronidase and extracted continuously with ether for 48 hours. To portions of each urine extract in 0.5 ml phosphate buffer pH 7.4 was added ~ 2000 count/min of [^{3}H]estriol, while alternate samples received 2000 count/min of [^{3}H]estradiol or [^{3}H]estrone, to allow monitoring of recoveries. The samples were extracted twice with 6 ml benzene, and then twice with 2 ml of ethyl acetate. The benzene extract was placed on a 600 mg alumina column (Activity III, 6 percent H_2O). Material eluted with 4 percent ethanol in benzene contained estrone and estradiol. One half of this was analyzed for estradiol and the other half for estrone by means of highly specific antiserums. The ethyl acetate extract was chromatographed on an identical column. Material eluted with 0.3 percent methanol in ethyl acetate was analyzed for estriol. The purpose of the alumina chromatography was to remove substances which interfered with the radioimmunoassays. All results are reported as micrograms of steroid per gram of creatinine (mean ± standard error). For each steroid, 30 matched pairs were analyzed. The results, when submitted to the Wilcoxon-Rouk test, exhibited statistical significance corresponding to the paired t-test.

Time of sample	Estradiol	Estrone	Estriol
Full cycle			
High-risk subjects	5.8 ± 0.3	7.9 ± 0.5	12.5 ± 1.3
Controls	6.8 ± 0.3	9.8 ± 0.6	12.0 ± 1.5
(High-risk − control)	−1.0 ± 0.3	−1.9 ± 0.7	0.4 ± 1.9
t	−3.11	−2.83	0.23
P	<.005	<.01	N.S.
Follicular stage			
High-risk subjects	6.4 ± 0.3	8.9 ± 0.7	11.9 ± 1.4
Controls	7.3 ± 0.4	9.8 ± 0.6	11.1 ± 1.7
(High-risk − control)	−0.9 ± 0.4	−0.9 ± 0.8	0.8 ± 2.1
t	−2.47	−1.11	0.36
P	<.02	N.S.	N.S.
Luteal stage			
High-risk subjects	5.7 ± 0.4	7.8 ± 0.5	13.5 ± 1.3
Controls	6.7 ± 0.4	10.1 ± 0.7	13.0 ± 1.5
(High-risk − control)	−0.9 ± 0.4	−2.36 ± 0.8	0.5 ± 1.9
t	−2.45	2.88	0.27
P	.02	<.01	N.S.

the estrogen glucuronides and sulfates were combined in the urinary measurements. The possibility that a change in conjugation might be the singular endocrine feature distinguishing women at risk for familial cancer is attractive, since it would suggest that the risk factor is linked not to the amount of estrogen secreted but to its metabolic fate, which is dependent on an enzymatic spectrum under genetic control. The conjugative control of the enterohepatic circulation of estrogens and the greater biological impact of the sulfates relative to the glucuronides (*14*) offer just two of the possible mechanisms by which conjugative changes can be expressed as risk factors for breast cancer. Examination of the urinary samples from our study for their estrogen sulfate content should reveal whether these conjugates are indeed higher in the women at high-risk for familial breast cancer.

A dominant theme of the participation of estrogens in the etiology of breast cancer has been the "estriol hypothesis," which postulates that estriol in contrast to estrone and estradiol is protective against breast cancer (*15-17*). Much recent biological and epidemiological evidence has cast serious doubt on this hypothesis (*18-20*), and our study has also failed to support it. The urinary and plasma estriol concentrations of the high-risk subjects are either higher or similar to those of controls, and the decrease in urinary estrone and estradiol gives them a greater estriol ratio or proportion rather than the lower values called for by the estriol hypothesis.

Our results describe a definitive endocrine difference that can be associated with increased risk for familial breast cancer. Whether the same abnormality is associated with risk for nonfamilial breast disease is an intriguing question, the answer to which would require a prospective study of formidable dimensions because of the need to sample urine throughout the menstrual cycle. In an effort to reduce such a potential study to more manageable proportions we have analyzed our estrogen data in terms of daily differences. This analysis has revealed that the significant differences in estradiol excretion are limited to the periovulatory and immediate postovulatory periods, while those of estrone excretion are even more limited to a 3-day postovulatory period. It seems that the differences found originate in the metabolism of the preovulatory secretory surge of estradiol and are reflected in the subsequent urinary excretion of the products. Careful monitoring of the menstrual cycle would permit the selection of single urine collections for the detection of urinary estrogen differences. The dependence of the urinary estrogen differences on the phase of the menstrual cycle emphasizes the absolute necessity for measuring the urinary hormones on specific days of the cycle in any further studies of this type.

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Racial Differences in Blood Pressure Control

Abstract. *Children from an entire biracial geographical population were examined for blood pressure. A sample of 278 children, stratified by diastolic blood pressure, was reexamined 1 to 2 years later. Dopamine β-hydroxylase, renin activity, and resting heart rate were observed in black and white children. In the group with high blood pressure, whites had higher heart rates and greater renin activity than blacks. Dopamine β-hydroxylase concentrations in blacks were lower than in whites over the entire spectrum of blood pressure levels. High blood pressure seems to have a different metabolic background in the two races which may influence the early natural history of essential hypertension. Therefore, the rationale of prevention, and possibly treatment, of early hypertension in blacks and whites may differ.*

It is well known that the mortality and prevalence of hypertension in the United States are higher in black persons than in whites (*1*). In young adult black patients with hypertension, a preponderance of low-renin hypertension has also been described (*2*). In adult subjects with no hypertension, the sodium excretion in response to a sodium load was found to be slower in blacks than in whites (*3*). The nature of these differences is not clear. In an attempt to distinguish genetic from environmental causes of hypertension we investigated school children in a biracial community at an early age, when complications of the disease are less likely to be established.

During the 1973–1974 school year 3524 children (ages 5 to 14 years, 63 percent white, 37 percent black) were examined in the Bogalusa Heart Study (*4*). Resting blood pressure, systolic and diastolic (fourth phase), was measured three times by each of three observers: two used a mercury sphygmomanometer and one an automatic blood pressure recorder. In a statistical analysis only three age groups of children were included, namely, those ages 6 to 7, 9 to 10, and 12 to 13. For each of these groups the four race-sex combinations were considered separately, resulting in 12 subgroups. For each subgroup, the rank of the median diastolic blood pressure values from each observer was assessed, and these three ranks were added for each child. The sum score formed the basis for a stratified random sampling, sex- and race-specific, with weighting of the extreme pressures. This sample of 368 children was reexamined in 1975 and 1976 in more detail, grouped into five strata labeled 1 (low blood pressure) to 5 (high blood pressure). The sample represented all children in the extreme 2 percent for strata 1 and 5, and a 70 percent random

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