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A new approach for an estimation of the equilibrium stratum corneum water content

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Background/purpose: Water content is the most vital parameter governing the overall function of the epidermal stratum corneum (SC). Thus, knowledge of the *in vivo* absolute water content of the SC is of great interest.
Methods: We have investigated a non-invasive method for the estimation of *in vivo* SC water content based on transepidermal water loss measurements combined with desorption studies of SC *in vitro*, by means of a dynamic vapour sorption setup where relative humidity (RH) and temperature are controlled.
Results: The SC equilibrium water content of the volar forearm in our study was estimated to be $80 \pm 7\,\mu g/cm^2$. The estimate of the water content seems to decrease slightly with increasing ambient RH.
Conclusion: The estimated water content is a bit lower than what can be expected to be realistic. A calibration against ambient RH is most probably needed if our method is to be applied over a broad range of values of the RH in the ambient air.

Key words: stratum corneum – water content –TEWL

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Water is the single most vital parameter governing the function of the epidermal stratum corneum (SC) and other keratinised tissues, and a knowledge of the hydration state therein is of general interest (1, 2). The corneum hydration state has been shown to be an indicator in the determination and evaluation of non-visible skin diseases such as atopic eczema (3). We want to investigate the possibility of finding an objective measuring method that estimates *in vivo* water content and hydration state of the SC. Such a method will not only be diagnostic, but also a vital tool in the treatment of skin diseases. In this paper, we investigate and discuss the possibility of a direct, non-invasive, and rapid method for the estimation of *in vivo* SC water content. In the recent decades, different methods have been tried out not only for SC (4–7) but also for other keratinised tissues such as nail (8). However, no calibration against absolute water content is yet completed. This paper is an attempt towards such a calibration.

We want to use transepidermal water loss (TEWL) measurements of the human skin *in vivo* in order to estimate the steady-state water content of the SC. This water content is present in the SC due to the passive and continuous transport of water from the viable skin towards the ambient and usually much drier air. The method presented in this paper is a continuation of a preliminary study where TEWL was measured on occluded skin, and SC water content was estimated as the area under the curve of the exponentially decaying TEWL with time (9). Our hypothesis is that the SC equilibrium water content can be found in a similar manner if the SC is allowed to evaporate water from its steady state. This evaporation is not possible to achieve *in vivo* due to the on-going water transport through the skin.

In this paper, we measure the steady-state value of TEWL and find the evaporation constant, which this water would evaporate with, if the equilibrium water content were to evaporate freely, i.e. with no influence from the underlying epidermal skin and its high water content. The latter is performed *in vitro* in a controlled moisture chamber. Mathematically, we can thus extract the steady-state SC water content by assuming that it would, hypothetically, evaporate with the same rate as observed *in vitro* in completely dry environments.

Materials and Methods

All TEWL measurements were performed with a Tewameter TM 300 from Courage and Khazaka Electronics GmbH, (Cologne, Germany), and an illustration of the principle of the measurements is shown in Fig. 1. The measurements were performed on the volar forearm for purely practical reasons and because a rapid measuring procedure is desired (i.e. instead of measuring TEWL at the heel also), standard procedures were followed in order to produce reliable results (10, 11). The test subject, a 30-year-old Caucasian female, had healthy and normal-looking skin. The test lab kept a stable temperature of 21 °C and the relative humidity (RH) was continuously monitored and showed only small fluctuations during the time when TEWL was measured. The RH values during the measurements are listed in Table 1.

The influence on TEWL from changes in temperature and from sweating is well known (12, 13). The SC temperature was held stable by providing a stable environmental temperature with no flow of air or influence from other perturbing effects. Sweating, which in opposition to TEWL, is an active transport of water through the sweat ducts. This was avoided by maintaining the temperature well below the temperature where sweating occurs and also by reducing all physical activities before the measurements to a minimum level (1, 14).

The desorption data of the human SC were measured with a dynamic vapour sorption (DVS) from Surface Measurement Systems Limited (London, UK). The SC was taken from the heel of the two test subjects, one male and one female, by means of an Aesculap dermatome (Braun, Tuttlingen, Germany). The SC pieces were about 0.5×0.5 cm in size, and their thickness 0.2 mm, which was the thinnest possible slices to be prepared by the dermatome. The DVS contains a sensitive microbalance weight with a resolution of 0.1 μg, and the apparatus was programmed so that the temperature was kept stable during the entire measurement. The RH was kept at a level of zero (by means of dry nitrogen gas through the sample chamber), the temperature was held constant at 25 °C, and the water in the SC sample was allowed to evaporate until the sample reached equilibrium with its dry environment. The constraint for the equilibrium to be reached was that the sample mass change was <20 p.p.m per min.

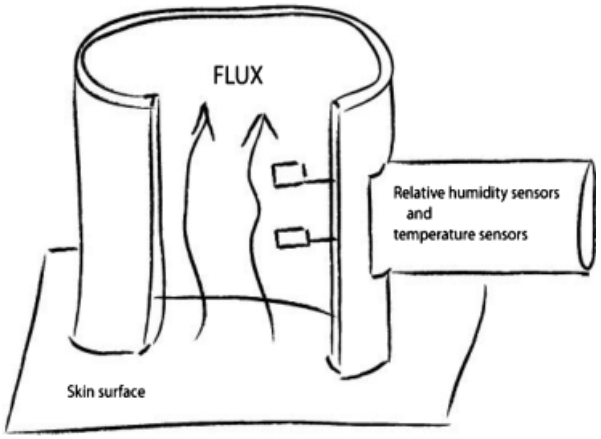

Fig. 1. Illustration of the measurement principle, Tewameter TM 300.

Mathematical model

TEWL is the amount of diffused water through the SC membrane due to the net action of minimising the concentration gradient between the viable epidermis (wet) and the ambient air (usually much drier). This water loss will decay exponentially with time after the SC is occluded (3, 15), not vanishing completely, but rather stabilising on a baseline value, T_b representing the continuous flux of water from skin into its environments. The amount of steady-state *in vivo* SC water content can be estimated by means of T_b and the desorption constant k that governs the decrease of SC water content when it is set to stabilise under 0% RH in the DVS. The decrease of the water content is expected to be exponential with time due to Fick's law of diffusion, thus following a similar behaviour as SC water content after occlusion (9). The *in vivo* steady-state water content (WC) of the SC is

$$\mathrm{WC} = \frac{T_b}{k} \qquad (1)$$

If the water content is to be calculated for other parts of the skin than the heel, the difference in thickness has to be accounted for. The SC samples from the heel were 0.20 mm thick. However, we measured T_b at the volar forearm where the SC is much thinner with a thickness in the range of 15 μm (16, 17). Thus, there is a correction factor of 200/15 with respect to the evaporation constant in Eq. (1), because its value is inversely proportional to the thickness of the SC membrane. Also, the SC sample was able to evaporate its water content on both of its major sides when located in the DVS, but only across one side (i.e. towards the ambient air) when TEWL was measured *in vivo*. The effect of the *in vitro* evaporation of water

through the sides of the SC samples is neglected in our model due to the fact that the thickness is much smaller than either of the other two spatial dimensions of the samples. The effective evaporation constant, k_{eff} for the skin region under consideration includes these corrections and for the specific case when the volar forearm is considered, it turns into

$$k_{\text{eff}} = \tfrac{20}{3}k \tag{2}$$

where k is the evaporation constant that is found experimentally by means of the DVS setup on SC samples taken from the heel. Thus,

$$\text{WC} = \frac{T_{\text{b}}}{k_{\text{eff}}} \tag{3}$$

Results

Figure 2 shows desorption of an average of two SC samples taken from the heel. The mass, relative to the 0% RH equilibrium mass, is plotted as a function of time and shows an exponential decay in accordance with Fick's theory of diffusion. The evaporation constant for desorption is 0.01478/min with an R^2 of 0.9581. The baseline TEWL data are listed in Table 1, and the baseline transport of water across the SC is seen to have relatively stable values, despite the fact that the RH is altered from one day to the other. The average value of the baseline water transport is $T_{\text{b}} = 4.7 \pm 0.40\,\text{g/hm}^2$, which is in the expected range according to the literature (3, 18). The volar equilibrium water content under the TEWL probe is then estimated to be $80 \pm 7\,\mu\text{g}$ when the probe area of $1\,\text{cm}^2$ has been accounted for. We see from

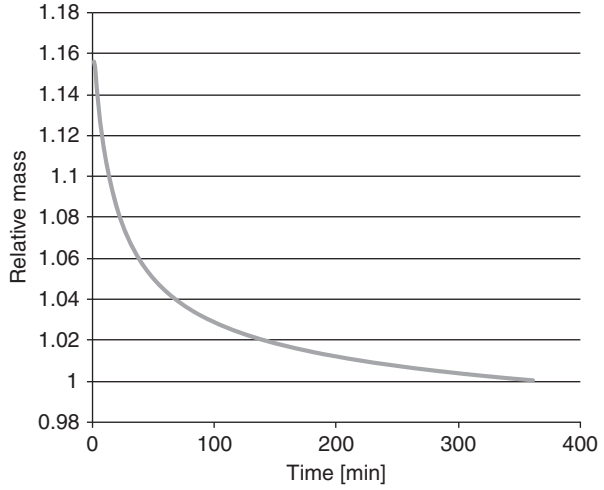

Fig. 2. Relative mass given as a function of time during the in vitro *desorption. The stratum corneum sample is taken from the heel.*

TABLE 1. Results of the transepidermal water loss measurements and the estimation of stratum corneum water content

	Morning	Noon	Afternoon
Day 1			
Skin temperature	27.8	27.4	28.1
% RH	25.8	27.2	28.1
T_{b} (g/hm^2)	4.40	4.86	4.28
WC (mg/cm^2)	0.0744	0.0822	0.0724
Day 2			
Skin temperature	26.7	27.0	28.0
% RH	17.2	15.9	14.6
T_{b} (g/hm^2)	5.10	4.88	4.50
WC (mg/cm^2)	0.0863	0.0825	0.0760

RH, relative humidity; T_{b}, the continuous flux of water from skin into its environments; WC, water content.

Table 1 that there is a tendency that an increase in the ambient RH during the TEWL measurements results in slightly lower estimates of the water content, although this tendency is too weak to draw any conclusions. However, an increase in the ambient RH results in a reduction of the water concentration gradient from epidermis towards the environments and is therefore expected to reduce the transport of water across the SC.

Discussion

In this paper, we have investigated the possibilities of a rapid and non-invasive method for estimating the equilibrium SC water content by means of TEWL measurements. In particular, we considered the skin on the volar forearm and found the water content there. The best estimate of the content of water free to evaporate is $80 \pm 7\,\mu\text{g/cm}^2$. This value is a bit less than what would be expected. A volar forearm SC thickness of about $15\,\mu\text{m}$, which has a hydration in the range of 15–30%, yields a water content of approximately $0.23–0.45\,\text{mg/cm}^2$ skin area. However, the SC is reported to contain at least three different phases of water (3, 19–21), where the most tightly bound phase consists of water molecules that are strongly bound to the lipid polar head groups (22). This primary water remains in the SC even at 0% RH (23) and constitutes about 5.5–10% (gram water per gram dry skin) (21, 22). Thus, it seems reasonable that this tightly bound water is not accounted for in our calculations, and must therefore be added in order to find the total estimate of SC *in vivo* equilibrium water content. This effect improves our estimate, but a precise knowledge of the amount of this water content would still be required.

Our method is temperature dependent because the baseline transport of water through the SC increases with temperature. Thus, the skin surface temperature should be equal or close to the stable temperature during the DVS measurements *in vitro*. The correspondence between TEWL and skin surface temperature is given by Mathias et al. (12) and should be used to correct the differences between skin temperature when baseline TEWL is found and the temperature in the DVS sample chamber. The increase in estimated water content with temperature in our model is as expected because an increase in skin temperature will result in a higher content of water in the SC as long as RH remains unchanged. A change in the RH in the ambient air will most probably alter the level of the SC hydration and thus, our method needs to be calibrated against the RH level of interest. Also, the baseline value of TEWL is expected to vary with the RH, although our results in Table 1 show little variance with RH within the range we performed our TEWL measurements. However, if this method is to be applied over a broad range of RH, a calibration is most probably needed. This would complicate our model and should be a topic for future studies.

One cannot exclude that some of the gap between estimate and expected values in this manner is due to the fact that SC from the heel is used to calibrate the hypothetical desorption rate of the equilibrium water content of the volar forearm (or other SC parts). Matolsy et al. (24) report on differences in keratin composition between various regions of the skin that may influence our calibration.

References

1. Blank IH. Factors which influence the water content of the stratum corneum. J Invest Dermatol 1952; 18: 433–440.
2. Blank IH. Further observations on factors which influence the water content of the stratum corneum. J Invest Dermatol 1953; 21: 259–271.
3. Fluhr J, Elsner P, Berardesca E, Maibach HI., (eds) Bioengineering of the skin, water and the stratum corneum. Boca Raton: CRC Press, 2005.
4. Foreman MI. A proton magnetic resonance study of water in human stratum corneum. Biochem Biophys Acta 1976; 437: 599–603.
5. Jacques SL. A linear measurement of the water content of human skin using a microwave probe. Proc IEEE EMBS 1979; 26: 533.
6. Potts RO, Guzek DB, Harris RR, McKie JE. A non-invasive in vivo technique to quantitatively measure water concentration of the stratum corneum using attenuated total-reflectance infrared spectroscopy. Arch Dermatol Res 1985; 277: 489–495.
7. Egawa M, Kajikawa T. Changes in the depth profile of water in the stratum corneum treated with water. Skin Res Technol 2009; 15: 242–249.
8. Martinsen OG, Grimnes S, Nilsen SH. Water sorption and electrical properties of a human nail. Skin Res Technol 2008; 14: 142–146.
9. Johnsen GK, Haugsnes AB, Martinsen ØG, Grimnes S. Stratum corneum in vivo water content from TEWL-measurements. Proc 30 IEEE EMBS 2008; 1-8: 3166–3169.
10. Rogiers V. EEMCO guidance for the assessment of transepidermal water loss in cosmetic sciences. Skin Pharmacol Appl Skin Physiol 2001; 14: 117–128.
11. Nilsson GE. Measurement of water exchange through skin. Med Biol Eng Comput 1977; 15: 209–218.
12. Mathias T, Wilson DM, Maibach HI. Transepidermal water loss as a function of skin surface temperature. J Invest Dermatol 1981; 77: 219–220.
13. Pinnagoda J, Tupker RA, Agner T, Serup J. Guidelines for transepidermal water loss measurement. Contact Dermatitis 1990; 22: 164–178.
14. Pinnagoda J, Tupker RA, Coenrads PJ, Nater JP. Transepidermal water loss: with and without sweat gland inactivation. Contact Dermatitis 1989; 21: 16–22.
15. Rosado C, Pinto P, Rodrigues LM. Modelling TEWL desorption curves: a new practical approach for the quantitative in vivo assessment of skin barrier. Exp Dermatol 2005; 14: 386–390.
16. Blank IH, Moloney J, Emslie AG, Simon I, Charles A. The diffusion of water across the stratum corneum as a function of its water content. J Invest Dermatol 1984; 82: 188–194.
17. Holbrook KA, Odland GF. Regional differences in the thickness (cell layers) of the human stratum corneum: an ultrastructural analysis. J Invest Dermatol 1974; 62: 415–422.
18. Scott RC, Oliver GJA, Dugard PH, Singh HJ. A comparison of techniques for the measurement of transepidermal water loss. Arch Derm Res 1982; 274: 57–64.
19. van Hal DA, Jeremiasse EE, Junginger HE, Spies F, Bouwstra JA. Structure of fully hydrated human stratum corneum: a freeze fracture electron microscopy study. J Invest Dermatol 1996; 106: 89–95.
20. Kasting GB, Barai ND. Equilibrium water sorption in human stratum corneum. J Pharm Sci 2003; 92: 1624–1631.
21. Potts RO. Stratum corneum hydration: experimental techniques and interpretations of results. J Soc Cosmet Chem 1986; 37: 9–33.
22. Pieper J, Charalambopoulou G, Steriotis T, Vasenkov S, Desmedt A, Lechner RE. Water diffusion in fully hydrated porcine stratum corneum. Chem Phys 2003; 292: 465–476.
23. Takenouchi M, Suzuki H, Tagami H. Hydration characteristics of pathologic stratum corneum – evaluation of bound water. J Invest Dermatol 1986; 87: 574–576.
24. Matolsy AG, Matolsy MN, Cliffel PJ. Characterisation of keratin polypeptide of normal and psoriatic horny cells. J Invest Dermatol 1983; 80: 185–188.

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