Effect of hormone replacement therapy on epidermal barrier lipids

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Synopsis

Current evidence of the effect of hormone replacement therapy (HRT) on skin lipids of postmenopausal women is scanty and indirect. Here, we report the ultrastructural differences in epidermal lipids between postmenopausal subjects who were and were not on HRT and a comparison is made with younger subjects. Biopsies were obtained from arms and legs, in a blinded, no-treatment, study conducted on postmenopausal subjects who were and were not on HRT and younger subjects. The ultrastructure of skin lipids and the lipid coverage of underlying corneocytes were compared for biopsies obtained from different subjects. Qualitative assessment as well as quantitative estimation of lipid-covered regions of corneocytes shows that skin lipids do not cover corneocytes effectively in postmenopausal women who are not on HRT. However, women who are on HRT show significantly improved lipid coverage of corneocytes, which is comparable with the younger subjects. This implies that HRT should improve the lipid coverage and skin condition of postmenopausal women.

Résumé

Aujourd'hui, on ne dispose que de preuves rares et indirectes de l'effet de la thérapie de remplacement d'hormones (HRT) sur les lipides de la peau des femmes post-ménopausées. On passe en revue ici les différences ultrastructurales des lipides épidermiques entre sujets post-ménopausés sous HRT ou non, et on effectue une comparaison avec des sujets plus jeunes. On préleve les biopsies au niveau des bras et des jambes sur des sujets post-ménopausés sous HRT ou non et sur des sujets plus jeunes dans le cadre d'une étude en aveugle sans traitement. On compare l'ultrastructure des lipides de la peau et la couverture lipidique des corneocytes sous-jacents dans les biopsies obtenues sur les divers sujets. L'évaluation qualitative et quantitative de la couverture lipidique des corneocytes montre que les lipides de la peau ne recouvrent pas les corneocytes efficacement chez les femmes ménopausées qui ne sont pas sous HRT. Toutefois, on observe chez les femmes sous HRT une amélioration significative de la couverture lipidique des corneocytes comparable à celle des sujets plus jeunes. Ceci implique que le HRT devrait améliorer la couverture lipidique et l'état de la peau des femmes post-ménopausées.

Introduction

The effect of oestrogens on skin ageing is well documented [1]. For example, a multicomunity-based study of postmenopausal women showed that oestrogen use prevents dry skin and skin wrinkling [2]. Ultrasonography measurements have shown significant increase in the thickness of epidermis and dermis after 12 months of hormone
replacement therapy (HRT) treatment [3]. This increase in skin thickness with HRT correlated with increase in bone thickness, which is an established benefit of HRT [4]. A steep increase in skin extensibility with menopause was observed in a study of 43 post- and premenopausal women. HRT prevented this increase, suggesting a preventive effect by oestrogen on skin extensibility [5].

Correlation between skin barrier changes and oestriadiol changes during regular menstrual cycle, in younger women, suggests the involvement of oestrogen in skin barrier properties [5]. Further, a role for oestrogen in the development of fetal skin is suggested in a study [6] that showed that the administration of oestrogen to female pregnant rats accelerated fetal barrier development of the pups. Moreover, skin explants in oestrogen-supplemented medium in vitro showed better barrier formation.

Such evidence suggests the role of oestrogen in skin’s barrier properties. However, the direct effect of HRT on the structure and presence of epidermal lipid barrier has not been studied. It is of interest to characterize the differences in skin condition between pre- and postmenopausal subjects who are and are not on HRT, in both an exposed area (outer lower leg, dry skin prone area) and a relatively unexposed area (inner upper arm, normal skin). Therefore, here we report the ultrastructural comparison of skin lipids in postmenopausal women who are and are not on HRT with that of younger women.

Methods

Design and procedures

This was a no-treatment study, consisting of three cells with 15 female subjects per cell. The test sites were the outer lower leg (either right or left), and the inner upper arm (either right or left). Test sites were visually evaluated for dryness and erythema, and transepidermal water loss (TEWL) measurements were taken. All subjects were required to be in good health with a leg dryness score of ≥ 2.0. Postmenopausal subjects were of age 55–70 and had been postmenopausal for a minimum of 5 years. The postmenopausal subjects (age 55–70) who were on HRT were required to have used a non-bleed form of HRT (oestrogen + progesterone) at a constant dose for a minimum of 5 years. Younger subjects, on the other hand, were of age 20–30 and were required to have a regular menstrual cycle. One 4 mm full thickness punch biopsy was collected from each test site. Ultrastructural analysis of lipids using transmission electron microscopy was performed on a set of 36 biopsies (one each from arms and legs) from 18 randomly selected subjects (six subjects per cell).

Sample preparation

Following excision, each biopsy was rinsed and dissected into c. 0.5-mm pieces in 2.5% buffered (in 100 mM sodium cacodylate, pH 7.0) glutaraldehyde overnight. After washing with the sodium cacodylate buffer the samples were postfixed in 0.2% ruthenium tetroxide in 100 mM sodium cacodylate buffer. Following buffer wash, the samples were dehydrated in graded acetone solutions, infiltrated and embedded in Spurr resin. Sections of 70 nm were deposited on carbon-coated grids, stained using lead and uranium salts, and examined in JEOL 1200 EX transmission electron microscope (JEOL USA, Peabody, MA, U.S.A.).

Image processing

Image analysis was performed on micrographs recorded at 7500× magnification. A series of scanned micrographs, from randomly selected sections obtained from different sample blocks, was subjected to quantitative lipid analysis. The corneocytes in the stratum corneum (SC) are covered by epidermal lipids, which provide a barrier to the movement of water. The barrier properties of skin are governed by the integrity of lipids and on the lipid coverage of corneocytes; e.g. interaction with surfactants causes a change either in the lamellar packing of the lipids or lipid loss causing an increase in TEWL [7]. The lipid coverage of cells was measured using the projected micrographs of ruthenium tetroxide-fixed epidermal sections. Measurements were carried out using Image Pro software Application (Media Cybernetics, Siler Spring, MD, U.S.A.) on micrographs recorded at 7500× magnification from at least three randomly chosen tissue blocks. In order to improve the differentiation between lipids and underlying corneocytes, an edge detection variance filter (7 × 7 pixel) was applied to the image. If necessary, the presence/absence of lipids was confirmed by observing the ultrastructure at higher magnification (60k×). Filtered images were used to trace lipids
and underlying corneocytes to determine the length of the corneocyte and the lipid-covered region, in projection. The ratio of lipid length to corneocyte length, called lipid ratio length, provided a measure of the lipid coverage of underlying corneocytes. Outer two to three layers of corneocytes were used for computing lipid ratio. Perfect value of ratio length would be one, implying complete coverage of corneocytes. A decrease from this value indicates a loss of lipid-covered region.

**Results**

**Stratum corneum and desmosomes**

Ultrastructural studies were performed on biopsies from arms and legs harvested from seven subjects belonging to postmenopausal (group 1), six subjects from postmenopausal group who were on HRT (group 2) and six subjects belonging to 20–30 age group (group 3). In general, leg biopsies, from all the three groups, showed larger number of cell layers in SC compared with that in arm (Fig. 1). But, the average number of cell layers (in SC) in legs was somewhat higher for group 1 than that for the other two groups, which were comparable. Number of corneocyte layers, in arm, was comparable for both, with and without HRT postmenopausal subjects. Younger subjects, however, showed overall a larger number of cell layers in SC from arms. Of the three groups of subjects, the premenopausal group (group 3) showed the thickest SC (arms) compared with the postmenopausal and postmenopausal + HRT groups.

Stratum corneum in leg skin showed cohesive and compactly packed cells with well-preserved desmosomes. However, loosely packed cells, with disintegrated desmosomes were present in skin samples harvested from arms. As mentioned earlier, although the arm samples from group 3 (young) subjects had more cell layers in SC, the desmosomes were found to be normal.

**Lipid morphology**

Lipids when fixed with RuO₄ show distinct presence of periodic bright and dark bands. However, if lamellae are too few or disordered, the periodic bands are not visible. The high magnification views of lipids from arms and legs from various subjects belonging to the three groups are shown in Figs 2–4. The lipids in subjects belonging to group 3 showed well-ordered lamellar structures (Fig. 2), showing periodic pattern of dark and light bands.

All the group 3 subjects showed well-preserved lipids. Postmenopausal subjects (group 1), on the other hand, showed significant loss of lipids (Fig. 3). The lipids were, in general, either too few to form lamellar structures or were disordered. On the other hand, the lipids in postmenopausal subjects, who were on HRT (group 2), displayed marked similarity (Fig. 4) to the ultrastructure of lipids visualized in group 3, young subjects (Fig. 2).

**Lipid ratio length measurements**

Extent of lipid-covered regions of corneocytes was determined by computing the ratio length of lipids as defined earlier. Measurements were conducted from at least two randomly picked regions of each biopsy. Figure 5a shows a low magnification micrograph of corneocytes and lipids. Although the presence of overlying lipids can be discerned in this micrograph, lipids are readily visualized by applying variance filter to the image as is shown in Fig. 5b. Total length of lipids and that of underlying corneocytes was determined using filtered micrographs for each group.

Data of lipid ratio length for arms and legs were analysed using ANOVA F-protected LSD (least significant difference) multiple comparison method using SAS PROC GLM software (SAS Institute, Carg, NC, U.S.A.). The mean lipid ratio length for HRT-treated subjects (0.70) was found to be significantly different from that for postmenopausal women who were not on HRT (mean 0.48,
For group 1, younger women the mean lipid ratio length (0.77) was also significantly different from postmenopausal women ($P = 0.001$). However, no significant difference was found between postmenopausal women who were on HRT and younger women.

Similarly, for legs, significant differences were found between the mean ratio length for postmenopausal women who were (0.74) and were not (0.51) on HRT ($P = 0.024$). As for arms, there were significant differences between younger women (0.68) and the ones who were not on HRT ($P = 0.1$). The combined (arm and leg) mean (SEM) values, for each group, are shown in Fig. 6. Group 1, young subjects, had highest mean lipid ratio length, closely followed by postmenopausal subjects who were on HRT. Postmenopausal subjects who were not on HRT showed significantly less value of ratio length compared with other two groups. Clear differences in ratio length, therefore, are evident between postmenopausal subjects and the subjects belonging to other two groups.

**Figure 2** Representative views of lipid lamellae in biopsies harvested from arms and legs from young premenopausal subjects. Most of the cells were covered with well-ordered lamellar lipids.
Transepidermal water loss measurements showed somewhat higher TEWL for postmenopausal subjects who were not on HRT and was comparable for younger subjects and the subjects who were on HRT (Fig. 7). A comparison of TEWL and lipid ratio length (Fig. 8) suggests the presence of an inverse relationship between the two – however, with weak correlation.

**Discussion**

The present study was designed to examine the effect of HRT on skin lipids of postmenopausal subjects. The treatment group was compared with those postmenopausal subjects who were not on HRT and also with younger, premenopausal subjects. The premenopausal subjects showed the presence of well preserved and ordered lipid lamellae throughout the SC. The

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**Figure 3** In postmenopausal who were not on hormone replacement therapy the lipids were irregular, few and/or disordered as seen in the representative views from biopsies harvested from arms and legs of the these subjects.
Figure 4 In postmenopausal women who were on hormone replacement therapy (HRT), the cells in skin biopsies harvested from arms and legs were extensively covered with well-ordered lipids, in contrast to the women who were not on HRT.

postmenopausal subjects who were not on HRT had severely degraded lipids, implying that either lipids were not present in sufficient quantity to form lamellae structures or the lamellae were not well ordered. HRT-treated subjects showed significant improvement in the extent of lipid coverage of cells and the lipids were found to be similar to those in younger subjects.

Stratum corneum provides the main barrier to water loss from skin. Disruption to the barrier results in enhanced TEWL. Besides the integrity of the barrier, the water loss depends upon age (though evidence is conflicting, [8]), sex and anatomical site [9] skin thickness and probably on the lipid composition, which changes with age [10]. Although not considered significant, there did appear to be a trend towards a lower TEWL for postmenopausal subjects who were on HRT compared with those subjects who were not on HRT. Likewise, a similar trend was observed between
the lipid ratio length and TEWL. This is in contrast with an earlier study [11] in which older subjects were found to have a somewhat lower TEWL values compared with younger subjects, however, the status of HRT usage for the subjects was not known. It is tempting to suggest that there indeed are differences in TEWL between non-HRT and HRT-treated subjects which correlates with their lipid ratio lengths, however, it needs to be established using a larger set of subjects.

Figure 5 Low magnification micrograph (a) shows the presence of lipids (arrows), which are highlighted by applying an edge detection filter (b) to facilitate computation of lipid-covered regions.

Figure 6 Average lipid ratio length (SEM) for arms and legs for the three groups showed significant differences between postmenopausal women who were on hormone replacement therapy and the ones who were not.

Figure 7 Transepidermal water loss (TEWL) measurements for the three groups show somewhat higher TEWL for the postmenopausal women who were not on hormone replacement therapy (HRT) compared with the subjects who were on HRT.

Figure 8 A comparison of transepidermal water loss (TEWL) and lipid ratio lengths for various subjects/sites shows a weak correlation between TEWL and lipid ratio.

References


