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Daily Variations in Skin Surface Properties Using Mixed Model Methodology

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Key Words
Bioengineering · Daily variation · Mixed effect model

Abstract
In order to explore the variations over the course of a day in certain skin biophysical properties, a study was conducted on 8 female volunteers. An assessment of several skin biophysical properties was carried out on the face and the volar forearm every 4 h over a period of 48 h. The biophysical parameters were assessed on the face for sebum secretion, skin surface pH, skin colour, transepidermal water loss, capacitance and skin surface temperature. The same parameters were measured on the volar forearm (excepted for sebum secretion). A statistical analysis based on mixed effect models was conducted. Four models, with different covariance structures, were successively tested. The analysis allowed us to identify a structure that repeated itself over time in the same way over each 24-hour period for capacitance on the forearm and for sebum secretion, skin surface pH and skin colour (L* and a* parameters) on the face. Mixed effect methodology is a powerful tool to analyse longitudinal data involving correlations among repeated measurements made on the same subject.

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Introduction

The skin is an organ that adapts constantly to its environment and to internal factors within the body itself. The daily variation in skin biophysical properties has previously been studied using different methodologies: cosinor, analysis of variance (ANOVA) and t-test [1–6]. The cosinor method enables the detection of rhythms over different periods like circadian (24 h) and ultradian (12 h, 8 h) rhythms [7]. However, it requires the major assumption that the data obtained can be reasonably well represented by a cosine curve, in this way non-sinusoidality limits the applicability of the method. On the contrary, ANOVA and t-test are not affected by the shape of the rhythm, but in most cases, ANOVA and t-test were used without taking into account the correlation among the measurements made on the same subject. In our study, 8 biophysical skin parameters were measured on the face and 7 on the forearm in 8 subjects every 4 h over 2 periods of 24 h. To explore the variations in biophysical parameters over the course of a day, an analysis in two steps had previously been performed on these data [8]. First, an ANOVA was performed to test an eventual period effect. When no variation between the 2 days was found, data were pooled on a 24-hour basis. Then, changes as a function of time were expressed as a percentage of the mean of the 24 h, and finally, a cosinor analysis and a second ANOVA were used to study the effect of time in biophysi-

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cal parameters. When variations between the 2 days were found, no conclusion was drawn. The purpose here was to investigate the daily variations in skin biophysical properties using the mixed model methodology. This method is generally used to test the effect of time when the data are repeated measurements. It allows us to take into account the individual effects and the correlation among the measurements made on the same subject without the assumption of any sinusoidal shape. Four models, with different covariance structures, were successively tested to study the daily variation of each biophysical parameter.

Materials and Methods

Subjects

Eight healthy Caucasian women naturally menstruating, in the luteal phase of their menstrual cycle (cycles of 28 ± 2 days) during the study, aged between 21 and 32 years (mean ± standard deviation, 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, and had not taken any oral contraceptive for at least 3 months or medication for 15 days prior to and during the study. All the subjects were non-smokers. Alcohol, hot beverages and spicy food were not permitted during the study. The subjects maintained a social and ecologic synchronization with diurnal activities with light on at 8.00 a.m. (± 1 h) and light off at midnight (± 1 h) during the 48-hour study. This schedule was close to their spontaneous individual behaviour. Standardized meals were served at fixed hours. Volunteers were hosted in rooms under controlled and recorded environmental conditions (temperature 20.0 ± 0.5°C and relative humidity 53.2 ± 4.7%). Moreover, the subjects followed strict skin care instructions for the body and the face 1 week prior to and during the study. In particular, they did not apply any cosmetics or make-up during the 12 h prior to and during the study. Furthermore, they did not apply water on the investigated skin areas during the study.

Skin Biophysical Parameters

The biophysical parameters were assessed on the face for skin capacitance expressed in arbitrary units (Corneometer® CM820, Courage & Khazaka Electronic GmbH, Köln, Germany), sebum secretion expressed as a percentage of Sebutape® surface covered by sebum droplets (Sebutape®, Cuderm Corp, Dallas, USA, and image analysis system Quantiseb®, Monaderm, Monaco), skin surface temperature expressed in degrees Celsius (Differential Thermometer PT 200, IMPO Electronics, Denmark), transepidermal water loss expressed in g/m²·h (Tewameter® TM210C, Courage & Khazaka Electronic GmbH, Köln, Germany), skin surface pH (pHmeter® PH900, Courage & Khazaka Electronic GmbH) and skin colour (Chromameter® CR-300, Minolta, France) reported in CIELab (L*a*b*) colour space mode [9], which expresses colour with three chromametric coordinates on three axes: the L* axis (black-white), the a* axis (green-red) and the b* axis (blue-yellow). The same parameters were assessed on the volar forearm except for sebum secretion.

Time Measurements

A first measurement was performed at midday on the first day of the study, then the measurements were divided into two 24-hour periods, six measurements being taken every 4 h: at 4.00 p.m., 8.00 p.m., midnight, 4.00 a.m., 8.00 a.m. and midday.

Statistical Analysis

To study the variations of biophysical parameters over time, an analysis based on mixed effect models was performed [10–13] (MIXED procedure, SAS® software, release 8.2.). This method enables the testing of the effect of time (fixed effect), taking into account the variations both between and within subjects and in particular the correlation between the measurements taken on the same individual at different times (random effects). The covariance structure of such a model is generally unknown. To perform a test of fixed effects, which would be robust, different ways are possible. One way is to apply the robust approach of Liang and Zeger [14] for repeated records based on the sandwich estimator of the least squares estimator of fixed effects. The sandwich estimator is robust even if the covariance structure is wrongly specified. Unfortunately, this approach cannot be applied here because of the small number of subjects in our sample. Another possible way is to perform such tests over a large range of possible covariance structures for longitudinal data [15, 16] and to retain consistent results. This approach was used here. The objective of our study was to identify a structure that repeated itself over time in the same way over each 24-hour period. To this aim, the effects of Time, Period and interaction Period × Time were tested with different covariance structures (4 models) for each skin biophysical property.

Let Y_{it} be the response variable measured on subject i = 1, 2,..., 8 at time t. Here, time t is represented by the combination of Period (j) by Time (k) within period (for the sake of simplicity, we will refer to 'Time within period' as 'Time' in the rest of the document). The model can be written as:

$$Y_{it} = \mu_t + \varepsilon_{it}.$$

The systematic (or fixed) part of the model μ_t can be written as $\mu_t = \mu_{jk} = \mu + \alpha_j + \beta_k + (\alpha\beta)_{jk}$, i.e. as the sum of Period (α_j), Time (β_k) and period × time [$(\alpha\beta)_{jk}$] interaction effects. The null hypothesis tested here corresponds to the absence of interaction: $(\alpha\beta)_{jk} = 0$, i.e. H0: $\mu_{jk} = \mu + \alpha_j + \beta_k$.

ε_{it} is the random part of the model and the four covariance structures of ε_{it} were as follows.

Model 1: 'subject effect + measurement error': $\varepsilon_{it} = s_i + e_{it}$

In this model, s_i is the random effect of subject i (i = 1, 2,..., 8), which is assumed to be normal, independently and identically distributed with mean 0 and variance σ_s^2, in short: $s_i \sim$ iid $N(0, \sigma_s^2)$.

Similarly, the measurement error e_{it} is assumed $\sim$ iid $N(0, \sigma_e^2)$.

The variance covariance structure V of the 12 measurements $Y_i = \{Y_{it}\}$ can be written as $V = \sigma_s^2 J + \sigma_e^2 I$, where J is a (12 × 12) matrix with all entries equal to 1 and I the (12 × 12) identity matrix. This model generates a typical intra-class (or compound symmetry) structure with homogenous variances of measurements over time and a constant correlation

$$r_{tt'} = \frac{\sigma_s^2}{\sigma_s^2 + \sigma_e^2}$$

between any pair of them.

Model 2: 'first-order autoregressive [(AR(1)]': $\varepsilon_{it} = \omega_{it}$

The random part is modelled here as a first-order autoregressive [AR(1)] stochastic process, such that variance $(\omega_{it}) = \sigma^2$ for any t, and covariance $(\omega_{it}, \omega_{it'}) = \sigma^2\rho^{|t-t'|}$ for $t \neq t'$. This model supposes that the covariance between observations depends only on the lag of time between them $|t - t'|$, and decreases exponentially with this lag. In matrix notation, V can be written as $V = \sigma^2H$, where $H = \{r_{tt'}\}$ with $r_{tt'} = \rho^{|t-t'|}$, if $t \neq t'$, and $r_{tt'} = 1$ if $t = t'$.

Model 3: 'AR(1) + measurement error': $\varepsilon_{it} = \omega_{it} + e_{it}$

This is a first-order autoregressive model as previously defined for model 2, plus a 'pseudo-measurement' error resulting in $V = \sigma^2H + \sigma_e^2I$ and correlation

$$r_{tt'} = \frac{\sigma^2\rho^{|t-t'|}}{(\sigma^2 + \sigma_e^2)}.$$

Model 4: 'subject effect + AR(1)': $\varepsilon_{it} = s_i + \omega_{it}$

This model combines a subject effect (s_i) and an AR(1) process, resulting in $V = \sigma_s^2J + \sigma^2H$ and

$$r_{tt'} = \frac{\sigma_s^2 + \sigma^2\rho^{|t-t'|}}{(\sigma_s^2 + \sigma^2)}$$

Notice, as claimed in Foulley et al. [15], that model 3 reduces to model 2 for $\sigma_e^2 = 0$ and to model 1 for $\rho = 1$. Similarly, model 4 reduces to model 2 for $\sigma_s^2 = 0$ and to model 1 for $\rho = 0$.

The dispersion parameters involved in V were estimated via restricted (or residual) maximum likelihood; these estimations were in turn used to calculate the generalized least squares estimators of location parameters (or fixed effects) involved in μ_t. The null hypothesis about the absence of interaction between the effects of Period and Time was tested using a Fisher-Snedecor (F) approach. Comparison among random effect models was based on a version of Schwarz's Bayesian information criterion (BIC) [10] equal to $-2 \times BIC = -2L + k\log(n - p)$, where L is the restricted log likelihood, k is the number of variance components in the model, n is the number of observations and p is the number of explanatory non-redundant variables. The model with the smallest $-2 \times BIC$ value is preferable.

Our aim was to identify a significant Time effect without a significant interaction between Time and Period. As measuring times were regularly spaced out, when the Time effect was found to be significant, compliance with linear, quadratic and cubic trends was tested using the orthogonal polynomial method [17]. When a significant interaction between Time and Period was revealed, the data of each period were analysed separately.

Results

Among the 4 models tested, model 2 [AR(1)] was in most cases the worst according to the $-2 \times BIC$ value for the biophysical parameters on the face (table 1) and on the forearm (table 2). Models 1, 3 and 4 had approximately the same $-2 \times BIC$ value and model 1 was preferred due to a lower number of parameters.

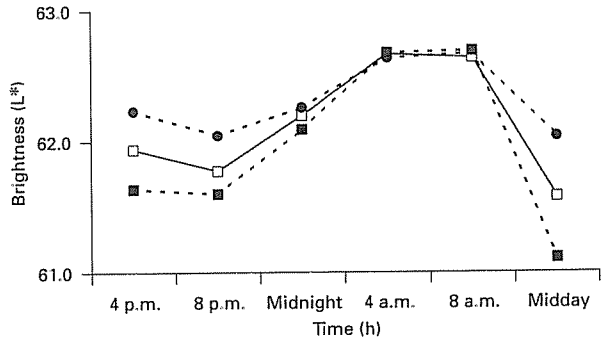

Fig. 1. Observed mean values of brightness (L*) on the face according to the 6 measurement times (period 1: ●, period 2: ■). The orthogonal polynomial method revealed a significant cubic model. The adjusted curve (□) is superimposed on the graph.

Brightness (L) on the Face*

The results of model 1 showed a non-significant interaction and significant Period and Time effects (table 1), measurements taken in the first period being higher on average than those taken in the second period, and the measurements taken at midnight, 4 a.m. and 8 a.m. being higher than those taken at midday (fig. 1). The orthogonal polynomial method revealed a significant cubic model. The adjusted curve was calculated for the 6 times of measurement. A graph was then drawn superimposing the values observed and those predicted by the model (fig. 1).

Red Intensity (a) on the Face*

The model 1 revealed a significant Time effect (table 1), measurements taken at 8 a.m. being lower than those taken at midday and 8 p.m. (fig. 2). The orthogonal polynomial method revealed a significant cubic model. A graph superimposing the average values observed and those predicted by the model was produced (fig. 2).

Sebum Secretion on the Face

The results of model 1 showed a significant Time effect (table 1), measurements taken at midnight, 4 a.m. and 8 a.m. being significantly lower than those taken at 4 p.m. (fig. 3). The orthogonal polynomial method revealed a significant quadratic model. The adjusted curve was calculated. A graph superimposing the average values observed and those predicted by the model was then drawn (fig. 3).

Skin Surface pH on the Face

The results of model 1 revealed a significant Time effect (table 1), measurements taken at 4 a.m. being signif-

Table 1. Results of the four models for each skin biophysical parameters on the face

Biophysical parameters on the face	Models	F value[a]			−2L[b]	−2 BIC[c]	Time trend[d]
		period	time	period × time			
Brightness (L*)	1	4.56*	5.21***	0.92	256.8	261.0	cubic
	2	0.27	3.74**	0.61	293.0	297.2	cubic
	3	4.56	5.21**	0.92	256.8	261.0	cubic
	4	4.65*	5.23***	0.92	256.8	263.0	cubic
Red intensity (a*)	1	0.76	2.84*	2.09	225.8	230.0	cubic
	2	0.08	2.67*	2.27	239.2	243.3	cubic
	3	0.44	3.09*	2.35	223.5	229.7	cubic
	4	0.54	2.77*	2.21	223.6	229.8	cubic
Yellow intensity (b*)	1	11.98***	1.97	1.23	157.7	161.9	–
	2	0.57	1.75	1.75	191.1	195.3	–
	3	11.98*	1.97	1.23	157.7	161.9	–
	4	11.38**	1.99	1.27	157.7	163.9	–
Sebum secretion	1	0.24	6.07***	0.99	519.6	523.8	quadratic
	2	0.03	5.09**	1.51	534.8	539.0	quadratic
	3	0.16	6.45***	1.09	518.5	524.8	quadratic
	4	0.18	5.96***	1.25	517.3	523.5	quadratic
Skin surface pH	1	1.77	2.85*	1.75	82.8	87.0	quadratic
	2	0.24	3.72**	1.83	80.4	84.6	–
	3	0.67	3.99**	2.1	67.1	73.3	quadratic
	4	0.84	3.53**	1.96	72.7	78.9	quadratic
Skin capacitance	1	27.20***	2.25	0.95	584.2	588.3	–
	2	4.15	1.55	0.71	599.6	603.8	–
	3	15.67**	2.33	1.05	580.9	587.1	–
	4	23.86***	2.14	0.96	583.9	590.1	–
Transepidermal water loss	1	0.97	4.77***	2.43*	429.9	434.1	–
	2	0.17	4.69**	1.84	445.8	450.0	–
	3	0.51	5.71***	2.82*	424.6	430.8	–
	4	0.82	4.92***	2.38*	429.3	435.5	–
Skin surface temperature	1	0.05	1.66	3.66**	254.1	258.3	–
	2	0.01	1.81	2.54*	267.6	271.8	–
	3	0.05	1.68	3.65**	254.1	260.3	–
	4	0.05	1.74	3.52**	253.9	260.1	–

p-values of the F test: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

[a] F-statistic for mixed model.

[b] −2 × restricted log likelihood.

[c] −2 × Schwarz's Bayesian information criterion = $-2L + k\log(n - p)$ where L is the restricted log likelihood, k is the number of variance components in the model, n is the number of observations and p is the number of explanatory non-redundant variables.

[d] Time trend found with the orthogonal polynomial method.

icantly lower than those taken at 8 a.m. and midday (fig. 4). The orthogonal polynomial method revealed a significant quadratic model. A graph superimposing the average values observed and those predicted by the model was then drawn (fig. 4).

Other Biophysical Parameters on the Face

Significant Period × Time interactions were identified for transepidermal water loss and skin surface temperature (table 1), revealing different variations according to the period concerned. Thus, the data of each period were

Table 2. Results of the 4 models for each skin biophysical parameters on the forearm

Biophysical parameters on the forearm	Models	F value[a]			−2L[b]	−2 BIC[c]	Time trend[d]
		period	time	period × time			
Brightness (L*)	1	3.62	0.84	0.78	234.9	239.1	–
	2	0.20	0.54	1.20	259.1	263.2	–
	3	2.00	0.86	0.90	232.4	238.6	–
	4	2.89	0.84	0.89	233.9	240.1	–
Red intensity (a*)	1	0.79	1.72	1.97	209.0	213.1	–
	2	0.06	1.08	1.54	235.2	239.4	–
	3	0.62	1.77	2.00	208.7	215.0	–
	4	0.75	1.7	1.95	208.9	215.1	–
Yellow intensity (b*)	1	2.23	1.18	0.34	176.4	180.6	–
	2	0.12	1.21	0.66	196.9	201.1	–
	3	1.70	1.22	0.35	176.2	182.5	–
	4	1.56	1.18	0.45	173.6	179.8	–
Skin surface pH	1	0.46	1.51	3.22*	−0.9	3.3	–
	2	0.11	2.57*	2.18	9.6	13.8	–
	3	0.32	2.42	3.48*	−13.0	−6.8	–
	4	0.45	2.06	2.79*	−4.8	1.4	–
Skin capacitance	1	0.03	2.99*	1.57	501.7	505.8	cubic
	2	0.00	6.27***	1.00	510.2	514.4	cubic
	3	0.01	4.03**	1.68	494.5	500.7	cubic
	4	0.02	4.72***	1.34	494.4	500.7	cubic
Transepidermal water loss	1	0.03	6.15***	2.53*	300.5	304.6	–
	2	0.05	2.5*	2.73*	318.2	322.3	–
	3	0.04	6.34***	2.84*	298.6	304.8	–
	4	0.04	5.41***	2.86*	299.3	305.6	–
Skin surface temperature	1	1.70	6.6***	4.29**	205.1	209.3	–
	2	0.89	5.32***	3.39*	217.1	221.3	–
	3	1.41	6.68***	4.43**	204.7	211.0	–
	4	2.14	6.67***	4.48**	203.9	210.2	–

p-values of the F-test: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

[a] F-statistic for mixed model.

[b] −2 × restricted log likelihood.

[c] −2 × Schwarz's Bayesian information criterion = −2L + k log(n − p) where L is the restricted log likelihood, k is the number of variance components in the model, n is the number of observations and p is the number of explanatory non-redundant variables.

[d] Time trend found with the orthogonal polynomial method.

analysed separately for transepidermal water loss and skin surface temperature. For both parameters, a significant Time effect was found at the first period, and in contrast, no Time effect was detected at the second period (data not shown). No significant Time effect was identified for the yellow intensity (b*) and the skin capacitance (table 1).

Biophysical Parameters on the Forearm

Only a significant Time effect was shown for skin capacitance [the measurements taken at 8 a.m., midday, 4 p.m. and midnight being significantly lower than those taken at 8 p.m. (fig. 5)]. No significant Time effect was observed for the three skin colour parameters. A significant Period × Time interaction was found for transepidermal water loss, skin surface temperature and skin surface pH once again revealing different time variations

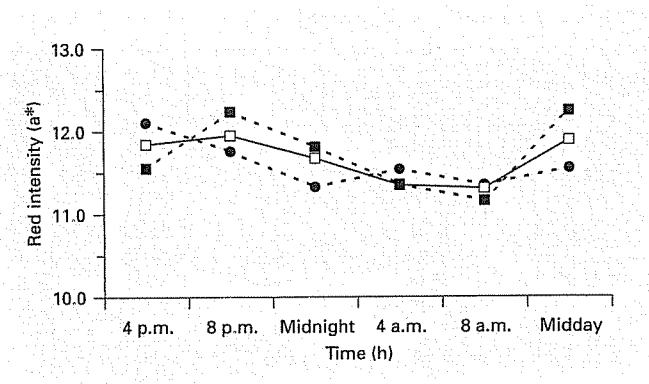

Fig. 2. Observed mean values of red intensity (a*) on the face according to the 6 measurement times (period 1: ●, period 2: ■). The orthogonal polynomial method revealed a significant cubic model. The adjusted curve (□) is superimposed on the graph.

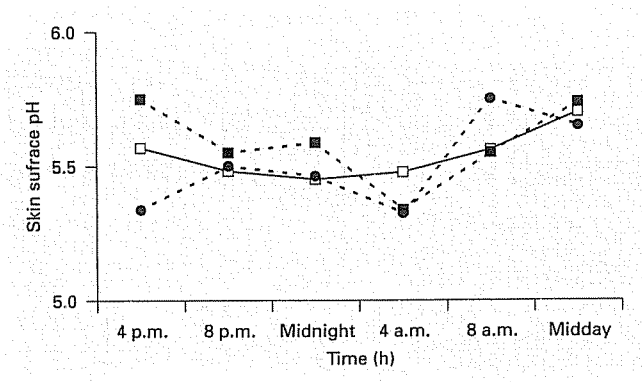

Fig. 4. Observed mean values of skin surface pH on the face according to the 6 measurement times (period 1: ●, period 2: ■). The orthogonal polynomial method revealed a significant quadratic model. The adjusted curve (□) is superimposed on the graph.

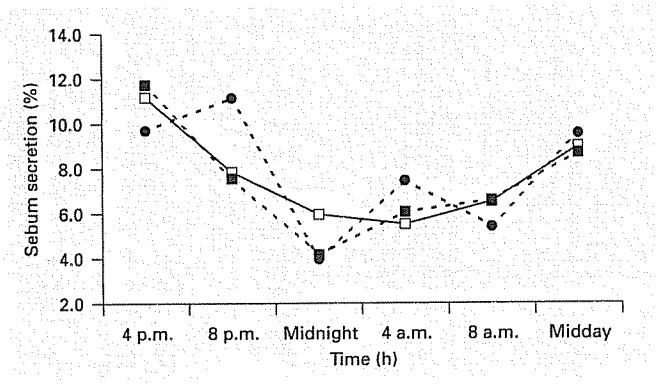

Fig. 3. Observed mean values of sebum secretion on the face according to the 6 measurement times (period 1: ●, period 2: ■). The orthogonal polynomial method revealed a significant quadratic model. The adjusted curve (□) is superimposed on the graph.

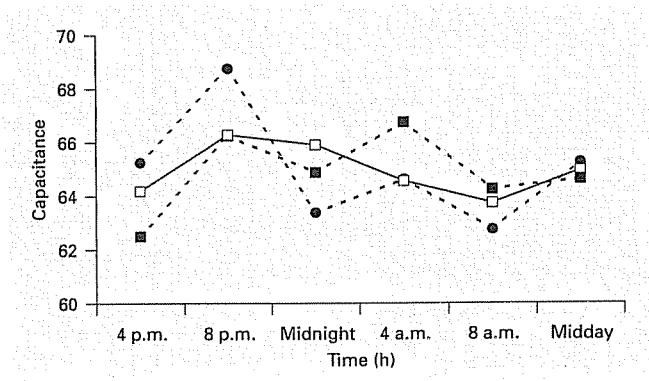

Fig. 5. Observed mean values of skin capacitance on the forearm according to the 6 measurement times (period 1: ●, period 2: ■). The orthogonal polynomial method revealed a significant cubic model. The adjusted curve (□) is superimposed on the graph.

according to the period concerned. The data of each period were consequently analysed separately for the three parameters. For transepidermal water loss, a significant Time effect was found at both periods. A cubic model was revealed for each period: for the first period, the measurements taken at 4 a.m., 8 a.m., 4 p.m. and 8 p.m. being higher than those taken at midnight, and the measurements taken at 4 a.m. and 8 a.m. being higher than those taken at 8 p.m., midnight and midday for the second period (data not shown). For skin surface temperature and skin surface pH, a significant effect of time was found at the second period but not at the first period (data not shown).

Discussion

The statistical analysis based on mixed effect models allowed us to identify a structure that repeats itself in the same way for each 24-hour period for brightness (L*), red intensity (a*), sebum secretion and skin surface pH on the face, and for skin capacitance on the forearm. Significant Period × Time interactions were detected for transepidermal water loss and skin surface temperature on face and forearm revealing different time variations according to the periods concerned.

Among the 4 models tested, model 2 [AR(1)] always provided the worst results according to the −2 × BIC val-

ue for all the biophysical parameters on the face and on the forearm, except for the skin surface pH on the face. This model implies that the correlation between two measurements made on the same subject decreases exponentially with the lag of time between them. However, the correlation observed between the measurements made on the same subject in our study does not follow this pattern and consequently model 2 was not retained. Models 1, 3 and 4 gave approximately the same $-2 \times$ BIC values. Models 3 and 4 suppose, as model 2, that a part of the correlation between the measurements made on the same subject decreases exponentially with time. Moreover, this correlation does not vanish with time due to either a measurement error (model 3) or a random subject effect (model 4). Although models 1, 3 and 4 could be good choices and give consistent results on our data, model 1 was preferred due to its lower number of parameters.

For brightness (L*), no Time effect was found on the forearm; in contrast, a cubic model was found on the face: the measurements taken at midnight, 4 a.m. and 8 a.m. being higher than those taken at midday. In the previous analysis (cosinor and ANOVA methods), a significant Period effect was found on the face; therefore, no conclusion was drawn and no Time effect was found on the forearm (data not published). To our knowledge, diurnal variations in skin colour on the face have never been published before. Chilcott and Farrar [18] have studied diurnal variations in skin colour on the forearm and reported a decrease between 9 a.m. and 5 p.m. On the contrary, we did not observe significant changes on the forearm; however, the experimental conditions, the time points for the measurements and the duration of the two studies were different. For red intensity (a*), no Time effect was revealed on the forearm, while a cubic model was found on the face: the measurements taken at 8 a.m. being lower than those taken at midday and 8 p.m. The cosinor analysis revealed a circadian rhythm only on the face with a peak between 2 and 8 p.m. (not published). Chilcott and Farrar [18] found a significant increase in red intensity (a*) on the forearm between 9 a.m. and 5 p.m. We did not find any significant change on the forearm; nevertheless, we found, as they did, that the variations in brightness (L*) are opposed to the variations in red intensity (a*).

A quadratic model was found for sebum secretion on the forehead: the measurements taken at midnight, 4 a.m. and 8 a.m. were significantly lower than those taken at 4 p.m. The cosinor analysis found circadian variations with a peak at midday and a trough at midnight [8], the results of an ultradian rhythm being superimposed on a circadian one. In the literature, several authors [1, 3, 5] have shown a circadian variation of sebum secretion. Our data are in line with these reports, which always show higher values at the beginning of the afternoon.

A quadratic model was found for skin surface pH on the face: the measurements taken at 4 a.m. were significantly lower than those taken at 8 a.m. and midday. In contrast, a significant Period $\times$ Time interaction was found for the skin surface pH on the forearm, with a significant Time effect at the second period and no Time effect at the first period. Due to this result, no conclusion can be drawn on the forearm. A cosinor analysis previously performed on these data did not detect any circadian or ultradian rhythms either on the face or on the forearm [8]. Nevertheless, the ANOVA method showed a significant Time effect on the face, with a nocturnal trough located around 4 a.m. As opposed to our findings, Yosipovitch et al. [6], using the cosinor analysis, did not observe any significant change on the forehead but a diurnal peak on the forearm. We do not have a satisfactory explanation for these findings other than that the experimental conditions, the time points for the measurements and the duration of the two studies differed.

We did not find any significant Time effect for skin capacitance on the face. In contrast, we found a Time effect on the forearm. A cubic model was found: the measurements taken at 8 a.m., midday, 4 p.m. and midnight being significantly lower than those taken at 8 p.m. In the previous analysis conducted on these data on the face, a Period effect was found; therefore, the cosinor analysis was not performed [8]. An ultradian rhythm was detected on the forearm by the cosinor method with three peaks: the main one at 8 p.m. and smaller ones at midday and 4 p.m. In the literature, capacitance is one of the most widely used techniques to assess the hydration state of the skin surface. Circadian variations have been studied in a recent study by Yosipovitch et al. [6] who failed to detect any Time effect of skin capacitance on the face and the forearm.

Our results showed a significant Period $\times$ Time interaction for transepidermal water loss on the face, with a significant Time effect at the first period, and in contrast, no Time effect at the second period. Thus, no conclusion can be drawn. For the forearm, a significant Period $\times$ Time interaction was also revealed. For both periods, the measurements taken at 4 a.m. and 8 a.m. were higher than those taken at midnight, but the magnitudes were different (data not shown). In the previous analysis, circadian rhythms were detected for transepidermal water loss on both areas [8]. On the face, two peaks at 8 a.m. and 4 p.m. and a trough between 8 p.m. and midnight were detected.

On the forearm, two peaks at 8 a.m. and 4 p.m. and two troughs at midday and midnight were revealed. Spruit [19] found that transepidermal water loss on the forearm skin was higher in the afternoon than in the morning and Yosipovitch et al. [6] found a circadian rhythm of transepidermal water loss with a peak in the late afternoon. In contrast, Reinberg et al. [20] reported a circadian rhythm of transepidermal water loss on the forearm skin with a trough at 2 p.m. and a nocturnal peak. Again differences in the study protocols prohibit a direct comparison of the results.

A significant Period × Time interaction was revealed for skin surface temperature on both areas. On the face, the separate analyses showed a significant Time effect at the first period, and in contrast, no Time effect at the second period. On the forearm, a Time effect was found at the second period but not at the first period. These results do not permit a conclusion. In the previous analysis, time-dependent changes were detected for skin surface temperature only on the forearm with a trough around midday and two peaks at 4 p.m. and 4 a.m., corresponding to an ultradian rhythm superimposed on a circadian one [8]. These results on the forearm were compatible with those recently reported by Yosipovitch et al [6].

In the previous analysis conducted on these data, a Period × Time interaction could not be tested as data were systematically pooled on a 24-hour basis when no Period effect was found. Therefore, a significant Period × Time interaction was detected in the present analysis regarding transepidermal water loss and skin surface temperature, which leads to questions about the findings of the previous analysis (cosinor analysis and ANOVA). Further studies will be necessary in order to confirm, refine and complete these results. These studies must be conducted with closer measurement points (every 3 h, or even every 2 h) over a period (two consecutive 24-hour periods or more) and should be analysed using mixed effect models. This methodology is, however, clearly appropriate for repeated measurements and enables us to accommodate unbalanced designs and take into account missing values in the dataset. In conclusion, Mixed effect models are a powerful tool to analyse longitudinal data provided there are enough individuals involved in the follow-up to estimate the between-subject components of variance and covariance.

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