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# A Comparison of Menstrual Cycle Profiles of Salivary Progesterone in British and Thai Adolescent Girls

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**Menstrual-cycle profiles of salivary progesterone concentration, obtained by radioimmunoassay of daily samples collected throughout the cycle, were obtained from Thai ($n$ = 232) and British ($n$ = 130) adolescent girls up to 4 years postmenarche. These profiles were graded from 1 to 5 ranging, respectively from concentrations at the detection limit of the assay to profiles generally observed for the mature premenopausal woman.Contingency table analysis of the grade frequencies for Thai–British pairs of girls matched for chronological age and age at menarche ($n$ = 2 × 90) demonstrated that British girls had more mature cycles (22/90) than Thais (11/90) ($P < 0.05$) particularly in the first 2 years postmenarche ($P < 0.01$). For these matched pairs of girls there was no evidence to support the view that girls with an early age of menarche develop their profiles more quickly following menarche than those with a late age of menarche, as previously reported and which was thought to be important in the development of breast cancer. The findings of this study also suggest that adolescent girls in Britain develop their menstrual cycle profiles of salivary progesterone more quickly than their Thai counterparts and this may be of value in formulating hypotheses regarding any role that ovarian progesterone secretion may have on subsequent breast cancer risk.**
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## INTRODUCTION

IT IS generally accepted that early menarche, late first full-term pregnancy and late menopause are factors which are associated with increased breask cancer risk [1–3]. Furthermore, it is generally assumed that this risk is related to the number of consecutive normal menstrual cycles before pregnancy or menopause and is, therefore, implicitly related to the exposure of the breast to hormones secreted by the ovary. The effect of age at first full-term pregnancy on the development of breast cancer, however, is complicated and data on breast cancer incidence, for nulliparous and parous women, suggest various mechanisms may be implicated in breast cancer development [4–7]. These three risk factors have formed the basis of a model by Pike and colleagues [8], that relates the rate of ageing of breast tissue to age-specific incidence rates for breast cancer. Although this model allowed for a decline in breast tissue ageing as the menopause is approached, unfortunately no similar assumption was made to account for the development of regular cycles during adolescent years. Studies from the Tenovus Institute [9] have shown that the establishment of regular 'endocrinologically-mature' menstrual cycles in adolescent girls is a complex issue which requires more detailed investigation. It was surprising that even at 4 years postmenarche, the proportion of girls ovulating was only of the order of 60%. Furthermore, if the establishment of early regular normal cycles is associated with increased breast cancer risk [10] then it might be expected that adolescent girls from countries where the breast cancer incidence is low, such as Thailand [11], would have relatively less mature cycles compared with their counterparts in Britain [12], where the risk is much higher. The aim of this study was to compare menstrual cycle profiles of salivary progesterone in adolescent girls from both Britain and Thailand during the first 4 years following menarche.

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## SUBJECTS AND METHODS

*Subjects*

Adolescent girls, up to 4 years postmenarche, attending school in Britain ($n$ = 130) or Thailand ($n$ = 232) provided daily 08.00 h samples of unstimulated saliva for one complete menstrual cycle. No attempt was made to select girls beforehand, although girls were asked not to participate in the study if they were users of oral contraceptives. The girls provided samples complete with dates of collection and also their dates of menarche and birth. Of these, it was possible to match 90 British–Thai pairs of girls for both chronological and menarchal ages. For these matched subjects, the mean chronological age, menarchal age and time span postmenarche (number of years following menarche) of the British girls were 14.8 (1.3), 12.9 (0.9) and 2.0 (1.2) years, respectively. The corresponding data for the Thais were 14.8 (1.3), 12.9 (0.9) and 1.9 (1.2) years. Standard deviations are in parentheses.

An additional 96 'sets' of 'monthly' data were collected but were rejected because of incomplete data, incomplete samples or that some girls were more than 4 years postmenarche. These 96 sets of incomplete data were rejected prior to any statistical analysis, and a subsequent review of these sets concluded that no known significant selection bias had been introduced into the study by their omission.

*Sample Collection*

Subjects were provided with a plastic box containing sample tubes held in a polystyrene insert. Pupils were told not to brush their teeth before providing a sample so as to avoid the risk of blood contamination, but to simply rinse their mouth with water to remove debris, wait a few minutes and then to dribble into the sample tube until about 4 ml were collected. A record/calendar card was contained in the box to assist the girls in identifying the date of sample collection and the day of their menstrual cycle which was associated with the sample collected. Samples were stored at −20°C and later packed in solid $CO_2$ for transport to the Tenovus Institute where they were assayed for progesterone.

*Salivary progesterone assay*

Details of this radioimmunoassay have been described [13], including data on the specificity of the antisera and accuracy of the method. Based on gas chromatographic–mass spectrometric criteria [14] the assay is judged to have an accuracy of 96%. The between- and within-assay coefficients of variation were generally less than 10% [15].

*Quality control and stability of salivary progesterone assay*

These have been described in a preliminary report [15]. Internal quality control procedures were used to monitor the constancy of the mean progesterone concentration, and the associated imprecision, in each of three pools spanning the analytical range of interest (about 100–500 pmol/l). The cause of any assays judged to be out-of-control, albeit on few occasions, was investigated, remedied and samples were re-assayed. Additionally, selected menstrual cycle profiles of salivary progesterone obtained from assays carried out at the start of the analytical programme were re-assayed at the end, some 15 months later. The results showed a small deterioration in the concentration of salivary progesterone on storage which was insufficient to affect the grading of the menstrual cycle profiles.

*Assessment of salivary progesterone profiles*

Five distinct identifiable menstrual cycle profiles of salivary progesterone have been recognised [9] in adolescent girls as shown in Fig. 1. These profiles have been termed 'grades'.

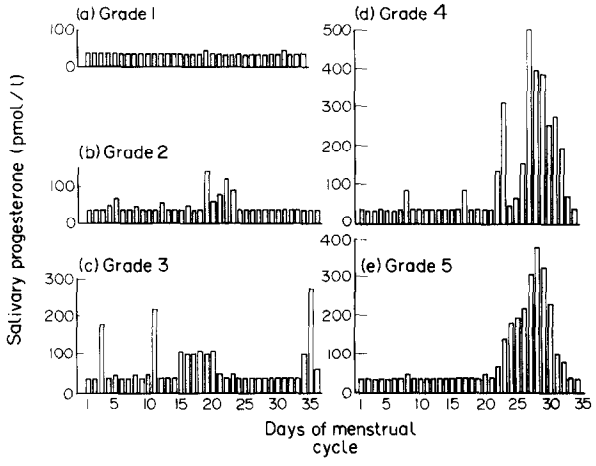


**Fig. 1. Five identifiable menstrual cycle profiles (Grades) of salivary progesterone concentration previously found in adolescent girls [9] which were used to characterise those found in this study of Thai and British adolescent girls. Grades are defined in 'Subjects and Methods'. Figure reproduced with permission from Chronobiology International [9].**

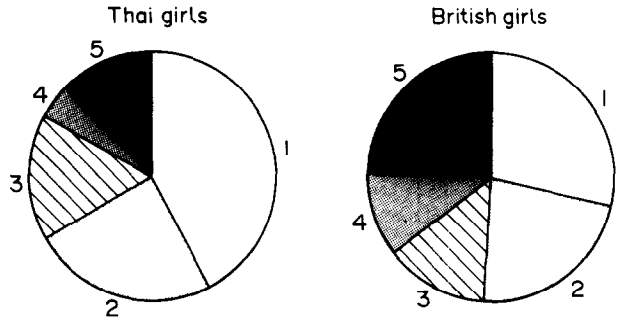


**Fig. 2. Pie-charts which illustrate the frequencies of grades of menstrual cycle profiles of salivary progesterone concentration in Thai and British subjects ($n = 2 \times 90$) matched for chronological and menarchal ages. The type of grade is given on the perimeter of the chart against the segment relating to its relative frequencies. Details of chronological and menarchal ages and years postmenarche are given in 'Subjects and Methods'.**

Grade 1 represents a quiescent profile in which all daily samples have progesterone concentrations which are close to the limit of detection of the assay at about 40 pmol/l; grade 2 has 'spikes' of progesterone concentration not exceeding 150 pmol/l set against a grade 1 background; grade 3, displays a greater activity with 'spikes' of progesterone now reaching 300 pmol/l and showing a tendency to aggregate on adjoining days; grade 4 has a biphasic profile of progesterone concentration occurring in the second 14-day time span of the cycle, and the magnitude of the sustained progesterone concentration approaches that of grade 5, which is the typical luteal-phase profile of the healthy premenopausal women. Each of the 362 profiles were assigned grades by two independent assessors and only 17 discrepancies were found; even then they differed by only one level of grade and for these a consensus grade was found. The inter-rater agreement based on the weighted Cohen's Kappa statistic was excellent and close to unity.

*Statistical analysis*

Contingency table analysis, producing a $\chi^2$ statistic, was used to assess the statistical significance of grades of menstrual-cycle profiles of salivary progesterone for various groups of interest. Contingency table analysis was carried out in three ways as follows, provided the frequencies of grades in each cell were sufficient: (i) all five grades were compared and gave a $\chi^2$ statistic with four degrees of freedom ($\chi^2_4$); (ii) because grade 3 is intermediate between the more immature and mature grades and possibly subject to a higher degree of misclassification, it was omitted from the analysis, the remaining four grades providing a $\chi^2$ statistic with three degrees of freedom ($\chi^2_3$); and (iii) the frequencies of grade 1 and 2 were combined together so as to represent a more general immature grade, $G_{IM}$; similarly, frequencies in grade 4 and 5 were combined to represent a more general mature grade of salivary progesterone profile and designated $G_M$. Frequencies in $G_{IM}$ and $G_M$ could be compared in a $2 \times 2$ contingency table giving rise to a $\chi^2$ statistic with one degree of freedom ($\chi^2_1$).

## RESULTS

*Development of mature 'luteal-phase' salivary progesterone profiles*
*Paired Thai–British girls matched for chronological and menarchal ages*

*General comparison of 'luteal-phase' development.* Figure 2 summarises the frequencies of grades of menstrual cycle salivary progesterone profiles found in Thai and British adolescent girls

matched for both menarchal and chronological ages ($n = 2 \times 90$) and constrained to the first 4 years following menarche; herein these girls are referred to as 'matched subjects'. The good degree of matching is indicated by the statistics given in the 'Subjects and Methods' section, which also contains details of the statistical analysis. Statistically significant differences were found between the grade frequencies for Thai and British girls, particularly for grades $G_{IM}$ and $G_M$ ($\chi^2_1 = 7.94$, $0.01 > P > 0.001$). The British girls had twice the number of the mature (grade 5) profiles (22/90) and approximately two thirds of the most immature (grade 1) profiles (26/90) than their Thai counterparts, which were 11/90 and 38/90, respectively.

*The effect of time postmenarche.* The predominance of the mature grade of salivary progesterone profile (grade 5) found in the British girls, was more evident (Figs 3 and 4) in the first 2 years following menarche. The difference was confirmed when grades $G_{IM}$ and $G_M$ were compared (Thai versus British girls, $n = 2 \times 51$, gynaecological age, 0–2 years, $\chi^2_1 = 6.98$, $0.01 > P > 0.001$). Little bias existed in the chronological and menarchal ages and years postmenarche which were 14.04 (0.95), 13.07 (1.00) and 0.96 (0.54) years, respectively for the Thai girls and correspondingly 14.05 (1.02), 12.99 (0.99) and 1.06 (0.57) years for the British girls; standard deviations are in parentheses. Although this difference between Thai and British girls was also apparent in the 2–4 years postmenarche group, it did not quite reach statistical significance ($P > 0.10$).

*The effect of 'early' versus 'late' menarchal age.* It would seem reasonable that if girls who have an early menarche do develop their normal menstrual cycles more rapidly following menarche, then on separation of subjects into 'early' versus 'late' menarchal

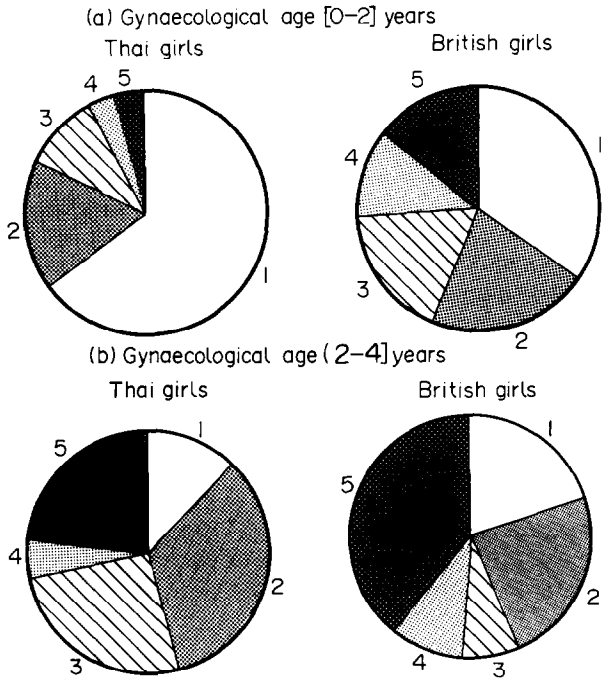


**Fig. 3. Similar to Fig 2, except pie-charts represent data subdivided according to the number of years following menarche. Details of 'ages' are given in the 'Results' section. The 'bracket' convention is as follows: [0–2] includes subjects who are 2 years postmenarche or less, whereas [2–4] refers to subjects who are greater than 2 years and up to, and including 4 years postmenarche. This convention applies to Figs 4 and 5.**

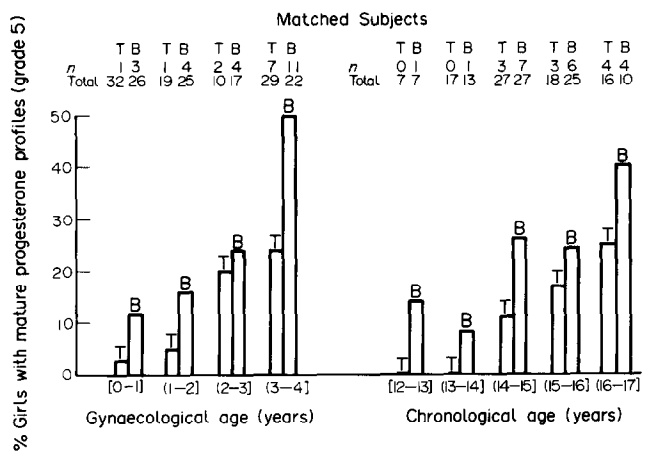


**Fig. 4. Histograms of the frequency of grade 5 menstrual cycle profiles of salivary progesterone, which represent 'mature ovulatory' cycles plotted as a function of years postmenarche and chronological age. Data are for matched Thai (T) and British (B) subjects given in Fig. 2.**

age, they should show different distributions of menstrual cycle salivary progesterone profiles. The matched subjects from both countries were divided into two equal populations according to their menarchal age, and the frequencies of grades of salivary progesterone profiles were analysed in the four subpopulations.

*Thai girls.* The frequencies of grades of salivary progesterone profiles were therefore compared for the two Thai populations ($n = 2 \times 45$), separated according to their median menarchal age of 13.01 years. Those less than 13.01 were designated to be of 'early age of menarche' and those greater, 'late age of menarche'. When the frequencies of grades relating to $G_{IM}$ and $G_M$ were compared for each population, there was a borderline statistical significance ($P \sim 0.05$) but interestingly it was the girls with the 'late age of menarche' who possessed relatively more grade 5 profiles. The subpopulations of Thai subjects were not matched between themselves for chronological age and years postmenarche, the respective age and time span being 14.27 (1.31) and 2.09 (1.18) years for the 'early age at menarche' group, and 15.38 (1.11) and 1.70 (1.27) years for the 'late age at menarche' group. This borderline increase in mature cycles for the 'late age of menarche' group may not be solely a menarchal effect, but a compromise situation arising from a chronological difference of +1.1 years offset by a difference in years postmenarche of −0.39 years.

In the Thai population of girls, it is evident that the frequencies of the more mature grades (grades 4 and 5), were small (Figs 2 and 4), although there is a trend towards more mature profiles with increasing years postmenarche.

*British girls.* These were organised similarly around their median menarchal age of 13.0 years. No statistically significant difference was observed between the two groups. As with the Thai girls, the British were not quite matched between subpopulations. Their chronological age and years postmenarche were 14.23 (1.33) and 2.03 (1.25) years for the 'early age at menarche' compared with corresponding values of 15.48 (1.00) and 1.87 (1.09) years for the 'late age at menarche' group. There was a small bias of +0.16 years in years postmenarche in the younger menarchal age group, although they were chronologically younger by 1.25 years.

In summary, the inter-country differences demonstrate that when compared with Thais (i) British girls have a statistically

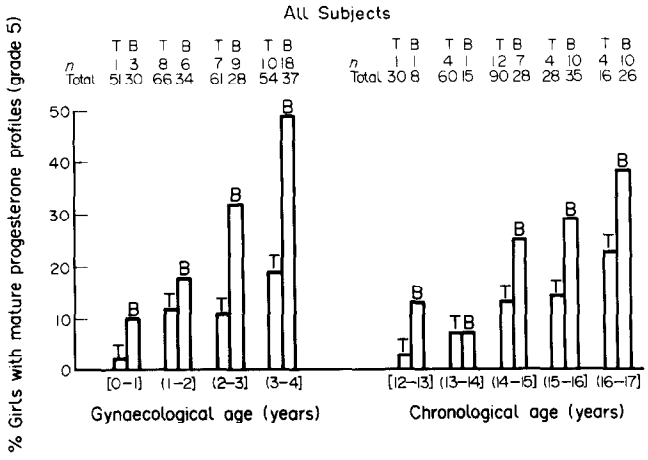


**Fig. 5. Similar to Fig. 4, except nearly all Thai and British subjects are represented as indicated.**

significant increased number of salivary progesterone profiles that are similar to that of the mature healthy premenopausal woman, (ii) this difference is most significant in the first 2 years following menarche, and (iii) this difference is also largely independent of the age at menarche. These conclusions apply to Thai–British pairs of girls who have been matched for chronological and menarchal ages, and who have been within the first 4 years following menarche.

*Analysis of data from all Thai ($n$ = 232) and British ($n$ = 130) girls*

*The effect of time postmenarche.*

*Thai girls.* The frequency of grades of salivary progesterone profiles were compared for those girls with time spans of 0–2 years or 2–4 years postmenarche, with sample sizes of 117 and 115, respectively. There was a highly significant difference in grade frequencies ($P < 0.001$), with the 2–4 year age group having a preponderance of mature profiles (Fig. 5), and fewer immature profiles. This is consistent with data from the matched pairs (Fig. 4). The mean chronological and menarchal ages and the time span postmenarche for the 0–2 year age group were 13.70 (0.92), 12.55 (1.02) and 1.15 (0.53) years, respectively and for the 2–4 year group, 14.80 (1.11), 11.87 (1.00) and 2.93 (0.59) years. Although the 2–4 year group were on average 1.1 years older, with menarche 0.68 years earlier, nevertheless there appears to be a strong time–postmenarche effect as shown in Fig. 4.

*British girls.* The subjects were divided into two groups, 0–2 and 2–4 years postmenarche, comprising 65 girls in each group. There was a highly significant difference between the frequencies of all grades ($P < 0.025$): when frequencies for grade 3 were omitted, or when frequencies for $G_{IM}$ and $G_M$ were used, the significance was even higher ($0.01 > P > 0.001$). The mean chronological and menarchal ages and the time span postmenarche for the 0–2 year age group were 14.31 (1.25), 13.23 (1.13) and 1.08 (0.58) years, respectively and corresponding values for the 2–4 group were 16.16 (0.95), 13.06 (0.95) and 3.10 (0.55) years. The menarchal ages for both groups are comparable but there is a bias of 1.91 chronological years.

*The effect of 'early' versus 'late' menarchal age.*

*Thai girls.* The girls were separated into two groups, depending on their menarchal age relative to the median value for the population of 12.22 years. Each group, therefore, contained 116 girls. In terms of frequencies of the grades of salivary progesterone profiles for $G_{IM}$ and $G_M$, there were no statistically significant differences between 'early' versus 'late' age at menarche. The mean chronological and menarchal ages and the time span postmenarche for the group of girls with an age of menarche less than 12.22 years were 13.71 (0.96), 11.35 (0.62) and 2.35 (0.97) years, respectively and corresponding values for those with the menarchal age greater than 12.22 years were 14.78 (1.08), 13.07 (0.64) and 1.71 (1.03) years.

Interestingly, a statistical significant difference ($P < 0.025$) for the preponderance of grade 2 profiles in the younger menarche age group was found and remains unexplained.

*British girls.* Similarly, the British girls were divided into two groups ($n = 2 \times 65$) according to their median age of menarche of 13.05 years. No statistical differences were observed between the frequencies of grades. The mean chronological and menarchal ages and time postmenarche for the younger menarchal age group were 14.58 (1.43), 12.34 (0.64) and 2.24 (1.26) years, respectively and corresponding values for the older menarchal age group were 15.93 (1.10), 13.95 (0.68) and 1.99 (1.05) years.

## DISCUSSION

It could be conjectured that girls living in developing countries have poor standards of nutrition and low calorific intake, a situation which might lead to a less well-developed hypothalamic–pituitary–ovarian axis or even a late age of menarche. This situation, coupled with prevailing social circumstances conducive to early pregnancy, would result in a short exposure of the developing breast to ovarian hormones and subsequently a low breast cancer risk. This concept underlies the purpose of the present investigation, which was designed to test the hypothesis that British girls (high risk) have more mature cycles than their Thai counterparts (low risk), particularly when matched for chronological and menarchal ages. It is rather surprising that the development of mature cycles may take several years following menarche, and the underlying mechanisms require elucidation. The reason for 'East–West' differences in breast cancer risk is complicated but greater body weight or height appear to be associated with increased risk. A Japanese–Dutch study [16] of women with breast cancer and controls attributed inter-country differences in risk to greater body weight (37%) and height (51%); consequently hormonal factors controlling growth and body mass are of interest. The extent of premenopausal obesity [7] and amenorrhoea or oligomenorrhoea in perimenopausal years are likely to affect breast cancer risk and suggest complementary studies to the present investigation concerned with the adolescent rather than perimenopausal 'window' for breast cancer initiation.

Salivary progesterone assay provides a useful means by which the functional capacity of the corpus luteum, in normal women or in those with defects in the hypothalamic–pituitary–ovarian axis, can be monitored [17]. The assay can also be used to investigate the development of this axis before, during and after menarche [9, 18]. In contrast to urine or plasma, saliva is a medium which is easily collected and accepted by subjects. Consequently, sufficient data can be easily generated so as to adequately reflect the total progesterone concentrations found in plasma [19, 20], which is in contrast to sparse data generally obtained using alternative media.

Previous studies of pre- and post-menarchal girls have identified five types of salivary progesterone profiles [9], grades 1–5, which represent progressive progesterone secretion ranging from 'background' (grade 1) to the profile resembling that of the

healthy premenopausal women (grade 5). This grading scheme was used to score salivary progesterone profiles obtained in this study.

The results clearly demonstrate that when Thai and British girls are matched for chronological and menarchal ages, and constrained within a postmenarchal age of 4 years, the British girls have a preponderance of mature salivary progesterone profiles and consequently fewer immature cycles, than their Thai counterparts. This effect is statistically more pronounced during the first 2 years following menarche. In each matched population there is an increase in mature progesterone profiles with respect to a longer time postmenarche and chronological age. For example, only about 3% of Thai girls had grade 5 profiles within 1 year postmenarche compared with about 25% after 4 years. Similarly, for the same time spans postmenarche, corresponding data for British girls were 12% and 50%.

There was no evidence from our study, albeit transverse, to suggest that girls with a younger age of menarche are associated with an early onset of ovulatory cycles. This is in contrast to the findings of Apter and Vihko [21]. These investigators followed-up their population of adolescent girls in Finland, twice, at 1.5 yearly intervals and also over a wider postmenarchal time span. They took samples of blood at specific periods of the cycle, days 6–9 and 20–23 and/or days 27–30. In particular, the ratio of progesterone concentration in the sample towards the end of the cycle to that near the beginning was used by these investigators to assess whether the cycles were 'ovulatory'. Relative to this, an ancillary assessment of 30 randomly selected grade 5 cycles from British girls was made. Data were re-evaluated as if single samples had been taken on day 8 and day 22. Using a conservative ratio of '6', less than the '10' of Apter and Vihko [21], only 14 cycles were confirmed as being 'mature' and 9 were equivocally confirmed: more importantly, 9 were judged anovulatory, which was clearly not the case when the complete profiles were examined. Consequently, our data draw attention to the potential difficulties arising from infrequent sampling, as is generally the case using blood for analysis, and conversely, to the advantages of making use of saliva. False positive 'ovulatory' cycles may also arise from 'spikes' of progesterone concentration when single samples of blood are used [9, 22].

The most important conclusion from this study is that, following menarche, British girls develop their 'cycles' more quickly than Thai girls. Instead of focussing on the age of menarche as a risk factor for breast cancer, it may be appropriate to consider the stages of ovarian development as judged from salivary progesterone measurements, particularly the age at which the 'cycle' reaches maturity.

It has been stated that breast cancer belongs to a group of hormone-dependent cancers referred to as Western diseases [23] because incidence and mortality are high in the Western world compared with Asian countries such as Thailand, Japan or the Philippines. Diet and lifestyle are major factors thought to be implicated in the development of these diseases, and considerable epidemiological evidence [24–26] would support this viewpoint. The effects of dietary components, or standards of nutrition, on pubertal development are not properly understood [27], but the maturing neuroendocrine system has been implicated in their expression, and may well influence the elaboration of the gonadotrophin-releasing hormone pulse generator and the production of gonadotrophins, and bring about changes in the responsiveness of the hypothalamus to circulating plasma gonadal steroid hormones secreted by the ovary.

Physical activity may also be an important factor in the development of the hypothalamic–pituitary–ovarian axis. It has been reported that young women participating in vigorous exercise, such as competitive running, swimming or ballet dancing, have increased incidence of oligomenorrhoea or amenorrhoea [28], which are indicative of considerable endocrine disturbances. Under less rigorous conditions such as non-competitive running, progesterone secretion appears to be lowered [29]. Indeed, breast cancer incidence appears to be reduced in college athletes compared with their non-athletic counterparts [30], which supports the hypothesis that ovarian function and the incidence of breast cancer are associated.

Finally, future studies of adolescent girls and young women should investigate the development of the hypothalamic–pituitary–ovarian axis, with a longitudinal design using salivary progesterone assays, and determine the effects that body weight, dietary intake and exercise may have on its elaboration; thereafter attention may be focussed on breast cancer prevention.


1. Staszewski J. Age of menarche and breast cancer. *J Natl Cancer Inst* 1971, **47**, 935–940.
2. MacMahon B, Cole P, Brown J. Etiology of breast cancer: a review. *J Natl Cancer Inst* 1973, **50**, 21–42.
3. Trichopoulos D, MacMahon B, Cole P. Menopause and breast cancer risk. *J Natl Cancer Inst* 1972, **48**, 605–613.
4. Nicholson RI, Wilson DW, Colin P, *et al.* Influence of pregnancy on the time of presentation of primary breast cancer. In: Angeli A, Bradlow HL, Dogliotti L, eds. *Endocrinology of the Breast: Basic and Clinical Aspects, Annals of the New York Academy of Sciences* 1986, **464**, 463–465.
5. Shore RE, Pasternack BS, Bulbrook RD, *et al.* Endocrine and environmental factors in breast cancer: The case for prospective studies. In: Bulbrook RD, Taylor Jane D, eds. *Commentaries on Research in Breast Disease*, 1983, **3**, 1–31.
6. Henderson BE, Ross R, Bernstein L. Estrogens as a cause of human cancer: The Richard and Hinda Rosenthal Foundation Award Lecture. *Cancer Res* 1988, **48**, 246–253.
7. Pike MC, Forman D. Epidemiology of cancer. In: L.M. Franks, N.M. Teich, eds. *Introduction to the Cellular Molecular Biology of Cancer*. Oxford, Oxford University Press, 1991, 49–97.
8. Pike MC, Krailo MD, Henderson BE, *et al.* 'Hormonal' risk factors, 'breast tissue age' and age-incidence of breast cancer. *Nature* 1983, **303**, 767–770.
9. Wilson DW, Read GF, Hughes IA, *et al.* Hormone rhythms and breast cancer chronoepidemiology: salivary progesterone concentrations in pre- and post-menarchal girls and in normal premenopausal women. *Chronobiol Int* 1984, **1**, 159–165.
10. Henderson BE, Pike MC, Casagrande JT. Breast cancer and the oestrogen window hypothesis. *Lancet* 1981, **ii**, 363–364.
11. Parkin DM, Ed. In: *Cancer Occurrence in Developing Countries*. IARC Scientific Publications, No 75. Lyon: International Agency for Research on Cancer, 1986.
12. Whelan SL, Parkin DM, Masyer E, eds. In: *Patterns of Cancer in Five Continents*. IARC Scientific Publications, No 102. Lyon: International Agency for Research on Cancer, 1990.
13. Truran PL, Read GF, Morton MS, *et al.* An alternative radioligand for immunoassay of salivary progesterone. *Clin Chem* 1985, **31**, 1091–1092.
14. Leith HM, Truran PL, Gaskell SJ. Quantification of progesterone in human saliva. *Biomed Environmental Mass Spectrometry* 1986, **3**, 257–261.
15. Danutra V, Turkes A, Read GF, *et al.* Progesterone concentrations in samples of saliva from adolescent girls living in Britain and Thailand, two countries where women are at widely differing risk of breast cancer. *J Endocrinol* 1989, **121**, 375–381.
16. de Waard F, Cornelis JP, Aoki K, Yoshida M. Breast cancer incidence according to weight and height in two cities of The Netherlands and in Aichi prefecture, Japan. *Cancer* 1977, **40**, 1269–1275.
17. Riad-Fahmy D, Read GF, Walker RF, *et al.* Determination of ovarian steroid hormone levels in saliva. An overview. *J Reprod Med* 1987, **32**, 254–272.
18. Read GF, Fahmy DR, Wilson DW, *et al.* A new approach for

breast cancer research: assays for steroids in saliva. In: Bulbrook RD, Taylor DJ, eds. *Commentaries on Research in Breast Disease*. New York, Alan R. Liss 1983, 3, 61–92.
19. Zorn JR, McDonough PG, Nessman C, *et al.* Salivary progesterone as an index of the luteal function. *Fertil Steril* 1984, **41**, 248–253.
20. Wang DY, Knyba RE. Salivary progesterone: relation to total and non-protein-bound blood levels. *J Steroid Biochem* 1985, **23**, 975–979.
21. Apter D, Vihko R. Early menarche, a risk factor for breast cancer, indicates early onset of ovulatory cycles. *J Clin Endocrinol Metab* 1983, **57**, 82–86.
22. Truran PL, Leith HM, Read GF. Transient increases in progesterone in daily and 2-hourly saliva specimens from adolescent girls. *J Endocrinol* 1986, **111**, 513–518.
23. Adlercreutz H. Western diet and Western diseases: some hormonal and biochemical mechanisms and associations. *Scand J Clin Lab Invest* 1990, **50**, Suppl 201, 3–23.
24. Armstrong B, Doll R. Environmental factors and cancer incidence and mortality in different countries with special reference to dietary practices. *Int J Cancer* 1975, **15**, 617–631.
25. Buell P. Changing incidence of breast cancer in Japanese-American women. *J Natl Cancer Inst* 1973, **51**, 1479–1483.
26. Kolonel LN, Hankin JH, Nomura AMY. Multiethnic studies of diet, nutrition and cancer in Hawaii. In: Hayashi Y, Nagao M, Sugimura T, *et al.* eds. *Diet, Nutrition and Cancer*. Tokyo, Japanese Societies Press, 1986, 29–40.
27. Kirkwood RN, Cumming DC, Aherne FX. Nutrition and puberty in the female. *Proc Nutrition Soc* 1987, **46**, 177–192.
28. Frisch RE, Wyshak G, Vincent L. Delayed menarche and amenorrhea in ballet dancers. *N Engl J Med* 1980, **303**, 17–19.
29. Ellison PT, Lager C. Exercise-induced menstrual disorders. *N Engl J Med* 1985, **313**, 825–826.
30. Frisch RE, Wyshak G, Albright NL, *et al.* Lower lifetime occurrence of breast cancer and cancers of the reproductive system among former college athletes. *Amer J Clin Nutr* 1987, **45**, 328–335.

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# Attributable Risks for Oesophageal Cancer in Northern Italy

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**The population attributable risk for oesophageal cancer in relation to cigarette smoking, elevated alcohol use and low beta-carotene intake has been estimated with 300 cases and 1203 controls in Greater Milan. In males 71% of oesophageal cancers were attributable to smoking, 45% to elevated alcohol use and 40% to low beta-carotene consumption. The corresponding figures were 32%, 10% and 29% in females and 61%, 39% and 38% in total. The overall estimate, including the joint effect of the three factors, was 90% in males, 58% in females and 83% in total. The discrepancies between the sums are due to the assumption of a multiplicative model and to the great percentage of oesophageal cancers attributable to each single factor. Cigarette smoking is the major known cause of oesophageal cancer and the three factors account for practically all the difference between male and female mortality rates. Elimination of smoking, reduction of alcohol consumption and enrichment of diet with fruit and vegetables would make oesophageal cancer a rare disease in Italians of both sexes.**
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## INTRODUCTION

AMONG COMMON neoplasms, oesophageal cancer is the one showing the largest geographical variation, with a several hundred times difference between high risk areas in Iran and low risk ones in north Africa, and also within Europe there is a 25-fold ratio between the highest risk areas in France and the lowest ones in Scandinavia and Eastern Europe [1, 2].

Elevated alcohol and tobacco consumption can explain most of the excess risk in Europe or North America [3]. A study conducted in the French department of Ille-et-Vilaine, for instance, found that over 87% of the cases could be explained by alcohol and tobacco [4]. However, alcohol and tobacco cannot explain the high risk areas in Asia or South Africa [3, 5], and have a quantitatively different role in developed countries, too. Thus, factors other than alcohol and tobacco, such as, for instance, a diet poor in fresh fruit and vegetables, may influence—to a variable degree—oesophageal cancer risk in Europe [6, 7].

We have therefore tried to quantify the role of tobacco, alcohol and dietary deficiencies on oesophageal cancer risk in males and females in a northern Italian population, using data from a large case-control study.

## SUBJECTS AND METHODS

Since January 1984 we have been conducting a case-control study of oesophageal cancer in the greater Milan area, whose