Analysis of Circadian and Ultradian Rhythms of Skin Surface Properties of Face and Forearm of Healthy Women

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Biologic rhythms of cells and organisms are well documented and have been extensively studied at the physiologic and molecular levels. For the skin, many circadian changes have been investigated but few systematic studies comparing skin at different body sites have been reported. In this study we investigated facial and forearm skin circadian rhythms in eight healthy Caucasian women. Noninvasive methods were used to assess skin capacitance, sebum excretion, skin temperature, transepidermal water loss, and skin surface pH on fixed sites of the face and the volar forearm during a 48 h span under standardized environmental conditions. Using the cosinor or ANOVA methods, circadian rhythms could be detected for sebum excretion (face), transepidermal water loss (face and forearm), skin temperature (forearm), pH (face), and capacitance (forearm). No circadian rhythmicity was found for the other biophysical parameters. In addition to the 24 h rhythm component, rhythms with periods of 8 h were found for sebum excretion, of 8 and 12 h for transepidermal water loss (face and forearm), and of 12 h for skin temperature (forearm). Our study confirms that rhythms of skin surface parameters are readily measurable and that these rhythms differ between different sites. Furthermore, we demonstrate for the first time that, for transepidermal water loss (face and forearm), sebum excretion, and skin temperature (forearm), in addition to circadian rhythms, ultradian and/or component rhythms can be detected. ***Key words: bioengineering/chronobiology/circadian rhythm/ultradian rhythm. J Invest Dermatol 117:718–724, 2001***

Biologic rhythms are defined as physiologic changes occurring over time with a reproducible waveform (Halberg and Reinberg, 1967). The period of rhythms encountered in biology may range from a fraction of a second, to a few hours, to about 24 h (circadian rhythms) and even longer (Scheving, 1959, Kahn *et al*, 1968; Gelfant *et al*, 1982; Haus and Touitou, 1992). Circadian rhythms have been extensively described at different levels of physiologic organization (Haus and Touitou, 1992). In addition, they have attracted considerable interest in the management of cancer patients (Levi, 2000) and have been associated with autoimmune and lymphoproliferative diseases in mice (Conti and Maestroni, 1998). In recent years molecular bases of circadian rhythms have been identified at the cellular level in prokaryotes and eukaryotes, and the regulation of biologic processes by circadian clocks has become a quickly expanding field of research (Dunlap, 1999).

Circadian rhythms of biophysical skin parameters (Burton *et al*, 1970; Spruit, 1971; Timbal *et al*, 1972; Cotterill *et al*, 1973; Gautherie, 1973; Lee *et al*, 1977; Marotte and Timbal, 1981; Verschoore *et al*, 1993; Reinberg, 1997) as well as the functional response of the skin to histamine (Reinberg *et al*, 1965, 1969, 1990, 1996) have been documented in the past by several investigators. Most of these studies have been performed on body skin, i.e., on the back and the volar forearm. Compared to these two regions, facial skin anatomy is more complex (Lévêque *et al*, 1987) and the face is one of the sites most exposed to environmental factors. Therefore its rhythmicity may differ from the other body areas. Circadian rhythms of facial skin have not been extensively documented as yet and the studies available are either restricted to one or two parameters (Burton *et al*, 1970; Timbal *et al*, 1972; Cotterill *et al*, 1973; Reinberg *et al*, 1990; Verschoore *et al*, 1993) or model the rhythmicity after unconventional sampling schedules (Yosipovitch *et al*, 1998).

Therefore in this study we attempted to investigate simultaneously around-the-clock changes in a set of five biophysical skin parameters on the face and the forearm over a time span of 48 h.

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Abbreviation: TEWL, transepidermal water loss.

MATERIALS AND METHODS

Subject and environmental control Eight healthy Caucasian women naturally menstruating, aged from 21 to 32 y (mean ± SD, 24 ± 3), were included in the study after having signed an informed consent. They had no history of ongoing or previous skin diseases. They were neither pregnant nor breast feeding, with no oral contraceptive for at least 3 mo and no medication for 15 d prior to and during the study. The influence of the menstrual cycle on skin circadian rhythms has been documented in the past (Reinberg and Smolensky, 1983). Therefore the subjects were all chosen to be in the luteal phase of their menstrual cycle (28 ± 2 d cycles) during the study. All study subjects were nonsmokers. Alcohol, hot beverages, and spicy food were not permitted during the study.

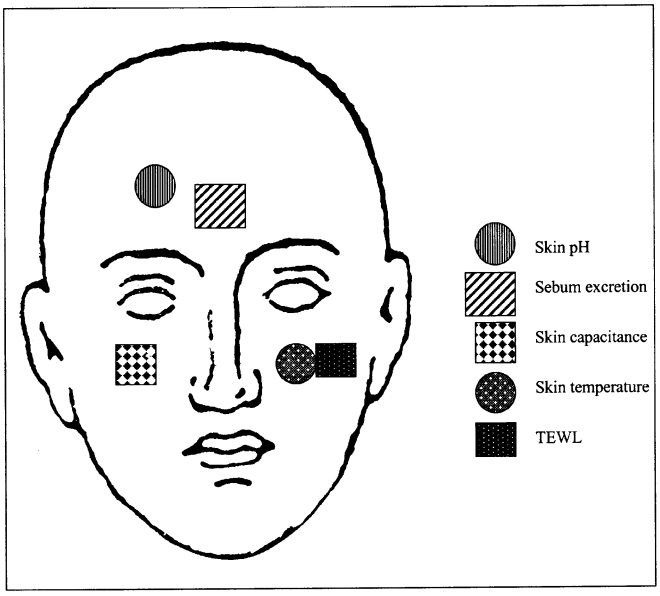

Figure 1. Sites of measurements of biophysical parameters on the face.

Subjects maintained a social and ecologic synchronization with diurnal activity with light on at 08.00 (± 1 h) and light off at midnight (± 1 h) during the 48 h study. This schedule was close to their spontaneous individual behavior. Study subjects were wearing day and night the same light cotton clothes with short sleeves leaving forearms free and were allowed to move freely in the study room. The light source consisted of fluorescent tubes Luxline-ES F 36 W/183, 120 cm (Sylvania, Erlangen, Germany), delivering only visible light (400–700 nm). Two tubes were located at the head of the bed within a distance of approximately 1.20 m from the face and 1.50 m from the volar forearm of the subject. The intensity of light reaching the skin site was about 250 lux for the face and 200 lux for the forearm.

Standardized meals were served at fixed hours. Volunteers were hosted in rooms under controlled and recorded environmental conditions (temperature 20.0°C ± 0.5°C and relative humidity 53.2% ± 4.7%). Non-strenuous activities such as reading, writing, and watching TV were allowed.

For standardization purposes, study subjects followed strict skin care instructions for the body and the face 1 wk prior to and during the study. In particular, they did not apply any cosmetics or make-up at least during the 12 h prior to and during the study. Moreover, they did not apply water on the investigated skin area during the study.

Variables and time series acquisition Measurements were performed at 4 h intervals on fixed predetermined sites of the face (**Fig 1**) and the volar forearm of subjects in recumbent position, their forearms in a horizontal position.

Skin biophysical parameters Skin capacitance was measured using a Corneometer CM820 (Courage & Khazaka Electronic, Köln, Germany) and expressed in arbitrary units as the mean of three recordings on adjacent sites. Measurements were performed according to the EEMCO guidance for assessment of stratum corneum hydration (Berardesca, 1997).

Sebum excretion was evaluated using Sebutape (Cuderm, Dallas, TX) applied on the skin surface for 1 h after cleaning the skin surface with 70° alcohol. After removing, the Sebutape was stored at −10°C until analysis with an automatic image analysis system (Quantiseb, Monaderm, Monaco). The results were expressed as percentage of Sebutape surface covered with sebum droplets, reflecting the quantity of excreted sebum in the 60 min span.

Face skin temperature was recorded with a cutaneous thermometer (Differential Thermometer PT 200 from IMPO Electronics, Denmark). It was expressed in degrees Celsius as the mean of five consecutive measurements.

Transepidermal water loss (TEWL), as a measurement of stratum corneum barrier function, was assessed using a Tewameter TM210C (Courage & Khazaka Electronic). At each test time a single measurement per area was performed according to the European Society of Contact Dermatitis recommendations and expressed in g per m^2 h (Pinnagoda *et al*, 1990).

Skin surface pH was measured with a PHmeter PH900 (Courage & Khazaka Electronic). It was expressed as the mean of two measurements performed on two adjacent skin areas.

Other physiologic variables Free salivary cortisol (μg per dl) as a marker rhythm to check subjects' synchronization (Touitou and Haus, 1992) was determined by enzyme-linked immunosorbent assay (BIO-Advance, Emerainville, France).

Statistics To minimize interindividual differences, changes as a function of time were expressed as a percentage of the 24 h individual mean for each study subject and skin parameter. The 48 h span was selected to visualize circadian and other rhythms and to check their stability from day 1 (first 24 h span) to day 2 (second 24 h span). Day-to-day variations for each variable were tested by ANOVA. When no variation between the 2 d was found, data were pooled on a 24 h basis. Then, changes as a function of time were expressed as a percentage of the 24 h mean and displayed as a plexogram.

Two complementary statistical methods were used to test 24 h (circadian), 12 h, and 8 h (ultradian) periodicities. Both cosinor and three-way ANOVA were used consecutively for the analyses. Three-way ANOVA was used to test time-dependent variations as a group phenomenon. The cosinor (Nelson *et al*, 1979) was used to obtain and quantify the best-fitting cosine function approximating all data for trial periods (τ) of 24 h, 12 h, and 8 h. The least squares method was used to quantify parameters characterizing each rhythmic function. A rhythm was detected when the amplitude (half peak to trough difference) of the cosine function differed from zero with $p < 0.05$. In these cases, the cosinor provided the acrophase (peak time location of the cosine curve) and its amplitude (half of the peak to trough difference) with their respective 95% confidence limits as well as the 24 h adjusted mean (*M*).

When confidence limits of the acrophase were larger than ±2 h the results of the cosinor method were not considered (De Prins and Waldura, 1993) because the experimental curve is not suited to the model of a cosine function (Reinberg *et al*, 1998).

Table I. Detection of time-dependent changes of skin surface biophysical parameters in eight study subjects detected by ANOVA (with $p < 0.05$)

Variables	Anatomical site	p value
Sebum excretion	Face	0.03
TEWL	Face	0.00005
	Forearm	0.00001
Temperature	Forearm	0.00001
PH	Face	0.03
Capacitance	Face	0.04
	Forearm	0.004

RESULTS

Day-to-day variability of biophysical parameters No significant day-to-day variability was found for the biophysical parameters investigated, except for skin capacitance on the face (data not shown). This allowed us to pool the data of all study subjects for the 48 h period on a 24 h basis (plexogram) except for the latter parameter.

Detection of time-dependent changes by three-way ANOVA Time-dependent changes were detected by ANOVA (**Table I**) for sebum excretion ($p < 0.03$), TEWL on the face ($p < 0.00005$) and the forearm ($p < 0.00001$), temperature on the forearm ($p < 0.00001$), pH on the face ($p < 0.03$), capacitance on the face ($p < 0.04$) and the forearm ($p < 0.004$), and free salivary cortisol ($p < 0.00001$).

Detection of circadian and ultradian rhythms by cosinor To investigate the nature of the time-dependent changes we used the cosinor method. As reported previously (Haus *et al*, 1988), a circadian rhythm for free salivary cortisol was

Table II. Detection of circadian rhythms for skin biophysical parameters by the cosinor method

Skin variables	Anatomical site	Trial period	p value[a]	Amplitude[b] (95% CL)	Acrophase[c] (95% CL)
Sebum excretion	Face	24 h	0.001	30.0 (± 24.4)	13.18 (± 3.30)
TEWL	Face	24 h	0.0005	9.5 (± 5.9)	11.38 (± 2.30)
	Forearm		0.03	5.6 (± 5.3)	6.00 (± 4.30)
Temperature	Forearm	24 h	0.0008	1.0 (± 0.8)	0.48 (± 3.50)

[a]p value of the circadian rhythm detection.
[b]Amplitude = half peak to trough difference. CL, confidence limits.
[c]Acrophase = peak time location expressed in hours and minutes number of study subjects = 8.

Table III. Detection of ultradian rhythms for skin biophysical parameters by the cosinor method

Skin variables	Anatomical site	Trial period	p value[a]	Amplitude[b] (95% CL)	Acrophase[c] (95% CL) (first peak location)
Sebum excretion	Face	8 h	0.01	4.5 (± 1.4)	5.50 (± 0.20)
Temperature	Forearm	12 h	0.00006	1.2 (± 0.8)	5.10 (± 1.40)
TEWL	Face	12 h	0.05	6.0 (± 5.0)	5.20 (± 1.70)
		8 h	0.0005	4.5 (± 3.4)	6.00 (± 0.40)
	Forearm	12 h	0.0001	8.9 (± 4.7)	5.50 (± 1.10)
		8 h	0.0006	4.1 (± 2.8)	6.00 (± 0.20)
Capacitance	Forearm	8 h	0.0001	10.9 (± 3.8)	2.10 (± 3.30)

[a]p value of the circadian rhythm detection.
[b]Amplitude = half peak to trough difference. CL, confidence limits.
[c]Acrophase = first peak time location expressed in hours and minutes. With 12 h periods two peaks occurred in the 24 h scale at +12 h (or –12 h) from the first acrophase given in the table. With 8 h periods three peaks occurred in the 24 h scale, namely at +8 h and +16 h (or –8 h and 6 h) from the first acrophase location given in the table. Number of study subjects = 8

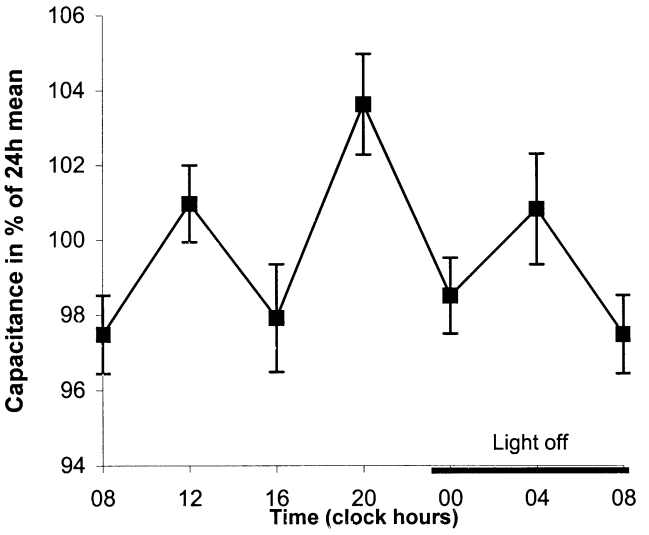

Figure 2. Circadian variations of skin capacitance on the forearm. Measurements were performed on the volar forearm of the eight study subjects (see **Fig 1**) at 4 h intervals during 48 h. As no variations of skin capacitance measurements were found between the 2 d, data were pooled on a 24 h basis. For each subject, time point values were expressed as percentages of 24 h individual mean. Then, the mean values of these variations for the study sample (*black squares*, mean ± SEM) were displayed to express time-dependent changes of skin capacitance (plexogram). A time dependence was found with three peaks, the main one at 20:00 and smaller ones at 12:00 and 04:00. No circadian rhythm was detected by the cosinor method. The light off period is indicated as a bold line on the time axis.

detected with a peak time around 08:50 (±1 h 30 min), a nocturnal trough, and an 80% amplitude change with reference to the 24 h mean ($p < 0.00001$) (figure not shown). This confirmed that the study subjects were synchronized.

For the skin, circadian rhythms (**Table II**) were detected for sebum excretion ($p < 0.001$), TEWL on face and forearm ($p < 0.0005$ and $p < 0.03$), and skin temperature on the forearm ($p < 0.0008$). Rhythms with periods of 12 h (**Table III**) were detected for TEWL on the face ($p < 0.05$) and the forearm ($p < 0.0001$) and the skin temperature on the forearm ($p < 0.00006$).

Additional rhythms with periods of 8 h were also detectable for sebum excretion on the face ($p < 0.01$), capacitance on the forearm ($p < 0.0001$), and TEWL on the two zones (face $p < 0.0005$ and forearm $p < 0.0006$) (**Table III**).

The plexograms are shown in **Figs 2–7**. When a circadian rhythm was detected by cosinor analysis (**Figs 3**, **4**, **5**, **7**) the cosine functions with $\tau = 24$ h were superimposed on the respective plexograms. The ultradian rhythms detected are not shown.

The 24 h mean values of skin capacitance for the study subjects ranged from 53 to 80 arbitrary units on the forearm (not shown). The plexogram showed three peaks (**Fig 2**) at 12:00, 20:00, and 04:00. No circadian rhythm was detected by cosinor but an 8 h ultradian rhythmicity was detected (**Table III**) with a peak time around 02:00 (±3 h 30 min).

As a measure for the sebum excretion, the 24 h mean value for the Sebutape surface covered with sebum droplets ranged from 1.0% to 18.7% for the study subjects (not shown). The plexogram (**Fig 3**, *black squares*) showed a peak at 12:00 and a trough at 0:00. By cosinor, a circadian rhythm with a peak time around 13:20 (± 3 h 30 min) was found ($p < 0.001$) (**Table II**, **Fig 3**, *dotted line*). The amplitude of the cosine function was about 30% (± 24.4) with regard to the 24 h mean. The large confidence interval (**Table II**) of the acrophase indicates that the curve pattern is far from being a cosine function. On the plexogram an additional small peak occurred at 4:00 leading to a biphasic curve pattern (**Fig 3**, *black squares*). This biphasic pattern is due to the presence of a rhythm component with $\tau = 8$ h (**Table III**) in addition to the prominent circadian rhythm.

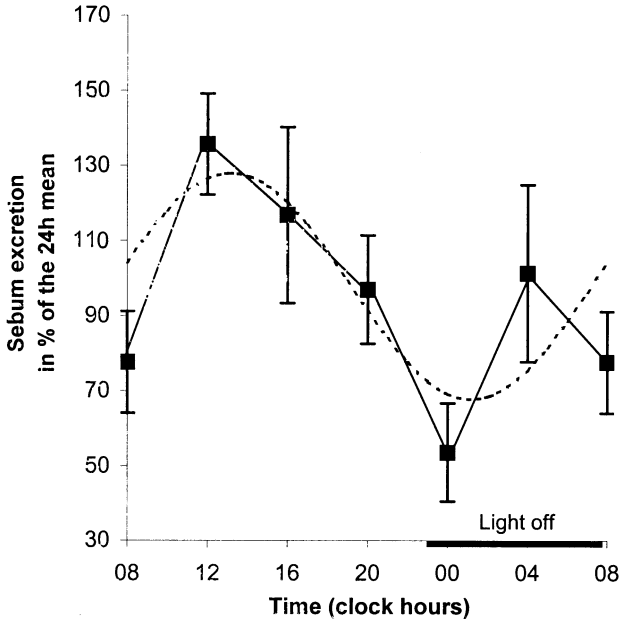

Figure 3. Circadian variations in sebum excretion on the forehead. Sebum excretion was determined on the forehead (see **Fig 1**) of the eight study subjects at 4 h intervals during 48 h. As no variations of sebum excretion measurements were found between the 2 d, data were pooled on a 24 h basis. For each subject, time point values were expressed as percentages of 24 h individual mean. Then, the mean values of these variations for the study sample (*black squares*, mean ± SEM) were displayed to express time-dependent changes of skin sebum excretion (plexogram). Time dependence was detected with a peak at 12:00 and a trough at 0:00. Analysis by the cosinor method detected a circadian rhythm (see also **Table II**) and provided the best-fitting curve that models the circadian variations for the 24 h period. This curve (*dotted line*) is superimposed on the corresponding plexogram. The light off period is indicated as a bold line on the time axis.

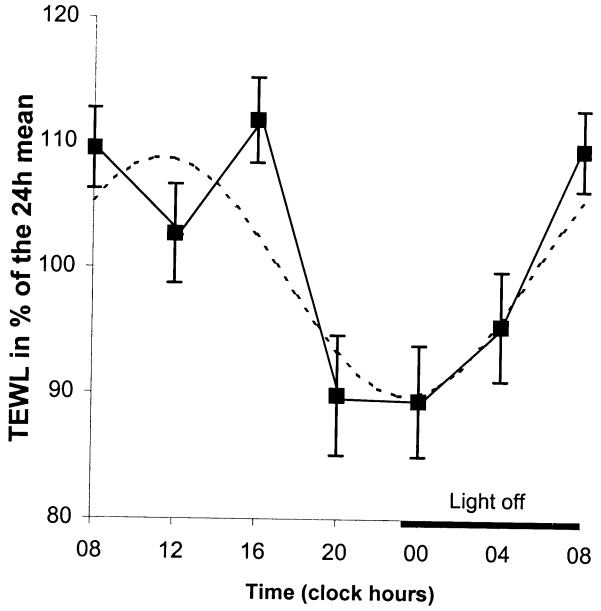

Figure 4. Circadian variations of TEWL on the cheeks. TEWL was measured on the left cheek (see **Fig 1**) of the eight study subjects at 4 h intervals during 48 h. As no variations of TEWL measurements were found between the 2 d, data were pooled on a 24 h basis. For each subject, time point values were expressed as percentages of 24 h individual mean. Then, the mean values of these variations for the study sample (*black squares*, mean ± SEM) were displayed to express time-dependent changes of TEWL on the cheek (plexogram). Time dependence was detected with two peaks at 8:00 and 16:00 and a trough between 20:00 and 0:00**.** Analysis by the cosinor method detected a circadian rhythm (see also **Table II**) and provided the best-fitting curve that models the circadian variations for the 24 h period. This curve (*dotted line*) is superimposed on the corresponding plexogram. The light off period is indicated as a bold line on the time axis.

The 24 h mean TEWL of the study subjects ranged from 9.9 to 19.2 g per m^2 h on the face and from 5.9 to 10.4 g per m^2 h on the forearm (not shown). For the face the plexogram of the TEWL showed peaks at 8:00 and 16:00 and a trough from 20:00 to 0:00 (**Fig 4**, *black squares*). Cosinor analysis detected a peak time at about 11:40 (±2 h 30 min), which is located between the two peaks of the plexogram. The amplitude was about 9.5% (± 5.9) with regard to the 24 h mean. Again large confidence intervals for acrophase detection were found (**Table II**). This is probably due to the fact that the 24 h curve is far from being a cosine function as it shows a two peak curve pattern.

The forearm skin showed a TEWL plexogram pattern slightly different from that of the face (**Fig 5**, *black squares*) with two peaks located at 08:00 and 16:00. This difference of pattern may be due to the coexistence of additional periodicities. Cosinor with a trial period of 24 h provided a peak time at 06:00 (± 4 h 30 min) and the amplitude was 5.6% (± 5.3). With the large confidence interval, however, this peak probably represents a mathematical artefact.

In addition to the 24 h rhythmicity, ultradian periodicities with periods of 12 h and 8 h were detected by cosinor for TEWL of both face and forearm (**Table III**). The detection (with $p < 0.05$) of periods less than 24 h corresponds to the detection of an ultradian rhythm if the amplitude is larger than the circadian amplitude. If the amplitude of the ultradian period is smaller than the circadian one, it means that a component period is present but cannot be considered as a rhythm. As shown in **Table III** the time of occurrence of peaks of the ultradian 12 h are almost similar in facial skin (5:20 and 17:20) and forearm skin (5:50 and 17:50); the same tendency was found with a trial period of 8 h (6:00, 14:00, and 22:00 for the two zones). On the forearm, the amplitude of the 12 h rhythm (8.9%) is larger than that of the 24 h rhythm (5.6%). The opposite is true for facial skin where the circadian amplitude (9.5%) is larger than the ultradian one (6%). This means that TEWL exhibits a prominent circadian rhythm on the face and a prominent 12 h rhythm on the forearm. Differences in the ultradian components between face and forearm skin may explain observed differences in the curves depicted in **Figs 4** and **5**.

The 24 h mean values of skin surface pH for the study subjects ranged from 4.9 to 6.3 on the face and from 5.4 to 6.6 on the forearm (not shown). Skin surface pH was found to be time dependent (ANOVA with $p < 0.03$) only on the face, with a nocturnal trough located around 04:00 (**Fig 6**). It showed a plateau during daytime. The nocturnal trough to diurnal plateau difference was around 5% of *M*. Cosinor did not detect any circadian or ultradian rhythm for this parameter.

The 24 h mean values of skin temperature ranged from 29.9°C to 33.0°C on the face and from 29.7°C to 31.1°C on the forearm (not shown). Skin temperature showed time-dependent changes only on the forearm (ANOVA with $p < 0.00001$) with a trough at midday and two peaks (**Fig 7**, *black squares*), a nocturnal one around 04:00 and a diurnal one around 16:00. A circadian rhythm with $\tau = 24$ h was detected by cosinor only at the forearm level with a peak time around 00:45 (± 3 h 50 min) and a peak to trough difference of 2% (± 0.8) with reference to the 24 h mean. Again the large confidence interval (**Table II**) indicates that the curve is far from being a cosine function. The presence of an ultradian periodicity with a trial period of 12 h ($p < 0.00006$) may contribute to the experimental curve pattern (**Table III**).

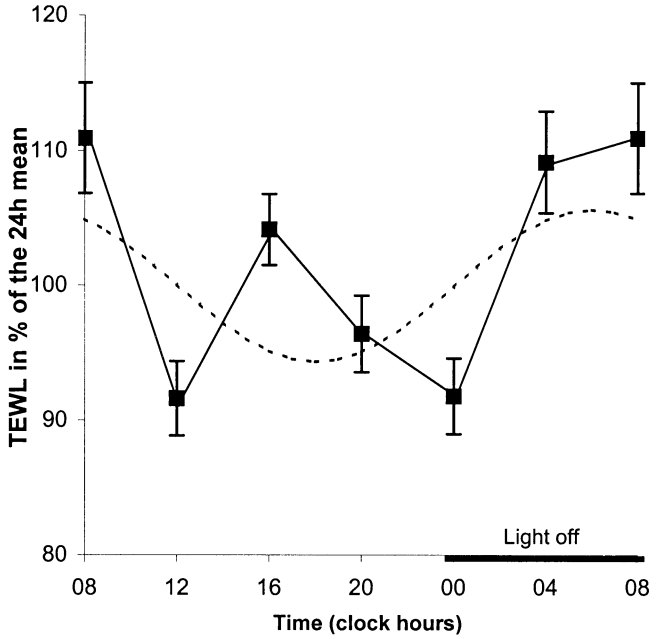

Figure 5. Circadian variations of TEWL on the forearm. TEWL was measured on the volar forearm (see **Fig 1**) of the eight study subjects at 4 h intervals during 48 h. As no variations of TEWL measurements were found between the 2 d, data were pooled on a 24 h basis. For each subject, time point values were expressed as percentages of 24 h individual mean. Then, the mean values of these variations for the study sample (*black squares*, mean ± SEM) were displayed to express time-dependent changes of TEWL on the forearm (plexogram). Time dependence was detected with two peaks at 8:00 and 16:00 and two troughs at 12:00 and 0:00. Analysis by the cosinor method detected a circadian rhythm (see also **Table II**) and provided the best-fitting curve that models the circadian variations for the 24 h period. This curve (*dotted line*) is superimposed on the corresponding plexogram. The light off period is indicated as a bold line on the time axis.

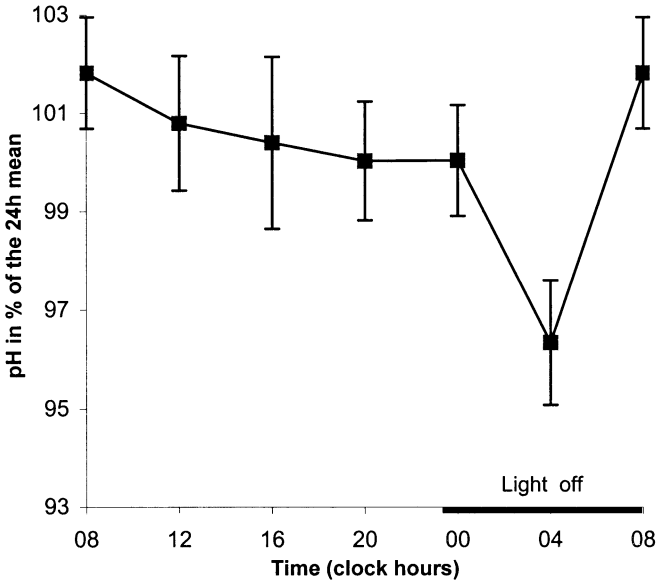

Figure 6. Circadian variations of pH on the forehead. The pH was measured on the forehead (see **Fig 1**) of the eight study subjects at 4 h intervals during 48 h. As no variations of pH measurements were found between the 2 d, data were pooled on a 24 h basis. For each subject, time point values were expressed as percentages of 24 h individual mean. Then, the mean values of these variations for the study sample (*black squares*, mean ± SEM) were displayed to express time-dependent changes of pH (plexogram). A nocturnal trough located around 04:00 was clearly detectable. No circadian rhythm was detected by the cosinor method. The light off period is indicated as a bold line on the time axis.

DISCUSSION

Circadian changes of biologic functions have recently attracted increasing attention due to their potential importance for drug delivery in chronopharmacology as well as chronotherapy. In this study we investigated the circadian variations of several biophysical skin variables by noninvasive methods at the skin surface.

Sebum excretion is one of the best-studied parameters of facial skin. Several authors (Burton *et al*, 1970; Verschoore *et al*, 1993) have demonstrated circadian rhythmicity of sebum excretion with a peak around midday. Our data are in line with these reports showing a prominent peak at noon. In addition, we detected an ultradian periodicity of 8 h, which has not been reported previously. Increase in skin temperature is known to increase sebum excretion in the range of 10% per 1°C (Cunliffe *et al*, 1970). Intraindividual variations of skin temperature on the face were minimal during our study, however, and neither circadian nor ultradian rhythms were found for this variable. In agreement with previous reports (Burton *et al*, 1970; Cotterill *et al*, 1973; Verschoore *et al*, 1993) this finding suggests that circadian variations of sebum excretion are not linked to variations of skin temperature. Other possible causes for the rhythmicity of sebum excretion are currently not known. Attempts to match the sebum excretion changes with free testosterone, dehydroepiandrosterone sulfate, delta-4-androstenedione, cortisol, or melatonin blood levels have been unsuccessful (Rony and Zakon, 1944; Pochi and Strauss, 1974; Verschoore *et al*, 1993). Other mechanisms, such as the regulation of androgen receptors, have been proposed as possible candidates for the regulation of sebum production (Verschoore *et al*, 1993). No evidence for this assumption has been provided as yet, however.

Circadian oscillations of body temperature have been known for a long time and a circadian rhythm with a late afternoon peak and a nocturnal trough has been reported (Aschoff, 1970; Refinetti and Menaker, 1992). Our data on skin temperature also showed time dependence on the forearm with a trough around 12:00 and two peaks at 16:00 and 04:00. Cosinor analysis results were compatible with those recently reported by others (Yosipovitch *et al*, 1998). Attempts to compare our data in detail with those of earlier studies on skin temperature were frustrating, however, because results as well as the experimental conditions differed considerably (Spruit, 1971; Mansfield *et al*, 1973; Marotte and Timbal, 1981). This large variability for such a common biophysical parameter as skin temperature underlines the desperate need for standardized protocols for chronobiologic studies on the skin.

Surprisingly and in contrast to what we observed on the forearm, we did not find temperature changes on the cheeks. The different behavior of the two body sites under the same environmental conditions is most probably due to physiologic differences, in particular to vascularization and vascular reactivity.

Capacitance is one of the most widely used techniques to assess the hydration state of the skin surface. Circadian variations of skin capacitance have been suggested previously by Gabard and Treffel (1994), whereas Yosipovitch *et al* (1998) in a recent study failed to detect time dependence of capacitance on the face and the forearm. In our study a day-to-day difference was found for the results on the face. This difference resulted from the data obtained on one subject (not shown) and prevented a conclusion from being drawn. In contrast to the face, we found time dependence of capacitance on the forearm and detected ultradian rhythmicity, which has not been reported before.

TEWL is a well-accepted *in vivo* indicator of skin barrier function (Pinnagoda *et al*, 1990). In previous studies different circadian rhythms have been reported for this parameter: Spruit (1971) found that TEWL on the forearm skin was higher in the afternoon than in the morning and Yosipovitch *et al* (1998) found a

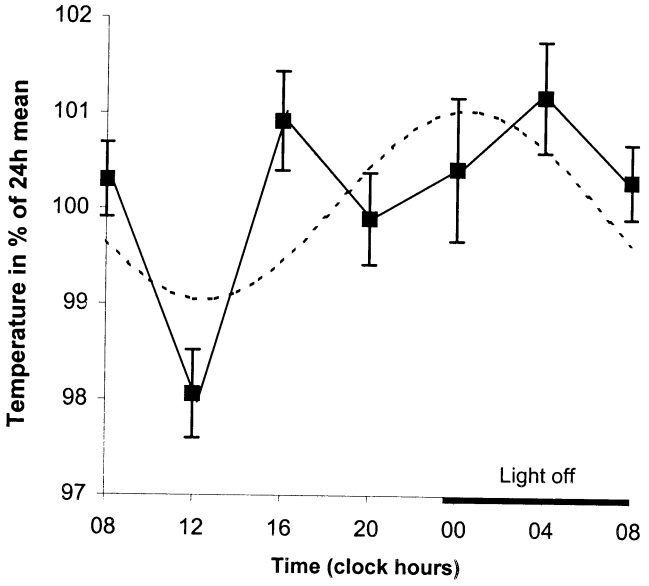

Figure 7. Circadian variations of temperature on the volar forearm. Measurements were performed on eight subjects at 4 h intervals on the volar forearm (see **Fig 1**) during a sampling span of 48 h. As no variations of temperature on the volar forearm between the 2 d were found, data were pooled on a 24 h basis. For each subject, time point values were expressed as percentages of 24 h individual mean. Then, the mean values of these variations for the study sample (*black squares*, mean ± SEM) were displayed to express time-dependent changes of skin temperature (plexogram). Time dependence was detected with a trough at 12:00 and two peaks**,** a nocturnal one around 04:00 and a diurnal one around 16:00. Analysis by the cosinor method detected a circadian rhythm (see also **Table II**) and provided the best-fitting curve that models the circadian variations for the 24 h period. This curve (*dotted line*) is superimposed on the corresponding plexogram. The light off period is indicated as a bold line on the time axis.

circadian rhythm of TEWL with a peak in the late afternoon. In contrast, Touitou *et al* (1994) and Reinberg *et al* (1996) reported a circadian rhythm of TEWL on the forearm skin with a trough at 14:00 and a nocturnal peak. Again differences in the study protocols, i.e., 4 h sampling over a 48 h span *versus* 2 h sampling over two time periods of 12 h, as well as differences of the environmental temperature and relative humidity during the studies prohibit a direct comparison of these data. With our protocol we found a bimodal rhythm for TEWL both on the cheeks and the forearm, with two peaks located at 08:00 and 16:00. In addition to circadian rhythms, ultradian rhythms with $\tau = 12$ h and $\tau = 8$ h were also detected. Whereas the amplitudes of the 8 h rhythm were comparable between the two study sites, the 12 h rhythm amplitudes were higher on the forearm than on the cheeks. The fact that the amplitude of the 12 h rhythm on the cheeks was even higher than the 24 h rhythm strongly supports the notion that the ultradian component indeed contributes to the circadian changes of TEWL.

A difference of TEWL circadian behavior between face and forearm has been reported also by Yosipovitch *et al* (1998). Unfortunately these authors provided only the results of cosinor analysis without showing raw data or a chronogram, which makes a direct comparison impossible. Cosinor analysis of the circadian changes of TEWL in both studies showed the acrophases at different time points, i.e., 11:30 and 6:00 *vs* 24:00 and 18:00 for the face and the forearm, respectively. The large confidence intervals for the acrophases, however, indicate that these circadian rhythms are far from being cosine functions and therefore the results obtained by cosinor with a trial period of 24 h might be misleading and should not be used for interpretation of the data (Reinberg *et al*, 1998). In contrast to the 24 h periods, cosinor analysis of our data with trial periods of 8 and 12 h showed smaller confidence intervals for the acrophases supporting the assumption that these components indeed contribute to the pattern of circadian changes observed. As to a possible explanation for differences of TEWL behavior between the forearm and the cheeks, again regional physiologic variations such as vascularization and distribution of eccrine glands are most likely candidates modifying TEWL rhythmicity. The finding that on the cheeks a rhythm of TEWL was detectable in the absence of significant changes in skin temperature (see above) indicates that, for circadian variations of this parameter also, skin temperature is not a main determining factor.

Apart from the contribution of sweat-derived lactic acid and free amino acid of the stratum corneum (Dikstein and Zlotogorski, 1989), factors accounting for skin surface acidity are not well defined (Dikstein and Zlotogorski, 1994; Ohman, 1994). pH measurements on the face but not the forearm showed a distinct 24 h rhythm with a nocturnal dip and a diurnal plateau. The fact that a rhythm in pH was detected only on the face suggests that structural differences of the epidermis at different sites, including sebum secretion and distribution of eccrine glands, which are more numerous at the facial level (Szabo, 1958), might be involved. As our findings were obtained under stable environmental conditions with strict skin care instructions and no physical activity of the study subjects, exogenous influences on sweat excretion can virtually be excluded. Whether or not the pH changes correspond to a circadian change of sweat composition will require further studies. As opposed to our findings, Yosipovitch and coworkers did not observe changes on the forehead but observed a diurnal peak on the forearm by cosinor analysis. We do not have an explanation for these discrepant findings other than that the experimental conditions differed as discussed above.

In conclusion we have confirmed that under controlled environmental conditions rhythmic changes of skin surface parameters can readily be measured. In addition, we have demonstrated for the first time that, besides circadian rhythms, ultradian rhythms or components of 8 and 12 h can be detected also for some of the skin biophysical parameters. The finding that rhythms may differ with respect to the different skin sites investigated suggests that anatomical and/or physiologic variabilities contribute to the circadian changes.

As to practical consequences of our results one has to be aware of the fact that, except for sebum excretion, which showed a peak to trough difference of 60%, the rhythmic changes of the other parameters were rather small, ranging from 2% to 20%. The skin is constantly adapting to changes of environmental conditions such as temperature, humidity, and solar irradiation, which influence several of the skin biophysical parameters measured in our study (Goh, 1995; Rohr and Shrader, 1998). In addition physical and emotional stress also have a bearing on the skin and influence these parameters (Pinnagoda *et al*, 1990). Therefore in ''real life'' very likely changes due to these exogenous and endogenous factors will mask some of the subtle circadian changes. To what extent the physiologic circadian changes explored here contribute to time-dependent functional changes such as the penetration of local anesthetics (Bruguerolle *et al*, 1991) and nicotinates (Reinberg *et al*, 1995) remains to be explored.

REFERENCES

Aschoff J: Circadian rhythm of activity and body temperature. In: Hardy JD, Gagge AP, Stolwijk JAJ, eds. *Physiological and Behavioral Temperature Regulation*. Springfield, IL: Charles C. Thomas, 1970:pp 905–919

Berardesca E: EEMCO guidance for the assessment of stratum corneum hydration. *Skin Res Technol* 3:126–132, 1997

Bruguerolle B, Giaufre E, Prat M: Temporal variations in transcutaneous passage of drugs: the example of lidocaine in children and rats. *Chronobiol Internat* 8:277–282, 1991

Burton JL, Cunliffe WJ, Shuster S: Circadian rhythm in sebum excretion. *Br J Dermatol* 82:497–502, 1970

Conti A, Maestroni GJ: Melatonin rhythms in mice: role in autoimmune and lymphoproliferative diseases. *Ann N Y Acad Sci* 840:395–410, 1998

Cotterill JA, Cunliffe WJ, Williamson B: Variation in skin surface lipid composition and sebum excretion rate with time. *Acta Derm Venereol* 53:271–274, 1973

Cunliffe WJ, Burton JL, Shuster S: The effect of local temperature variations on the sebum excretion rate. *Br J Dermatol* 83:650, 1970

De Prins J, Waldura J: Sightseeing around the single cosinor. *Chronobiol Internat* 10:395–400, 1993

Dikstein S, Zlotogorski A: Skin surface hydrogen ion concentrations pH. In: Lévêque JL, ed. *Cutaneous Investigation in Health and Disease*. New York: Marcel Dekker, 1989:pp 59–78

Dikstein S, Zlotogorski A: Measurement of skin pH. *Acta Derm Venereol Supplement* 185:18–20, 1994

Dunlap JC: Molecular bases for circadian clocks. *Cell* 10:271–290, 1999

Gabard B, Treffel P: Hardware and measuring principle: the NOVA DPM 9003. In: Elsner P, Berardesca E, Maibach HI, eds. *Bioengineering and the Skin: Water and the Stratum Corneum*. CRC Press, 1994:pp 177–197

Gautherie M: Circadian rhythm in the vasomotor oscillations of skin temperature in man. *Internat J Chronobiol* 1:103–139, 1973

Gelfant S, Ozawa A, Chalker DK, Smith JG: Circadian rhythms and differences in epidermal and in dermal cell proliferation in uninvolved and involved psoriatic skin *in vivo*. *J Invest Dermatol* 78:58–62, 1982

Goh CL: Seasonal variations and environmental influence on the skin. In: Serup J, Jemec GBE, eds. *Handbook of Non-Invasive Methods and the Skin*. CRC Press, 1995: pp 27–30

Halberg F, Reinberg A: Rythmes circadiens et rythmes de basses frequencies en physiologie humaine. *J Physiol* (suppl.) 59:117–200, 1967

Haus E, Nicolau GY, Lakatua D, Sackett-Lunden L: Reference values for chronopharmacology. *Ann Rev Chronopharmac* 4:333–424, 1988

Kahn G, Weinstein GD, Frost P: Kinetics of human epidermal cell proliferation: diurnal variation. *J Invest Dermatol* 50:459–462, 1968

Lee RE, Smolensky MH, Leach CS, McGovern JP: Circadian rhythms in the cutaneous reactivity to histamine and selected antigens, including phase relationship to urinary cortisol excretion. *Ann Allergy* 38:231–236, 1977

Lévêque JL, Grove G, De Rigal J, Corcuff P, Kligman AM, Saint Léger D: Biophysical characterization of dry facial skin. *J Soc Cosmet Chem* 82:171–177, 1987

Levi F: Therapeutic implications of circadian rhythms in cancer patients. *Novartis Found Symp* 227:119–136, 2000

Mansfield CM, Carabasi RA, Wells W, Borman K: Circadian rhythm in the skin temperature of normal and cancerous breast. *Internat J Chronobiol* 1:235–243, 1973

Marotte H, Timbal J: Circadian rhythm of temperature in man. Comparative study with two experimental protocols. *Chronobiologia* 8:87–100, 1981

Nelson W, Tong Y, Lee JK, Halberg F: Methods for cosinor rhythmometry. *Chronobiologia* 6:305–323, 1979

Ohman H, Vahlquist A: In vivo studies concerning a pH gradient in human stratum corneum and epidermis. *Acta Derm Venereol* 74:375–379, 1994

Pinnagoda J, Tupker RA, Agner T, Serup J: Guidelines for transepidermal water loss (TEWL) measurement. A report from the Standardization Group of European Society of Contact Dermatitis. *Contact Dermatitis* 22:164–178, 1990

Pochi PE, Strauss JS: Endocrinologic control of the development and activity of human sebaceous glands. *J Invest Dermatol* 62:191–201, 1974

Refinetti R, Menaker M: The circadian rhythm of body temperature. *Physiol Behav* 51:613–637, 1992

Reinberg A: Rythmes circadiens de la peau humaine: valeur adaptative de son organisation temporelle aux variations périodiques de l'environnement. In: Schmitt D, ed. *Biologie de la Peau Humaine*. INSERM Paris, 1997:pp 267–283

Reinberg A, Smolensky MH: *Biological rhythms in Medicine*. New York: Springer Verlag, 1983

Reinberg A, Sidi E, Ghata J: Circadian reactivity rhythms of human skin to histamine or allergen and the adrenal cycle. *J Alleg* 36:273–283, 1965

Reinberg A, Zagula-Mally Z, Ghata J, Halberg F: Circadian reactivity rhythm of human skin to house dust, penicillin and histamine. *J Alleg* 44:292, 1969

Reinberg A, Koulbanis C, Soudant E, Nicolaï A, Mechkouri M: Circadian changes in the size of facial skin corneocytes of healthy women. *Ann Rev Chronopharmacol* 7:331–334, 1990

Reinberg A, Soudant E, Koulbanis C, Bazin R, Nicolaï A, Mechkouri M, Touitou Y: Circadian dosing time dependency in the forearm skin penetration of methyl and hexyl nicotinate. *Life Sci* 57:1507–1513, 1995

Reinberg A, Touitou Y, Soudant E, Bernard D, Bazin R, Mechkouri M: Oral contraceptives alter circadian rhythm parameters of cortisol, melatonin, blood pressure, heart rate, skin blood flow, transepidermal water loss and skin amino acids of healthy young women. *Chronobiol Internat* 13:199–211, 1996

Reinberg A, Le Fur I, Tschachler E: Problems related to circadian rhythms in human skin and their validation. *J Invest Dermatol* 111:708–709, 1998

Rohr M, Shrader K: Climatic influence on cosmetic skin parameters. In: Baran R, Maibach HI, eds. *Textbook of Cosmetic Dermatology*. London: Martin Dunitz, 1998:pp 1–15

Rony HR, Zakon SJ: Effect of androgen on the sebaceous glands of human skin. *Arch Dermatol Syph* 48:601–604, 1944

Scheving LE: Mitotic activity in the human epidermis. *Anat Rec* 135:7–14, 1959

Spruit D: The diurnal variation of water vapor loss from the skin in relation to temperature. *Br J Dermatol* 84:66–70, 1971

Szabo G: The regional frequency and distribution of hair follicles in human skin. In: Montagna W, Ellis RA, eds. *Biology of Hair Growth*. Academic Press, 1958:pp 33–38

Timbal J, Colin J, Boutelier C, Guieu JD: Evolution circadienne des températures cutanées de l'homme au repos à la neutralité thermique. *Biologie, Comptes Rendus* 4–5:512–516, 1972

Touitou Y, Haus E: Principles of clinical chronobiology. In: Touitou Y, Haus E, eds. *Biologic Rhythm in Clinical and Laboratory Medecine*. Berlin: Springer-Verlag, 1992:pp 6–34

Touitou Y, Soudant E, Koulbanis C, Reinberg A, Bazin R, Nicolaï A, Mechkouri M: Circadian rhythms in a set of biochemical and biophysical skin variables (including transepidermal water loss) documented with non-invasive methods in healthy young women. In: Haus E, ed. *6th International Conference on Amelia Island, FL, July 1994, Biological Rhythm and Medications*, Abstract book, XI-5

Verschoore M, Poncet M, Krebs B, Ortonne JP: Circadian variations in the number of actively secreting sebaceous follicles and androgen circadian rhythms. *Chronobiol Internat* 10:349–359, 1993

Yosipovitch G, Xiong GL, Haus E, Sackett-Lunden L, Ashkenazi I, Maibach HI: Time-dependent variations of the skin barrier function in humans: transepidermal water loss, stratum corneum hydration, skin surface pH and skin temperature. *J Invest Dermatol* 110:20–23, 1998