The Correlation Between Transepidermal Water Loss and Percutaneous Absorption

6

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Contents

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6.1 Introduction

6.1.1 What Is Transepidermal Water Loss?

Transepidermal water loss (TEWL) is the outward diffusion of water through the skin (Oestmann et al. 1993). An evaporimeter determines TEWL by measuring the pressure gradient of the boundary layer resulting from the water gradient between the skin surface and ambient air. TEWL measurements can reflect the general health of the skin via the assessment of skin barrier function and also assess treatment effectiveness or skin barrier repair by monitoring the change in TEWL over time (Nilsson. 1997; Pinnagoda et al. 1990). However, TEWL measurements cannot be simply compared across multiple experiments. TEWL measurements are subject intra-individual variation based on the anatomic site where the TEWL was measured and interindividual variation based on the extent of skin perspiration and skin surface temperature of the individual tested. In addition, TEWL measurements can be affected by experimental conditions such as the air convection, the ambient air temperature and air humidity of the room where the TEWL measurement was taken, and the method and type of instrument used to measure TEWL. Although TEWL can be influenced by many variables, experiments show

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that evaporimeter measurements generally are reproducible in vitro and in vivo (Pinnagoda et al. 1989, 1990; Elkeeb et al. 2010; Fluhr et al. 2006).

6.1.2 What Is Percutaneous Absorption?

Percutaneous absorption refers to the rate of absorption of a topically applied chemical through the skin. A compound's absorption rate is important for determining the effectiveness and/or potential toxicity of topically applied compounds. Since many topical formulations are used on diseased skin, where the integrity of the permeability barrier is in doubt, the dose absorbed into the body could vary greatly (Bronaugh and Stewart 1986). One rate-limiting step of a compound's absorption through the skin is the rate of diffusion through the stratum corneum (SC). The rate of absorption through the SC cannot be described by a zero- or first-order mathematical rate equation because the SC is a complex system variable in its penetration properties. Many factors contribute to the percutaneous absorption of a given chemical, such as methodology (including the effects of application time, method of measurement), physicochemical properties of the topical compound, interindividual variation (including the effects of skin condition, age of individual, and blood flow), and intra-individual variation (including the differences between anatomic sites) (Noonan and Gonzalez 1990; Wester 1993).

6.1.3 What Is the Significance of a Correlation Between TEWL and Percutaneous Absorption?

The extensive procedure required to measure percutaneous absorption versus TEWL enhances the desire to find a correlation between the two measurements in order to more easily assess skin barrier function and should aid in the understanding and development of penetration enhancers. In a review by Levin and Maibach in 2005, nine studies investigating the correlation between TEWL and percutaneous absorption were reviewed. Of the nine studies reviewed, a majority demonstrated a significant quantitative correlation, and a few found no quantitative correlation. At that time it was thought that the correlation between TEWL and percutaneous absorption may not hold for in vitro experimentation models, extremely lipophilic compounds, or possibly experiments performed on animal skin. Since then, several other studies have been published investigating the relationship between TEWL and percutaneous absorption using a very lipophilic compound (Hui et al. 2012), in vitro models (Elkeeb et al. 2010; Hui et al. 2012; Elmahjoubi et al. 2009), and animal skin (Elmahjoubi et al. 2009), and all studies have demonstrated a significant quantitative correlation.

In the next section, we review 12 studies investigating the correlation between TEWL and percutaneous absorption.

6.2 Pertinent Studies Investigating the Correlation Between TEWL and Percutaneous Absorption

Oestmann et al. (1993) investigated a correlation between TEWL and hexyl nicotinate (HN) penetration parameters in man. Penetration of HN was indirectly measured by means of laser Doppler flowmetry (LDF), which quantifies the increase in cutaneous blood blow (CBF) caused by penetration of HN, being a vasoactive substance. Lipophilic HN was chosen over hydrophilic methyl nicotinate because HN is a slower penetrant, hence, making it easier to distinguish an intact barrier from an impaired barrier.

LDF parameter initial response time (t_o) and the time to maximum response (t_{max}) were compared with corresponding TEWL values, and a weak quantitative negative correlation was found ($r=-0.31$, $r=-0.32$). This correlation suggests that when an individual's response time, t_o, was fast, the skin barrier was impaired. The weak negative correlation found that maybe LDF is not

as reproducible as other methods of measuring percutaneous absorption. Further research should investigate this weak correlation between TEWL and penetration of HN.

Lamaud et al. (1984) investigated whether TEWL correlated to the percutaneous absorption of the lipophilic compounds (hydrocortisone). Penetration of 1 % hydrocortisone and TEWL rates were recorded for the hairless rats in vivo before and after UV irradiation (660 J/cm^2). The results demonstrated a correlation between TEWL and the percutaneous absorption of hydrocortisone both before and after UV irradiation for application periods up to 1 h. In part two of the experiment, drug penetration was evaluated by urinary excretion 5 days after a single 24 h application of hydrocortisone on normal, stripped, or UV-irradiated skin of hairless rats. In this experiment the quantity of the drug eliminated correlated with the level of TEWL for up to 2 days for all skin conditions suggesting that TEWL can predict the changes of skin permeability to lipophilic drugs in normal and damaged skin.

Lavrijsen et al. (1993) characterized the SC barrier function in patients with various keratinization disorders using two noninvasive methods: measuring outward transport of water through skin by evaporimetry, i.e., TEWL, and the vascular response to HN penetration into the skin determined by LDF. Three of the five types of keratinization disorders studied, i.e., autosomal dominant ichthyosis vulgaris, X-linked recessive ichthyosis, and autosomal recessive congenital ichthyosis, have impaired barrier function and are a type of ichthyosis, while for the other two keratinization disorders studied, dyskeratosis follicularis and erythrokeratoderma variabilis, there were no prior information available on barrier impairment. In this experiment the two methods of barrier function assessment, TEWL and LDF, were correlated for all skin diseases and normal skin as a control.

TEWL measurements and the LDF parameter, $t_{o,}$ showed a high negative correlation in those with skin disease ($r=-0.64$) and a weaker negative correlation among the control healthy skin group ($r=-0.39$). Because TEWL reflects the steady state flux of a compound across SC and parameter, t_o is a function of the duration of the lag phase (not a steady state measurement), and this study suggested that these two methods, because they are measuring different things, should not be considered as exchangeable alternatives but rather as complementary tests to assess barrier function. On the basis of results of this chapter, however, it could be concluded that TEWL and HN penetration injunction are suitable methods to monitor skin barrier function in keratinization disorders.

Rougier et al. (1988) attempted to establish the relationship between the barrier properties of the horny layer using percutaneous absorption and TEWL measurements and discern the surface area of the corneocytes according to anatomic site, age, and sex in man.

The penetration of benzoic acid (BA) was measured in vivo at seven anatomic sites and compared to its TEWL value measured on the contralateral site. The amount of BA penetrated was measured through urinary extraction up to 24 h after application. It was discovered that irrespective of the anatomic site and gender, a linear relationship ($r=0.92$, $p<0.001$) existed between total penetration of BA and TEWL.

Comparing corneocyte surface area to permeability, Rougier et al. (1988) also found a general correlation of increasing permeability for both water and BA with decreasing corneocyte size. The smaller the volume of the corneocyte, the greater the intercellular space available to act as a reservoir for topically applied molecules, resulting in a higher absorption (Dupuis et al. 1984). This thinking is in accord with other studies who have shown that the smaller the capacity of the reservoir, the less the molecule is absorbed (Dupuis et al. 1984; Rougier et al. 1983, 1985, 1987a, b). In order to determine the influence of age on corneocyte size, Rougier et al. (1988) investigated the corneocyte size in the upper-outer arm for three groups of six to eight male volunteers: (1) 20–30, (2) 45–55, and (3) 65–80 years. No variation in corneocyte size up to 55 years was observed. The mean corneocyte size for the 20–30-year cohort was 980 ± 34 μm^2, and for the 45–55-year cohort, a value of 994 ± 56 μm^2 was recorded. The group aged 65–80 years did, however, show significantly larger corneocytes ($1{,}141 \pm 63$ μm^2)

relative to the other groups. Relatively small numbers of subjects were used by Rougier et al. (1988) which may explain the discrepancies when compared with data from other more recent studies (Leveque et al. 1984). Generally it is now understood that corneocytes generally increase in size with age (Machado et al. 2010) and TEWL and percutaneous absorption also increases with age (Roskos and Guy 1989); therefore, it seems that corneocyte size cannot explain the permeability changes in mature skin.

Rougier et al. (1988) used a detergent scrub technique to collect corneocytes at different anatomic sites from a group of six to eight male volunteers, aged 20–30 years. The rank order of the corneocyte surface area was forearm (ventral elbow) = forearm (ventral-mid) = arm (upper-outer) = abdomen > forearm (ventral-wrist) > postauricular > forehead. However when Rougier et al. (1988) investigated corneocyte size by anatomic site, he found that for certain anatomic sites where corneocyte size was similar (980–1,000 μm^2), there were large differences in permeability. Therefore, while percutaneous absorption and TEWL are quantitatively correlated, corneocyte size only partially explains the difference in permeability between the different anatomic sites and different age of the skin.

Lotte et al. (1987) examined the relationship between the percutaneous penetration of four chemicals (acetylsalicylic acid, benzoic acid, caffeine, and sodium salt of benzoic acid) and TEWL in man as a function of anatomic site. The amount of chemical penetrated was measured by urinary excretion for up to 24 h after application. For a given anatomic site, the permeability varied widely in relation to the nature of the molecule administered due to the physicochemical interactions which occur between the molecule, vehicle, and SC. For all anatomic sites investigated, irrespective of the physicochemical properties of the molecules administered, there was a linear relationship between TEWL and percutaneous absorption.

Aalto-Korte et al. (1993) attempted to find the precise relationship between TEWL and percutaneous absorption of hydrocortisone in patients with active dermatitis. Percutaneous absorption of hydrocortisone and TEWL was studied in three children and six adults with dermatitis. All the subjects had widespread dermatitis covering at least 60 % of the total skin area. Plasma cortisol concentrations were measured by radioimmunoassay before and 2 and 4 h after hydrocortisone application. TEWL was measured in six standard skin areas immediately before application of the hydrocortisone cream. Each individual TEWL value was calculated as a mean of these six measurements.

The concordance between the post application increment in plasma cortisol and the mean TEWL was highly significant resulting in a correlation coefficient of $r = 0.991$ ($p < 0.001$). In conclusion this study found a highly significant correlation between TEWL and percutaneous absorption of hydrocortisone.

Tsai et al. (2001) investigated the relationship between the permeability barrier disruption and the percutaneous absorption of various compounds with different lipophilicity. Acetone treatment was used in vivo on hairless mice to disrupt the normal permeability barrier, and in vivo TEWL measurements were used to gauge barrier disruption. The hairless mouse skin was then excised and placed in diffusion cells for the in vitro percutaneous absorption measurements of five model compounds: sucrose, caffeine, hydrocortisone, estradiol, and progesterone. The partition coefficient or lipophilicity of these compounds and compounds used in the subsequent studies are summarized in Table 6.1.

The permeability barrier disruption by acetone treatment and TEWL measurements significantly correlated with the percutaneous absorption of the hydrophilic and lipophilic drugs sucrose, caffeine, and hydrocortisone. However acetone treatment did not alter the percutaneous penetration of the highly lipophilic compounds estradiol and progesterone, hence, suggesting that there is no correlation between TEWL and the percutaneous absorption of highly lipophilic compounds. The results imply the need to use both TEWL and drug lipophilicity to predict alterations in skin permeability.

Chilcott et al. (2002) investigated the relationship between TEWL and skin permeability to tritiated water (^3H_2O) and the lipophilic sulfur mustard (^{35}SM) in vitro. No correlation was found

between basal TEWL rates and the permeability of human epidermal membrane to ^3H_2O ($p=0.72$) or sulfur mustard ($p=0.74$). Similarly, there was no correlation between TEWL rates and the ^3H_2O permeability on full-thickness pig skin ($p=0.68$). There was also no correlation between TEWL rates and ^3H_2O permeability following up to 15 tape strips ($p=0.64$) or up to four needle stick punctures ($p=0.13$). Taken together these results from this experiment indicate that under these experimental circumstances (i.e., in vitro human and pig skin) TEWL cannot be used as a measure of the skin's permeability to topically applied lipophilic or hydrophilic compounds.

Elkeeb et al. (2010) compared TEWL to the percutaneous absorption/flux rate of ^3H_2O in in vitro dermatomed clinically healthy human cadaver skin using three different evaporimeters to measure TEWL. Measurements were taken at baseline (i.e., at the start of the experiment) and then again at several time points over 24 h. The evaporimeters included an open chamber evaporimeter A (TEWameter® TM 210 (Courage and Khazaka, Cologne, Germany)) and two closed chamber evaporimeters B (VapoMeter™ (Delfin Technologies, Kuopio, Finland)) and C (AquaFlux AF200, Biox Systems, Ltd, London, UK). Open chamber evaporimeters are open to the ambient air, while closed chamber evaporimeters are closed systems that are not open to the environment. There has been controversy over the years as to whether open and closed chamber evaporimeters are equivalent in given accurate and precise TEWL measurements TEWL. Baseline TEWL measurements with evaporimeters A ($p=0.04$, $r^2=0.34$) and C ($p=0.00$, $r^2=0.50$) correlated with the percutaneous absorption or flux rate of tritiated H_2O, while evaporimeter B showed no statistically significant correlation ($p=0.07$, $r^2=0.31$). However, the pattern of changing TEWL values over 24 h was similar to that of the percutaneous absorption or tritiated water flux for all three evaporimeters A, B, and C ($p=0.04$, $r^2=0.34$,). The reason why evaporimeter B showed no significant correlation for baseline TEWL measurement remains unknown. Elkeeb et al. (2010) state that the results of this experiment imply the validity of using both open and closed chamber evaporimeters in the evaluation of skin barrier function.

Atrux-Tallau et al. (2007) demonstrated significant correlation between TEWL and the percutaneous absorption of caffeine (a hydrophilic compound) during an ex vivo experiment on heat separated epidermis and dermatomed human skin ($p<0.001$, $r^2=0.88$). Since caffeine is a hydrophilic compound and has a relatively small molecular weight of 194 Da, it was not surprising to the authors that the permeation behavior resembles that of tritiated water (22 Da).

Hui et al. (2012) investigated the correlation between TEWL and the percutaneous absorption of clonidine (a lipophilic compound) and ^3H_2O (a hydrophilic compound) in in vitro human cadaver skin. The partition coefficient of clonidine is reported in Table 6.1. TEWL measurements were made with a closed chamber TEWL meter (AquaFlux AF200). With the goal of discerning the potential differences in the correlation between TEWL and lipophilic clonidine, the correlation between TEWL and hydrophilic ^3H_2O percutaneous absorption and general differences in the percutaneous absorption of clonidine and ^3H_2O, the flux rate, skin distribution, and total amount of absorption for clonidine and tritiated water were recorded and compared. Statistical analysis indicated that the baseline TEWL values weakly correlated with the flux of [14C]-clonidine ($p<0.03$, $r^2=0.36$) and ^3H_2O ($r^2=0.34$, $p=0.04$). The correlation between fluxes of ^3H_2O and [14C]-clonidine was moderate (correlation coefficient = 0.675, $p<0.001$). In addition, TEWL and permeation data of ^3H_2O expressed as a percent dose of the amount in the receptor fluid correlated well throughout the experiment. However, the permeation curve of [14C]-clonidine as a percent dose in the receptor fluid differed from that of ^3H_2O and TEWL. The difference in the curves is likely secondary to differences in the hydrophilic/lipophilic properties of clonidine versus water. Therefore as Hui suggests, it may be necessary to combine the TEWL values with factors such as molecular weight and/or hydrophilicity/lipophilicity to gauge percutaneous absorption.

Elmahjoubi et al. (2009) investigated TEWL (using the AquaFlux evaporimeter) and the

Table 6.1 A summary of the compounds used in the correlation studies, their octanol-water partition coefficient, solubility classification, and whether or not their percutaneous absorption correlated with TEWL (Oestmann et al. 1993; Nilsson 1997; Elkeeb et al. 2010; Hui et al. 2012; Elmahjoubi et al. 2009; Lamaud et al. 1984; Lavrijsen et al. 1993; Rougier et al. 1988; Lotte et al. 1987; Aalto-Korte et al. 1987; Tsai et al. 2001; Chilcott et al. 2002; Atrux-Tallau et al. 2007)

Compound	Partition coefficient (log $P_{octanol/water}$)	Classification	Correlation
Sucrose	−3.7	Hydrophilic	Yes
Caffeine	−0.02	Hydrophilic	Yes
Water	1	Hydrophilic	Yes
Acetylsalicylic acid	1.13	Hydrophilic	Yes
Sulfur mustard	1.37	Lipophilic	No
Hydrocortisone	1.5	Lipophilic	Yes
Benzoic acid	1.87	Lipophilic	Yes
Sodium benzoate	1.87	Lipophilic	Yes
Estradiol	2.7	Highly lipophilic	No
Progesterone	3.9	Highly lipophilic	No
Hexyl nicotinate	4	Highly lipophilic	Yes (weak)
Clonidine	5.4	Highly lipophilic	Yes (weak)

percutaneous absorption/flux of ^3H_2O in full-thickness in vitro porcine skin both at baseline and after physical and chemical barrier disruption in multiple different experiments. The aim of these experiments was to further investigate the relationship between TEWL and ^3H_2O flux using the AquaFlux evaporimeter® (Bio Systems Ltd, USA) and to evaluate the use of porcine skin in vitro as a model to study the human skin barrier.

The first experiment investigated the relationship between basal TEWL rates and ^3H_2O flux in in vitro healthy full-thickness porcine skin. The results showed that basal TEWL values were linearly correlated with basal ^3H_2O flux values ($r^2=0.80$, $n=63$).

The second experiment examined the effect of physical barrier disruption with skin punctures on TEWL measurements. The results did not show a perfect correlation between skin punctures and TEWL measurements. TEWL increased significantly after the first skin puncture and then remained constant for punctures 2, 3, and 4. Another large increase in TEWL was seen with the fifth puncture. However no changes in TEWL values were seen with the sixth or seventh puncture suggesting that a threshold may have been reached after the fifth puncture.

The third and fourth experiments examined TEWL changes after chemical barrier disruption with surfactants. In the third experiment, anionic surfactants of differing alkyl chain lengths were applied to the full-thickness porcine skin in vitro to determine if measuring TEWL values could discern between mild and severe perturbations to the barrier function. TEWL was largely unaffected following cutaneous exposure to short and long alkyl chain surfactants and, however, was significantly elevated over control levels following exposure to those with intermediate chain lengths. Exposure to sodium lauryl sulfate (SLS), with an intermediate 12 carbon alkyl chain, produced the greatest increase in TEWL.

In the fourth experiment, the effect of varying SLS concentration, volume, and contact time on the TEWL in vitro in porcine skin was measured. The results showed a linear trend between TEWL and SLS concentration in the 0–1 % w/v concentration range. However, following treatment with 5 % w/v SLS, TEWL readings were only slightly higher than those following treatment with 1 % w/v surfactant. A linear correlation was also demonstrated between TEWL and surfactant solution volume ($r^2=0.87$), which was statistically significant ($p<0.01$). TEWL also increased as a function of increasing SLS treatment time, when concentration was fixed at 1 % w/v and volume fixed at 200 µl.

In conclusion, Elmahjoubi et al. (2009) found that baseline TEWL values correlated with the percutaneous absorption of ^3H_2O in vitro in healthy

porcine skin and the TEWL measurements linearly correlated with the exposure of porcine skin in vitro to increasing concentrations, time, and volumes of SLS. TEWL measurements did not demonstrate a linear correlation between skin punctures (i.e., skin damage) and TEWL. The authors feel that TEWL measurements in vitro in porcine skin may serve as a model for future studies in this area in contrast to the previous findings by Chilcott et al. (2002).

6.3 Discussion of the Assumptions Made in the Studies Investigating the Correlation Between TEWL and Percutaneous Absorption

Many of the experiments investigating TEWL and percutaneous absorption make large assumptions which could affect the results and hence be the source of controversy. For example, Tsai et al. (2001) and Chilcott et al. (2002) assume that in vitro measurements of TEWL and percutaneous absorption are equivalent to in vivo measurements, while Lamaud et al. (1984) assume that animal skin may serve as a permeability model for human skin. Great sources of error and variation can also be induced depending on the measurement device used to record TEWL rates and the choice of the compound and/or method used to measure percutaneous absorption rates. Because we do not completely understand the qualitative relationship between TEWL and percutaneous absorption, it is hard to determine which assumptions made during the experiment could be affecting the correlation results. This section investigates the probable causes that could influence the results of the correlation experiments. Provided in Table 6.2 is a summary of the major assumptions from 12 studies discussed in this chapter.

6.3.1 Using In Vitro Methods to Model In Vivo Experiments

Skin permeation can be measured in vivo or in vitro by using excised skin in diffusion cells. In theory, studies using in vitro or ex vivo are feasible models for in vivo experiments because passage through the skin is a passive diffusion process and the stratum corneum is nonliving tissue. Many studies comparing in vivo and in vitro TEWL and percutaneous absorption measurements have been conducted, and the results from those experiments support the contention that reliable measurements can be obtained from in vitro studies (Elkeeb et al. 2010; Noonan and Gonzalez 1990; Hui et al. 2012; Elmahjoubi et al. 2009; Nangia et al. 1993; Brounaugh et al. 1982b). While the consensus is that in vitro experiments are reasonable models for in vivo human experiments, some experiments note significant differences between these methods for measuring skin permeation. The most significant study by Bronaugh and Stewart (1985) found that the effects of UV irradiation could not be duplicated using an in vitro experimentation model, hence, suggesting that in vitro experiments examining the TEWL and percutaneous absorption after barrier damage may not be an acceptable model for correlation with in vivo studies. In vitro damage to the SC barrier may not be an accurate model to in vivo SC damage because in vivo exposure to skin irritants results in a cascade of reactions that do not occur in vitro in human cadaver skin (Nangia et al. 1993).

Chilcott et al. (2002) investigated the correlation between TEWL and percutaneous absorption in vitro after inducing different types of barrier damage. This was one of the rare studies which did not observe a correlation between TEWL and percutaneous absorption after barrier damage. It is possible that in vitro methodology in the experimental design may be responsible for the lack of correlation of TEWL to skin damage reported in this study. However, Fluhr et al. (2006) suggest that the conditions used in the study of Chilcott et al. (2002), i.e., the use of heat-split human epidermis and non-pigmented pig skin that had been stored for up to 14 days and penetration studies which extended over 96 h post-heat separation, likely contributed to their results. Fluhr (2006) states that the extracellular lipid matrix and corneocytes of the SC were potentially compromised from the heat separation. However, it is this author's opinion that even if the barrier was compromised by heat separation, these changes in barrier function should have been reflected both in the TEWL and percutaneous absorption

Table 6.2 A summary of the major assumptions made by the studies discussed in this chapter (Aalto-Korte et al. 1993; Atrux-Tallau et al. 2007; Chilcott et al. 2002; Elkeeb et al. 2010; Hui et al. 2012; Elmahjoubi et al. 2009; Lamaud et al. 1984; Lavrijsen et al. 1993; Lotte et al. 1987; Nilsson 1997; Oestmann et al. 1993; Rougier et al. 1988; Tsai et al. 2001)

Reference	In vivo vs in vitro (percutaneous absorption)[b]	Skin type	Percutaneous absorption measurement method	Compound[c]	Healthy skin vs damaged skin	Correlation results
Oestmann et al. (1993)	In vivo	Human	LDF	Lipophilic	Healthy	Yes
Lamaud et al. (1984)	In vivo	Animal	Urinary	Lipophilic	Both	Yes
Lavrijsen et al. (1993)	In vivo	Human	LDF	Lipophilic	Damaged	Yes
Rougier et al. (1988)	In vivo	Human	Urinary	Lipophilic	Healthy	Yes
Lotte et al. (1987)	In vivo	Human	Urinary	Hydrophilic and lipophilic	Healthy	Yes
Aalto-Korte et al. (1993)	In vivo	Human	Plasma cortisol level	Lipophilic	Damaged	Yes
Tsai et al. (2001a)[a]	In vitro	Animal	Diffusion cell	Hydrophilic and lipophilic	Damaged	Yes
Tsai et al. (2001b)[a]	In vitro	Animal	Diffusion cell	Highly lipophilic	Damaged	No
Chilcott et al. (2002)	In vitro	Both	Diffusion cell	Hydrophilic and lipophilic	Both	No
Elkeeb et al. (2010)	In vitro	Human	Diffusion cell	Hydrophilic	Healthy	Yes
Hui et al. (2012)	In vitro	Human	Diffusion cell	Hydrophilic and lipophilic	Healthy	Yes
Atrux-Tallau et al. (2007)	Ex vivo	Human	Diffusion cell	Hydrophilic	Healthy	Yes
Elmahjoubi et al. (2009)	In vitro	Animal	Diffusion cell	Hydrophilic	Both	Yes

[a]Reference Tsai et al. was divided into two experiments in this table since the study found a correlation between TEWL and percutaneous absorption with some compounds and no correlation with others

[b]TEWL in vivo and in vitro measurements are considered equivalent. We are only concerned with how percutaneous absorption measurements were performed

[c]Compounds were classified by their octanol-water partition coefficient, log $K_{octanol/water}$. See Table 6.1. Compounds possessing log $K_{octanol/water}$ values less than one are considered hydrophilic, while compounds with log $K_{octanol/water}$ higher than three were considered very lipophilic

and hence should have correlated if both measured variables truly reflect skin barrier function.

However since Chilcott et al.'s original publication in 2002, many studies demonstrating the correlation between TEWL and percutaneous absorption have been conducted in in vitro models (Elkeeb et al. 2010; Hui et al. 2012; Elmahjoubi et al. 2009), and it is more likely that the results of Chilcott et al. (2002) were an exception rather than the rule.

6.3.2 Using Animal Skin to Model Human Skin

Comparing the skin morphology and absorption of chemicals through human versus animal skin, it is clear that human skin is unique in both aspects and should be used for the most meaningful results (Bronaugh and Franz 1986). Yet an experiment by Bronaugh et al. (1982a) found that depending on the compound and the vehicle

used, permeability values obtained using animal skin can be well within an order of magnitude of the permeability values for human skin.

Independently, in vitro methods and animal skin models prove to be reliable models to predict percutaneous absorption in human skin in vivo. Therefore it seems logical to assume that the in vitro condition and the use of animal skin may be used in unison to accurately model in vivo absorption through human skin. However Rougier et al. (1987a, b) documented a distinct difference between animal studies performed in vivo versus animal studies performed in vitro when compared to the absorption of compounds through human skin in vivo. This experiment compared the permeability of human skin to the hairless rat (Walker et al. 1983) and the hairless mouse (Bronaugh and Stewart 1986) skin using molecules of widely different physicochemical properties. The results show that on in vivo animal or human skin, for whatever the molecule tested, the permeability ratios remained relatively constant, while in vitro they do not. Therefore, when application conditions are strictly identical in humans and in animals, it may be possible to predict percutaneous absorption in human skin in vivo by measuring in vivo absorption through animal skin, but not using in vitro animal absorption. The inaccurate results obtained when conducting experiments in vitro using animal skin may have affected the results studies by Tsai et al. (2001) and Chilcott et al. (2002) which were the only two studies using in vitro animal skin and showing no correlation between TEWL and percutaneous absorption.

However, Laumaud et al. (1984) conducted their study in porcine skin in vivo, and Elmahjoubi et al. (2009) conducted their study in porcine skin in vitro, and both found a correlation between TEWL and percutaneous absorption. This suggests that other factors than using animal in vitro model may have played a role in the lack of correlation found in studies by Tsai et al. (2001) and Chilcott et al. (2002). However, there is no doubt that there are distinct differences between animal skin and human skin when used as a model for human absorption, whether these differences are large enough to invalidate that the use of animal skin as a model for experimentation seems unlikely. However, further research may be warranted.

6.3.3 Differences in TEWL Measurement Methods

TEWL meters or evaporimeters can have an open or closed chamber system. Open chamber TEWL meters are open to the environment, and therefore their measurements are influenced by environmental factors such as room temperature or humidity. Closed chamber devices are closed systems that are not dependent on environmental variables. With adequate control of environmental variables, open chamber TEWL meters can provide reliable and reproducible measurements that are comparable to closed chamber TEWL meters (Pinnagoda et al. 1990, 1989; Elkeeb et al. 2010; Fluhr et al. 2006). Yet, only a limited number of comparisons between different types of TEWL meters have been described in the literature until the last few years, and TEWL meters are known to differ in their measurement range, speed, repeatability, and reproducibility (Hui et al. 2012).

Elkeeb et al. (2010) and Fluhr et al. (2006) performed studies which exemplify the general comparability of TEWL meters, but also exemplify their differences. As mentioned in the previous section, Elkeeb et al. (2010) compared TEWL to the percutaneous absorption/flux rate of $^{3}H_2O$ in in vitro human cadaver skin using three different evaporimeters: open chamber evaporimeter A (TEWameter® TM 210, Courage and Khazaka, Cologne, Germany; Acaderm Inc., Menlo Park, CA, USA) and two closed chamber evaporimeters B (VapoMeter™, Delfin Technologies, Kuopio, Finland) and C (AquaFlux AF200, Biox Systems, Ltd, London, UK). TEWL values correlated at baseline and over the 24 h experiment for evaporimeters A and C. However TEWL values of evaporimeter B only correlated with evaporimeters A and C during the experiment and did not correlate at baseline.

An experiment by Fluhr et al. (2006) compared many different TEWL meters in vivo in human and murine skin and ex vivo in

murine skin. TEWL rates obtained with two closed chamber systems (VapoMeter™ (Delfin Technologies, Kuopio, Finland) and H4300 (NIKKISO YSI CO., Ltd, Tokyo, Japan)) and one closed-loop system (MEECO; MEECO, Warrington, PA, USA) under different experimental in vivo conditions were compared with data from four open-loop instruments, i.e., TEWameter® TM 210, TEWameter® TM 300 (Courage and Khazaka, Cologne, Germany), DermaLab (Cortex Technology, Hadsund, Denmark), and EP 1 (ServoMED, Stockholm, Sweden). Through his experiments, Fluhr demonstrated the ability of most of TEWL meters to detect minor, moderate, and severe changes in barrier dysfunction; however, none of the devices could detect minor improvements in barrier function, and there were differences in the TEWL meters' ability to detect differences between severe and very severe barrier dysfunction. However, analysis of all the data collected demonstrated a weak correlation between a few TEWL meters, but an overall good correlation between all the TEWL meters.

An additional study by Farahmond et al. (2009) found similar results to Fluhr et al. (2006) when studying the differences between two closed chamber TEWL measurement instruments. These instruments were designed based on different measurement principles and demonstrated slight differences in their ability to detect changes in skin barrier function despite that the values of all three instruments correlated well with each other ($p < 0.001$).

These studies by Elkeeb et al. (2010), Fluhr et al. (2006), and Farahmond et al. (2009) reveal that there are potential limitations to TEWL meters in experimentation and the TEWL meter must be chosen carefully based on the proposed study design. In general, TEWL meters produced comparable and reliable results; however, in both Elkeeb et al.'s (2010) and Fluhr et al.'s (2006), experiments there were reported TEWL measurements that did not significantly correlate with other measurements. These variations in measurement have the potential to influence experimentation.

6.3.4 Influences of Percutaneous Absorption Measurement Methods

The major factor affecting percutaneous absorption measurements is the used methodology (Bronaugh and Maibach 1989; Wester and Maibach 1992). Methods used for percutaneous absorption measurements are not equal and hence can give different results. Table 6.2 column 3 summarizes the percutaneous absorption measurement methodology used in these correlation studies.

The most common method for determining percutaneous absorption in vivo is measuring the radioactivity of excreta following topical application of a labeled compound. Determination of percutaneous absorption from urinary radioactivity does not account for metabolism by the skin but has been proven to be a reliable method for absorption measurements and is widely accepted as the "gold standard" when available.

The most commonly used in vitro technique involves placing excised skin in a diffusion chamber, applying radioactive compound to one side of the skin and then assaying the radioactivity in the collection vessel on the other side of the skin (Bronaugh and Maibach 1991). The advantages of using this in vitro technique are that the method is easy to use and that the results are obtained quickly. The disadvantage is that the fluid in the collection bath which bathes the skin is saline, which may be appropriate for studying hydrophilic compounds, but is not suitable for hydrophobic compounds. If the parent compound is not adequately soluble in water, then determining in vitro permeation into a water receptor fluid will be self-limiting.

When conducting in vitro experiments, animal skin often substitutes human skin. Because animal skin has different permeability characteristics than human skin, one should be careful which type of animal skin is used (see section on animal vs human skin). In addition, proper care should be taken in skin preparation of excised skin to not damage the skin barrier integrity. Anatomic site is also important, since the skin from different sites shows different permeability as well as using many different donor skin samples.

The only two experiments which did not find a correlation between TEWL and percutaneous absorption, Tsai et al. (2001) and Chilcott et al. (2002), were experiments that measured percutaneous absorption in vitro. Perhaps using a diffusion cell to measure percutaneous absorption is the reason for not finding a correlation.

Oestmann et al. (1993) and Lavrijsen et al. (1993) used laser Doppler flowmeter (LDF) to measure HN penetration. LDF measures the increase in cutaneous blood flow (CBF) caused by the penetration of HN, a vasoactive substance. One problem with this method is that LDF measurements are not only dependent on the amount of HN absorbed but also on the individual's vasoreactivity, gender, and age. This may be the reason why Oestmann et al. (1993) and Lavrijsen et al. (1993) obtained only a weak correlation between TEWL and percutaneous absorption of HN. Another disadvantage of this method is that LDF measurements have many sources of variation which make it difficult to compare inter-laboratory results.

6.3.5 Influence of the Lipophilicity or Hydrophilicity of the Compound Studied

The percutaneous absorption rate and/or total absorption of a compound varies greatly depending on the compound and its lipophilicity. Yet, many of the papers reviewed did not consider how lipophilicity of the test compound would affect percutaneous absorption and hence affect the correlation between TEWL and percutaneous absorption. Feldmann and Maibach (1970) measured both the total absorption and maximal absorption rate for 20 different compounds of different lipophilicities. The range for total absorption for the 20 compounds tested demonstrated a difference greater than 250 times in total absorption amounts, while the 20 compounds that had a difference in maximum absorption rate were greater than 1,000-fold (Feldmann and Maibach 1970). Because of the extreme range of absorption for topically applied compounds, it seems reasonable to assume that the correlation between TEWL and percutaneous absorption may not be independent of the physicochemical properties of the compound applied. Namely, can TEWL measurements predict the skin barrier's permeability changes to both hydrophilic and very lipophilic compounds?

Correlation between TEWL and percutaneous absorption was found in many studies, such as Oestmann et al. (1993), Lamaud et al. (1984), Lavrijsen et al. (1993), Lotte et al. (1987), Aalto-Korte et al. (1993), Tsai et al. (2001a), Elkeeb et al. (2010), Elmahjoubi et al. (2009), Hui et al. (2012), and Atrux-Tallau et al. (2007), which suggest that TEWL can predict the changes in skin permeability to topically applied hydrophilic and lipophilic drugs. However, Tsai et al. (2001b) found that the percutaneous absorption of the highly lipophilic progesterone and estradiol did not correlate with TEWL.

The most common lipophilicity scale of molecules is defined by the octanol-water partition coefficient ($K_{oct/w} = \log(p_{oct/w})$). Presented in Table 6.1 are the compounds used in the aforementioned studies, their octanol-water partition coefficient, their solubility classification, and whether or not their percutaneous absorption correlated with TEWL.

Looking closely at Table 6.1, the highly lipophilic compounds were the compounds that demonstrated a weaker correlation or no evidence of a correlation between percutaneous absorption and TEWL, while the moderately lipophilic compounds such as hydrocortisone and benzoic acid and the hydrophilic compounds did show a correlation. This should be further investigated. As stated previously, it may be necessary to use both TEWL and drug lipophilicity to predict alterations in skin permeability.

Conclusion

In 2005, Levin and Maibach reviewed nine studies investigating the correlation between TEWL and percutaneous absorption of actives. At that time seven of the nine studies demonstrated a quantitative correlation, yet two studies did not. Those studies that did not confirm a quantitative correlation (Tsia et al. 2001b; Chilcott et al. 2002) or only observed a weak correlation (Oestmann et al. 1993; Hui

et al. 2012; Lavrijsen et al. 1993) used different experimental methods, such as an in vitro model, animal skin, or extremely lipophilic compounds compared with the studies which found a quantitative correlation. The conclusion at this time was that those assumptions and differences in experimental design were likely responsible for the lack of correlation. Since then, new studies have been published investigating the use of lipophilic compounds, in vitro models, and animal skin as models for in vivo human skin barrier study (Elkeeb et al. 2010; Hui et al. 2012; Elmahjoubi et al. 2009). These studies have demonstrated significant correlation between TEWL and percutaneous absorption in vitro in human and animal skin for both lipophilic and hydrophilic compounds (Elkeeb et al. 2010; Hui et al. 2012; Elmahjoubi et al. 2009). In this updated overview, 10 of the 12 studies discussed here found some degree of correlation between TEWL and percutaneous absorption. It is uncertain why these two studies found no correlation; however, it seems likely after looking at the compiled data in Table 6.1 that TEWL can serve as a prediction for percutaneous absorption in both in vivo and in vitro models and in human and animal skin and those studies which did not report a correlation between TEWL and percutaneous absorption were the exception rather than the rule. Furthermore, it may be that evaporimeter choice may play a more important role in experimental design than previously assumed.

Taken together, the weight of evidence confirms a relationship between TEWL and percutaneous penetration of actives, yet, much remains to be understood.

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