Characterisation of breast skin temperature rhythms of women in relation to menstrual status


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Abstract. Circadian breast skin temperature rhythms were characterised throughout the menstrual cycle, for various locations on the left breast of ambulatory women. All subjects exhibited highly significant circadian rhythms (P < 0.001). Changes in rhythm parameters, such as the mesor, amplitude and acrophase, were observed during the menstrual cycle. No consistent trend in these rhythm parameters was observed between subjects in relation to menstrual cycle stage. Experimental and statistical techniques used to characterise circadian rhythms in pre-menopausal women were applied to a post-menopausal woman with primary breast cancer. Comparison of rhythm parameters associated with the tumour area and corresponding site on the contralateral breast showed abnormal thermal characteristics such as elevated mesor values, decreased amplitude as well as changes in the timing of the acrophase. These properties may be exploited for the early detection of breast cancer. The project also involved the design and testing of an ambulatory device, known as the 'chronobra', for the measurement of breast skin temperature. The performance of the chronobra was in close agreement with reliable, conventional equipment. The chronobra now allows studies of breast skin temperature rhythms associated with breast disease to be extended.

Experience of human breast cancer has clearly indicated that malignant cells may disseminate at any stage of the disease and not merely when the tumour has reached a certain critical or detectable size (Duncan & Kerr 1976). Early detection of a small tumour may well improve prognosis, but research must be directed to the recognition of 'pre-neoplastic discriminants' of breast biology. Familial relationships (Macklin 1959; Anderson 1976), the different incidences of breast cancer in Western and Asian populations and the risk factors associated with age at first term-birth (MacMahon et al. 1970) or early menarche suggest that high risk groups might be identified. Furthermore, Jensen and her colleagues (Jensen et al. 1976; Brem et al. 1978) have described recognisable pre-neoplastic lesions in the human breast.

There would appear to be a possibility that early detection of breast cancer may be achieved with the aid of an ambulatory monitoring device for the measurement of breast skin temperature (Simpson 1977) provided the appropriate statistical techniques can be used to interpret the critical aspects of the data.

This investigation has the ultimate aim of determining whether specific temporal pathologies precede tumour development and if so, whether this can be exploited for diagnosis. The present report concerns the characterisation of circadian and cir-
catrigintan breast skin temperature rhythms of pre-menopausal women exhibiting normal ovulatory cycles and their relationship to hormonal profiles. The study was directed towards the accumulation of breast temperature data, from groups of subjects, making use of conventional equipment previously described (Wilson et al. 1979). These 'normal' data can be presented in the form of cosinor diagrams (Halberg et al. 1967, 1972) and time-qualified reference ranges termed chronodesms (Halberg et al. 1978) against which results from other groups of women, possibly with early breast cancer, benign breast disease or 'normal' subjects from high or low risk groups, can be compared.

The eventual correlation of the breast temperature circadian and circatriginant rhythms with rhythms of hormone concentrations in plasma and saliva should provide valuable data on the hormonal control of breast physiology. The practical limitations to collecting this type of chronobiological information necessitated the development of an ambulatory device which can automatically measure breast skin temperature and store the data over a substantial time-span. A device known as the 'chronobra' has been developed and basic information on this garment and associated electronics is described in this report.

Materials and Methods

Subjects
Nine pre-menopausal, clinically healthy subjects, aged 18–37, who were research staff volunteers from the Tenovus Institute, were studied either throughout the whole (4 subjects), or a large proportion of (5 subjects) a normal menstrual cycle. The latter group was studied during the late follicular-early luteal phase (days 8–23 of the cycle). Breast skin temperature measurements were made on these subjects, as they carried out their normal daily routines. Nocturnal-rest schedules were generally between 23.00 and 07.00 h. Blood and saliva samples, for hormone assay, were collected at 09.00 h on selected days. Oral temperatures were taken daily upon wakening but before rising. On one post-menopausal patient with primary breast cancer, temperature measurements were made at 30 min intervals over a 96 h time-span, prior to surgery. Temperatures were recorded using the manual conventional procedure, as described below, with the patient in a hospital bed.

Measurement of breast skin temperature
(a) Manual procedure. Each day, miniature semi-conductor, temperature sensors (4 mm dia. with the reverse side insulated) connected through fine electrical wire to small plugs, were attached by the subjects to each of the 4 quadrants of the left breast. The axes were defined vertically by the cephalo-caudal line and horizontally by the parasternal to mid-axillary line, the nipple being located at the origin. One sensor was attached to the sternum just above the sinus sternum, and another at a point approximately level with the nipple on the mid-axillary line of the left lateral chest wall. Areas of skin against which the sensors were placed, were carefully washed to remove dead tissue. Sensors were attached using hypoallergenic surgical tape (Microspore 3M Co. Ltd., St. Paul, Minnesota, USA). Temperature readings from the 6 sensors were recorded by plugging each in turn into an electronic thermometer (range 32–38°C in 0.05°C divisions: Light Laboratories, Brighton, England) as shown in Fig. 1A. Readings were generally taken at 30 min intervals throughout the course of the study and various activities, such as dietary intake, were recorded. All the sensors were interchangeable within 0.1°C.

(b) Automatic procedure. An ambulatory device, the chronobra, has been designed to automatically monitor and store breast skin temperature. The prototype, shown in Fig. 1B–D, currently provides for 16 channels of data, has a memory capacity of up to 8K bit words and a wide range of sampling times. The device is initialised and functionally checked by an interface unit, (Fig. 1E) which also effects extraction and storage of data from the memory system of the chronobra for subsequent analysis by a main frame- or mini-computer.

Hormone analyses
Blood samples were centrifuged for 5 min at approximately 1000 g within 10 min of collection and the plasma stored at −20°C. Saliva was also stored at −20°C after collection. Plasma luteinizing hormone (LH) and follicle stimulating hormone (FSH) were assayed by double antibody radioimmunoassays shown to be specific for these hormones (Groom et al. 1971). Concentrations of progesterone and 17α-hydroxyprogesterone in saliva were determined by procedures recently developed in the Institute (Walker et al. 1978, 1979a,b). Plasma oestradiol-17β concentration was measured by the routine Supraregional Assay Service Laboratory in the Institute.

Numerical analysis
Breast skin temperature data were subjected to rhythmmetry (Halberg et al. 1967, 1972, 1977) in which a cosine function of the form

\[ y(t) = M + A \cos(2\pi t + \phi) + \varepsilon(t) \]

was used, with the patient in a hospital bed.
was fitted to the data using the method of least squares. The statistical analysis provided values and fiducial limits for the mesor, M, (mean of the fitted rhythm), amplitude, A, (this is a measure of one half the extent of the rhythmic change in the cycle estimated by the cosine function) and acrophase, \( \phi \), (the lag from local midnight to the crest time of the fitted cosine function), respectively. The breast skin temperature at a time \( t \) is given by \( y(t) \), the period of the rhythm is denoted by \( \tau \) and \( \epsilon(t) \) is the uncertain or uncontrolable or unobservable errors assumed to be independent normal variables with a mean of zero and similar variance. The percentage variability accounted for by the cosine fit together with the \( P \)-value for the statistical significance of the rhythm were also calculated.

The possibility that circadian rhythm characteristics may change during the menstrual cycle had also to be considered (Simpson & Halberg 1974), and such rhythm parameters were assessed using serial section analysis. A ‘window’ of fixed dimension was selected and data contained therein, were fitted by a cosine function using a fixed 24-hour period. Values of the rhythm parameters were then calculated. The window, located initially at the beginning of the data train, was then moved in fixed increments along the train and the whole process continued until all data had been scanned. The process is analogous to the more generally used ‘moving’ average technique (Chatfield 1975).

Circadian rhythms of breast skin temperature were displayed graphically in the form of a cosinor diagram with an attendant table summarising the statistical analysis.

**Results**

This report describes interim progress in the development and use of the chronobra, which automatically monitors changes in breast skin temperature over relatively long time-spans. At the same time, the potential value of circadian and circatrigintan rhythms of breast skin temperature is clearly illustrated by the preliminary results.

It was established in early experiments using the manual procedure, that the breast skin tempera-

tures for corresponding sites on the left and right breast were similar and consequently, all subsequent measurements were made on the left breast.

Breast skin temperature data were also collected by the manual procedure from subjects studied throughout one menstrual cycle and measurements were taken every 30 min during wake-span. The effect of a gap in the sampling of breast skin temperatures from 23.00 to 07.00 h does produce rhythm parameters which are somewhat different from those collected over a full 24 h. Nevertheless the results obtained are comparable to each other. Fig. 2 shows the analysis of this type of data in the form of a single cosinor diagram for each sensor from one of the subjects studied. Representation of circadian breast skin temperature rhythms is shown in the diagram. The acrophase is displayed by the position of the pointer on the clock and its value is given in both clock time and degrees; the fiducial limits are also indicated. The length of the pointer is scaled according to the amplitude of the rhythm. The table provides a statistical summary of the cosine fit.

All subjects \( n = 4 \) studied for the complete menstrual cycle exhibited a statistically significant circadian rhythm \( (P < 0.001) \) with a period of approximately 24 h. The overall mean values for the breast mesor, amplitude and acrophase for these women exhibiting ovulatory cycles, assessed by plasma and saliva hormone concentrations, were 34.13°C, 0.63°C and −316° respectively. All circadian rhythms of breast skin temperature had acrophases approximating to 21.00 h. The acrophase associated with the mid-axillary line occurred approximately one hour earlier and the rhythm had an amplitude that was usually lower than that of the breast. Subjects studied during the late follicular-early luteal phase of the cycle displayed similar rhythm parameters as those previously described.

Changes in circadian rhythm parameters through the menstrual cycle are illustrated in Fig.
3. Spans of 48, 72, 96 and 120 h (windows) were incremented by 24 h intervals for the serial section analysis and values for the circadian rhythm parameters were calculated for each window throughout the data train. An example of a computer print-out of results from a 120 h serial section analysis of temperature measurements obtained from the lower inner quadrant of the left breast of one subject investigated for 34 days is shown in Fig. 3.

This subject was studied from day 19 of one cycle to day 24 of the next. Hormone analyses indicated that ovulation occurred on day 14. Circadian variation in breast skin temperature can be clearly seen

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**Fig. 2.**

Cosinor diagram of circadian breast skin temperature rhythms 9 days before and 7 days after the onset of menses. Temperatures were recorded at 30 min intervals during wake-span on a subject with sensors located on the lower inner, lower outer, upper outer and upper inner breast quadrants, the left lateral chest wall and the sternum denoted by the key as A-F respectively. Definitions of the rhythm parameters and descriptions of the cosinor diagram are found in the text and references.

<table>
<thead>
<tr>
<th>VARIABLE AND ELLIPSE IDENT</th>
<th># OF DATA</th>
<th>MESOR</th>
<th>SEM</th>
<th>AMPLITUDE AND (95% LIMITS)</th>
<th>ACROPHASE AND (95% LIMITS)</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A LOW INNER</td>
<td>324</td>
<td>35.02</td>
<td>0.08</td>
<td>-0.69 (0.51, 0.88)</td>
<td>-313 (-292, -339)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>B LOW OUTER</td>
<td>316</td>
<td>35.22</td>
<td>0.08</td>
<td>-0.74 (0.56, 0.96)</td>
<td>-306 (-286, -328)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C UP OUTER</td>
<td>323</td>
<td>34.78</td>
<td>0.07</td>
<td>-0.72 (0.55, 0.90)</td>
<td>-303 (-287, -324)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>D UP INNER</td>
<td>327</td>
<td>34.75</td>
<td>0.06</td>
<td>-0.62 (0.45, 0.81)</td>
<td>-294 (-278, -318)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>E CONTROL OUT</td>
<td>332</td>
<td>34.52</td>
<td>0.06</td>
<td>-0.74 (0.59, 0.90)</td>
<td>-305 (-290, -323)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>F CONTROL IN</td>
<td>336</td>
<td>35.28</td>
<td>0.05</td>
<td>-0.57 (0.41, 0.74)</td>
<td>-284 (-270, -302)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
in the chronogram (Fig. 3a) which is a plot of the raw temperature data as a function of time. The temperature varied daily from 32°C at 07.00 h to 36°C at 21.00 h. The chronogram indicated a circatrigintan rhythm peaking around the time of menstruation, with a nadir around day 8. Values for the significance of the rhythm ($P < 0.001$), the mesor (33–36°C), the mesor plus amplitude and acrophase, together with standard error, are also displayed in Fig. 3b–d. In an individual, and for each quadrant, these values can be seen to change through the menstrual cycle. Sampling frequency (Fig. 3e) is also displayed in the computer output for each section taken through the menstrual cycle.

Fig. 4 illustrates data on one subject showing hormone concentrations in both saliva and plasma.

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**Fig. 3.**

Computer print-out summarising a 120 h serial section analysis of circadian breast skin temperature data (°C). Temperatures were measured at 30 min intervals during wake-span and obtained from the lower inner quadrant of the left breast of a pre-menopausal women studied for 34 days. (a) Chronogram. This is a plot of breast skin temperature with time. (b) Plot of the significance of the circadian rhythm ($P$). (c) Plot of circadian mesor (lower) to which the amplitude has been added (upper) together with respective standard errors. (d) Plot of circadian acrophase and 95% confidence limits. (e) Number of temperature measurements collected in 120 h. All calculations of the rhythm parameters are based on a circadian rhythm with a 24 h period.
oral temperatures and mean mesor temperatures for the breast. The characteristic LH and FSH peaks follow the usual oestradiol-17β surge prior to ovulation. Post-ovulatory rises of progesterone and 17α-hydroxyprogesterone concentrations in saliva were observed in all these subjects, again characteristic of normal ovulatory cycles. Although in this example, a rise in oral temperature after ovulation was clearly demonstrated, this was not the case in all subjects and it would seem that oral temperature is not a reliable indicator of ovulation, particularly in untrained subjects. Detailed salivary hormone analysis will be related to those temperature measurements. The application of rhythmometry of the data obtained from this preliminary study of subjects for one menstrual cycle indicates that while circatrigintan rhythms are evident, the relatively wide range of possible values for the rhythm parameters suggests that interpretation would be more meaningful from data accumulated from at least three consecutive menstrual cycles.

The performance of the chronobra was assessed...
against temperature data obtained by the manual procedure in one subject for five weeks. The chronobra was arranged such that 4 sensors were in contact with the left breast. Sensors for the manual procedure were attached immediately adjacent to those of the chronobra and the performance of both systems recorded. Cosinor analysis of the full sets of data were comparable indicating that values for the circadian rhythm parameters were similar (unpublished data). Investigations are currently underway in the Institute to accurately calibrate the chronobra using thermometers certified by the British Standards Institution.

A preliminary study of breast rhythm parameters of a post-menopausal woman with primary breast cancer was encouraging. Sensors were attached to the skin over the tumour and to corresponding sites on the contralateral breast. Temperatures were recorded by the manual procedure and the rhythm characteristics are shown in Fig. 5. The acrophase of the circadian rhythm associated with the tumour is significantly different to that of the contralateral breast as shown in Fig. 6 and occurs approximately 5 h earlier. More detailed studies are in progress to attempt to exploit such differences as a means of detecting early breast cancer.

**Discussion**

The data reported clearly demonstrate the presence of circadian breast skin temperature rhythms in pre-menopausal subjects, studied during wake-span, who were on a normal daily routine. In
Cosinor diagram of circadian breast skin temperature rhythm parameters for duplicate sensors over the tumour and similar sites on the contralateral breast. The mean temperature data for the cancerous and contralateral breast are shown in Fig. 5.

<table>
<thead>
<tr>
<th>VARIABLE AND ELLIPSE IDENT.</th>
<th>N OF DATA</th>
<th>MESOR</th>
<th>SEM</th>
<th>AMPLITUDE AND (95% LIMITS)</th>
<th>ACROPHASE AND (95% LIMITS)</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. CANCEROUS 1</td>
<td>191</td>
<td>36.11</td>
<td>0.02</td>
<td>0.23 (0.16 , 0.30)</td>
<td>-283 (-265 , -301)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>B. CANCEROUS 2</td>
<td>191</td>
<td>36.24</td>
<td>0.02</td>
<td>0.28 (0.22 , 0.34)</td>
<td>-272 (-258 , -286)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C. CONTRALATERAL 1</td>
<td>191</td>
<td>34.73</td>
<td>0.05</td>
<td>0.63 (0.47 , 0.79)</td>
<td>-342 (-327 , -357)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>D. CONTRALATERAL 2</td>
<td>128</td>
<td>34.92</td>
<td>0.06</td>
<td>0.90 (0.68 , 1.12)</td>
<td>-360 (-346 , -13)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Fig. 6.**

Contrast, evidence for a well characterised circartrigintan rhythm is equivocal on the data collected over a time-span of only one menstrual cycle.

The cosinor diagrams described, provide a valuable means by which potential differences between a 'normal' and diseased breast might become evident. Just as a biopsy might be used to assess early breast pathology, so might a thermopsy be used to detect neoplasia before the disease becomes clinically manifest. This preliminary study, now described, provides encouraging evidence that the chronobra may detect early cancer, since in the patient investigated, a definite phase shift of nearly five hours was present in the temperature rhythm associated with the tumour. A major study of patients with breast cancer is now in progress in the hope of establishing the validity of these preliminary data and initial analyses suggest that an 'abnormal' rhythm, relating to the presence of cancer, can be detected.
Such studies support the early work of Mansfield and his colleagues (Mansfield et al. 1973) who studied surface temperatures over the normal and cancerous breast with a view to monitoring hormone therapy. They described certain changes in the rhythm parameters when the control and affected side were compared. Similar support for this concept was provided by surface tumour temperature data obtained by radiotelemthermometry (Gautherie & Gros 1971). It is obvious that detailed, carefully controlled studies are required to establish the biological characteristics of the developing tumour, which might allow for early detection, or ultimately identify women with a high risk of developing breast cancer.

The detailed study, now described, set out to define some of the rhythm criteria which should be considered in the establishment of screening programmes. When circadian mesors for selected spans of the data were plotted through the menstrual cycle, there was often a nadir around midcycle as previously reported (Wilson et al. 1979), and circatrigintan rhythms, based solely on the P-value for a cosine fit, were demonstrated (P < 0.001).

To determine whether or not changes in breast skin temperature occur after ovulation, certain improvements to the protocol have been implemented in a study of a group of Cardiff women practising 'natural' family planning methods. The span of the study has been extended to a minimum of three months and the temperature data are being collected using the prototype chronobra which has produced data similar to that employing the manual procedure. The value of salivary steroid analysis to monitor ovulation and the endocrine changes in relation to menstruation is obvious. The assay for oestradiol-17β in saliva, recently developed at the Institute, will also be used. Circadian temperature rhythms in relation to hormone changes as reflected in the concentration of steroid in saliva, and prolactin in plasma, will form a part of the next phase of the study. The fact that the concentration of steroid in saliva is a measure of the free, non-protein bound level in plasma further improves the physiological nature of the study. Special attention is being directed to the design of the chronobra and its insulation in order to substantially reduce environmental noise and this may enable any biorhythms present to be elicited more readily.

This report describes preliminary data which have been obtained from early studies of breast temperature rhythms and it forms the guide-line for the development and use of chronodesms in breast cancer screening.

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References


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