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Vulvar Transepidermal Water Loss (TEWL) Decay Curves

Effect of Occlusion, Delipidation, and Age

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The kinetics of water desorption after water loading was evaluated by means of transepidermal water loss (TEWL) decay curves. The effects of delipidation and ageing on the desorption of the water biomolecule was investigated in the vulva and on the forearm. All decay constants were analysed by curve stripping analysis and found to fit a bi-exponential decay. There was no significant difference in decay constant when compared by site. However, the terminal decay constant differed significantly after delipidation in both vulva and on forearm ($p<0.02$, $p<0.04$, respectively) in premenopausal women. Significant differences were also noted at post-menopausal forearm and vulva sites after delipidation for the terminal decay constant ($p<0.006$, $p<0.02$, respectively). Delipidation probably alters bound water in the process of sorption. Differences in decay constant when compared between pre- and post-menopausal women were noted only at the vulva for β_1 decay constant ($p<0.05$). β_2 of the forearm was not affected by menopause. Data are also presented to support the notion that structural lipids of the stratum corneum may have a water-holding property. *Key word: Menopause.*

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Human vulvar skin is a specialized skin type. Its unique anatomical position offers a sequestered environment protected from the insults of ultraviolet, temperature, and wind. Certain functional attributes have been assembled through regional comparisons. Transepidermal water loss (TEWL) measurements in comparison with flexor forearm are significantly elevated (1). Yet no differences in stratum corneum water content quantified by microwave probe have been discernible (2). How are such differences in TEWL accountable? Furthermore, women not infrequently complain of a vulvar wetness sensation, irrespective of emotion. Is this phenomenon a function of elevated TEWL in a closed environment, or is vulva water desorption fundamentally different than that of the forearm? This study evaluated the kinetics of water desorption in the hope of shedding light on the unique physiological response of the vulva to the water biomolecule, by utilizing TEWL decay curves. In addition, since the vulva is responsive to sex hormones (3, 4), this technique allows one to evaluate the effects of cessation of hormone production at menopause. TEWL decay curves have previously been used to evaluate moisturizers and skin barrier function (5–8).

MATERIALS AND METHODS

Subjects

Two groups of healthy women, volunteer subjects, pre- and post-menopausal, aged 22–35 years and 48–70 years, respectively were studied. All subjects gave informed consent. Subjects who were pregnant, lactating, or had underlying skin or gynecological disorders were excluded. No subjects were taking oral contraceptives or hormonal supplement. Measurements were made on the labia majora and antecubital area (5 cm from antecubital crease). The subjects were asked not to bathe on the morning of the study. All measurements were performed between the months of late September to November at a room temperature of 22–24°C and relative humidity ranging from 40 to 52%, though not fluctuating more than 1–3% during a study period. Every attempt was made to minimize convection of air in the exam room.

Treatments

The hairs on the forearm and labia majora of female subjects were clipped gently with scissors. Then each site was treated with 10 cc of acetone/ether (1 : 1; v/v) mixture for 30 min as described by Imokawa et al. (9). An untreated control site was chosen on the contralateral side. Each site was occluded with polypropylene chambers 2 cm in diameter (Hilltop Research, Cincinnati, Ohio) for 3 h. The gauze padding within the Hilltop chamber was loaded with 0.4 cc of distilled water and subsequently applied to the particular site.

Measurements

Skin temperature was monitored by telethermometer (Yellow Spring Instrument Co., USA) and ranged from 31–33°C. All

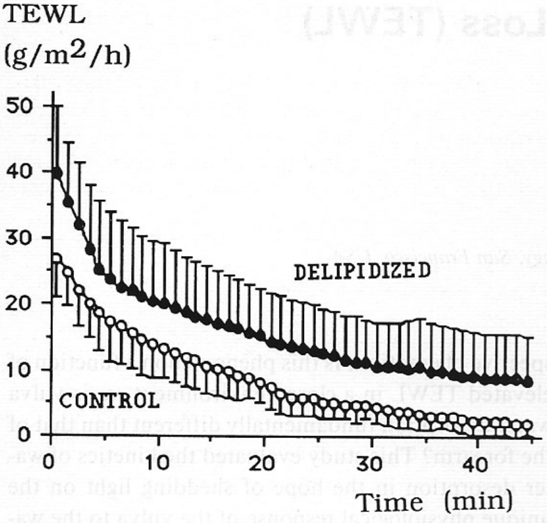

Fig. 1. Typical transepidermal water loss decay curves fitted to biexponential equation, $y=A_0e^{-\beta_1 t}+B_0e^{-\beta_2 t}$. A_0, B_0 are coefficients, β_1, β_2 are decay constants of the initial and terminal phase of the decay curve respectively, and t is time in minutes. Each error bar represents one standard deviation for 10 subjects.

TEWL measurements were corrected for temperature by the methods of Mathias et al. (10). TEWL decay curve values were measured in the following fashion: After removal of the chamber, each site was lightly blotted with tissue paper. TEWL measurements were made with an Evaporimeter (Servo Med AB, Stockholm, Sweden) (11). Readings were taken every 30 s, beginning usually a minute after removing of the plastic chamber and completed by 45 min. All subjects were acclimatized for 20 min to the examining room prior to measurements. The lights were dimmed and visitors excluded to minimize emotional sweating. Vulva TEWL measurements were made while the subject lay on the gynecological table.

Stratum corneum water content (WC)

WC was measured by a capacitance device (Corneometer, CM 820 PC, Courage and Khazaka Electronics, West Germany) (12, 13).

Curve fitting analysis

The TEWL values were analysed to fit a bi-exponential mathematical model. The general form of the bi-exponential equation is:

$$y=A_0e^{-\beta_1 t}+B_0e^{-\beta_2 t}$$

where
A_0, B_0 = coefficients
β_1, β_2 = decay constants 1 and 2 respectively
t = time (min).

Curve fitting was performed on Apple II GS computer using JANA Exponential Curve Stripping program (Statistical Consultants, Inc., Louisville, Ky.) (14). All TEWL measurements were corrected for temperature and background baseline values subtracted prior to analysis.

Statistical analysis

Statistical methods used to demonstrate differences were calculated by Student's *t*-test.

RESULTS

TEWL decay curves

Fig. 1 depicts a typical TEWL decay curve for the premenopausal vulva, untreated (control) and treated site. This curve was generated after values at each corrected time point were averaged. Delipidation caused the average TEWL values to rise at each time point. It also altered the shape of the decay curve in the terminal phase of desorption (see details in text below). Individual curves (not shown) may not have appeared smooth due to small fluctuations in measurements. This was especially true when decay curves were constructed for the vulva. Occasionally, small bursts of increased TEWL measurements were observed. This may have been eccrine sweating and was excluded from calculations when noted.

TEWL decay constants

Table I displays the calculated decay constants generated by JANA Curve Stripping Analysis. These values gave a closer fit than uni-exponential generated values, as compared by *R*-values. β_1 decay constant, always larger than β_2, represents the first phase of desorption decay curve. Delipidation appears to change the shape of the terminal portion of decay curve. Significant differences were seen in β_2 when compared with treated and untreated sites. This difference was observed both on vulva and on forearm, in both pre- and post-menopausal women. No differences were observed when decay constants were compared by region in pre- or post-menopausal women.

Water content

Delipidation, as outlined by the above methods, induced a dry, chafed appearance to the skin that lasted approximately 72 h. Measurements of water content were made at the end of desorption (45 min) of untreated and treated sites. It appears that delipidation induced a decrease in water content (see Table II). However, this decrease in water content may have occurred because the barrier property from stratum corneum lipids was removed, thereby enhancing water loss.

Table I. *Exponential decay constants for pre- (n=10) and post-menopausal (n=9) women in units of min^{-1} ± one standard deviation*

β_2 decay constants of un- and treated arm in both pre- and post-menopausal women differed significantly ($p<0.04$, $p<0.02$, respectively) when tested by Student's paired *t*-test. This difference in β_2 was also observed at the vulva in pre- and post-menopausal women ($p<0.02$, $p<0.006$, respectively). β_1 of either site was not significantly different with respect to site or treatment. β_2 from delipidized sites were not significantly different in pre- versus post-menopausal women ($p>0.15$ for arm, $p>0.17$ for vulva). β_1 from delipidized sites of vulva were significantly different in pre- versus post-menopausal women ($p<0.05$)

	Pre-menopausal		Post-menopausal	
Site	β_1	β_2	β_1	β_2
Arm	0.25±0.21	0.033±0.030	0.35±0.32	0.045±0.027
Arm (delipidized)	0.15±0.07	0.015±0.014	0.12±0.06	0.008±0.001
Vulva	0.26±0.24	0.048±0.024	0.26±0.25	0.035±0.009
Vulva (delipidized)	0.50±0.39	0.028±0.018	0.18±0.12	0.018±0.013

DISCUSSION

The classic works by Blank have demonstrated the paramount role of the water biomolecule on the physical behaviour and attributes of skin (15, 16). Most notably, water is a major plasticizer of the human stratum corneum. We investigated the behaviour of this molecule in the desorption process in vivo. The sorption and desorption of water from the stratum corneum has been studied primarily with in vitro technology. Scheuplein & Morgan characterized the desorption of water by a microbalance technique (17). They introduced the notion of bulk (free) and bound water, observing that the rate of desorption is initially high, representing the loss of bulk water, followed by an abrupt, slower phase of desorption. Similar results have been demonstrated by other investigators using different techniques (18, 19). Although seemingly obvious, the process of desorption is not the mere reverse of hydration (also called sorption); in fact, the desorption of water from the stratum corneum is dependent on the hydration history (15). Interestingly, if one examines the flux of water at equilibrium conditions, experimental calculations show a near constancy of flux over the relative humidities in the range 0–80% (20). We attempted to study the kinetics of water desorption in vivo on the volar forearm and in vulva after water loading. The TEWL decay curves regardless of site, age, or treatment by delipidation of the stratum corneum decayed in an exponential fashion. When analysed by curve-stripping analysis, the decay curves fit a mathematical model of bi-exponential decay. All curves fit bi-exponentially better than a uni-exponential decay reflected by *R*-values > 0.80.

The heterogeneous group of lipids that comprise the intercellular space of the stratum corneum are regulators of barrier function (21). The delipidation technique employed in this study removed lipids beyond the surface. After delipidation, the TEWL values, as expected, were elevated compared with control sites. The TEWL values were higher throughout the curve when compared with control sites (see Fig. 1), reflecting the loss of barrier function provided by the stratum corneum lipids.

Analysis of the desorption curve decay constants revealed that β_1 was not affected by the delipidation procedure. This is consistent with the observations of Scheuplein's work in vitro (17). They observed that the removal of lipids by their treatment did not alter

Table II. *Averaged stratum corneum water content in pre-menopausal (n=10), and post-menopausal women (n=9) ± one standard deviation*

	Water content (aqua meter units)			
Site	Pre-menopausal		Post-menopausal	
Arm	68±11	$p<0.01$	66±8	$p<0.001$
Arm (delipidized)	47±13		42±9	
Vulva	69±14	$p<0.01$	68±15	$p<0.002$
Vulva (delipidized)	51±12		39±11	

the initial phase of desorption. We observed, however, a difference in β_2 after delipidation. This occurred on both the forearm and vulva. The β_2 decay constants also appeared to decrease in value with delipidation. Currently, it is not clear to us why this decrease occurred. We know that the decay constant β_2 represents the terminal portion of the TEWL decay curve where water is slowly desorbed. It is felt that this water reflects the long-term process of hydration and structural alterations must occur to expose new binding sites for the water biomolecule. The putative cellular structure to undergo alteration is keratin (22). Why would delipidation affect such a process? It is possible that one or more of the lipids of the stratum corneum may 'aid' in the stabilization of the water structure in concert with the other components. Delipidation with organic solvents induces dry skin (23). In response to water loading, it appears that the water-holding capacity of delipidized skin is diminished. Water content is diminished at delipidized sites, in comparison with control sites, on the volar forearm and vulva ($p<0.001$, $p<0.002$) in post-menopausal (see Table II). This difference was also seen in pre-menopausal women. This supports the notion that stratum corneum lipids are involved in the water-holding capacity of the stratum corneum (24).

The skin is a target organ for hormonal steroids (25). The vulva has steroid receptor capacity and metabolism higher than in other skin regions (25, 26). Menopause may constitute a biological event with which to examine estrogen withdrawal and its effect on skin. The vulva becomes delicate and parchment-like after middle age. In addition, the number of hair follicles become sparse. TEWL does not appear to vary with age in normal adult skin until after 60 years of age, when there is a significant decrease (27). We could not demonstrate any differences in TEWL decay curves between pre- and post-menopausal skin, except for β_1 of the vulva. This difference was not observed on the forearm. Could estrogen possibly play a role in barrier function in the vulva? The significance of this remains unclear at this time. There were no demonstrable differences in TEWL decay constants from site to site.

Further work needs to be done to delineate the unique properties of the vulva. The vulva may offer a localized site on which to study ageing of the skin, at least as regards the effects of hormonal cessation. This work will hopefully shed light on the physiology of the vulvar skin and be of benefit in the preparation of topical drug and skin care formulations. Studies are being made to fit a physical model to this mathematical model.

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