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Changes with Age in the Moisture Content of Human Skin

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A technique to measure the dynamic mechanical properties of human skin in vivo is described. The technique measures the propagation and attenuation of shear waves in skin tissue over a range of frequencies (8–1016 Hz). Results show that both the propagation velocity and attenuation of shear waves in skin are highly dependent upon the water content of the stratum corneum. The technique was used to measure the dynamic mechanical properties of the skin on the back of the left hand for a group of 16 men ranging in age from 24–63 years. The results suggest that aged skin has a lower water content than the skin of younger men.

The assessment of skin aging through changes in its mechanical properties has been the subject of many investigations [1–4]. These studies have focused exclusively on full-thickness skin, however, with little if any attention paid to the role of the stratum corneum in skin aging in vivo.

We have developed an in vivo technique that measures the propagation and attenuation of low frequency (8–1016 Hz) shear waves in skin tissue proximal to the surface [5]. Our results suggest that at frequencies up to a few hundred hertz the technique measures the dynamic mechanical properties of the stratum corneum. At higher frequencies the dynamic mechanical properties of the underlying skin tissue predominate. Thus, the technique is sensitive to changes in both the stratum corneum and the underlying tissue.

In order to ascertain the relative change in the stratum corneum and underlying tissue in skin aging, we have employed our in vivo technique to measure the dynamic mechanical properties of human skin using a group of male subjects ranging in age from 24–63 years.

METHODS

Our technique for measuring in vivo dynamic mechanical properties in human skin has been described in detail previously [5]. In brief, the technique utilizes an apparatus consisting of two transducers, each attached to a stylus which lightly rests on the skin. One electromechanical transducer is driven transversely by a periodic noise source at constant amplitude (0.06 mm peak to peak) over the frequency range of 8–1016 Hz. The resultant shear wave is propagated through the skin and measured with a second transducer at known distances from the first. The phase differences and relative amplitudes between the signals from the two transducers are measured using a spectrum analyzer (Hewlett-Packard model 3582A). The second transducer is mounted on a slide so that measurement may be made over a range of separation distances. From the change in phase with distance at each frequency the propagation velocity (Vp) is calculated. Similarly, from the change in amplitude with distance the damping length (D) is calculated at each frequency. The damping length is reciprocally related to the attenuation and represents the distance over which the amplitude falls to about 37% ($1/e$) of its original value. The data are obtained as both Vp and D as a function of frequency.

The Vp and D were measured for skin on the back of the left hand of a group of 16 men ranging from 24–63 years of age. Data were obtained under two experimental conditions: either ambient conditions ("dry"), or after soaking the hand in water for 5 min followed by removal of the excess water ("wet"). At least 3 separate determinations were made under each condition, with a 1-min reimmersion between each "wet" experiment. All experiments were performed in late winter when the indoor ambient conditions were 20–25°C and 25–35% relative humidity. None of the subjects had experienced excessive sun exposure since the previous Fall.

RESULTS

Fig 1 shows a plot of Vp vs frequency for 1 individual subject. The data represent an average obtained from 7 experiments, each consisting of at least 2 determinations, over a 2-week period of time. The standard error of the mean (SE) was calculated ($n = 7$) at each frequency. Over the entire frequency range, the coefficient of variation (COV—the ratio of the SE to the mean value) never exceeded 10%. Above 200 Hz the COV was less than 5%. The upper curve represents data obtained under ambient conditions ("dry") while the lower curve represents data obtained at the same site after a 5-min immersion in water ("wet").

The data of Fig 1 show a curve in which the Vp goes through a minimum at an intermediate frequency. In both instances, the Vp and frequency at which the minimum occurs (f_{min}) are reduced under "wet" conditions. It is important to note that in both the "wet" and "dry" conditions Vp approaches the same value at high frrequency.

Fig 2 represents the D data obtained simultaneously with the Vp data shown in Fig 1. As before, the data represent an average of 7 experiments, each of at least 2 determinations, over a 2-week period. Over the entire frequency range the COV never exceeded 10%. Above 200 Hz the COV was always less than 5%. At low frequencies the "dry" data exhibit larger D values, while at high frequencies "wet" data show larger D values. In addition, both curves show a minimum which shifts to lower frequency under "wet" conditions. The data of Figs 1 and 2 are nearly identical to previously published values obtained under identical conditions, with a male human subject [5].

The technique was repeated on 16 men ranging in age from 24–63 years. In each case the data were qualitatively similar to the data shown in Figs 1 and 2. The subjects were grouped by age and the average value for Vp and D calculated at each frequency. In each age group, for both Vp and D, the COV never exceeded 15% at any frequency. Fig 3 shows a plot of the average Vp vs frequency for "wet" data in each age group. These data show several important age-related changes: (a) the Vp increases with increasing age. The change is greatest at low frequencies and negligible at high frequencies. (b) the f_{min} increases with increasing age.

In similar manner, the average D vs frequency data were plotted against age for the same group of individuals under

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Abbreviations:
- ATR-IR: attenuated total reflectance infrared
- COV: coefficient of variation
- D: damping length
- f_{min}: minimum frequency
- Vp: propagation velocity

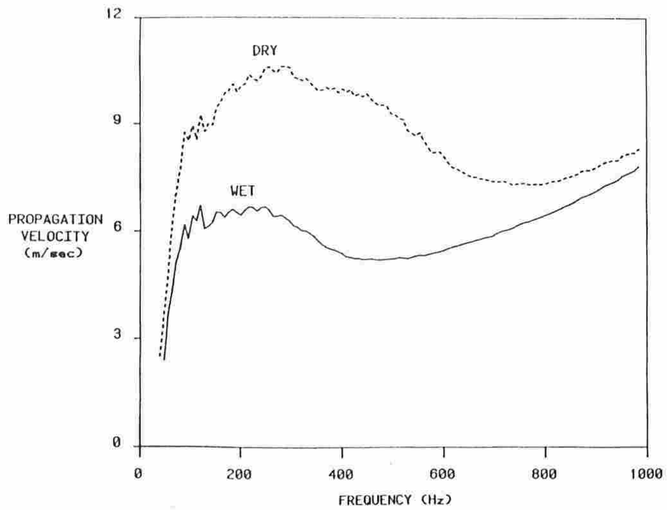

FIG 1. The propagation velocity vs frequency for shear waves in the skin on the back of the hand of 1 individual. Data were obtained under ambient conditions (*DRY*) or after soaking the hand in water for 5 min (*WET*). The data represent the average of 7 determinations, each consisting of at least 2 experimental runs, over a 2-week period.

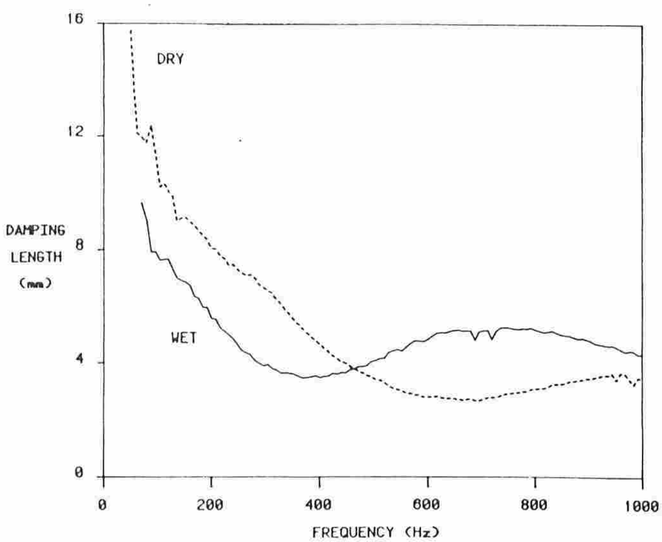

FIG 2. The damping length vs frequency for the attenuation of shear waves in the skin on the back of the hand of 1 individual. All conditions are identical with those of Fig 1.

"wet" conditions (Fig 4). These results show several important, seemingly age-related changes: (a) At low frequencies, D increases with increasing age while at high frequencies, D decreases with increasing age. (b) The f_{min} value increases with increasing age.

Fig 5 shows a plot of f_{min} vs average age for each of the groups. The upper line represents the best fit to the data obtained from Vp measurements (Fig 3), while the lower line represents the best fit obtained from D measurements (Fig 4). The error bars in Fig 5 represent the SE obtained from the f_{min} values for each individual in the age group. Note that for both measurements the COV is much less than 10%. In each case, the data fit to a straight line with a correlation coefficient (r) greater than 0.98 and slopes of 6.1 ± 0.4 Hz/year and 5.5 ± 0.4 Hz/year, respectively, the Vp and D data (p-null < 0.005). Thus, by either measurement, the f_{min} increases with increasing age with an average value near 6 Hz/year.

Similar results are obtained when comparing "dry" data. Fig 6 shows the average Vp vs frequency obtained for groups of young (20–39 years; n = 7) and old (40–63 years; n = 9) males.

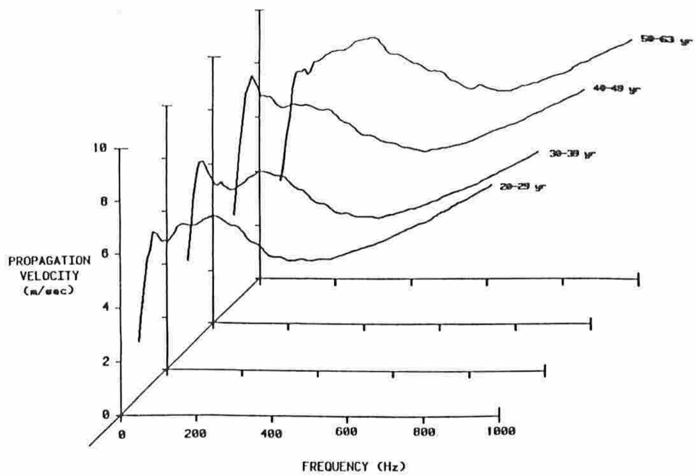

FIG 3. The average propagation velocity vs frequency for shear waves in the skin on the back of the hand. Data were obtained under "wet" conditions and grouped by age: 20–29 years (n = 3), 30–39 years (n = 5), 40–49 years (n = 4), 50–63 years (n = 4).

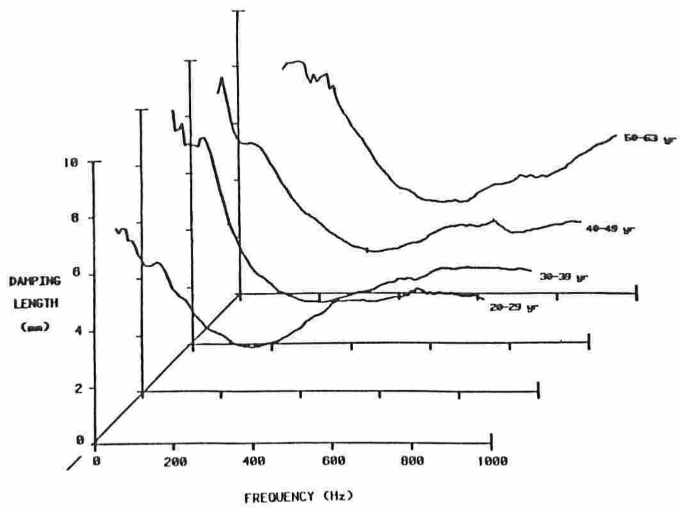

FIG 4. The average damping length vs frequency for the attenuation of shear waves in the skin on the back of the hand. Conditions are identical with those of Fig 3.

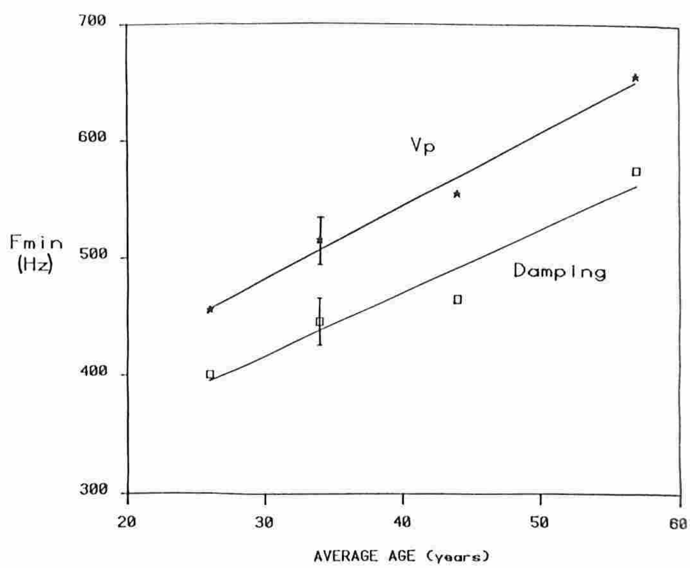

FIG 5. The average minimum frequency (f_{min}) vs average age for each group of men. The f_{min} values were obtained for each individual and the average determined for all individuals within an age group. The *error bars* represent SE.

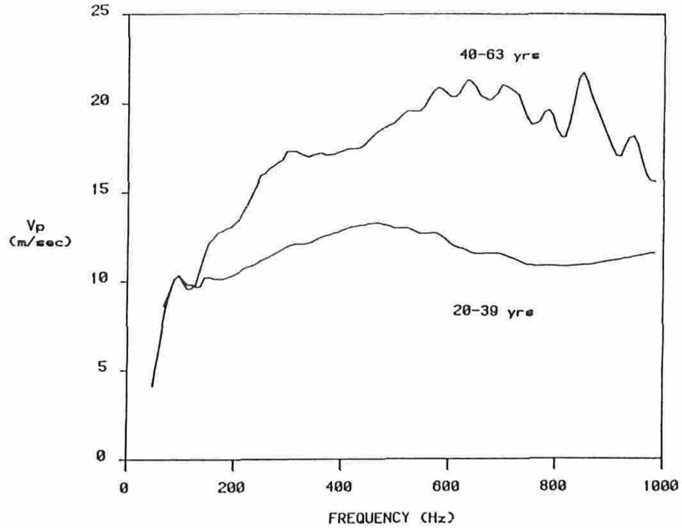

FIG 6. The average propagation velocity (*Vp*) vs frequency for shear waves in the skin on the back of the hand. Data were obtained for 16 men ranging in age from 24–63 years under "dry" conditions and grouped by age.

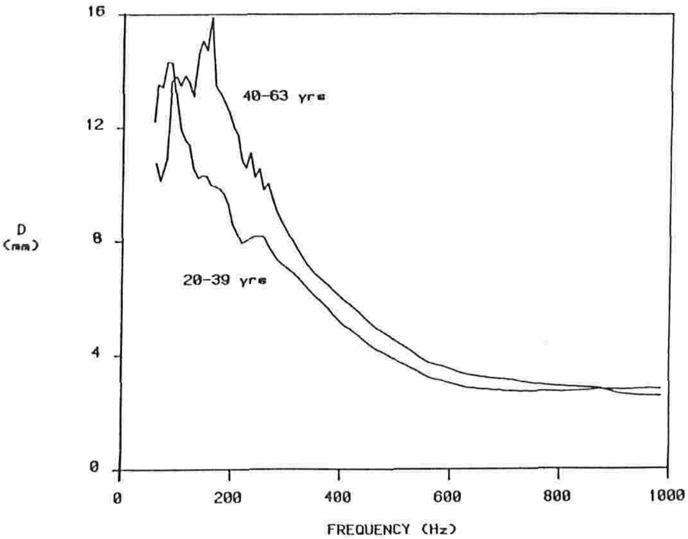

FIG 7. The average damping length (*D*) vs frequency for shear waves in the skin on the back of the hand. Conditions are identical to those of Fig 6.

As with "wet" data both Vp and f_{min} increase with increasing age. Fig 7 shows the average D vs frequency calculated for the same two age groups. Once again f_{min} increases with increasing age.

DISCUSSION

One possible explanation for the Vp and D vs frequency curves for human skin could be a moisture-dependent transition from surface to subsurface shear waves [5]. In brief, this hypothesis is supported by numerous lines of evidence.

1. The low frequency Vp data are identical to results obtained when surface shear waves were propagated as "ripples" in human skin [6].

2. Using a technique to measure in-plane vibrations of the human skin in vivo, Christensen and coworkers showed that the stratum corneum alone was responsible for the low-frequency (1–10 Hz) properties [7].

3. Differences in Vp between "wet" and "dry" data are maximal at low frequencies, yet negligible at higher frequencies. Since absorbed water is most readily bound to the outer layers, these differences suggest that the low-frequency domain is associated with propagation through the stratum corneum. The absence of difference in Vp at high frequencies suggest that this region is associated with wave propagation through tissue beneath the stratum corneum.

4. The data of Fig 3 indicate that the damping length goes through a minimum as a function of frequency. The results obtained from in vitro investigations of moisture-dependent changes in the dynamic mechanical damping (inversely related to the damping length) of human stratum corneum show that damping exhibits a maximum near 70% relative humidity [8, 9]. Since the water content of the stratum corneum in vivo increases with depth in the tissue, the in vitro results indicate that the damping length should go through a mimimum with increasing depth. Thus, the damping length vs frequency results presented here suggest that the tissue is being probed to increasing depth with increasing frequency.

5. The technique of attenuated total reflectance infrared (ATR-IR) spectroscopy has been used by numerous investigators to directly measure the water content in the outermost layers of the stratum corneum [10–12]. This technique has been utilized, in conjunction with the shear wave propagation techniques described in this report, to measure in vivo changes in the stratum corneum due to differences in ambient relative humidity. These results (R. Potts, data to be published) show that the water content measured by the ATR-IR method is negatively correlated with the Vp. As the frequency of shear wave propagation increases, however, the correlation between water content and Vp decreases. Thus, these results demonstrate that increased moisture content of the stratum corneum results in a decreased Vp.

Taken together, these results suggest that at low frequencies propagation occurs through a relatively dry layer near the surface, while as frequency increases the thickness of the propagation layer, and hence, the average water content, increases.

Regardless of the theoretical interpretation of these data, the results show a systematic change in both Vp and D when water is absorbed by human skin. In particular, the results indicate that f_{min} shifts to a lower value after the skin is wetted. In addition, the magnitude of Vp and D change with altered water content of the skin. The results show the Vp decreases after the skin has been immersed in water for 5 min. The damping length, however, shows a decrease at low frequencies but an increase at high frequencies with increased water content of the skin. Similar results were obtained for all individuals tested. The rapid nature of these moisture-dependent changes strongly suggests that they occur primarily in the stratum corneum. It seems unlikely that the rate of water transmission through the stratum corneum would permit the tissue beneath to absorb enough water in 5 min to affect such large changes in physical properties.

These results are useful in interpreting the age-dependent changes in Vp and D. The data of Figs 3, 5, and 6 show an increase in f_{min} with increasing age, suggesting that aged skin contains less water than young skin. Furthermore, the results show an increase in Vp with increasing age at low frequencies, yet little change at high frequencies. These changes are similar to the results shown in Fig 1 upon a transition from "wet" to "dry" skin, suggesting tht aged skin appears drier than young skin. In addition, the largest change in Vp occurs at low frequencies, suggesting that the difference in water content is predominantly in the stratum corneum.

A similar conclusion can be drawn by comparing age-related changes (Figs 4, 5, 7) to moisture-dependent changes (Fig 2) in D. The results of Figs 4, 5, and 7 show that f_{min} increases with increasing age, while results of Fig 2 suggest that f_{min} increases with decreasing moisture content of the stratum corneum. Taken together, these results suggest that aged skin has a lower moisture content than does young skin. In addition, the low-frequency D values increase with age while at high frequencies the opposite is true. Again, the results of Fig 2 show that this is similar to the results seen when "wet" skin becomes "dry." Finally, as discussed in #4 above, the shape of the damping length vs frequency curve is most likely related to changes in D with increasing moisture (depth) within the stratum corneum. Increasing age results in a shift of the damping length curve to higher frequency (i.e., greater depth), thus suggesting that a region of constant hydration occurs at greater depth in aged stratum corneum.

Since age-related differences of similar magnitude are seen in both "wet" and "dry" skin, it seems likely that the variation with age is due predominately to decreased moisture content and not due to differences in the rate of water absorption. In addition, these results suggest that the site of altered water content is the stratum corneum. On the other hand, little change is seen in the high-frequency range associated with wave propagation through the underlying tissue. This does not preclude changes in the underlying tissue with age, but rather suggests that the moisture-dependent changes occur predominantly in the stratum corneum.

Recently, Tan and coworkers measured the in vivo skin thickness of a large population of men using pulsed ultrasound [13]. Their results show a decrease in thickness of skin on the forearm with increasing age. Their observation of decreased skin thickness seems to be concordant with our conclusion that the water content of skin decreases with age. Parallel changes in thickness with water content have been measured for keratinized tissue [14] and are commonly observed for a variety of proteinaceous materials.

While the concept of aged skin being "drier" than young skin is not novel, few substantive data are to be found in the literature to support this hypothesis. Hegner and coworkers [15,16] have reported on the use of an in vivo conductometric method to assess stratum corneum "humidity" as a function of age. Their results indicate average changes of less than 5% in "humidity" over the 21- to 80-year range, but there is no indication that this change is statistically significant.

The magnitude of change in moisture content with age is not large. Comparison of Figs 1 and 2 with Figs 3 and 4 suggest that the change in "wet" skin over 40 years is les than the change seen in young skin going from about 30% ambient relative humidity to a 5-min immersion in water.

Aging of human skin is accompanied by numerous changes in both biologic and physical properties, many of which may affect the propagation and attenuation of shear waves through this tissue (i.e., water content, extent of cross-linking, stratum corneum thickness, stratum corneum renewal rate, etc.) Our results show that the shear wave technique is primarily sensitive to changes in the moisture content of the stratum corneum and that changes in propagation and attenuation with age are very similar to reversible changes which occur upon loss of water from the stratum corneum. These results, while not ruling out other biophysical changes, suggest that skin aging is accompanied by a decrease in the moisture content of the stratum corneum.

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