Time-Dependent Variations of the Skin Barrier Function in Humans: Transepidermal Water Loss, Stratum Corneum Hydration, Skin Surface pH, and Skin Temperature

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Although circadian rhythms have been described for many human functions, there are minimal data on circadian rhythms related to skin physiology. This study investigated the circadian rhythmicity of skin variables related to skin barrier function in humans. We measured transepidermal water loss, stratum corneum moisture, skin surface pH, and skin temperature in 16 healthy volunteers (nine men and seven women, aged 23–53 y). Subjects were sampled every 2 h in two sessions over a 24 h span. Twelve samples were obtained for each variable in the following sites: forehead, forearm, upper back, and shin. We used cosinor analysis and ANOVA to validate observed differences. Time-dependent rhythms were detected in most skin variables except in stratum corneum hydration. We found a statistically significant circadian rhythmicity characterized by cosinor analysis in transepidermal water loss, skin surface pH, and skin temperature on the forearm, forehead, and shin. Peak–trough differences occurred in all locations. The values of the same variables measured at different sites correlated positively, whereas the values of the different variables did not. These results suggest that skin permeability is higher in the evening and night than in the morning. These data may be clinically relevant in several aspects applied to skin physiology and topical drug application.

Key words: circadian rhythms/cosinor analysis/skin physiology.

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Circadian rhythms have been extensively described (Haus and Touitou, 1990); these physiologic and metabolic changes are usually synchronized by environmental light and darkness (Wetterberg, 1994). Studies on circadian rhythms related to skin variables have mainly investigated diurnal variation of eccrine sweating and epidermal cell proliferation (Timbal *et al*, 1975; Stephenson *et al*, 1984; Frentz *et al*, 1991).

The epidermis, especially the stratum corneum, protects against water loss (Elias, 1988), serves as a barrier preventing entrance of microorganisms, and has an acidic pH ranging from 4 and 6.5 (Dikstein and Zlotogorski, 1994). The stratum corneum is also of prime importance for topical drug permeability. Many skin care cosmetics and topical medications are applied at night, based on clinical impressions rather than experimental data.

We studied the circadian rhythms of the following functions on different anatomic sites over a 24 h span using rhythmometric methods: stratum corneum water content, transepidermal water loss (TEWL), stratum corneum pH, and skin temperature.

MATERIALS AND METHODS

Subjects Sixteen healthy subjects, nine men and seven women, aged 23–53 y (mean 38.2 ± 9.5 y), who gave informed consent, participated in a two session study 1 wk apart. The daytime session was conducted from 10:00 to 18:00 and the night time study was conducted from 20:00 to 08:00.

All subjects followed similar diurnal activity patterns. The climatic and environmental temperatures during the two studies (May) were comparable.

All subjects were asked to refrain from washing with soap, using detergents, or applying cosmetics 12 h prior to the test to minimize the effect of these substances on skin moisture, TEWL, and skin pH. The experiments were performed in a controlled environment with temperature at 22°C ± 1°C and humidity between 40 and 60%. During the study the subjects were lightly dressed in short pants and short sleeved shirts. All examinees shared the same meals and comparable mental and physical activities and rest periods during both sessions, and had a 30 min adaptation period before each sampling. Measurements were conducted on the forehead, upper back, forearm, and shins. Each subject was sampled at 2 h intervals in the two sessions combined for 24 h. Twelve samples were obtained for each variable in each site for these two sessions, except for TEWL in six subjects who were not examined in a daytime session.

TEWL TEWL was measured with an evaporimeter (Tevameter, TM 210 Courage and Khazaka, Aca Derm, Menlo Park, CA). The probe, a small hollow cylinder (10 mm diameter, 20 mm height), was held on the skin surface until stable TEWL was established (≈1 min). The results were expressed as g per m^2 per h. We used the guidelines for measurement of TEWL of the European Society of Contact Dermatitis (Pinnagoda *et al*, 1990).

Stratum corneum hydration Measurements of stratum corneum hydration were performed with a corneometer (Cm 820 PC Courage and Khazaka Electronie GmbH, Cologne, Germany) that registered the electrical capacitance of the skin surface as an indicator of stratum corneum hydration, which is dependent on the high dielectrical constant of water content relative to other skin components (Tagami *et al*, 1980). The capacitance was expressed digitally in arbitrary units.

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Abbreviation: TEWL, transepidermal water loss.

Skin surface pH Skin surface pH was measured with a pH meter (pH 900 M, Courage and Khazaka, Aca Derm) with a flat glass electrode as described previously (Yosipovitch *et al*, 1993).

Skin temperature Skin temperature was measured with a digital infrared thermometer (OS202 Omega Engineering, Stanford, CT) with resolution of 0.10.

Statistical analysis The circadian arithmetic mean of each subject's data for pH, TEWL, stratum corneum hydration, and skin temperature at each time was determined. Means and standard errors were calculated for each variable at each time point. The means of the group were then graphed by time as a chronogram. Each subject's data were analyzed for circadian rhythmicity by the single cosinor procedure, which determines, by least squares fit, the cosine curve best fitting the data (Nelson *et al*, 1979). This method detects a rhythm by rejection of the null hypothesis for amplitude in a frequency selected by the investigator. If a rhythm was described by this technique the method determines the rhythm parameters: the MESOR (rhythm adjusted mean); amplitude (half the peak trough difference of the best fitting cosine curve); and, as indicator of the timing of the rhythm, acrophase (the calculated peak time of the cosine curve best fitting the data). The rhythm parameters obtained by single cosinor serve as input for a population mean cosinor describing the rhythm of the group with its variance estimates. Details of this statistical method are found in the report by Nelson *et al* (1979). Because there were no significant differences noted in all variables between men and women, the data were combined. Time-dependent differences were confirmed by one-way ANOVA. For comparison of rhythm parameters, the Bingham Test (Bingham *et al*, 1982) was used for acrophase comparison.

Regression analysis was performed for correlation analysis between different parameters in the same site.

Peak–trough differences were examined by Fisher least significant difference test and Schefe F test.

RESULTS

TEWL is time dependent in all sites examined Time-dependent rhythms were detected in TEWL on the forearm, forehead, upper back, and shin by ANOVA (**Table I**). A circadian rhythm was described by population mean cosinor on the forearm and forehead, as shown in **Table II** and **Fig 1**. For the majority of the subjects, the maximal values were obtained in the evening at all sites (≈20:00) and the minimum levels (trough) were obtained in the morning (08:00–10:00), except on the shin where two peaks were noted, one around 12:00 and the other around 04:00, and two nadirs at 22:00 and at 08:00. Relative peak to trough differences were as follows (g per m^2 per h): forehead 36/9, forearm 16/9, shin 18/10, upper back 31/12, all differences were statistically significant $p < 0.05$.

The acrophase for skin TEWL on the forearm was significantly different from that of the forehead (Bingham Test, $p = 0.0001$).

Circadian rhythms of skin pH were detected on shin and forearm but not on other sites Time-dependent rhythms were detected by ANOVA in skin pH on the forearm and shin (**Table I**), and a circadian rhythm by population mean cosinor was described on the shin and forearm (**Table II** and **Fig 2**).

For the majority of subjects the maximal values were obtained at all body sites in the afternoon (between 14:00 and 16:00) and the minimum in the evening (around 20:00). Relative peak to trough differences were statistically significant ($p < 0.05$) and were as follows: forehead 5.29/4.93, forearm 5.44/4.87, upper back 5.5/5.14, shin 5.5/4.8.

The acrophase for skin surface pH was similar on all body sites (Bingham Test).

No time-dependent rhythms of stratum corneum hydration were detected The stratum corneum hydration did not show time-dependent variations by ANOVA or by cosinor over the 24-h range.

Peak–trough differences were found in the shin, forearm, and forehead. The maximal values were at ≈16:00, and minimal values around 22:00. Relative peak to trough differences were statistically significant ($p < 0.05$).

Skin temperature displayed time-dependent rhythms on all body sites Time-dependent rhythms were detected on all body sites by ANOVA (**Table I**).

Table I. Significant time-dependent rhythms of TEWL, skin surface pH, and skin temperature in different body areas by one-way ANOVA

Site	Function	F test	p value
Forearm	TEWL	2.43	0.04
Forehead	TEWL	9.28	0.0001
Upper back	TEWL	1.8	0.05
Shin	TEWL	2.2	0.001
Forearm	pH	2.74	0.002
Shin	pH	1.88	0.04
Forearm	temperature	6.75	0.0001
Forehead	temperature	4.14	0.0001
Upper back	temperature	2.53	0.005
Shin	temperature	5.65	0.0001

Circadian rhythms in skin temperature were detected by population mean cosinor on the forearm, forehead, and shins (**Table II** and **Fig 3**).

Peak–trough differences were found at all locations. The maximal values were noted on the forehead and upper back at ≈18:00; the minimal values were noted around 10:00. On the forearm and shin the maximal values were noted around 02:00 and the minimal values at 10:00. Relative peak to trough differences were statistically significant ($p < 0.05$) as follows: forehead 33.3/32, forearm 32.8/30.6, upper back 33.3/32.2, shin 32.7/30.6.

The acrophase values of skin temperature at different body sites were comparable (Bingham Test). No correlation was found between TEWL and skin temperature using linear regression.

DISCUSSION

This study demonstrated a 24 h rhythm in many variables of human skin barrier function. Marked circadian differences were noted in skin temperature, TEWL, and skin surface pH at most anatomic sites.

The water evaporation rate as reflected by TEWL increased significantly in the evening at all sites and was minimal during the morning. The relative differences between peak and trough ranged between 71 and 415%. These changes could not be attributable to skin temperature, because skin temperature during this period did not correlate to TEWL. Spruit (1971) found significant differences in water vapor loss and skin temperature measurements at 08:00 and 16:00. The values at 16:00 were higher. Spruit therefore suggested that the diurnal variation in water vapor loss from the skin is a function of a diurnal variation in skin temperature. Based on this study, the guidelines for TEWL state that diurnal variation in TEWL are probably small and of no practical significance (Pinnagoda *et al*, 1990). Henane *et al* (1977) observed that changes in body temperature and body TEWL in neutral environments showed opposite trends during REM sleep. Both studies were limited to two time points, which does not allow circadian rhythm characterization. The current study, by using more extended sampling and rhytmometric techniques, found significant differences in day and night TEWL.

There is no evidence that the diurnal measurements of TEWL were the result of diurnal variation in sweating. In the resting position, profuse thermal sweating is only elicited at relatively high room temperature over 28–30°C and, because the examiners were not exposed to any physical exercise during the study period and the room temperature was well controlled on 22°C, which is considered ideal for measurements of TEWL (Pinnagoda *et al*, 1990), diurnal sweating could not explain these results.

The substantial differences of peak and trough could not be disregarded. The clinical relevance of these findings is apparent especially in regard to topical drug application and skin irritation. TEWL is thought to be an *in vivo* index of the efficiency and integrity of the stratum corneum (Pinnagoda *et al*, 1990). The higher TEWL in the evening suggests that the epidermal barrier is decreased during this time, and thus topical drug permeability may increase significantly. Reinberg *et al* (1990) demonstrated in a controlled study using rythmometric procedures that an evening application of a facial cosmetic cream seemed more effective than a morning application. The suggested

Table II. Significant diurnal rhythms of 24 h period in TEWL, skin surface pH, and skin temperature by population mean cosinor

Site	Function	Mesor	Amplitude	Acrophase hours	95% CI	p value
Forearm	TEWL	13.99	2.03	18:00	10:30–19:30	0.005
Forehead	TEWL	19.653	9.24	24:00	21:40–0200	0.001
Forearm	pH	5.184	0.191	13:40	08:30–16:20	0.009
Shin	pH	5.242	0.166	11:00	05:30–14:30	0.02
Shin	temp	32.052	0.523	23:30	20:20–01:20	0.002
Forehead	temp	32.77	0.385	21:50	18:40–24:40	0.001
Forearm	temp	31.948	0.721	01:30	23:30–03:20	0.001

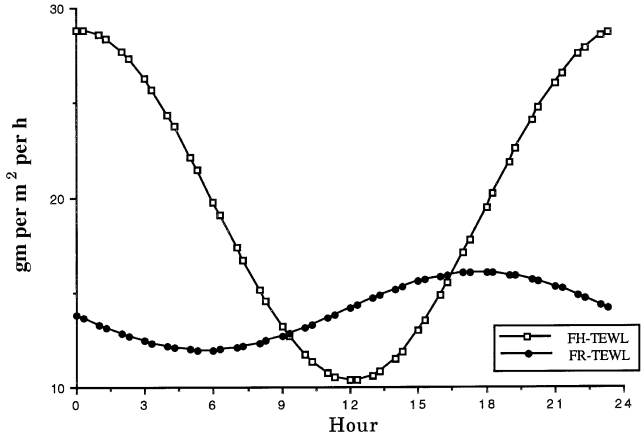

Figure 1. TEWL displays circadian rhythm on both forearm and forehead. The pooled data of forearm (FR) and forehead (FH) were analyzed by best cosine curve fitting, using a 24 h period (see *Materials and Methods* for further details).

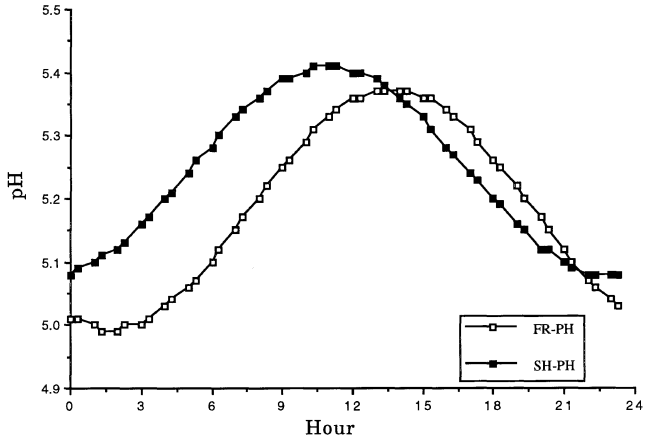

Figure 2. Skin surface pH displays circadian rhythm on forearm and shin. The pooled data of the forearm (FR) and shin (SH) were analyzed by best cosine curve fitting, using a 24 h period.

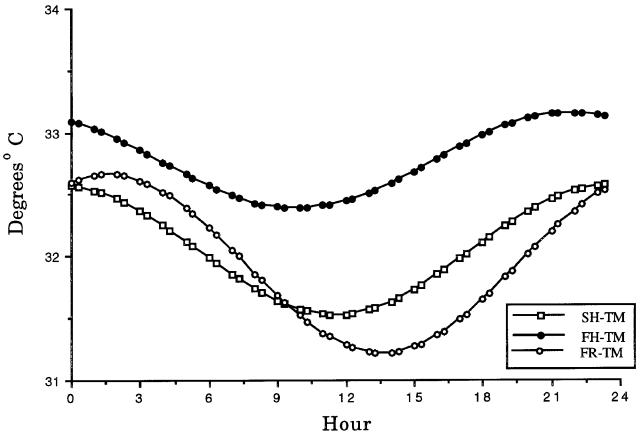

Figure 3. Skin temperature displays circadian rhythm on forearm, forehead, and shin. The pooled data of the forearm (FR), forehead (FH), and shin (SH) were analyzed by best cosine curve fitting, using a 24 h period.

explanation for these differences was the existence of an unknown circadian cellular or metabolic phenomena in the epidermis. Our findings that the TEWL significantly increases during the evening could explain these differences.

The difference in acrophase of skin TEWL between the forearm and forehead could be explained by regional differences. The demonstration of regional circadian rhythmicity has been shown in DNA synthesis in the epidermis at different body sites (Zagon *et al*, 1996).

Skin care agents (e.g., moisturizers) have a local effect on the epidermis. The importance of applying them during the evening, especially on the face and forehead, should be further explored.

The fact that body temperature oscillates on a daily basis has been known for centuries (Refinetti and Menaker, 1992). The clinical relevance of the high skin temperature during the night time could relate to pruritus, where itching usually exacerbates in the late afternoon and evening. Although it has not been previously investigated, it is possible that these exacerbations are related to increased skin temperature.

This study demonstrated a 24-h rhythm of skin surface pH in the forearm and shins. The relative differences between peak and trough were 7–16%. The skin surface pH is an acid mantle of pH 4–6.5. The factors that regulate human skin surface acidity are not clear (Dikstein and Zlotogorski, 1994).

Human stratum corneum has a pH gradient from an acidic pH to a neutral pH (Ohman and Vahlquist, 1994). Therefore, the circadian rhythm in skin surface pH may be a reflection of circadian variations in enzyme function in the human stratum corneum. A high pH is frequently correlated with high TEWL (Thune *et al*, 1988), but in our study no correlation was found between the circadian rhythms in TEWL and those of skin pH. Whereas drug absorption process and skin irritation can be altered by the skin surface pH, no study is available on the effect of changes of cutaneous pH on drug absorption.

Significant time-dependent variables were found by ANOVA in more variables than with the population mean cosinor analysis. These differences may be explained by the presence of rhythms with shorter periods (less than 24 h = ultradian) or nonsinusoidal circadian variations. Our analysis examined only sinusoidal rhythms with about 24 h periods.

Chronopharmacology involves the timing of administration of medications: drugs may have different therapeutic effects or side-effects if given in the morning or in the evening. This field has been evolving recently (Labrecque and Belanger, 1991) leading to consideration of chronopharmacologic variables in cancer, immunologic, and endocrinologic treatments. Our study provides further support that skin barrier functions are predictably time dependent and skin chronopharmacology should be also considered. These data provide significant opportunity for thought: the mechanisms subserving these changes require explanation.

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