Thyroid-Associated Periorbitopathy

Eyebrow Fat and Soft Tissue Expansion in Patients With Thyroid-Associated Orbitopathy

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Objective: To compare soft tissue and fat volumes in the supraorbital area of healthy patients and patients with thyroid-associated orbitopathy (TAO) using 3-dimensional reconstruction software.

Methods: The superiolateral orbital area was delineated on a bony framework. Three-dimensional reconstruction and volumetric calculation of the retro-orbicularis oculi fat (brow fat), galeal fat (including the retro-orbicularis oculi fat), and soft-tissue muscle were performed.

Results: We analyzed 100 computed tomographic scans from 48 patients with TAO and 52 control subjects. All patients showed an age-related increase of fat volumes. The mean total eyebrow volume was greater in patients with TAO vs healthy control subjects (P < .001). Galeal fat (P = .02) and retro-orbicularis oculi fat (P = .01) volumes were significantly higher in patients with TAO vs control subjects. Soft-tissue muscle volume decreased with age in healthy females but remained constant in the aging female group with TAO. Both total volume and brow thickness did not appear to change with age in healthy patients but exhibited an increase in the female population with TAO.

Conclusions: This study brings into focus the clinico-pathologic entity of thyroid-associated periorbitopathy. Three-dimensional evaluation of computed tomographic scans can provide information on volumetric changes in the eyebrow profile of patients with TAO. Further investigation of the biologic and morphologic changes of eyebrow fat and soft tissue in patients with TAO may help better characterize, classify, and guide their treatment.

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Despite advances in understanding the cellular and molecular biology of autoimmune diseases, the pathogenesis of thyroid-associated orbitopathy (TAO) is still incompletely understood. The leading hypothesis focuses on the targeting and activation of orbital fibroblasts, a critical agent involved in the pathologic changes of TAO.1,2 The degree of expanded adipogenic potential of fibroblast cells within an altered cytokine milieu accounts for the extent of altered orbital morphologic features in patients with TAO.3-5 Recent evidence has shown that eyebrow fibroblasts display similar biological and structural changes; thus, leading to the brow fat enlargement appreciated clinically in several patients with TAO.6 This contributes to the aesthetic disfigurement of the supraorbital profile, which persists following orbital decompression or eyelid surgery and should be appropriately addressed in a staged surgical treatment plan.

The aim of this study was 2-fold: (1) to describe the volumetric differences between the eyebrow profile of patients with TAO and healthy control subjects using 3-dimensional reconstruction software, and (2) to introduce the concept of thyroid-associated periorbitopathy.

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Investigating the clinical features and biologic and morphologic changes of eyebrow fat and periorbital soft tissue should allow us to better characterize, classify, and guide treatment of patients with TAO.

METHODS

PATIENTS AND CT DATA

The medical records of patients followed up in the Division of Orbital and Ophthalmic Plastic Surgery at Jules Stein Eye Institute...
between January 1, 2001, and December 31, 2009, were reviewed and analyzed. Approval from the University of California at Los Angeles institutional review board was obtained. Inclusion criteria for healthy control subjects were patients who underwent investigative computed tomographic (CT) imaging of the orbit (0.5- to 3.0-mm slice increment) for unilateral pathologic characteristics (neoplastic, vascular, muscular, neurological, and lacrimal) or orbital floor fracture. Patients with extended orbital trauma (as verified by CT imaging and the photograph of the patient at presentation) or previous eyebrow surgery or trauma were excluded. Patients with TAO who underwent CT imaging of the orbit in our electronic database were included in the study. Demographic information (age, sex, and ethnicity), orbital pathologic characteristics at presentation, surgical history of the orbit and eyebrow, and Hertel exophthalmometry measurements were also retrieved from patients’ medical records. The supraorbital volumetric analysis was performed unilaterally only on the unaffected side for control subjects. For patients with TAO, the side to analyze was selected via randomization.

**CT IMAGING SEGMENTATION SOFTWARE**

Mimics version 9.12 (Materialise) is image-processing software with 3-dimensional visualization, and it is a reliable and validated tool for biomedical research. It enables the user to control and correct the segmentation of CT data. Reference CT images are imported and following image processing, analysis, and 3-dimensional reconstruction, volumetric calculations of the studied tissues (bone, muscle, and fat) are obtained. The method of tissue analysis and segmentation was adapted from the approach described by Mourits et al. First, a bony mask (226-2165 Hounsfield units [HU]) was created followed by the 3-dimensional reconstruction of the facial skeleton. On this bony framework, the superolateral orbital target area was defined from the supraorbital notch (2.5 cm lateral from the frontal midline) to the lateral orbital rim. The inferior limit was defined as the zygomaticofrontal suture, while from the frontal midline) to the lateral orbital rim. The inferior corrugator muscles embedded in the multiple galea layers.

The initial mask was created within the range of −200 to 100 HU. This range includes fat (−200 to −30 HU) and soft tissue with muscle (−30 to 100 HU). Segmentation was performed exclusively on the axial planes. Using the 3-dimensional cropping tool, the target area was isolated from the surrounding facial structures. This cropped mask was manually segmented on serial axial slices. The editing involved removing orbital structures included in the cropped 3-dimensional quadrant but did not relate directly to the bony superolateral orbital rim (Figures 2 and 3). The final edited mask defined the target area of interest.

The next step involved the creation of fat (−200 to −30 HU) and soft-tissue muscle (−30 to 10 HU) masks. These were intersected with the edited mask of the supraorbital construct (Boolean operation). The resulting mask defined the total fat tissue in the eyebrow area (galeal fat). This mask was duplicated and manually edited to isolate the brow fat or ROOF overlying the bony rim. Similarly, the soft-tissue muscle mask of the eyebrow was created by intersecting the soft-tissue muscle mask of the face with the edited mask of the supraorbital construct (Boolean operation). Following region growing (computer-assisted separation of nonconnected tissues), the software 3-dimensionally reconstructed the 3 masks of interest: soft-tissue muscle (any structure that is not fat) (Figure 1D), total galeal fat (Figure 1E), and brow fat or ROOF (Figure 1F). Volumetric calculations were obtained and expressed in cubic millimeters.

The imaging analysis was performed by a single operator with substantial experience using the software. The segmentation was performed exclusively on the axial CT scans. In addition, we evaluated the thickness of the lateral third of the eyebrow on the axial CT as a linear measurement projected at a right angle from the supraorbital ridge.

Univariate summary statistics as well as regression of continuous variables were performed.

### RESULTS

We analyzed 100 CT scans from 48 patients with TAO and 52 healthy control subjects. Computed tomography slice thickness varied between 0.5 and 3.0 mm, and image pixel size ranged from 0.252 to 0.488. The mean age was 49.3 years (range, 17-81 years) in the healthy group and 51.2 years (range, 23-78 years) in the TAO group. In the healthy group, 24 patients were male (46%) and 28 were female (54%). In the TAO group, 11 patients were male (23%) and 37 were female (77%).

Univariate summary statistics of all 5 continuous parameters are outlined in Table 1. Table 2 summarizes the average amount by which patients with TAO exceed healthy patients for each variable. Sex was controlled for in all 5 regressions. The mean total eyebrow volume (Figures 4 and 5) was markedly increased in patients with TAO showing a 826-mm³ point estimate increment vs the control (P < .001). All patients showed an age-related increase of fat but the patients with TAO had significantly higher fat volumes compared with control subjects. The galeal fat (P = .02) and ROOF (P = .01) volumes (Figures 6, 7, and 8) were significantly higher in patients with TAO (397-mm³ and 236-mm³ point estimate increment, respectively, vs control subjects).

### TAO vs HEALTHY FEMALE POPULATION

#### AGE TRENDS

Owing to the prevalence of Graves disease in female subjects, there was a disparity in the male distribution of the study group, with only 3 males with TAO older than 50 years of age. Because point estimates of the age slopes often diverged substantially between male and female patients, we adopted a sex-interaction regression model that allowed separate slopes to be estimated for the female population only. Separate age trends were calculated for healthy
Figure 1. The bony mask (226-2165 Hounsfield units) and 3-dimensional reconstruction of the facial skeleton. The longest vertical line represents the midsagittal computed tomographic plane (A, B, and C). The horizontal plane intersects the zygomaticofrontal suture and corresponds to the axial computed tomographic plane (A). Projection of the supraorbital target area on the 3-dimensional fat (yellow) and soft-tissue muscle (red) masks of the face (B and C). D, Three-dimensional reconstruction of the soft-tissue muscle projected on the bony orbital rim. E, Three-dimensional reconstruction of the galeal fat projected on the semitransparent soft-tissue muscle mask (red) of the face. F, Three-dimensional reconstruction of the retro-orbicularis oculi fat (ROOF) projected on the semitransparent soft-tissue muscle mask (red) of the face. Total eyebrow volume consisting of soft-tissue muscle (red) and galeal fat pad (yellow). On the anterior to the frontalis, we notice a thickened galeal fat layer that blends with the ROOF (G and H).
Figure 2. Initial mask created within the range of −200 to 100 Hounsfield units (HU). This range represented the fat (−200 to −30 HU) and the soft tissue with muscle (−30 to 100 HU). Using the 3-dimensional cropping tool option, the target area was isolated from the surrounding facial structures. This cropped mask was manually segmented on serial axial slices. Segmentation was performed exclusively on the axial plane. The editing involved erasing all the orbital structures included in the cropped 3-dimensional quadrant but did not relate directly to the bony superolateral orbital rim. The final edited mask defined the target area of interest (A−F). Fat (−200 to −30 HU) and soft-tissue muscle (−30 to 10 HU, red) masks were created and intersected with the edited mask of the supraorbital construct (Boolean operation). As a result of this intersection, a galeal fat mask was created. Subsequently, this mask was duplicated and manually edited to isolate the brow fat, which overlies the bony rim (G and H).
females and those with TAO and estimates of the average differences in the 5 variables between these 2 groups are given at ages 35, 50, and 65 years (Table 3).

Fat volumes increased with age in both healthy and diseased female populations, and the increase appears to be substantially higher in the TAO group. For both ROOF...

Figure 3. The duplicated mask manually edited to isolate the brow fat, which overlies the bony rim (A and B). Similarly, the soft-tissue muscle mask of the eyebrow (C-E) was created by intersecting the soft-tissue muscle mask of the face with the edited mask of the supraorbital construct (Boolean operation). Parts F and G represent the accurate definition of the final masks of interest. The linear measurement of the lateral eyebrow thickness was projected at a right angle from the supraorbital ridge (H).
and galeal fat, healthy patients and those with TAO did not differ significantly in average levels at age 35 (P = .14), but patients with TAO had significantly higher volumes than healthy female subjects at ages 50 and 65 years. Soft-tissue volume (Figure 9) appeared to remain constant as patients with TAO aged but decreased with age in healthy female subjects (P = .017). As with fat volume, healthy patients and those with TAO did not differ with respect to soft-tissue volumes at age 35 years, but patients with TAO had significantly higher levels than healthy patients of the same age at 50 and 65 years. Both total volume and brow thickness did not appear to change with age in healthy patients but exhibited a considerable, highly significant increase with age in the female population with TAO.

**EYEBROW VOLUME AND HERTEL MEASUREMENTS**

Regression analysis of Hertel measurements (Table 4) showed a statistically significant correlation between the amount of proptosis and the total eyebrow volume.
For every millimeter of proptosis, there was an 85.0-mm³ point estimate increment in total volume (P = .007) and 0.2-mm increase in brow thickness (P = .014) for patients with TAO. There was no evidence of significant correlation between galeal or brow fat and proptosis in

Figure 4. The mean total eyebrow volume was markedly increased in patients with thyroid-associated orbitopathy (TAO) showing a 826-mm³ point estimate increment vs control subjects (P < .001) (A and B). This trend was confirmed by the linear measurement of the lateral third of the eyebrow in millimeters, which was used as an internal control.

Figure 5. A man with thyroid-associated orbitopathy in active phase. Fat and soft-tissue expansion with marked increase of the total eyebrow volume (A and B). The supraimposed 3-dimensional image is tilted to allow better appreciation of the volumes.

Figure 6. The retro-orbicularis oculi fat and galeal fat volumes were significantly higher in patients with thyroid-associated orbitopathy (TAO). Fat volumes increased with age in both healthy and diseased female populations, an increase that appeared to be substantially higher in the TAO group. For both retro-orbicularis oculi fat and galeal fat, healthy patients and those with TAO did not significantly differ in average levels at age 35 years (P = .14), but patients with TAO had significantly higher volumes than healthy female subjects at ages 50 and 65 years.
Tissue remodeling as a result of TAO involves an activational cross talk among the recruited professional immune cells, infiltrating fibrocytes, and the resident fibroblasts. A unique set of environmental factors and signaling pathways lead to enhanced adipogenic

Table 3. Regression Analysis of Continuous Variables on Continuous Age in Female Subjects With Disease-Age Interaction

<table>
<thead>
<tr>
<th>Variable</th>
<th>Population</th>
<th>Point Estimate (SE)</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total volume</td>
<td>Healthy</td>
<td>98 (38.6)</td>
<td>19.9-176</td>
<td>.02</td>
</tr>
<tr>
<td></td>
<td>TAO</td>
<td>85.1 (30.2)</td>
<td>24.1-146</td>
<td>.007</td>
</tr>
<tr>
<td>Brow thickness</td>
<td>Healthy</td>
<td>0.201 (0.086)</td>
<td>0.027-0.375</td>
<td>.04</td>
</tr>
<tr>
<td></td>
<td>TAO</td>
<td>0.199 (0.078)</td>
<td>0.043-0.356</td>
<td>.01</td>
</tr>
</tbody>
</table>

Abbreviation: TAO, thyroid-associated orbitopathy.

Figure 7. A young woman with thyroid-associated orbitopathy and galeal eyebrow fat. Galeal eyebrow fat represents 91% of the total estimated volume (superimposed 3-dimensional image viewed from below) (A). This is demonstrated by the enlarged dome-shaped retro-orbicularis oculi fat (yellow) on the axial computed tomographic scan (B).

Figure 8. A 53-year-old woman with thyroid-associated orbitopathy and enlarged eyebrow fat component. The superimposed 3-dimensional view is tilted.

Table 4. Regression Analysis of Continuous Variables on Hertel Measurementsa

<table>
<thead>
<tr>
<th>Variable</th>
<th>Point Estimate (SE)</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brow fat</td>
<td>236 (66)</td>
<td>106-367</td>
<td>.001</td>
</tr>
<tr>
<td>Galeal fat</td>
<td>397 (125)</td>
<td>150-644</td>
<td>.002</td>
</tr>
<tr>
<td>Soft tissue</td>
<td>429 (107)</td>
<td>216-643</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Total volume</td>
<td>826 (143)</td>
<td>542-1111</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Brow thickness</td>
<td>1.83 (0.35)</td>
<td>1.13-2.52</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

a No additional variables were adjusted for any of the regressions. There was no statistically significant correlation of the amount of proptosis with the galeal or brow fat though for every millimeter of proptosis, there was a 98.0-mm³ point estimate increase in total volume (P = .015) and 0.2-mm increase in brow thickness (P = .25).

Figure 9. Soft-tissue volume appeared to remain constant as patients with thyroid-associated orbitopathy (TAO) aged but decreased with age in healthy female subjects (P = .017). As with fat volume, healthy patients and those with TAO did not differ with respect to soft-tissue volumes at age 35 years, but patients with TAO had significantly higher levels than healthy patients of the same age at ages 50 and 65 years. The expansive involvement of the soft-tissue muscle seems to counterbalance the atrophic changes of soft tissue observed in postmenopausal healthy female subjects.
proliferation, muscle enlargement, and amplified hyaluran synthase gene expression.\textsuperscript{19-21} The resulting orbital tissue expansion leads to significant disfigurement and frequently requires surgical decompression or immunomodulatory treatments.\textsuperscript{22}

Clinical observations have shown that patients with TAO exhibit a volumetric expansion of their eyebrow profile.\textsuperscript{23} This clinopathologic entity is seen by comparing premorbid to post-TAO photographs and often represents the basis for persistent disfigurement and patient dissatisfaction. Recent histopathologic and immunohistochemical evidence has demonstrated that the eyebrow fat compartments in patients with TAO show structural changes similar to the orbital fat.\textsuperscript{6} With a marked increase in collagen and fibrosis and by displaying the key biologic markers thyrotropin receptor and IGFR1b, eyebrow fibroblasts are potentially insinuated in site-specific immunoreactivity in the eyebrow tissues.

The use of a 3-dimensional construct of the eyebrow profile in both healthy patients and those with TAO aimed to describe the relative volumetric changes related to the disease and lay the basis for the recognition of the clinical entity of thyroid-associated periorbitopathy.

Higher fat volumes in patients with TAO likely reflect the markedly expanded adipogenic potential of the eyebrow fibroblasts. The phenotypic discrepancy of these cells, with an overexpression of the thyrotropin receptor and IGFR1b, suggests a divergent biologic potential, similar to the orbital fibroblasts, with enhanced proliferative and biosynthetic pathways.\textsuperscript{24,25} We recognize, though, that facial weight and body mass index (calculated as weight in kilograms divided by height in meters squared) differences between TAO and control groups might have influenced the results.

Of considerable mechanistic importance is that circulating Graves disease–IgG activates IGFR1b and relevant signal transduction pathways that upregulate all 3 isoforms of hyaluran synthase.\textsuperscript{3,26-27} This biologic component could represent the basis for the observed expansion of volume in female patients with TAO. The soft-tissue muscle eyebrow volume decreased with age in the healthy female group, likely related to menopause-induced estrogen deprivation.\textsuperscript{28-31} In contrast, this volume appeared to remain constant as patients with TAO aged, perhaps related to the enhanced synthetic rate of hyaluran and glycosaminoglycan accumulation in the eyebrow. Although female patients with TAO did not differ with respect to soft-tissue volumes at age 35 years, they had significantly higher levels than healthy patients of the same age at ages 50 and 65 years. These elevated parameters mirror the generalized activation and proliferative status of eyebrow fibroblasts in TAO. There was no significant correlation between the amount of proptosis and the galeal or ROOF fat. Therefore, the expanded eyebrow fat volume was not related to proptotic displacement of preseptal or orbital fat across the supraorbital rim.

Three-dimensional imaging analysis of patients with TAO brings into focus thyroid-associated periorbitopathy, an under-reported clinopathologic entity in patients with TAO. This study provides evidence for the expanded anatomic and clinical spectrum of site-specific immunoreactivity around the orbit in patients with TAO specifically the eyebrow region. As clinicians and scientists, we should integrate assessment of the eyebrow profile in our clinical evaluation of patients with TAO. In addition, the disfiguring eyebrow changes seen in some patients with TAO can lead to persistent patient dissatisfaction (ongoing quality-of-life studies with preliminary data confirming clinical observations) following the classic 3 stages of rehabilitation of TAO. Surgical management of affected patients might appropriately address the eyebrow region as well; for example, lipocountouring of the ROOF and possibly anterior galeal fat (stage 6).

In the future, translational research on involvement of the periorbital soft tissues may help us better characterize, classify, and guide surgical treatment of Graves orbitopathy. We suggest that both from a therapeutic standpoint regarding treatment of the aesthetic deformity and a diagnostic standpoint regarding a clinical parameter that can be useful in recognition and grading of TAO, measurement and understanding of thyroid-associated periorbitopathy can improve patient care and redefine the goals of rehabilitation.

Ophthalmic Images

Radial Keratoneuritis in Acanthamoeba Keratitis
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Jonathan Letsch, MD
Benoît Guignier, MD
Tristan Bourcier, MD, PhD

A spectral-domain optical coherence tomography examination was performed with the anterior segment module to provide images detailing radial keratoneuritis in a 30-year-old woman with microbiologically proven Acanthamoeba keratitis who used monthly disposable soft contact lenses.