Volumetric measurements in Graves’ orbitopathy
Regensburg, N.I.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Densities of orbital fat and extra ocular muscles in Graves’ Orbitopathy patients and controls

Noortje I Regensburg\(^1\), Wilmar M Wiersinga\(^2\), Tos TJM Berendschot\(^3\), Peerooz Saeed\(^1\), Maarten P Mourits\(^1\)

\(^1\) Orbital Centre; Dept. of Ophthalmology, Academic Medical Centre, University of Amsterdam, the Netherlands, \(^2\) Dept. of Endocrinology and Metabolism, Academic Medical Centre, University of Amsterdam, the Netherlands, \(^3\) University Eye Clinic Maastricht, the Netherlands

Abstract:

PURPOSE To investigate Computer Tomography (CT)-densities (densities) of orbital soft tissue volumes in patients with Graves’ Orbitopathy (GO) and to compare these with the densities of controls.

DESIGN Observational case series

PATIENTS AND METHODS Of 95 patients with GO and 150 controls, soft tissue volumes, mean densities, and ratios of fat volume/orbital volume (FV/OV) and muscle volume/orbital volume (MV/OV) were calculated with software. The 95% Confidence Intervals of the controls were used as reference values. The densities were plotted against age and volume ratios. For statistical analysis SPSS 16.0.2 was used; p-values were calculated with the following tests ANOVA, Pearson correlation, Kruskal-Wallis, Mann-Whitney and linear regression.

MAIN OUTCOME MEASUREMENT Differences in orbital soft tissue densities.

RESULTS In GO patients the mean orbital fat density was significantly higher than in controls (p≤0.001) and independent of age (p=0.23). The mean Extra Ocular Muscle (EOM) density of GO patients was within the range of controls and did not decrease with age (p=0.16) as it did in controls (p≤0.001). Mean fat density increased with decreasing fat volume (p=0.001). Mean EOM density increased slightly with increasing muscle volume (p=0.09). Muscle density correlated with fat density in both controls and GO patients.

CONCLUSION Orbital fat density in GO patients is significantly higher than in controls and negatively correlated to fat volume, but positively correlated to muscle volume and muscle density.
Introduction

In Graves’ Orbitopathy (GO) increased volume of extra ocular muscles (EOM) and of orbital fat are characteristic clinical findings, which can be observed and evaluated with Computed Tomography (CT) or Magnetic Resonance Imaging (MRI). The observed changes in orbital fat unleashed a growing interest. Orbital fat, as shown by Koornneef et al., is not merely space filling, but has a distinct shape of its own. Fibroblasts in orbital fat are induced to secrete excessive amounts of Gag’s (Glucosaminoglycan’s a strong hydrophilic molecule) and a subset differentiates into adipocytes. This mechanism leads to an increase in soft tissue volumes in the orbit and consequently to exophthalmus.

Previous studies of our group showed unexpectedly a rather low prevalence of 14 % of increased orbital fat in GO patients as calculated from CT- image stacks. Of 95 GO-patients, only 5% showed an increase of just fat volume (FV), 8% an increase in FV and muscle volume (MV), whereas 61% showed an increase in only EOM volume. These results are not in line with the experience of orbital surgeons performing orbital decompression in patients with GO, who usually assess large masses of fat bulging through the incised periorbit. Our detailed study of 3D-reconstructions of fat volumes in controls and in GO-patients revealed evident visual differences between both groups, attributable to differences in CT-density (density) of various tissues.

We hypothesize, that in GO patients not only orbital soft tissue volumes change, but also their densities. Therefore we measured orbital fat and EOM densities in GO patients and in controls.

Patients and Methods

Soft tissue volumes and densities were measured of 150 orbits without orbital disease in order to obtain reference values and in 190 orbits of 95 GO patients. To eliminate racial differences in orbital volumes, only Caucasians were chosen. GO patients were included if they were > 20 years of age (to exclude ongoing changes due to growth), Caucasians and were clinically diagnosed with GO by experienced ophthalmologists and endocrinologists during a combined thyroid-eye clinic.

Clinical symptoms of GO were described and classified using the NO SPECS classification (No signs and symptoms, Only signs, Soft tissue involvement, Proptosis, Extraocular muscle involvement, Corneal involvement and Sight loss) and the Clinical Activity Score (CAS). GO patients were excluded if they refused to participate, had biochemical overt hyperthyroidism (high FT4, suppressed TSH), were pregnant, were known drug abusers, had received previous treatment for their orbitopathy (radiotherapy/surgical decompression), had an incomplete set of CT images or CT-scans with uncorrected Gantry-tilt.
CT-scans were made with a Philips Mx8000 scanner using the following scan protocol: Orbital Helix, 120kV, 50 mAs, 1.3 mm thickness, 0.7 mm increment, pitch 0.875, filter B, FOV 150. The software program Mimics® converted the axial images also in coronal and sagittal reconstructions. The scans had been obtained in the course of the routine clinical care.

Although the local Ethical Committee considered this study not subject to consent, all participants were asked to sign an informed consent in advance. We followed the tenets of the declaration of Helsinki.

Orbital fat and muscle densities were computed in Hounsfield Units (HU) by the software program Mimics®. Orbital fat volume (FV) and muscle volume (MV) of the four EOM were calculated with computer assisted region growing and manual segmentation using the software Mimics® following the protocol described previously. To eliminate anatomical differences between subjects, the ratios fat volume/orbital bony cavity volume (FV/OV) and muscle volume/orbital bony cavity volume (MV/OV) were used. The most affected orbits of the 95 GO patients were used for further analysis in the present paper.

To reduce variation of fat and EOM density due to the location in the orbit (figure 1), we took the mean density of the total fat and EOM volume as segmented. Reference values of orbital fat and EOM were established from control orbits.

We then divided the GO patients into a group with a fat density at or below the 97.5 percentile (P≤97.5) of reference values and a group with a fat density of P≥97.5. Endocrinological and ophthalmological parameters of these two groups were compared. SPSS 16.0.2 was used for statistical analysis. Applied tests were ANOVA, Pearson correlations, Kruskal-Wallis and Mann-Whitney non parametric test (p-value of ≤ 0.05 was considered significant) and linear regression analysis.

Figure 1. Densities (in Hounsfield Units as mean ± SD) calculated from a defined area (in mm²) in an axial image of a computed tomography (CT) scan of medial rectus muscle (red) and orbital fat (turquoise). Density values may differ on different spots within muscle or fat tissue in the CT image.
Results

Figure 2 shows frequency histograms of mean densities in Hounsfield Units (HU) of orbital fat density and EOM volumes in controls and in 190 orbits of 95 Graves’ Orbitopathy patients. Mean orbital fat densities of GO patients were higher than in controls (mean HU ± SD are -70.3 ± 4.9 and -79.1 ± 5.4 respectively, p<0.001). EOM densities did not differ between both groups (mean HU ± SD are +24.0 ± 5.8 and 22.9 ± 6.5 respectively, ns).

![Figure 2](image)

The reference range of orbital fat and EOM densities as a function of age, calculated from the control orbits, is depicted in figure 3. In non-diseased orbits orbital fat densities are independent of age (y=-0.1466*x -78.358, r= -0.043, p=0.600), but EOM densities decrease with advancing age ( y= -0.217*x +33.344, r= -0.529, p=0.000). In 95 most affected orbits of GO patients orbital fat densities were -like in controls- not related to age (y= -0.01551*x -69.554, r= -0.038, p= 0.713), but EOM densities were – in contrast to controls- also independent of age (y=- 0.00671*x +24.377, r= -0.014, p=0.894). In 43% of the 95 GO patients orbital fat densities were above P97.5 of reference values, whereas EOM densities exceeding the P97.5 value were observed in just 3% (figure 3).

Figure 4 shows relationships between densities and volumes. The continuous lines depict the regression lines between densities and volume in control orbits. The observed negligible slopes indicate absence of an association between orbital fat and EOM densities and volumes in non diseased orbits. The interrupted lines refer to GO patients. Here a negative correlation is observed between fat volume (FV/OV) and both fat density (figure...
Figure 3. Scatter plots of the mean (CT)-density of orbital fat and extraocular muscles from the most affected orbits of 95 patients with Graves’ orbitopathy (GO) against age. Continuous lines indicate percentiles 2.5 and 97.5 of reference values obtained from measurements in 150 control orbits.

Table 1. Characteristics of patients with Graves’ orbitopathy in whom the orbital fat density was ≤ P97.5 vs. ≥ P.97.5 of the normal reference values (values given as median with interquartile range between brackets).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>orbital fat dens ≤97.5 (N=54)</th>
<th>orbital fat dens ≥97.5 (N=41)</th>
<th>p-value #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean density fat (HU)</td>
<td>-73 [-76- -71]</td>
<td>-66 [-68- -64]</td>
<td>0.000</td>
</tr>
<tr>
<td>Mean density muscle (HU)</td>
<td>22 [17-27]</td>
<td>28 [25-30]</td>
<td>0.000</td>
</tr>
<tr>
<td>FV/OV'</td>
<td>0.64 [0.55-0.69]</td>
<td>0.5 [0.47-0.65]</td>
<td>0.003</td>
</tr>
<tr>
<td>MV/OV''</td>
<td>0.20 [0.17-0.23]</td>
<td>0.4 [0.19-0.29]</td>
<td>0.010</td>
</tr>
<tr>
<td>Age (years)</td>
<td>50 [43-61]</td>
<td>51 [42-57]</td>
<td>0.781</td>
</tr>
<tr>
<td>Lidaperture (mm)</td>
<td>12 [10-13]</td>
<td>13 [12-14]</td>
<td>0.025</td>
</tr>
<tr>
<td>Proptosis (mm)</td>
<td>22 [20-24]</td>
<td>21 [20-23]</td>
<td>0.234</td>
</tr>
<tr>
<td>Abduction (degree)</td>
<td>48 [40-50]</td>
<td>45 [42-48]</td>
<td>0.129</td>
</tr>
<tr>
<td>Adduction (degree)</td>
<td>46 [43-49]</td>
<td>46 [42-50]</td>
<td>0.740</td>
</tr>
<tr>
<td>Elevation (degree)</td>
<td>40 [30-46]</td>
<td>41 [30-47]</td>
<td>0.912</td>
</tr>
<tr>
<td>Diplopia score*</td>
<td>1 [0-1]</td>
<td>1 [0-2]</td>
<td>0.807</td>
</tr>
<tr>
<td>CAS</td>
<td>2 [1-2]</td>
<td>2 [1-3]</td>
<td>0.804</td>
</tr>
<tr>
<td>TSH (mu/L)</td>
<td>0.93 [0.08-4.10]</td>
<td>0.88 [0.02-3.2]</td>
<td>0.470</td>
</tr>
<tr>
<td>FT4 (pmol/L)</td>
<td>15.1 [12.3-17.5]</td>
<td>15 [12.418.7]</td>
<td>0.657</td>
</tr>
<tr>
<td>Anti TPO (kU/L)</td>
<td>120 [39-1010]</td>
<td>160 [22-610]</td>
<td>0.825</td>
</tr>
<tr>
<td>TBII (U/L)</td>
<td>4.8 [2.4-16.2]</td>
<td>9.6 [2.6-16.7]</td>
<td>0.278</td>
</tr>
</tbody>
</table>

# by Mann-Whitney non parametric test. HU=Hounsfield units, 'FV/OV'=ratio fat volume/orbital volume, "MV/OV"= ratio muscle volume/orbital volume, °proptosis measured with a Hertel exophthalmometer in mm, °0=no diplopia , 1=intermittent diplopia, 2=inconstant diplopia, 3=constant diplopia. CAS=clinical activity score. TSH=thryoid stimulating hormone, FT4= free thyroxin 4, anti TPO= thyroid per oxidase antibodies, TBII=TSH binding inhibitory immunoglobulin's.
Figure 4. Scatter plots depicting relationships between orbital fat (FV/OV) and extraocular muscle (MV/OV) volumes and mean orbital fat and extraocular muscle densities. Continuous lines represent regression lines \(y=ax+b\) of relationships in control orbits, interrupted lines those in 95 GO orbits.

4a, controls: \(y=-0.001270\times+0.463, r=0.068, p=0.41\). GO, \(y=-0.008599\times+0.000276, r=-0.327, p=0.001\) and muscle density (figure 4b, controls: \(y=-0.001452\times+0.596, r=0.094, p=0.254\). GO, \(y=-0.00556\times+0.739, r=-0.252, p=0.014\). A positive correlation is observed between muscle volume (MV/OV) and both muscle density (figure 4c, controls: \(y=-0.000966\times+0.150, r=-0.39, p=0.63\). GO, \(y=-0.00189\times+0.178, r=0.175, p=0.090\) and fat density (figure 4d, controls: \(y=0.000246++0.167, r=0.083, p=0.0315\). GO, \(y=0.00272\times+0.416, r=0.211, p=0.040\). In other words, the lower the fat volume, the higher fat and muscle density and the higher the muscle volume, the higher the muscle and fat density. Mean densities in orbital fat compartment are positively related to
mean densities in EOM, both in control orbits (y=0.326x-86.531, r= 0.393, p<0.001) and in GO patients (figure 4e, y=0.421x -80.450, r=0.501, p<0.001).

We compared GO patients having a mean orbital fat density at or above the 97.5 percentile of the normal reference range with those below the P97.5 (table 1 page 62). Patients with high fat densities had less orbital fat volume and greater muscle volume. No differences were observed between both groups in recorded eye changes or thyroid function tests, except lid aperture which was higher in the group with fat densities above P97.5.

Discussion

This study shows that the mean density of orbital fat is higher in GO-patients compared to controls and does not change with age. This higher density suggests that not only the volume, but also the structure of the orbital fat undergoes changes in GO. Given the used HU (Hounsfield Units) for fat (-200 HU to -30 HU), the fat density in GO moves closer to water (HU value 0). As edema (water + water soluble materials) has HU of around 0, the increased density of the fat compartment in GO-patients may be the result of increased watery contents. One would expect to find a relationship between the mean fat density and the clinical activity score, for a higher CAS (more active GO) is supposed to be correlated with edema. However we did not find such a relationship. Low accuracy of the CAS to detect tissue edema and/or deposition of hitherto unidentified material may explain the increased density. Another explanation for the increased fat density may be compression of the fat cells themselves. Indeed, increased intraorbital pressure is a hallmark of GO as described by various authors. 12,13 In line with this is our finding that an increase in fat density is associated with decrease in fat volume and increase of muscle volume. Compression of orbital fat might be in agreement with clinical experience of orbital surgeons that upon incision of the periorbit large masses of fat bulge forward. One may further speculate that decreasing space in the orbit resulting from increased muscle volume causes the fat to be absorbed. The connective tissue strands remain intact. These strands have a higher X-ray absorption, thus explaining the increased density of the “fat compartment”. Finally in GO patients a reduced blood flow in the orbit has been reported. 14 The resulting orbital hypoxia may influence negatively adipogenesis by inhibiting triglyceride accumulation in adipose tissue. 15

In contrast to fat density, we found no significant change in GO patients of the mean muscle density. However the mean muscle density did not decrease with age as it did in controls. In other words we did find a relative increase of the mean muscle density in GO patients, because the age-dependant decrease of mean muscle density in GO patients disappears. We also assessed that with increasing muscle volume the mean muscle density increased as well. As the muscle volume is increased in as much as 61 percent of GO patients17, the question arises whether this increase is due to ‘hypertrophy ad
numerum’ or ‘hypertrophy ad magnitudinem’. If muscle fibers are swollen due to edema, one may expect the density to decrease (e.g. closer to 0). Since we found no decrease of mean muscle density, in this study no arguments can be found in favor of a ‘hypertrophy ad magnitudinem’. A relative increase of mean muscle density in GO patients may be caused by muscle fibrosis or muscle fiber degeneration. Here again orbital hypoxia may play a role. We know that EOM are extremely sensitive to hypoxia, since they possess a large amount of mitochondria and have a high oxygen need. Support for this hypothesis comes from Uhlenbrock who calculated the mean density of rectus muscles and found a relation between density and duration of the disease.

In order to assess whether the subgroup of GO patients with fat densities \( P \geq 97.5 \) differed clinically from the one with fat densities \( P \leq 97.5 \), we compared these two subgroups with regard to clinical parameters. Patients with fat densities above the P97.5 had less fat volume and more muscle volume, a higher muscle density and a larger eyelid aperture. This larger lid aperture is understandable when one considers the etiology of lid retraction in GO. An increased lid aperture is caused by an over-activity of the levator muscle (sometimes as a response to the stiff inferior rectus muscle) or by adhesions. Increased fat density occurs in the presence of increased muscle volume, an increased size of especially the inferior rectus muscle causes upper lid retraction.

Mean fat density increases in parallel with mean muscle density in both GO and controls. This means that there is a consistent balance between the soft tissue densities. It appears there is a similar cell infiltration or water absorption in fat and muscle tissues in GO.

**Conclusion**

This study shows, that the mean density of orbital fat is higher in GO-patients than in controls, which suggests that not only fat volume, but also fat structure undergoes changes in GO. Fat compression, absorption, infiltration and muscle fibrosis may explain the increased fat densities.
References


5. Fledelius HC. [Exophthalmometry and thyroid disease. The value of the Hertel measurement evaluated by a group of patients with thyroid diseases and a control group]. *Ugeskr Laeger.* 1994;156:6528-6531.


