Relationship Between the Depth of Facial Wrinkles and the Density of the Retinacula Cutis

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Objective: To identify whether there is a relationship between the depth of facial wrinkles and the density of the retinacula cutis in the subcutaneous tissue of the skin.

Design: Wrinkle depth was assessed with image analysis on the forehead and the lateral canthus of human cadavers. The density of the retinacula cutis was measured in Azan-Mallory–stained skin sections obtained around the wrinkles.

Setting: Gross Anatomy Section, Kagoshima University Graduate School of Medical and Dental Sciences.

Participants: Fifty-five male and female cadavers (35-93 years old).

Main Outcome Measures: The maximum depth of each wrinkle was used to represent the wrinkle’s degree. In the skin sections, the density of the retinacula cutis was measured around the deepest point of each wrinkle in a 1-mm-wide area (the wrinkle-specific area) and a 10-mm-wide area that included the wrinkle (the wrinkle-inclusive area).

Results: In both the wrinkle-specific and wrinkle-inclusive areas, the retinacula cutis densities became lower in the forehead and in the lateral canthus areas. When a wrinkle was shallow, the density was lower in the wrinkle-specific area than in the wrinkle-inclusive area. With wrinkle progression, the density difference between the wrinkle-specific and the wrinkle-inclusive areas gradually decreased until there was no apparent difference.

Conclusions: Facial wrinkles seem to develop above sites of reduced lower retinacula cutis density. As a wrinkle develops, the density decreases in both the wrinkle-specific and the wrinkle-inclusive areas, whereas the density difference between those areas vanishes.


Previously, it was found that the dermis under a wrinkle becomes thinner as the wrinkle deepens and that this thinning of the dermis stops when the dermis becomes thinner than half of its original thickness. It was also found that some wrinkles stopped deepening at this point, whereas some wrinkles developed further by invagination of the dermis into the layer of the subcutaneous tissue.1 Those findings led to the assumption that other structural changes are made in the subcutaneous tissue under those deep wrinkles.

The skin ligaments (retinacula cutis) are numerous, small, fibrous bands that extend from the deep surface of the dermis to the underlying deep fascia.2,3 The retinacula cutis was considered to maintain the morphologic features of the loose connective tissue with its palisade configuration. Iwanami and Tsurukiri4 reported that the structure of the retinacula cutis was distorted by the aging process, whereas Piéard and Lapière5 reported that the trabeculae in the retinacula cutis are broader and much shorter beneath a wrinkle compared with the surrounding skin. It was thought that these structural changes of retinacula cutis might relate to the dermal invagination and to the wrinkle progression. This present study was conducted to examine this possibility. First, the depth of wrinkles was assessed in skin from the forehead and the lateral canthus of human cadavers. Second, the density of the retinacula cutis was measured in Azan-Mallory–stained skin sections obtained.
around those wrinkles. Finally, potential relationships between the retinacula cutis density and the wrinkle depth were characterized.

**METHODS**

**STUDY PARTICIPANTS**

Fifty-five cadavers donated for medical education (from individuals ranging in age at death from 35-93 years) were embalmed with a formalin-phenol-alcohol-thymol solution and were stored in the repository of the Kagoshima University Graduate School of Medical and Dental Sciences. Consent was obtained from all donors and/or their relatives for the use of the body (including organs, tissues, and cells) for medical research (including anatomical examination, dissection, and other similar purposes) and education. All methods used in this study complied with the Post-mortem Examination and Corpse Preservation Act of Japan.

Two regions of facial skin were investigated. Regions approximately 10 mm above the superior margin of the right or left eyebrow (hereafter referred to as the forehead) and 5 mm lateral from the right or left lateral canthus (hereafter referred to as the lateral canthus) were investigated. Regions with moles, wounds, or scars were excluded from the investigation.

**MARKING AND PHOTOGRAPHY**

In the forehead region of each cadaver, a reference wrinkle was chosen. A 20 × 10-mm rectangle was drawn on the surface of the skin with its longer sides crossing perpendicular to the wrinkle. In the lateral canthal region of each cadaver, a wrinkle that originated from the lateral canthus was chosen. A point on the line 5 mm from the temporal side of the lateral canthus was marked. A similar rectangle was drawn by aligning its inner long side at the marked point. Finally, a close view of each rectangle was taken with a digital camera (D200; Nikon Corporation, Tokyo, Japan).

**CONSTRUCTION OF REPLICAS AND IMAGE ANALYSIS**

Using a hydrophilic vinyl silicone impression material (GC Exafine; GC Co Ltd, Tokyo, Japan), we obtained a replica from each rectangle with a 5-mm surrounding margin. The surface image of each replica was captured and then analyzed using a linear analyzing function of the 3-dimensional image analyzer (PRIMOS system; GF Messetechnik GmbH, Tetlow, Germany) as detailed previously. Among the multiple roughness parameters defined, the maximum roughness (R_max), which is the vertical distance between the highest peak and the lowest valley of the surface, was chosen to indicate the maximum depth of each wrinkle.

**HISTOLOGIC METHODS**

As previously described, a 10 × 20-mm block of cadaveric tissue, including the skin and facial muscle, was obtained from each marked area. The block was then embedded in paraffin, which in turn was cut into 6-µm sections in the sagittal plane. The sections were stained with Azan-Mallory stain. Images were captured using a light microscope (Olympus BX50; Olympus Corporation, Tokyo, Japan) and were then processed with an image analyzer (Image Pro Plus version 3; Media Cybernetics, Bethesda, Maryland). Blue-stained subcutaneous tissue was considered the retinacula cutis (Figure 1A).

**MEASUREMENT OF RETINACULA CUTIS DENSITY**

The retinacula cutis density, which is defined as the ratio of the retinacula cutis area in a measurement area, was obtained as follows. First, the deepest point of each wrinkle was iden-
identified in each histologic image by comparison with its surface photograph taken before collection of the specimen. If the deepest point was not detected on a captured image, the image was excluded from the analysis. Second, 2 measurement areas were set on the image in depth within the subcutaneous tissue, which is defined as the layer lower than any follicles and sebaceous and sweat glands and shallower than the facial muscle layer. One area was set in width within 1 mm from the deepest point and is defined as the wrinkle-specific area (Figure 1B), and another area was set in width within 10 mm from the deepest point and is defined as the wrinkle-inclusive area (Figure 1C). The retinacula cutis density was obtained from both areas.

STATISTICAL ANALYSIS

The regression curve with the highest coefficient of determination was selected among the linear, logarithmic, involution, and exponential approximations using the statistical function of Microsoft Excel (Microsoft Inc, Redmond, Washington). A paired $t$ test and an unpaired $t$ test were used to determine the significance of differences between the 2 groups.

RESULTS

SAMPLE SPECIMENS

Of the initial 55 cadavers in the study, 10 and 6 cadavers were excluded from each regional observation so that the forehead regions of 45 cadavers (27 men and 18 women) and the lateral canthal regions of 49 cadavers (31 men and 18 women) were characterized. The age distribution of these cadavers is given in Table 1. No difference in age distribution was found between male and female cadavers.

OBSERVATION OF THE RETINACULA CUTIS IN SUBCUTANEOUS TISSUE SPECIMENS

The thickness of the subcutaneous tissue varied according to the individual. The density of the retinacula cutis varied when wrinkles were shallow (Figure 2A and B and Figure 3A and B) but became constantly lower when wrinkles were deep (Figure 2C and D and Figure 3C and D). The retinacula cutis density tended to be lower in the wrinkle-specific area than in the wrinkle-inclusive area, and that tendency was common in the forehead and the lateral canthus.

RELATIONSHIPS BETWEEN THE RETINACULA CUTIS DENSITY AND Rmax

In the forehead and lateral canthus, a correlation was detected between the Rmax and the retinacula cutis density (Figure 4). The variation in the Rmax was reduced by excluding Rmax values greater than 0.6 mm from the analysis. The remaining data of the forehead were then divided into 2 groups: the shallow (0-0.3 mm) and the deep (0.3-0.6 mm) wrinkle groups. When data were compared within each group, the retinacula cutis density was significantly lower in the wrinkle-specific area than in the wrinkle-inclusive area in both groups. Subsequently, data of the lateral canthal wrinkles were divided into 3 groups: shallow (0-0.3 mm), middle (0.3-0.6 mm), and deep (>0.6 mm). The density of the retinacula cutis was significantly lower in the wrinkle-specific area than in the wrinkle-inclusive area in the shallow wrinkle group, but that difference was lost in the middle and the deep wrinkle groups (Table 2). No correlation with age was found for either the wrinkle depth or the retinacula cutis density (data not shown).

SEX DIFFERENCE

No sex-dependent difference was found in the Rmax values of the forehead and lateral canthus wrinkles (Table 3). The retinacula cutis densities of the forehead and lateral canthus were lower in the women than in the men. Other than this, no sex-dependent difference was found in this study.

COMMENT

This present study yielded 4 major findings. First, retinacula cutis density was less in the wrinkle-specific area than in the wrinkle-inclusive area. Second, retinacula cutis density of the wrinkle-inclusive area varied widely in skin specimens with shallow wrinkles but was consistently lower in specimens with deep wrinkles. Third, retinacula cutis density was significantly lower in women than in men, but no sex-dependent difference was found in wrinkle depth. Fourth, the density difference between the wrinkle-specific and wrinkle-inclusive areas were more marked in specimens with shallow wrinkles than in specimens with deep wrinkles.

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<th>Table 1. Age and Sex Distribution of the Study Cadavers</th>
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Figure 2. Representative images of forehead wrinkles (from the left, external appearance, 3-dimensional replica images, and tissue specimens). A, Shallow wrinkle in a 35-year-old woman (maximum roughness (Rmax), 0.11 mm; retinacula cutis density in the wrinkle-inclusive area, 12.1%; retinacula cutis density in the wrinkle-specific area, 13.8%). B, Shallow wrinkle in a 55-year-old man (Rmax, 0.14 mm; retinacula cutis density in the wrinkle-inclusive area, 29.5%; retinacula cutis density in the wrinkle-specific area, 14.2%). C, Middle-level wrinkle in a 67-year-old man (Rmax, 0.32 mm; retinacula cutis density in the wrinkle-inclusive area, 9.0%; retinacula cutis density in the wrinkle-specific area, 8.7%). D, Deep wrinkle in a 72-year-old woman (Rmax, 0.59 mm; retinacula cutis density in the wrinkle-inclusive area, 5.5%; retinacula cutis density in the wrinkle-specific area, 2.3%). Microscopic observations of the external appearance were made along the blue line. Three-dimensional replica images were obtained and analyzed at the same area as the external appearance. Color bar indicates the level of the surface. S indicates superior direction; I, inferior direction; asterisk, wrinkle point; E, epidermis; D, dermis; SC, subcutaneous tissue; and FM, facial muscle. Yellow dotted line indicates the border between dermis and subcutaneous tissue (Azan-Mallory stain, original magnification ×40).
Figure 3. Representative images of lateral canthus wrinkles (from the left, external appearance, 3-dimensional replica images, and tissue specimens). A. Shallow wrinkle in a 46-year-old man (maximum roughness \(R_{\text{max}}\), 0.26 mm; retinacula cutis density in the wrinkle-inclusive area, 12.4%; retinacula cutis density in the wrinkle-specific area, 11.3%). B. Shallow wrinkle in a 68-year-old man \(R_{\text{max}}, 0.27 \text{ mm};\) retinacula cutis density in the wrinkle-inclusive area, 19.8%; retinacula cutis density in the wrinkle-specific area, 11.9%). C. Middle-level wrinkle in an 81-year-old woman \(R_{\text{max}}, 0.43 \text{ mm};\) retinacula cutis density in the wrinkle-inclusive area, 9.2%; retinacula cutis density in the wrinkle-specific area, 7.3%). D. Deep wrinkle in an 81-year-old woman \(R_{\text{max}}, 1.5 \text{ mm};\) retinacula cutis density in the wrinkle-inclusive area, 3.9%; retinacula cutis density in the wrinkle-specific area, 2.3%). Explanation of the composition and abbreviations are given in the Figure 2 legend.
Hypertrophy of the extracellular matrix in the hypodermal septae, which was reported by Piérard and Lapière to exist at the sites of frown lines, was also found in our samples but was rare. Rather, the extracellular matrix appeared atrophied due to reduced retinacula cutis density. Previously, invagination of the dermis into the subcutaneous layer was found in approximately one-third of specimens with deep wrinkles. Because reduced retinacula cutis density can lead theoretically to the loss of supporting force to the dermis, the reduced retinacula cutis density might be a factor that makes the dermis vulnerable to this invagination. Furthermore, the density of retinacula cutis may be one of the factors that affects wrinkle depth. It was assumed that when skin is highly...
elastic, wrinkles remain shallow regardless of retinacula cutis densities (Figure 5A and C), and when skin loses its elasticity, wrinkles become deeper, depending on retinacula cutis densities (Figure 5B and D). Further confirmation of this assumption awaits an in vivo analysis of skin elasticity, retinacula cutis density, and wrinkle depth.

This study has 3 limitations. First, because this study used embalmed cadavers, the depths of wrinkles measured may be different from those that occur in vivo. This difference was considered negligible because the mean wrinkle depth and the value distribution obtained in this study were not different from those of age-matched living individuals.11 Second, the effects of collection, embalming, and fixation of the specimens on the morphologic features of the retinacula cutis are not negligible. However, the effects were minimized by comparing data of specimens obtained using the same procedure. Third, in cadaver skin, dynamic (temporal, reducible) and static (permanent) wrinkles cannot be distinguished from each other. However, because muscles in a cadaver are in a relaxed or nearly relaxed state, the wrinkles observed in this study are considered to be static ones.

In conclusion, retinacula cutis density was more dense in the wrinkle-inclusive area than in the wrinkle-specific area. Wrinkles seem to develop at the site of lower-density retinacula cutis. The decrease in retinacula cutis density in the wrinkle-specific and wrinkle-inclusive areas seems to be one of the factors that makes wrinkles deeper. We hope these findings further the understanding of how facial wrinkles form and, in turn, offer new avenues for the development of effective antiwrinkle treatments.

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Author Contributions: Drs Tsukahara, Tamatsu, and Shimada had full access to all of the data in the study and take responsibility for the integrity of the data and accuracy of the data analysis. Study concept and design: Tsukahara, Tamatsu, and Shimada. Acquisition of data: Tsukahara and Tamatsu. Analysis and interpretation of data: Tsukahara, Tamatsu, and Sugawara. Drafting of the manuscript: Tsukahara and Tamatsu. Critical revision of the manuscript for important intellectual content: Sugawara and Shimada. Statistical analysis: Tsukahara. Administrative, technical, and material support: Tamatsu. Study supervision: Tamatsu, Sugawara, and Shimada.

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Top-Accessed Article: On Beauty


“On Beauty,” a thoughtful philosophical examination of humankind’s obsession with beauty, discusses why physical beauty is so compelling and why we spend millions of dollars each year on procedures and products to enhance or restore it. This well-researched review summarizes the evidence that humans associate physical attractiveness with such desirable traits as goodness, health, and reproductive capacity. Beauty is valued in potential mates because such attributes as reliability, kindness, and intelligence have no physical markers. Physical beauty is so much easier to assess than what lies beneath. “On Beauty” provides valuable insight into the pervasive and cross-cultural attitudes toward beauty that fuel the recent dramatic expansion of the practice of cosmetic medicine and surgery.

From August 2009 through August 2010, this article was viewed 1786 times on the Archives of Dermatology Web site.

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