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Menopause increases the iron storage protein ferritin in skin

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Synopsis

Menstruation and desquamation are important routes for humans to excrete iron. Because menstruation is no longer available in postmenopausal women, in the present study, we examined whether iron accumulates more in postmenopausal skin than in premenopausal skin. Skin biopsy samples were obtained from six pre- and six postmenopausal Caucasian women. Iron levels in the form of ferritin were 42% higher, but vascular endothelial growth factor and total antioxidant capacity were 45% and 34% lower in postmenopausal skin (58.8 ±1.3 years old) than in premenopausal skin (41.6 ±1.7 years old), respectively. Moreover, *in vitro* cultured normal human epidermal keratinocytes had surprisingly high levels of ferritin when compared to immortalized human breast epithelial MCF-10A cells or human liver HepG2 cancer cells. Our results indicate that skin is a cellular repository of iron and that menopause increases iron in skin and, thus, may contribute to the manifestation of accelerated skin aging and photo aging after menopause.

INTRODUCTION

Menopause probably has the greatest impact on a woman's health. Structural and functional changes attributable to decreased estrogen as a result of menopause are visibly evident in the skin, which becomes thin and dry with increased wrinkle formation (1). Consequently, the skin becomes more vulnerable to environmental stressors, such as ultraviolet (UV) exposure (2). Previously, we reported that serum ferritin is significantly higher in postmenopausal women than in premenopausal women and is independent of diet or smoking habits (3). Although systemic estrogen decreases because of the cessation of ovarian function, we have recently shown that estrogen downregulates the iron hormone hepcidin leading to enhanced iron uptake (4). Thus, estrogen deficiency does not

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lead to iron buildup in postmenopausal women, yet iron in the form of ferritin increases as a result of decreasing menstrual periods (5).

Ferritin is an iron storage protein that can bind up to 4,500 atoms of iron per molecule of ferritin (3), and increasing evidence shows that ultraviolet A (UVA, 320–400 nm)-induced damage to the skin could be mediated through ferritin (6). For example, we recently found that ferritin sensitized primary human dermal fibroblasts to UVA, which resulted in a significant release of matrix metalloproteinase-1 (MMP-1) (7).

Iron is excreted through menstruation and skin desquamation (8). Because postmenopausal women no longer menstruate, here we examined whether postmenopausal skin accumulates more ferritin. We further compared ferritin levels in normal human epidermal keratinocytes (NHEK) to other cell types.

MATERIALS AND METHODS

HUMAN STUDIES

After consent from our institutional review board, clinical subjects were chosen based on a questionnaire that selected for healthy, nonlactating women on a normal American diet. Three millimeter punch human skin biopsies were obtained from six pre- and six postmenopausal Caucasian women by punching the upper, inner left, and inner right arms. After homogenization, the concentration of the protein extracts was determined by the bicinchoninic acid assay (Thermo, Rockford, IL). The remaining protein extracts were used for ferritin, vascular endothelial growth factor (VEGF), and total antioxidant capacity (TAC) analysis. Two data points (left and right arm) per subject were presented in the study.

Levels of ferritin were determined by an enzyme-linked immunosorbent assay (ELISA) as previously reported (3). Levels of VEGF were measured using an ELISA kit (Biosource Inc., Camarillo, CA). Levels of TAC were measured using the OxiSelect™ TAC assay kit (Cell Biolab, Inc., San Diego, CA). The results were expressed as nanogram ferritin, picogram VEGF, or absorbance [optical density (OD)] per milligram of total protein.

CELL CULTURE AND FERRITIN MEASUREMENT IN CELLS

NHEK were obtained and cultured in EpiLife® medium (Cascade Biologics, Portland, OR). For comparison, human hepatocarcinoma HepG2 cells with known high levels of ferritin and immortalized human mammary epithelial MCF-10A cells were also tested (ATCC, Manassas, VA). To unify the background levels of ferritin, cells were initially grown in their respective media but subsequently changed to Dulbecco's Modified Eagle Medium containing 1% fetal bovine serum (FBS) for at least 24 h before ferritin analysis.

STATISTICAL ANALYSES

All values are expressed as means ± standard deviation (SD). Student's *t* tests were used for comparison between experimental groups (p value < 0.05).

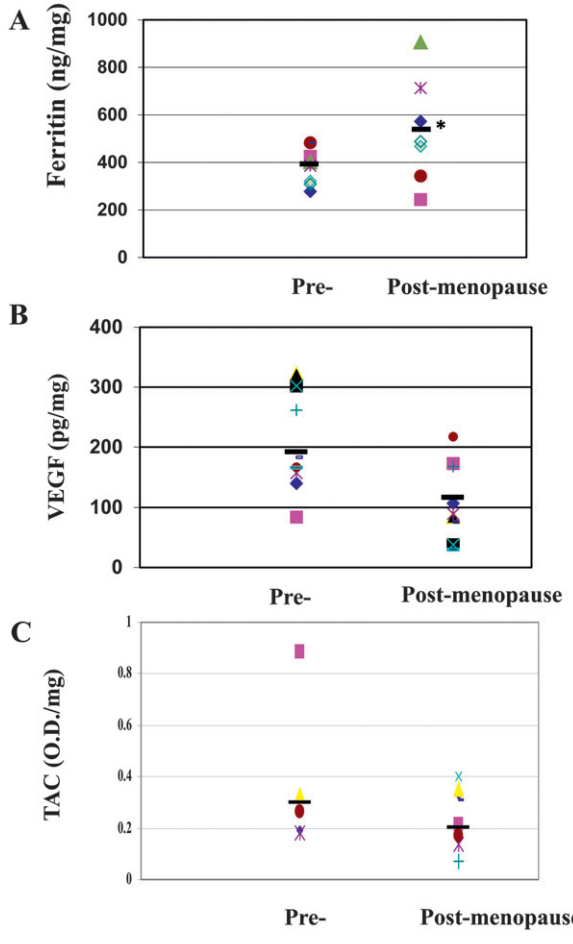

Figure 1. Differences in levels of ferritin (A), VEGF (B), and TAC (C) between skin biopsy samples of pre- and postmenopausal women. Mean age of postmenopausal women was 58.8 ± 1.3 ($n = 6$), and mean age of premenopausal women was 41.6 ± 1.7 ($n = 6$). (A) The mean level of ferritin for premenopausal subjects was 381.6 ng (±74.0 SD, $n = 12$), whereas for the postmenopausal subjects it was 542.4 ng (± 194.3 SD, $n = 10$). (B) The mean VEGF level in the premenopausal subjects was 198.0 pg (±75.0 SD, $n = 12$), and for the postmenopausal subjects it was 109.1 pg (±60.2 SD, $n = 10$). (C) The mean TAC in the premenopausal subjects was 0.32 (±0.20 SD, $n = 12$), and for the postmenopausal subjects it was 0.21 O.D (±0.18 SD, $n = 10$). Results were expressed as nanogram ferritin per mg protein, picogram VEGF per mg protein, and TAC as absorbance (OD) per mg protein. *Significant difference from premenopausal skin, $p < 0.05$.

RESULTS

We analyzed levels of ferritin, VEGF, and TAC in human skin biopsies. Premenopausal women had a mean age of 41.6(±1.7 years, $n = 6$, range 40–44). For postmenopausal women, the mean age was 58.8 (±1.3 years, $n = 6$, range 55–60 years). Two skin biopsy samples were collected from both arms of each subject, and a total of 24 skin samples was used in the present study. Figure 1A shows that the mean level of ferritin was significantly higher in postmenopausal skin (542.4 ng/mg protein ± 194.3, $n = 10$, range 243.5–905.8 ng/mg) than in premenopausal skin (381.6 ng/mg protein ± 74.0, $n = 12$, range 277.4–481.5 ng/mg, $p < 0.05$), a 42% increase. One outlier from postmenopausal

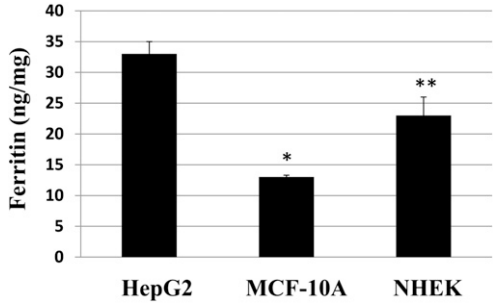

Figure 2. Comparison of background levels of ferritin among human liver HepG2 cells, immortalized human breast epithelial MCF-10A cells, and NHEK. Cells were originally grown in their respective media. After 24 h, 1% FBS was added to the media until cells reached 70–80% confluency. After washing, cells were scraped and lysed for total protein and ferritin measurements. Data were expressed as nanogram ferritin per milligram protein. *Significant difference from HepG2 cells ($p < 0.05$); **Significant difference from MCF-10A cells ($p < 0.05$).

skin with a left arm ferritin of 2,360.2 ng/mg protein and a right arm ferritin of 989.0 ng/mg protein was excluded in the analyses. Otherwise, the difference would be even more significant. The mean level of VEGF in postmenopausal skin was 109.1 pg/mg protein (±60.2, $n = 10$, range 30.0–217.1 pg/mg), lower but statistically insignificant than in premenopausal skin (198.0 pg/mg protein ± 75.0, $n = 12$, range 84.1–322.5 pg/mg) (Figure 1B). The mean level of TAC in postmenopausal skin (Figure 1C) was 0.21 OD/mg protein (±0.11, $n = 10$, range 0.11–0.39 OD/mg), also lower but statistically insignificant than in premenopausal skin (0.32 OD/mg protein ± 0.35, $n = 12$, range 0.2–0.94 OD/mg protein).

We then compared the levels of ferritin in keratinocytes to those in immortalized human breast epithelial MCF-10A cells and human liver cancer HepG2 cells. Figure 2 shows that ferritin in liver HepG2 cells, derived from an organ targeted by iron, was significantly higher (33 ± 2.0 ng/mg protein, $n = 6$) than in breast epithelial MCF-10A cells (13 ± 0.6 ng/mg protein, $n = 6$). To our surprise, the levels of ferritin in NHEK were 23 ± 5.1 ng/mg protein ($n = 4$), only slightly lower than those in HepG2 cells.

DISCUSSION

In conclusion, although reduced estrogen in skin is considered one of the main etiological factors in postmenopausal skin aging, we show here that menopause increases ferritin in postmenopausal skin by 42%, suggesting that skin becomes a main portal for the release of excess iron in menopause. Considering the increased cancer risk of hormone replacement therapy in postmenopausal women (9), targeting increased iron, independent of estrogen deficiency, presents a novel skin improvement therapy for this population. This finding is highly significant because human skin is constantly exposed to UV that can increase hydrogen peroxide levels in NHEK (10). Thus, as iron is released by ferritin after UVA irradiation (6), it is in the ferrous form, which can react with hydrogen peroxide to form highly reactive hydroxyl radicals leading to biomolecular damage (11). Thus, future treatments should include strategies that specifically chelate, remove, or block iron transport in noncytotoxic ways. In view of the previously reported protective effects of iron

chelators on skin (12) in which we showed the protective effects of a UVA-photo activated iron chelator, our results indicate that increased iron as a result of menopause may make postmenopausal skin more susceptible to chronological aging as well as photo aging in postmenopausal women. Reductions in TAC and VEGF also support increased vulnerability to oxidative stress and reduced wound healing, respectively, as a result of increased ferritin in skin. Finally, we observed levels of ferritin in NHEK comparable to that of HepG2 cells, a liver cancer cell that is rich in iron. Taken together, our study shows that menopause increases ferritin in skin and that this increased iron is a potential risk factor contributing to the manifestation of accelerated skin aging after menopause.

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