Urine Estrogen Profiles in European Countries with High or Low Breast Cancer Rates*

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Abstract—Urine estrogens of women in two age groups, 15–18 and 30–39, were
measured in four northern European countries where breast cancer rates are high, two
southern European countries where they are low, and in Finland, a northern country
where incidence rates are comparable to those of the southern countries and mortality
rates are intermediate. The estriol ratio (the ratio of the concentration of estriol to the
sum of those of estrone and estradiol) was lower in the high-risk countries than in the
low-risk areas in both age-groups and in both the follicular and luteal phases of the
menstrual cycle. In two of the four comparisons the difference was statistically
significant. This provides modest support to the hypothesis that a population's breast
cancer risk is inversely related to the estriol ratios of its young women. However, the
data from Finland are not consistent with this hypothesis, the Finnish women having
lower mean estriol ratios than the women in the high-risk areas. The pregnanediol levels
of the young Finnish women were also unusually low, suggesting a high frequency of
anovulatory menstrual cycles.

INTRODUCTION

THE INCIDENCE of breast cancer varies appreciably among European countries, although the variability is less than that which is seen world-wide. Thus, the highest and lowest recorded national incidence rates of this disease in Europe differ by two-fold while the highest rates in Europe and North America differ from those of some Asian and African countries by a factor of five. Comparisons of the urine estrogen profiles of young North American and Asian women showed an inverse correlation between a population's breast cancer risk and the 'estriol ratio'—the amount of estriol (E3) excreted relative to the sum of the amounts of estrone (E1) and estradiol (E2) [1–3]. The present study attempts to evaluate further this phenomenon by comparison of some populations that are racially more homogeneous but still show differences in breast cancer risk.

MATERIALS AND METHODS

Patients

Study centers were located in four high-risk populations of northern Europe (Denmark, Netherlands, Norway and Sweden) and in two low-risk populations in southern Europe (Greece and Yugoslavia). An additional center was in Finland which, although a northern country, has lower reported incidence and mortality from breast cancer than the other Scandinavian countries. The objective in each country was to enroll 40 women 15–18-yr old and 30 age 30–39 yr. In the 15–18 yr group,

Table 1. *Source of subjects in the study centers*

Center	Town or area	Source
Denmark	Aarhus	Young: mostly pupils from two schools. Older: staff of hospital; friends.
Netherlands	Utrecht	Young: students at two high schools. Older: women attending a population screening project for cervical cancer.
Norway	Tromsø	Young: students at a vocational school. Older: women attending a health clinic for check-up; friends.
Sweden	Malmö	Young: high school students. Older: mostly nurses and friends; a few healthy patients in a policlinic.
Finland	Joensuu, N. Karelia	Young: girls from a secondary school. Older: from the acquaintances of the study staff (and their acquaintances).
Greece	Athens	Young: students at one middle class high school. Older: mostly women coming to Social Security clinics for vaccination of children; staff; friends.
Yugoslavia	Ljubljana	Young: students at schools of nursing, midwifery and medical technology. Older: staff of several hospitals and affiliated institutions.

an attempt was made to have 10 subjects at each single year of age, but this was not achieved in all countries. All the young women were nulliparous and all the older women, were parous. the sources of the subjects are described briefly in Table 1.

The field work was carried out in 1973. Potentially eligible women, very few of whom were unwilling to participate, were interviewed. The following were reasons for exclusion: currently pregnant, pregnancy within the prior 6 months, unusual length of menstrual cycle, history of diabetes, thyroid disorder, cancer, breast lump or cyst, liver disease, uterine curettage (other than for miscarriage), ovarian surgery, pelvic surgery for any neoplasm, current or past systemic endoctrine therapy, oral contraceptive use within the previous 24 months, or current regular use of any medication.

Each eligible woman provided one urine collection during the follicular phase and one during the luteal phase, usually of the same menstrual cycle. In almost all instances, collection of the follicular specimen started on the evening of the 10th day, and of the luteal specimen on the evening of the 21st day of the cycle, and was completed 12 hr later.

Methods

After measurement of urine volume and specific gravity, the aliquots, identified only by number, were stored frozen and later shipped frozen to Melbourne. There, estrogens were measured by the modified Brown method [4] and pregnanediol was measured by the method of Cox [5]. For 10% of urine specimens, a second aliquot was sent under a different code number. Based on these blind duplicates, the coefficients of variation due to replication were 10% for E1, 15% for E2, 13% for E3, 4% for the estriol ratio, and 21% for pregnanediol. The estrogen profile is described by the estriol ratio, $E3/(E1 + E2)$. This ratio and the concentrations of the individual estrogens and pregnanediol have ap-

Table 2. *Average annual age-adjusted breast cancer incidence and mortality rates, per* 100,000 *female population, in the countries represented in the study*

Country	Incidence		Mortality	
	Period	Rate	Period	Rate
Denmark	1963–67	68.2	1971–75	37.2
Netherlands	1960–62	62.0	1971–75	38.7
Norway	1968–72	60.8	1969–73	25.3
Sweden	1966–70	72.8	1971–75	28.9
Finland	1966–70	44.9	1970–74	21.4
Greece	1965–67	53.3	1970–74	15.0
Yugoslavia	1968–72	38.2	1970–74	14.1

Except as otherwise noted, incidence rates are from Waterhouse *et al.* [6] and mortality rates from data given in the World Health Statistics Annual, Vol. 1. Incidence data for the Netherlands are from Doll *et al.* [7] and for Greece from Valaoras *et al.* [8]. Incidence data for Yugoslavia are for the Province of Slovenia only. Age-adjustment is to the European standard population of Waterhouse *et al.* [6].

Table 3. Characteristics of the young subjects and of their urine specimens by country and cycle phase

Characteristic	Northern European countries					Southern European countries Low breast cancer risk	
	High breast cancer risk				Finland		
	Denmark	Netherlands	Norway	Sweden		Greece	Yugoslavia
No. of women	40	41	39	40	39	46	43
Age (range)	15–18	15–18	15–18	15–18	15–18	15–18	15–18
Age (mean)	16.4	15.9	15.7	16.5	16.3	16.1	16.5
Age at menarche	12.8	12.8	12.6	12.9	13.0	12.3	12.8
Follicular phase							
Urine volume (ml)	459	417	446	375	506	412	310
Urine specific gravity	1022	1022	1024	1027	1023	1021	1025
Estrone (E1)	7.8	7.4	6.6	8.7	7.9	7.0	10.3
Estradiol (E2)	3.7	3.7	3.1	4.2	3.5	4.1	5.6
Estriol (E3)	6.1	8.0	6.5	8.5	6.6	7.7	11.7
Total estrogens	18.3	19.6	16.8	22.5	18.8	19.5	28.6
Estriol ratio	0.53	0.72	0.66	0.65	0.58	0.69	0.73
Luteal phase							
Urine volume (ml)	427	441	459	405	525	467	365
Urine specific gravity	1022	1022	1023	1026	1023	1021	1025
Estrone (E1)	15.7	13.9	12.0	14.7	14.2	10.8	16.4
Estradiol (E2)	7.3	7.0	6.1	7.6	7.1	6.4	8.4
Estriol (E3)	18.5	17.3	14.2	18.4	13.7	16.8	23.6
Total estrogens	43.7	39.7	33.9	42.3	36.4	35.1	50.7
Pregnanediol	1.4	1.0	0.8	1.3	0.7	1.2	1.7
Estriol ratio	0.81	0.82	0.78	0.83	0.64	0.97	0.94

Values of estrogen concentrations (μg/l) pregnanediol (mg/l) and estriol ratios are geometric means. All other values are arithmetic means.

proximately log-normal distributions and so are summarized as geometric means.

RESULTS

Table 2 shows breast cancer incidence and mortality rates reported in the seven countries represented in the study. In both incidence and mortality the four high-risk countries are clearly differentiated from the two low-risk countries. Finland is also different from the other northern European countries, having incidence rates similar to those of Greece and Yugoslavia and mortality rates intermediate between the two groups. If the data are limited to women under 55, so as to include generations more similar to the women studied (data not shown), the geographical differences remain—rates are highest in the four northern countries, and Finland occupies an intermediate position.

Data on the study subjects and their urine estrogens are given for the young group in Table 3 and for the older women in Table 4. In view of the equivocal status of Finland with respect to breast cancer risk and of the strikingly different observations for the data from Finland in this study, the data are first examined with the Finnish data disregarded.

The means of the estriol ratios for luteal specimens of young women in the four high-risk countries are all lower than those for the two southern low-risk countries. In the follicular specimens of young women, there is only one exception to this same phenomenon, the mean for Netherlands (0.72) being slightly higher than that for Greece (0.69). Among the older women, there are similar trends, but there are two exceptions—Netherlands among the follicular specimens and Denmark among the luteal. Altogether, of 16 possible comparisons of the ratio in a high-risk area with that in the lowest of the low-risk areas, the ratio is in the expected direction (lower in the high-risk area) in 13 and in 3 it is not. There is no instance of the contrary—that is, a higher ratio in a high-risk area than in both of the southern low-risk areas. Data combined for the high-risk and low-risk areas are shown in Table 5. The directions of differences in the estriol ratios are the same in all four possible comparisons and are significantly so ($P<0.05$) in two (follicular specimens in older women and luteal specimens in the younger group).

It is of interest that, in all comparisons except that for the luteal specimens of young women, the higher estriol ratios in the southern low-risk countries occur in spite of the

Table 4.   Characteristics of the older subjects and of their urine specimens by country and cycle phase

Characteristic	Northern European countries — High breast cancer risk				Finland	Southern European countries — Low breast cancer risk	
	Denmark	Netherlands	Norway	Sweden		Greece	Yugoslavia
No. of women	30	33	30	30	32	30	31
Age (range)	30–39	30–39	30–39	30–39	30–39	30–39	30–39
Age (mean)	34.6	35.7	35.1	33.5	34.3	34.9	34.2
Age at menarche	13.1	13.1	13.1	13.6	13.9	12.8	14.0
Follicular phase							
Urine volume (ml)	572	539	613	505·	522	569	466
Urine specific gravity	1019	1020	1021	1022	1023	1019	1023
Estrone (E1)	12.3	14.0	14.0	13.0	17.4	16.0	15.6
Estradiol (E2)	6.3	6.9	7.3	6.9	8.0	8.2	9.0
Estriol (E3)	13.0	16.3	13.2	11.7	12.0	23.9	18.8
Total estrogens	32.8	38.9	36.1	33.0	39.2	49.7	45.6
Estriol ratio	0.70	0.78	0.62	0.59	0.47	0.98	0.76
Luteal phase							
Urine volume (ml)	571	599	675	550	603	644	487
Urine specific gravity	1019	1019	1022	1020	1025	1019	1021
Estrone (E1)	13.2	11.6	14.2	13.4	15.8	14.2	17.9
Estradiol (E2)	6.2	5.8	6.9	7.0	7.2	6.9	9.7
Estriol (E3)	20.5	16.8	17.9	15.8	15.6	22.9	27.9
Total estrogens	41.4	36.2	40.9	38.3	40.2	45.7	58.3
Pregnanediol	2.6	2.2	3.4	2.6	2.6	2.6	2.9
Estriol ratio	1.05	0.96	0.84	0.77	0.67	1.08	1.00

See footnotes to Table 3.

Table 5.   Characteristics of the urine specimens of the study subjects in four high risk countries and two low risk countries, by age group and cycle phase

Characteristic	Young women		Older women	
	High risk northern countries	Low risk southern countries	High risk northern countries	Low risk southern countries
No. of women	160	89	123	61
Follicular phase				
Estrone (E1)	7.6	8.5	13.3	15.8
	(6.8–8.5)	(7.4–9.7)	(11.6–15.3)	(13.4–18.5)
Estradiol (E2)	3.7	4.8	6.8	8.6
	(3.3–4.1)	(4.2–5.4)	(6.0–7.9)	(7.3–10.1)
Estriol (E3)	7.2	9.4	13.5	21.1
	(6.3–8.2)	(8.1–11.0)	(11.4–16.2)	(17.6–25.4)
Total estrogens	19.2	23.5	35.2	47.6
	(17.2–21.4)	(20.6–26.9)	(30.6–40.7)	(40.9–55.5)
Estriol ratio	0.64	0.71	0.67	0.86
	(0.58–0.70)	(0.64–0.79)	(0.60–0.74)	(0.74–1.00)
Luteal phase				
Estrone (E1)	14.0	13.2	13.0	16.0
	(12.6–15.5)	(11.4–15.3)	(11.7–14.6)	(13.5–19.0)
Estradiol (E2)	7.0	7.3	6.5	8.2
	(6.3–7.7)	(6.4–8.3)	(5.8–7.2)	(6.9–9.7)
Estriol (E3)	17.0	19.8	17.6	25.3
	(15.1–19.3)	(17.0–23.0)	(15.3–20.2)	(21.3–30.5)
Total estrogens	39.7	41.9	39.1	51.7
	(36.0–44.1)	(36.6–47.9)	(35.2–43.5)	(44.6–60.4)
Pregnanediol	1.1	1.4	2.7	2.8
	(1.0–1.3)	(1.1–1.8)	(2.3–3.1)	(2.3–3.3)
Estriol ratio	0.81	0.96	0.90	1.04
	(0.74–0.89)	(0.85–1.08)	(0.80–1.01)	(0.90–1.21)

See footnotes to Table 3.
Values given in parentheses are 95% confidence limits.

fact that concentrations of E1 and E2 are higher than in the high-risk areas—that is, the concentrations of all three estrogens are higher but that of E3 is especially so. In the luteal specimens of young women the pattern is not so clear but it appears that, on average, the concentrations of E1 and E2 are at least not lower than those in the high-risk areas.

Among the four high-risk areas there is no clear pattern except for a tendency for the ratios to be high in the Netherlands, and, in the older women, in Denmark. There is no consistency to the direction of the difference between estriol ratios in the two southern low-risk areas.

In contrast to the results of the comparison of the high-risk with the southern low-risk areas, the data for Finland do not support the estriol ratio hypothesis. On the basis of the relatively low breast cancer incidence in Finland it would be predicted from the hypothesis that estriol ratios would be high in that center. Instead, the ratio in Finland is lower than that of any of the high-risk areas. The pregnanediol concentration is also low in many young women in Finland as reflected in the low mean value, suggesting a high frequency of anovular menstrual cycles. However, frequency of anovulation varies strikingly with age in this age-group and examination of this question will require more detailed analysis.

DISCUSSION

This comparison of high breast cancer risk northern European countries with low-risk countries in southern Europe provides data supportive of the hypothesis that a population's breast cancer risk is inversely associated with the urine estriol ratios of its young women [9]. The fact that the differences in estriol ratio between high-risk and low-risk areas are less than those previously seen in comparisons of Asian and North American populations [1] is consistent with the fact that the differences in breast cancer rates are less. Some exceptions to the overall pattern are to be expected even if the hypothesis is correct—the variable under study has a wide range of values and the individual study groups are relatively small. Further, no special effort was made in this study to select subjects in terms of demographic variables, such as socio-economic status, which is associated with considerable variation in the estriol ratio within a population [10].

In contrast, the comparison of data from Finland with those from other Scandinavian and northern countries is inconsistent. The inconsistency is even more remarkable when it is considered that a special effort was made to obtain the Finnish specimens from a rural area in Northern Karelia where it was judged that the factors responsible for the rapidly increasing breast cancer rates in Finland [11] would be least influential. The contrast between the expectation and the observation in the Finnish data is so strong as to suggest that there is some circumstance in Finland which is unusual and an understanding of which might be important to the elucidation of breast cancer etiology.

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