Skin indentation firmness and tissue dielectric constant assessed in face, neck, and arm skin of young healthy women

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Purpose: Our goal was to test the hypothesis that skin firmness correlates with skin hydration.

Methods: Dermal water was assessed by tissue dielectric constant (TDC) at 0.5 mm (TDC_{0.5}) and 2.5 mm (TDC_{2.5}) depths on four face sites and two arm sites of 35 women (25.0 \pm 1.6 years). Firmness was determined by force (mN) to indent skin to 0.3 mm (F_{0.3}) and 1.3 mm (F_{1.3}).

Results: $F_{0.3}$ was similar among face sites (avg = 16.2 ± 7.2 mN) but $F_{1.3}$ varied (avg = 32.5 ± 4.1 mN). TDC_{2.5} was similar among face sites (avg = 37.7 ± 4.2) but TDC_{0.5} varied (avg = 36.2 ± 4.8). $F_{1.3}$ of arm sites was similar (avg = 60.2 ± 18.6 mN) and both greater than $F_{1.3}$ of neck (28.3 ± 7.1 mN) and face. Regression analysis showed a near-zero correlation between forces and TDC at all sites.

Conclusion: The near-zero correlation may be due to low skin interstitial hydraulic resistance to mobile water movement in

ECHANICAL PROPERTIES of skin change with Mage and some pathological conditions. Examples include skin softness, elasticity, and fibrosis associated with lymphedema progression subsequent to cancer treatment (1, 2), radiation therapy (3–5), systemic sclerosis (6, 7), and wound healing (8-11) with some changes in some of these conditions linked to inflammatory mediators (12). Interestingly, although lymphedema-related fibrosis has been associated with increased interstitial protein, some data suggest roles for inflammatory mediators (13) indicating that accumulation of interstitial protein may not be a sufficient fibrotic causative mechanism (14) in which experimental data link fibrosis to further lymphatic dysfunction (15). Assessments of skin mechanical properties including skin softness or firmness and elastic features are thus potentially useful to detect healthy young skin. If true, then conditions in which dermal hydraulic conductance is reduced as in lymphedematous, diabetic, or aged skin are more likely show the hypothesized relationship. Our findings provide normalized reference values and suggest that such persons are an important population to study to test for a possible skin water-indentation force relationship and its utilization for early diagnosis.

Key words: skin dielectric constant – skin water – skin fibrosis – skin softness – edema – lymphedema

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skin changes that accompany the insidious progression of pathological process. They may also be useful to evaluate sequential normal agerelated changes (16–19) that occur for a variety of age-related mechanisms (20–22) and to track outcomes of therapeutic interventions designed to lessen pathological or age-related skin-related changes.

Various methods have been used to measure different aspects of skin's mechanical properties for widely differing purposes. These include methods that indent skin statically or dynamically to assess softness or viscoelasticity (16, 23–29), methods that stretch skin, mostly via suction methods (17, 18, 30–37), and various imaging methods including ultrasound (4). Many of these methods essentially depend on research-type instruments, either specially designed or often bench bound, that are either not generally available or are not suited to rapid mobile measurements in a busy and timepressured clinical environment.

Recently, two hand-held noninvasive portable devices have become available that may provide a more convenient and rapid assessment method that will permit rapid quantitative assessment of certain aspects of skin mechanical properties using indentation methods. These devices indent skin to nominal depths of 0.3 mm and 1.3 mm while automatically recording the force required for the indentation. This force then becomes a quantitative measure of the skin's resistance to deformation. The availability of these devices allows study of fundamental issues related to possible linkage between skin tissue water and skin mechanical properties. In this regard, it is widely stated that skin stiffness and firmness (softness) is largely influenced by its water content (38) although this concept is not without detractors (39) and reported improvements in skin hydration do not necessarily lead to improved elasticity (40). However, others have suggested that the water content of the dermis increases indentation resistance (41). It was our initial hypothesis that skin hydration, when measured in upper dermis and deeper, would in fact directly correlate with skin softness as assessed by tissue indentation resistance. Thus, a main goal of this study was to test this hypothesis by comparing indices of local skin water via tissue dielectric constant (TDC) measurements (42-49) with skin indentation forces measured at the same skin targets. Further, because the devices used to measure indentation forces are relatively new and little is known about the range of values to be expected, a secondary goal was to provide such background reference values for future potential clinical uses that might include assessments of facial skin conditions and skin fibrosis in persons treated for breast or head and neck cancer.

Methods

Subjects

Eligibility was restricted to females aged 18– 30 years who had not had prior injectable fillers in their face, neck, or arm. All participants were health professional students at Nova Southeastern University who were recruited around campus for this study. Research study goals and procedures were explained to all potential

participants and any who chose to participate signed a consent form that was approved by the university institutional review board. Subjects were 35 Caucasian women with ages between 23 and 29 years with age (mean \pm SD) of 25.0 ± 1.6 years. Body mass index (BMI) range was 16.9–34.9 kg/m² (22.8 \pm 4.1 kg/m²). Total body fat (TBF) percentage, measured as described subsequently, ranged from 14.2 to 49.8% (28.9 \pm 7.6) and total body water percent (TBW) ranged from 38.2 to 62.4% (52.5 \pm 5.3). A tentative 'healthy' TBF range for Caucasian women in the age range of the present study is 21-32% (50). Accordingly, 26 subjects (74.3%) would be classed as within the healthy range, 2 (5.7%) as under fat and 7 (20%) as over fat. Based on BMI criteria, 2 (5.7%) were classed as underweight (BMI <18.5 kg/m²), 27 (77.1%) were 18.5–24.9 kg/m², 3 (8.6%) were overweight (BMI 25–29.9 kg/m²), and 3 (8.6%) were classed as obese (BMI \geq 30 kg/m²). Each subject completed a Fitzpatrick skin questionnaire from which their Fitzpatrick score and skin type were determined (51). Scores and skin types were 25.4 ± 5.6 and 3.6 ± 0.9 , respectively. For skin type, 3 subjects were classed as type II (8.6%), 14 (40%) as type III, 13 (37.1%) as type IV, and 5 subjects as type V (14.3%). For the group, 19 (54.3%) were currently taking oral birth control and 6 (17.1%) had experienced moderate-tosevere facial acne previously. None were smokers. At measurement, time from the end of the last menstrual cycle was 13.6 \pm 10.5 days.

Anatomical sites for measurements

Sites measured in this study included four face sites (A, B, C, and D), two neck sites (N1 and N2) and two arm sites (F1 and F2). Locations of the face sites (Fig. 1) were as follows; A was 2 cm anterior to the midline of the tragus, C was 2 cm lateral and inferior to the commissure of the mouth, B was halfway between A and C, and D was 1 cm lateral and 2 cm superior to the commissure of the mouth. Neck sites N1 and N2 were 8 cm and 10 cm inferior to face site B. Arm sites A1 and A2 (not shown) were located on the anterior forearm 6 cm distal to the antecubital fossa (A1) and on the anterior biceps 6 cm proximal to the antecubital fossa (A2). All measured sites were on the right side of the body and all measurements were made with subjects seated.



Fig. 1. Measurement sites on the face and neck. Face site A is 2 cm anterior to the tragus, and site C is 2 cm lateral and inferior to the commissure of the mouth. Site B is located halfway between sites A and C, while site D is 1 cm lateral and 2 cm superior to the commissure of the mouth. Neck sites N1 and N2 are located 8 and 10 cm inferior to face site B, respectively. Not shown are arm sites A1 and A2 that were, respectively, 6 cm distal and 6 cm proximal to the antecubital fossa on the anterior arm.

Indentation force measurements

To measure skin tissue firmness, the indentation force in milliNewtons (mN) required to indent skin to 0.3 mm ($F_{0.3}$) or to 1.3 mm ($F_{1.3}$) was determined using two commercially available hand-held battery-operated devices. One was the ElastiMeter (0.3 mm) and the other the SkinFibroMeter (1.3 mm) both made by Delfin Technologies, Kuopio Finland. In use, skin is lightly touched whereupon a small indenter approximately 2 mm in diameter is caused to deform skin inwardly with the resultant force recorded and displayed on a window on the front of the device. Each device is equipped with internal sensors that accept measurements only within prescribed limits of force and velocity. This means that if an applied force is too large or applied too slowly or rapidly, the software contained within the device prompts to repeat the measurement until it is within the set limitations of the device. A single recorded value is obtained as the average of five acceptable sequential measurements made rapidly in succession. The time to make these five sequential measurements at a single site is about 5 s. In the present protocol, each site was measured completely five separate times. $F_{1,3}$ was determined at all sites (face, neck, and arm), whereas $F_{0.3}$ was determined only at face sites.

Tissue dielectric constant (TDC) measurements

Tissue dielectric constant, which is the ratio of the skin tissue dielectric constant to that of a vacuum, was measured at 300 MHz using two commercially available hand-held battery-operated devices that function in a manner similar to an open-ended coaxial transmission line (47, 49, 52). At 300 MHz, TDC values are sensitive to both free (mobile) and bound water within the measurement volume (53, 54). The devices are made by Delfin Technologies and are stated to measure TDC values to depths below the skin surface of about 0.5-1.0 mm (MoistureMeterEpiD) and 2.5 mm (MoistureMeterDCompact). Although both devices convert and show values as percentage water, the present results give the actually measured TDC values, which for reference is about 76 for water at 32°C. Many reports regarding the physics (46, 49, 55-57) and use of TDC measurements are available (42, 58–62), but the compact devices herein used are relatively newer and their reported use in the literature is more limited although values obtained are similar with those previously reported (58). In the present study, both TDC measuring devices were tested against known values for various ethanol/water concentrations to insure intrinsic accuracy. All such measurements agreed with published values within $\pm 2.5\%$. In use, the device is applied to the skin for about 8-10 s whereupon a 300-MHz signal that is generated within the device is transmitted to the tissue with a portion of the incident electromagnetic wave reflected in an amount that depends on the dielectric constant of the tissue. Herein, face (A-B-C-D) and arm sites (A1 and A2) were measured to depths of 0.5–1.0 mm (TDC_{0.5}) and 2.5 mm (TDC_{2.5}) five separate times. No TDC measurements were made on the neck.

Protocol and procedures

All indentation force and TDC measurements were made by the same investigator, while subjects were seated in a room in which measurements could be done with minimum distractions. Prior to measurement start and while subjects were acclimating in a seated

position, the study was explained, the informed consent was signed, and a Fitzpatrick skin scale questionnaire was completed. Thereafter, target sites were marked with a surgical pen for reference. During this and the prior acclimation time, subjects were comfortably seated with right forearm resting on a flat table. The first set of measurements was on the face with measurements proceeding from A to B to C to D. This measurement sequence A-B-C-D was repeated five times for each parameter in the following temporal order (1) $F_{1,3}$, (2) $F_{0,3}$, (3) $TDC_{0,5}$, and (4) $TDC_{2.5}$. The set of five face measurements for each parameter was completed before doing the next parameter measurement set. The complete facial measurement set took about 12 min. Neck F_{1,3} measurements were done next alternating between N1 and N2 until five complete sets were obtained. Finally, arm measurements were done alternating between A1 and A2 in the sequential order of $F_{1,3}$, $TDC_{0,5}$, and $TDC_{2,5}$ with each parameter measured five times prior to measuring the next parameter. The neck and arm measurements together took about 5 min to complete. At the end of these measurements, each subject stood barefoot on a body composition scale (Tanita BC-558, Tokyo, Japan) while gripping an electrode in each hand to determine their weight, TBF percentage, and total body water percentages (TBW). This device measures the electrical impedance of the body at 50 kHz which together with gender, age and height provide input to a company private algorithm that is based on a model representation of the body components to estimate fat and water.

Results

Face skin parameter values by location

As summarized in Table 1, face sites A and D had the greatest resultant forces for indentations

TABLE 1. Face skin parameter values by location

of 0.3 and 1.3 mm with $F_{0.3}$ and $F_{1.3}$ measured at A and D being significantly greater than force values measured at sites B and C (P < 0.01). The largest mean percentage differences among sites for $F_{1,3}$ were between sites A and C with a mean difference of 10.7 \pm 8.6% and between sites D and C for $F_{0.3}$ with a mean difference of 7.7 \pm 7.3%. At every measured facial site, the larger indentation force $F_{1,3}$ was significantly greater than the corresponding $F_{0,3}$ (P < 0.001). Face site A had the greatest $TDC_{0.5}$ value that was significantly greater than all other sites (P < 0.001). The largest mean percentage differences among sites for $TDC_{0.5}$ were between sites A and B with a mean difference of $6.5 \pm 7.3\%$. Contrastingly, face sites C and D demonstrated the largest TDC_{2.5} values which were significantly greater than those measured at sites A and B (P < 0.01). The largest mean difference was between sites C and B with a mean difference of $2.6 \pm 2.8\%$. At each site, $TDC_{0.5}$ differed significantly from $TDC_{2.5}$ (P < 0.01) although only face site A was TDC_{0.5} greater than $TDC_{2.5}$, whereas it was less than TDC_{2.5} at the other face sites (P < 0.01).

Forearm and neck skin parameter values

As summarized in Table 2, $F_{1.3}$ did not significantly differ between forearm and biceps 58.8 ± 21.5 vs. 61.6 ± 18.0 mN with an arm average value of 60.2 ± 18.6 mN. But, ear arm value was significantly greater than at neck sites which averaged 28.3 ± 7.1 mN, P < 0.001. $F_{1.3}$ also did not differ between the two measured neck sites separated by two cm. TDC_{0.5} and TDC_{2.5} also did not differ significantly between forearm and biceps with the average of the forearm and biceps arm values for $F_{0.5}$ and $F_{2.5}$ being 32.0 ± 4.2 and 27.0 ± 4.1 , respectively. TDC_{0.5} was significantly greater than TDC_{2.5} at both arm sites (P < 0.001).

Parameter	Location on face							
	A	В	С	D	Average			
F _{0.3}	$16.9\pm3.5^*$	14.7 ± 3.0	14.4 ± 4.3	18.8 ± 4.0*	16.2 ± 7.2			
F _{1.3}	$41.0 \pm 16.3^{*}$	$\textbf{27.2} \pm \textbf{7.6}$	25.3 ± 6.7	$36.5 \pm 8.6*$	32.5 ± 4.1			
TDC _{0.5}	40.2 \pm 5.3†	31.4 ± 8.5	$\textbf{36.8} \pm \textbf{6.2}$	36.4 ± 5.7	$\textbf{36.2} \pm \textbf{4.8}$			
TDC _{2.5}	35.9 ± 4.7	35.7 ± 4.2	$39.9 \pm 5.6 \ddagger$	$39.4 \pm 5.2 \ddagger$	$\textbf{37.7} \pm \textbf{4.2}$			

 $F_{0.3}$ and $F_{1.3}$ are resultant forces (mN) to indentations of 0.3 and 1.3 mm. $TDC_{0.5}$ and $TDC_{2.5}$ are tissue dielectric constants measured to effective depths of 0.5 and 2.5 mm. Values are mean \pm SD for N = 35 female subjects. Locations A, B, C, and D are as in Fig. 1. Force values at A and D were greater than at B and C (*P < 0.01). At all sites, $F_{1.3}$ was significantly greater than $F_{0.3}$ (P < 0.001). $TDC_{0.5}$ was greatest at site A (*P < 0.01) compared to all other sites). $TDC_{2.5}$ at sites C and D was larger than at sites A and B (*P < 0.01). At each site, $TDC_{0.5}$ differed significantly from $TDC_{2.5}$ (P < 0.01).

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TABLE 2. Arm and neck skin parameter values

Parameter	Anatomical location	Anatomical location							
	Forearm	Biceps	Arm AVG	Neck A	Neck B	Neck AVG			
F _{1.3}	58.8 ± 21.5	61.6 ± 18.0	60.2 ± 18.6*	29.2 ± 9.3	27.3 ± 11.3	28.3 ± 7.1			
TDC _{0.5}	$31.8\pm4.2\dagger$	$32.3\pm4.4\dagger$	$32.0\pm4.2\dagger$						
TDC _{2.5}	$\textbf{27.4} \pm \textbf{4.2}$	$\textbf{26.6} \pm \textbf{4.2}$	$\textbf{27.0} \pm \textbf{4.1}$						

 $F_{1.3}$ is the resultant force (mN) to 1.3 mm indentation and TDC_{0.5} and TDC_{2.5} are TDC values measured to effective depths of 0.5 and 2.5 mm. Values are mean \pm SD for N = 35 female subjects. Forearm is on anterior forearm and biceps is on anterior biceps. Neck A and neck B are lateral neck sites separated by 2 cm. $F_{1.3}$ did not significantly differ between the two arm sites or between the two neck sites, but $F_{1.3}$ on arm was significantly greater than on neck (*P < 0.001). Neither TDC_{0.5} nor TDC_{2.5} differed significantly between arm sites, but TDC_{0.5} was significantly greater than TDC_{2.5} (*P < 0.001) at both arm sites.

Correlations among parameters

Based on a total of 140 separate face measurements (35 subjects \times 4 face sites/subject), there was a highly significant linear positive correlation (r = 0.723, P < 0.001) between $F_{0.3}$ and $F_{1.3}$ (Fig. 2) in which the linear regression equation is given by $F_{1.3} = 1.75 F_{0.3} + 2.97 mN$. The correlation between $TDC_{0.5}$ and $TDC_{2.5}$ was also significant (r = 0.318, P < 0.001) with an overall linear regression equation given by $TDC_{0.5} =$ 0.429 TDC_{2.5} +19.9. However, no significant correlation between face $F_{0.3}$ or $F_{1.3}$ values and either of the measured face TDC parameter could be demonstrated (Fig. 3). Similar results were found for arm measurements which had a significant positive correlation (r = 0.777,P < 0.001) between TDC_{0.5} and TDC_{2.5} with a regression equation $TDC_{0.5} = 0.8TDC_{2.5} + 11$ but there was essentially no correlation between arm $F_{1,3}$ values and either TDC_{0.5} or TDC_{2.5} as shown in Fig. 4. Tests for correlations between any measured skin parameter and total body



Fig. 2. Facial indentation forces. Data points are from a total of 140 separate face indentation measurements (35 subjects × 4 face sites/ subject) to 0.3 mm ($F_{0.3}$) and 1.3 mm ($F_{1.3}$) expressed in mN. There was a highly significant linear positive correlation (r = 0.723, P < 0.001) between $F_{0.3}$ and $F_{1.3}$ in which the linear regression equation is given by $F_{1.3} = 1.75 F_{0.3} + 2.97 \text{ mN}$.

fat, total body water, and Fitzpatrick skin score also showed no statistical significance. But, as expected, total body fat percentage and body mass index were highly correlated (r = 0.785, P < 0.001).

Discussion

Parameter variations with menstrual cycle and Fitzpatrick score

The fact that there was no correlation between the time point within the menstrual cycle and measured TDC values that are reflective of skin water changes is consistent with a prior report (45) in which TDC values were assessed on the forearm of young women at various phases of their menstrual cycle. Therein no relationship was found between TDC values and the phase of menstrual cycle in which measurements were made. The present findings confirm this for the forearm values and extend this prior observation to include the four measured facial areas



Fig. 3. Absence of TDC–force correlation in face measurements. Values are TDC–F1.3 pairs for TDC depths of 0.5 mm (open square) and 2.5 mm (closed square). Regression lines indicate near-zero correlation among parameters.



Fig. 4. Absence of TDC–force correlation in arm measurements. Values are TDC–F1.3 pairs for TDC depths of 0.5 mm (open square) and 2.5 mm (closed square). As in the case of the face measurements, arm regression lines indicate near-zero correlation among parameters.

for shallow (0.5 mm) and deeper (2.5 mm) skin depths. Although we have failed to demonstrate such TDC changes, others have indicated that various skin dermatoses are linked to menstrual cycle phases (63). Further, the absence of a measureable menstrual cycle-related difference in this and prior studies is in opposition to the concept that estrogen, via an induced increase in dermal hyaluronic acid, causes an increase in dermal water (64). It may be that such estrogen-linked changes are sufficiently subtle as to not be detected by the present measurements. The present results also indicate no menstrual-related detectible relationship between indentation force as measured on face, neck, or forearm. As far as we are able to determine, no other indentation data of the type herein obtained are available for comparison. However, forearm skin extensional data that were compared between day 10 and day 25 of the menstrual cycle in 20 women of the age range herein used suggested the possibility of a change in extensional properties (65). The fact that we detected no correlation between any measured skin parameter is consistent with data that compared hydration of the stratum corneum with various forced extensional skin parameters (66).

Parameter variations among sites

The finding that indentation-related forces varied among face, neck, and forearm is not inconsistent with site dependence as demonstrated

by the presence of extensional and recovery differences of these sites in a subgroup of women (N = 30) with an age range similar to the present group (18). These authors used Cutometer technology to measure various extensional parameters at cheek, neck, and forearm and found significantly greater initial skin extension (Ue) at neck and least at cheek with recovery parameters least at cheek although most parameter values have been found to significantly correlate among sites (17). Herein, the face site most closely associated with the cheek is face site B that had an indentation force significantly less than found on either arm site although no significant difference between cheek and neck was observed. We conclude from this that although variations in skin mechanical properties are anatomical site dependent, the nature of this variability depends on whether extensional or compressional assessments are done. This observation would be consistent with prior comparisons that found weak correlations between forearm skin indentation and suction parameters (16). Further, the wide variation in indentation resistance found among the four facial sites in the present work especially for indentation depths of 1.3 mm indicates to us that care must be used to clearly specify facial site locations used in any comparison study.

Similar words of caution may be suggested for facial TDC measurements and for comparisons among widely separated anatomical sites especially at shallow measurement depths of 0.5 mm. Average facial TDC values varied by 28% between sites A and B when measured to a depth of 0.5 mm but were nearly identical at a depth of 2.5 mm. This suggests that a major source of variability between these sites resides in the dermal water content since the dermis is the main component measured to a depth of 0.5 mm, whereas measurements to 2.5 mm include varying portions of subcutaneous fat. Because fat holds less water, it causes a lowered TDC value and would likely buffer effects of pure dermal water variations. This then might smooth out net TDC values among facial sites as in fact was observed. In contrast to the facial TDC variations, TDC measurements between forearm and biceps were remarkably similar and demonstrated the expected decrease in TDC value with increasing depth attributable to the inclusion of larger amounts of low waterholding fat.

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Indentation force–TDC relationships

A major finding of the present study was the absence of any significant correlation between skin softness as measured by indentation force and TDC values measured at the same sites that served as a surrogate measure of skin water content. This finding was contrary to our hypothesized relationship in which we expected there to be a positive association between indentation force and TDC values. As is clear from Fig. 3, the correlation coefficients were near zero with a TDC range of at least 2:1; thus, the possibility of the present study not being sufficiently powered is an unlikely explanation. Thus, the absence of a relationship would appear to be real and a possible explanation is called for and would be useful.

Although it has been shown that epidermal relative hydration may be associated with alterations in extensional properties of skin (67–69), it appears that indentation is much more dependent on dermal properties and hydration state at least for small indentation depths (24). The present indentation depths of 0.3 and 1.3 mm are not large deformations, and given the essentially incompressible features of water, we would suggest the following as a possible explanation to account for the absence of the hypothesized relationship.

If water is free to move (mobile water), then skin's indentation resistance, herein quantified as indentation force for a fixed indentation depth, depends on the ease with which dermal water can flow or diffuse in response to the imposed indentation force. In healthy young skin tissue as herein evaluated although much is bound water (38, 70), resistance to water mobile water movement is low as compared to skin interstitium subjected to significant edema or collagen linkage as in early fibrosis or other skin conditions. Thus, we would speculate that despite the fairly wide range in measured TDC values among the young women evaluated, the dermal water ranges that these represent were not sufficiently large to substantially restrict water movement away from the sites of indentation. If true, then the indentation force would be largely dependent on other tissue factors and not the compressibility of larger amounts of water and there would be no correlation between TDC and indentation force. If this speculative explanation were true, then conditions in which the hydrodynamic conductance of the dermis interstitium was compromised would more likely demonstrate a direct relationship between dermal water content and indentation force. One such condition occurs in persons with lymphedema in which lymphatic flow pathways are compromised causing increased interstitial flow resistance (71). The present findings besides setting out reference values suggest that such persons would be an important population to study to further test for a possible skin water- indentation force relationship and its utilization for early diagnosis.

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