

Intra- and inter-individual variations in transepidermal water loss on the face: facial locations for bioengineering studies

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The volar forearm is the favored location for bioengineering studies. However, transepidermal water loss (TEWL), which is an important indicator of the function of the epidermal barrier, shows regional variations, and for the evaluation of cosmetic formulations, facial skin would be more suitable. In this study, we have compared 10 facial locations with 1 test site on the volar forearm for absolute TEWL values, reproducibility, and correlation. Measurements were performed over 3 consecutive days. The TEWL values of the facial test sites were higher compared to forearm values and did not correlate with the forearm. On the face, there was a good correlation between the values of the right compared to the left side of the face. The sites superior to the nervus supraorbitalis, the medial borders of the musculus masseter, and the center of the chin proved to be locations with relatively stable TEWL values. There was a greater variability at the center of the forehead and at the center of the cheeks. It is recommended that TEWL measurements on facial locations with stable TEWL values are used for the evaluation of cosmetic formulations.

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Transepidermal water loss (TEWL) is a non-invasive method, frequently used to assess stratum corneum integrity and as an indirect measure of barrier function (1). As a helpful complementary tool, TEWL is widely employed for the evaluation of cosmetic formulations, as well as skin moisturizers and repairs (2). It also seems to be an accurate and sensitive method to detect and quantify skin alterations caused by chemical irritants.

Irritation studies and evaluation of cosmetic formulations have mainly been performed on different forearm sites. It is known that, even on the forearm, different regions may have different skin reactivity (3–8), and results obtained by a skin test on 1 site are not directly applicable to another site. This holds especially for the regional variation in TEWL, which is probably related to varying skin structure, particularly the regional distribution of eccrine sweat glands (1, 4, 5, 8–10). Investigation of differences in TEWL of the face (mapping) have not been performed systematically.

The aim of the investigation was to obtain data on TEWL values in defined anatomical regions of

the face. The reproducibility (intra-individual variation) of TEWL values was validated in order to find anatomical sites with stable TEWL values, as assessment of the effect of topically applied cosmetic products on barrier function and skin hydration depends on the stability of TEWL.

Materials and Methods

Panelists

Measurements were performed in 11 healthy subjects, aged 19 to 53, mean age 33.9 years, 8 female and 3 male, with no history of atopic eczema or other skin diseases. Informed consent was obtained from all of them. During the 3-day study, the panelists were not allowed to apply any topical products: facial cleaning could only be performed with water.

Study design

Measurements were carried out in an air-conditioned room (room temperature 20–22°C, relative

humidity between 30%–45%, room temperature and humidity data being recorded during the measurements, applying the guidelines of the Standardization Group of the European Society of Contact Dermatitis (1)). All measurements were performed after a rest of 30 min for equilibration while the panelists were in a horizontal position. Since the room environment was comfortable (20–22°C) and the subjects physically inactive, the TEWL should closely reflect stratum corneum water flux without gross interference from sweating. The quantitative measurements of TEWL were carried out using the Tewameter TM 210 (Courage and Khazaka, Cologne, Germany). The TEWL was calculated automatically and expressed in $\text{g/m}^2 \times \text{h}$. The Tewameter probe was used in a holding device to avoid heating of the probe. The probe was rested on the skin and TEWL was continuously recorded for a period of 3 min. Mean values were obtained from 2 successive recordings for every test site. Measurements were performed in a special laboratory with constant temperature and humidity. The air-conditioning had been modified technically, such that there was no air convection. Skin temperature was measured at all test sites prior to TEWL measurements using a skin thermometer (Digimed H11S), and had to range between 28°C–32°C (11). The measurements were performed on 3 consecutive days at the same time of the day. The study took place in January to March.

Facial locations (Fig. 1)

TEWL was assessed on 10 anatomically-defined test sites on the face (8 sites allotted to both sides

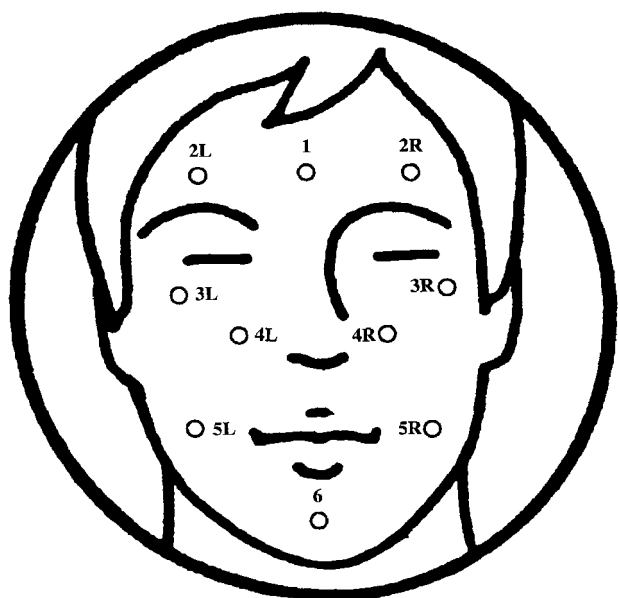


Fig. 1. Location of the facial test sites.

of the face to exclude prevalence of 1 side of the face) and on 1 test site on the volar forearm.

1. The center of the forehead.
- 2R. Superior to the right nervus (N.) supra-orbitalis at the same horizontal level as point 1.
- 2L. Superior to the left N. supraorbitalis at the same horizontal level as point 1.
- 3R. The center of the cheek bone right.
- 3L. The center of the cheek bone left.
- 4R. The center of the cheek right.
- 4L. The center of the cheek left.
- 5R. Medial border of musculus (M.) masseter crossing the cavity of the mouth right.
- 5L. Medial border of M. masseter crossing the cavity of the mouth left.
6. The center of the chin.
7. Middle part of the volar forearm 12 cm above the wrist skinfold.

The order of measurements was randomized. Comparison with the test site on the forearm was performed. To guarantee the exact anatomical locations for the duration of the study, each measuring point was marked with an UVA fluorescence-marker (Hermal-Epimarker D-9139–1).

Statistical evaluation

The following statistical analyses were performed within the statistical packages SAS® and SPSS®.

1. *Descriptive statistics.* The distributions of TEWL in panelists across the different locations are presented as boxplots. Boxplots show the median, the 25th percentile, the 75th percentile, and values that are far removed from the rest. The lower boundary of the box is the 25th percentile, the upper boundary the 75th percentile; the line inside the box represents the median. Lines are drawn from the ends of the box to the largest and smallest observed values that are not outliers. Cases with values that are between 1.5 and 3 box-lengths from the upper or lower edge of the box are called outliers (designated with the letter o). Mean TEWL values on the different sites were additionally calculated. Inter-individual variability was assessed by the coefficient of variation.

2. *Repeated measurements ANOVA* (analysis of variance) models were calculated to account for the fact that measurements were taken from the same panelists in time course and at different locations. We investigated the following. (a) The variations in TEWL over 3 consecutive days at the different locations, where importance of the time effect was assessed by an *F*-test. (b) The correlation

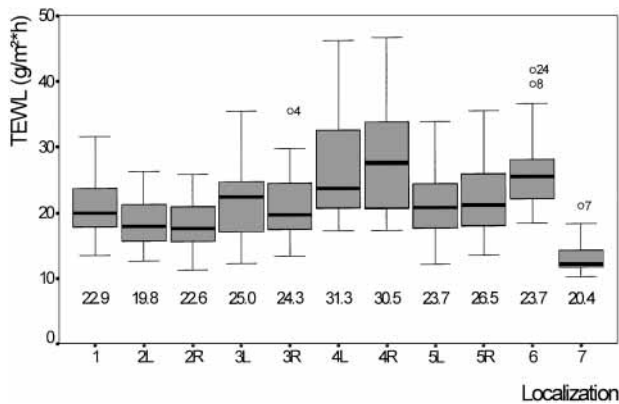


Fig. 2. Inter-individual variation. Distribution of TEWL in 11 panelists (over 3 consecutive days) at different locations. Data are illustrated as box plots. Top and bottom lines of each box show 75th and 25th percentile, middle line shows 50th percentile (median value). Vertical bars extend to upper and lower adjacent values, points outside this range being indicated by open circles. Variation in TEWL is additionally reported in terms of coefficient of variation (values below the boxes).

of TEWL measurements between the different anatomical sites, accounting for the time effect by averaging over daily values.

Results

Absolute values

Comparison of all test sites showed that the average TEWL of the middle part of the volar forearm (mean: 12.4 g/m²h) was clearly lowest within all panelists. The highest mean value was detected at the center of the cheek (right: 27.8 g/m²h, left: 27.0 g/m²h). The TEWL of the chin was nearly the same (25.8 g/m²h). The other TEWL values in the region of the face were found to be between 18 g/m²h and 22 g/m²h. The exact mean values were: cheek bone right: 20.9 g/m²h, cheek bone left: 21.3 g/m²h, M. masseter right 21.7 g/m²h, M. masseter left: 20.8 g/m²h, center of the forehead: 20.8 g/m²h, N. supraorbitalis right: 18.2 g/m²h, N. supraorbitalis left: 18.5 g/m²h. Additional information about the distribution of the TEWL values is provided by Fig. 2.

Locations symmetrical to a vertical axis were comparable as they yielded similar TEWL values. The TEWL values for the right side of the face showed only slight differences to values for the left side: cheek bone: 0.4 g/m²h, cheek: 0.8 g/m²h, M. masseter: 0.9 g/m²h, and N. supraorbitalis: 0.3 g/m²h.

Evaluation of inter-individual reproducibility

Regarding the inter-individual variation, there were no pronounced differences between the vari-

ous locations of the face and the test site on the volar forearm, as shown by coefficients of variation (Fig. 2). In spite of the fact that the forearm TEWL showed lower values and seemed to be less distributed as compared to the TEWL values of the face, the coefficient of variation (volar forearm 20.4) was within the same range as the other locations (range: 19.8–31.3).

Evaluation of intra-individual reproducibility

The individual reproducibility over time can be judged by the *p*-values from the *F*-tests for the hypothesis of no time effect. Low *p*-values indicate a variability over time (see Table 1). Most of the locations did not differ significantly over time, i.e. reproducibility was good.

In addition to the volar forearm, the regions superior to the N. supraorbitalis, the medial borders of the M. masseter, and the center of the chin proved to be locations with relatively stable TEWL values. On the other hand, there was greater variability at the center of the forehead and at the middle of the right cheek.

Correlation between different locations

Using the mean TEWL values over time, we investigated the correlation between all pairs of different locations. To avoid problems with inflating the type I error by multiple comparisons, we did not perform any significance tests on the correlation coefficients, but defined the strength of correlation as follows.

Correlation coefficient:

0.00–0.39 no correlation

0.40–0.59 weak correlation

Table 1. Intra-individual variation. Assessment of variation over time (3 days) for the different locations. Reported are *p*-values from *F*-tests of no time effect in a repeated measurement ANOVA model

	Location	<i>p</i> -values
1	center of forehead	0.04
2L	superior left	0.54
	N. superior	
2R	superior right	0.70
	N. superior	
3L	left cheek bone	0.17
	(center)	
3R	right cheek bone	0.15
	(center)	
4L	left cheek	0.30
4R	right cheek	0.04
5L	left M. masseter	0.40
5R	right M. masseter	0.25
6	center of chin	0.53
7	forearm	0.73

Table 2. Correlation of TEWL measurements (mean over 3 consecutive days) between different locations. Shown are the correlation coefficients adjusted for repeated measurements of sites in panelists. Intensity of grey scale reflects strength of correlation

2L	0.80										
2R	0.80	0.84									
3L	0.46	0.66	0.67								
3R	0.46	0.63	0.70	0.94							
4L	0.35	0.35	0.60	0.67	0.73						
4R	0.17	0.14	0.44	0.62	0.58	0.94					
5L	0.49	0.59	0.73	0.80	0.86	0.90	0.78				
5R	0.32	0.43	0.62	0.76	0.75	0.89	0.88	0.85			
6	0.44	0.26	0.50	0.56	0.56	0.84	0.76	0.74	0.61		
7	-0.26	0.18	-0.18	0.18	0.09	-0.26	-0.31	-0.17	-0.17	-0.28	
	1	2L	2R	3L	3R	4L	4R	5L	5R	6	

=strong correlation, $r \geq 0.8$
 =medium correlation, $0.6 \leq r < 0.8$
 =weak correlation, $0.4 \leq r < 0.6$

0.60–0.79 medium correlation

0.80–1.00 strong correlation

While inspecting the correlation between different locations, it was noticed that there was no correlation between the TEWL of the middle part of the volar forearm and the different regions of the face. Hence, the panelists who showed lower TEWL values at the forearm site did not necessarily show lower values in the regions of the face.

Furthermore, there was no or only a weak correlation between chin and the upper half of the face (middle of the forehead, N. supraorbitalis, cheek bone) and between the forehead and all other locations, except the N. supraorbitalis.

A strong correlation was found for all horizontal locations. Furthermore there was a medium to strong linear correlation between the cheek and the M. masseter. The values of the correlation coefficients are found in Table 2.

Discussion

The reliability of TEWL as a stable indicator of barrier function depends heavily on the intra-individual variation being smaller than the inter-individual variation (12). For the volar forearm as a region for bioengineering studies, this condition is generally accepted to be fulfilled. However, cosmetic formulations are mainly applied to the face. Therefore it would be preferable to investigate cosmetic products by TEWL measurements on facial skin. Prerequisite for this procedure is that the facial TEWL values are reproducible and reliable.

We investigated 10 facial locations and compared them to a test site at the middle part of the volar forearm. In correspondence with the literature (1, 5, 13, 14), we found higher absolute TEWL values on the face compared to the forearm in all panelists. There are various hypotheses regarding the higher TEWL values for the facial regions: a) the higher TEWL values might be due to the higher density of eccrine sweat glands on the face; b) additionally, a smaller surface of the corneum

cells of the facial stratum corneum in comparison to the corneum cells of the arm has been found (15), which might influence the barrier function; also, it has been discussed that regional variation of skin temperature might influence percutaneous absorption (13). However, according to further studies (1, 4, 16), this seems not to be the case regarding TEWL values at ambient room temperatures (20–22°C). At room temperature, the normal range of skin temperature between (28–32°C) is considered to vary too little to influence TEWL importantly (4, 16). In our study, no correlation between the skin temperature and higher TEWL values could be demonstrated.

Inter-individual variability was calculated by coefficient of variation, instead of more common measures like standard deviation or range, in an effort to make inter-individual variability comparable between the facial locations and the forearm. Inspection of the coefficient of variation is necessary, because the seemingly greater variability at facial locations is mainly due to an enhanced TEWL level. Regarding this fact, the inter-individual variability of the facial locations is not remarkably higher than the variability of the forearm.

Intra-individual variability was modeled by means of repeated measurement ANOVA to account for the fact that panelists were repeatedly measured over time. The commonly applied ordinary ANOVA would have led to a loss of power in the given situation (17). It could be shown that the time effect, which is the intra-individual variability, differs slightly in few facial locations. Our results suggest that measurement of TEWL on the face at selected locations can be considered a relatively stable parameter (periphery of forehead > lower parts of the face > central parts of the face > center of forehead).

Another repeated measurement ANOVA model was used to compare the TEWL values of the forearm and of the face because panelists were repeatedly measured regarding the different sites. In this case, we adjusted for the time effect by averaging

daily values. The use of an ordinary ANOVA model would have implied data from each panelist for different locations was independent, a condition that cannot be expected to be fulfilled (and further results from the calculations confirm heavy dependency). We found no correlation between TEWL values on the forearm and the face, but there was a good correlation of identical locations between the right and left side of the face.

The measurement of TEWL on the face can be a helpful and sensible tool in evaluating cosmetic formulas. This would be especially useful for testing of facial cosmetics, because different body regions have different absorption properties (18) and because a higher TEWL level (as shown for the face) can be seen as an indicator of a higher susceptibility to irritation (9). As shown in this study, the periphery of the forehead and the lower parts of the face have stable TEWL values and should be good anatomical locations for bioengineering studies.

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