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

Citation for published version (APA):

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Chapter 7

General discussion
General Discussion

Graves’ orbitopathy (GO) is characterized by changes in orbital soft tissues. This thesis studied these changes by measuring the soft tissue volumes with a standardized and validated tool.

1. Technical aspects of orbital volumetry

For the calculations of orbital soft tissue volumes in health and disease, Computed Tomographic (CT) or Magnetic Resonance Imaging (MRI) scans are used. In the Netherlands, for practical purposes, CT scans are preferred in the diagnosis of GO, because they do not only show us the size of the muscles, but also the structure of the bony orbital walls, which is needed in case of orbital bony decompression. Moreover, MRI scans take more time and are not suitable for claustrophobic patients. In our studies we used CT scans exclusively, since these were routinely made in all our patients with GO.

In the past, most orbital CT imaging was in direct transverse, coronal or sagittal planes. Volume measurement based on these 2-dimensional images suffered from subjective visual interpretation and from poor measurement of dimensions (e.g. muscle thickness) or areas (e.g. mid-orbital muscle area). These measurements depended on the location and orientation of the imaging plane, and could not adequately assess the total volume of the muscles.

Kwon et al. recently pointed out there are substantial differences between measurements in axial and coronal plane of orbital volume. Scanning in the axial plane is the preferred technique for our software, because of the possibility of thinner slices and the opportunity of good coronal and sagittal reconstructions. In our opinion, the only way to reach the highest possible accuracy is to work through axial, coronal and sagittal images and to display 3-D results in the 3-D displayed orbit in order to check the obtained measurements.

The introduction of thin slices and high spatial resolution of more advanced CT scanners made it possible to perform more precise measurements. Volume measurements were first based on manually drawn contours on X-ray film or on the display screen. These resulted in an error rate between 7-10%. However, since CT can distinguish between various tissues due to different tissue densities on the basis of X-ray absorption, tissue segmentation on CT scans (region growing) can be performed by computer assisted selection of all pixels with particular densities (HU) to include the preferred tissue. The error rate of this technique is around 3%. With our method of calculating volumes, the intraobserver variability of the principal observer was 0.98% for orbital volume (OV), 0.97% for fat volume (FV) and 2.6 % for muscle volume (MV).

The advantage of the region growing method is that it can correctly separate the regions that do not have the same predefined properties. A disadvantage is that the subsequent manual segmentation is time consuming. However, nowadays with fast computers it can be done reasonably quickly, e.g. within 45 minutes per orbit. Therefore, we used both
region growing and manual segmentation simultaneously in axial, coronal and sagittal images with the control of 3-D reconstruction.

1.1 Extra ocular muscle volumes
An overview of the methods used in segmentation of extraocular muscles (EOM) from CT and MRI scans is given by Bijlsma et al.11 Not only the methods of segmentation differed between researchers, also the plane (axial, coronal) in which they worked is different. Tian et al. found, that the muscle volumes differed significantly when measured in various planes and that the results could thus not be compared.12 In coronal views, the rectus muscles tend to become inseparable as the orbital apex is approached. Especially in GO when the muscles are enlarged, it is difficult to distinguish the individual muscle border.13 Our method of working in axial, coronal and sagittal images minimizes the difference in volumes from the real volume compared to volumes measured in only one plane.9

Even with improved imaging techniques the superior rectus muscle, superior ophthalmic vein and the levator palpebrae muscle, which are in a very close relationship, cannot be distinguished from each other. Most researchers measure these structures together as the levator-superior rectus complex which is the reason for a slightly larger muscle volume than the rectus muscle in reality has.12;14-17 More anteriorly in the orbit, the muscles transit to tendon before insertion on the globe. Volume measurement should stop at the tendon, but it is hard to point out this transition.13 However, the tendinous part is small and will have little effect on measurement accuracy.18 We decided to make our cut off point as the muscle touches the globe. This point can be clearly seen in the CT image stacks. At present, it is not possible to measure the exact full muscle volume. However, in our opinion, muscle volume on CT scans is best measured using a region growing algorithm, with manual segmentation accepting a slight overestimation of the true volume due to inevitable selection of non-muscular tissue.

HU values used for identification of extraocular muscles differed among different observers. For instance Forbes et al. used values from 0 to +135 HU, Gorman et al. from 0 to +200 HU and Zonneveld et al. from −30 to +160 HU.19-21 To be sure to include all pixels in the changed muscles in patients with GO, we measured the lowest and highest HU in our CT scans by thresholding each CT scan with the software Mimics® (Materialise, Leuven, Belgium). It turned out that EOM densities in our calculations were between -30 to and +100 HU.9;22

1.2. Orbital fat volumes
At the introduction of the HU values, the HU values of fat were set between -100 to -50 HU 23, since orbital fat absorbs less X-ray energy than water (HU=0) and more than air (HU=-1000). With thresholding we found the lowest value to be -200 HU and the highest -30 HU for the orbital fat in our patient group. [chapter 4]. Reports on fat volume calculations are found in the maxillo-facial surgical literature dealing with blowout fractures. In these reports the contralateral, not injured, orbit is always taken as the control orbit. As with EOM however, the method of calculation varies.12;18;21;24-26
In our 3D reconstructions of the orbital fat (Figure 1), one can distinguish the spaces in the fat occupied by the muscles, the optic nerve and the lacrimal gland. This is in line with the report of Koornneef in 1977, who showed by slicing orbital specimens, that the orbital fat has a distinct shape of its own.

Only a few volume measurements of the orbital fat using CT have been reported in GO. Since the methods of these measurements differed between the researchers, comparison of the observed can only be approximative. Better comparison is possible with the calculations of Ramieri et al., who used the same method, although they did not validate their method.

2. Physiological aspects of orbital volumetry

Reference ranges of orbital soft tissue volumes have been reported in several studies. Table 1 gives an overview of applied methods in obtaining intervals and their results. The large variations in the reported values are most likely due to the method used. Especially when the scan slices are thicker and multiplying areas with slice thickness will result in over- or underestimation of the volume.

In table 1 we did not insert the report of Forbes et al. from 1983, the report of Nishida et al. from 2001 and the report of Gorman 2002, because the numbers given for controls had almost the same values as in the other report of the same author, meaning that the same control group was used.

A number of limitations, however, prevent general use of these reference ranges. First, there are important racial differences. Asians have shallower orbits than Caucasians. Von Lanz et al. found higher mean orbital bony cavity volumes in Caucasian than in Japanese adults (27 cm$^3$ and 23 cm$^3$ respectively). Second, there may be gender and age specific differences in orbital volumes. Although volumes of men and women are often reported

![Figure 1. Medial and anterior view on the orbital contents, showing the fat body (green) of the right orbit with free space for muscles indicated in pink and optical nerve (dark blue)](image-url)
separately, age is usually not taken into account and race is often not mentioned. Therefore, the second aim of the present thesis was to provide age- and gender-specific reference values of orbital soft tissue volumes in adult Caucasians.

Orbital volume calculations are needed in plastic and reconstructive surgery to aid to the reconstruction of the orbit after fractures. The orbital volume of an adult is presumed to be in a static phase throughout life. However, Kahn et al. showed that the bony orbit like other bones in the body changed in shape during aging. We observed, however, that the volume of the bony orbit did not change in our control group. It turned out that men have bigger orbits with larger fat volumes and larger muscle volumes than women. We calculated the ratios of fat volume/orbital volume and muscle volume/orbital volume. This provided us with the proportion of the orbital volume that is occupied by fat and muscle respectively. Since these ratios were the same for men and women we eliminated gender differences.

We observed higher fat volumes with advancing age (FV ranged from 13.8 – 18.7 (p<0.001) in men and 11.9 – 17.2 (p<0.001) in women). This finding was unexpected, because most people assume that orbital fat slowly regresses in the last decades of life. The measurements of Darcy et al. supported our finding that fat volume increased with increasing age. In general, muscle mass in the body decreases with age; however, the volume of the EOM, as we observed it, did only slightly decrease in the course of life, and only in women, but not in men. By establishing reference values for the age groups between 20 and 70 years, we provided the possibility to distinguish between “normal” and enlarged volumes.

Table 1, Reported reference values for orbital fat volume (FV) and extra ocular muscle volume (MV) in non diseased human orbits

<table>
<thead>
<tr>
<th>Year</th>
<th>Author</th>
<th>Scan+slice (mm)</th>
<th>Method*</th>
<th>Select orbits</th>
<th>Control group</th>
<th>Ethnic years</th>
<th>Age ♀/♂</th>
</tr>
</thead>
<tbody>
<tr>
<td>1985</td>
<td>Forbes</td>
<td>CT 1.5</td>
<td>1</td>
<td>a</td>
<td>42</td>
<td>ns</td>
<td>28-76</td>
</tr>
<tr>
<td>1989</td>
<td>Krahe</td>
<td>CT 3</td>
<td>3</td>
<td>b</td>
<td>80</td>
<td>ns</td>
<td>48</td>
</tr>
<tr>
<td>2000</td>
<td>Tian</td>
<td>MRI 2</td>
<td>2</td>
<td>c</td>
<td>42</td>
<td>ns</td>
<td>32-63</td>
</tr>
<tr>
<td>2001</td>
<td>Gorman</td>
<td>CT 1.5</td>
<td>1</td>
<td>b</td>
<td>54</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>2002</td>
<td>Nishida</td>
<td>MRI 2</td>
<td>2</td>
<td>ns</td>
<td>13</td>
<td>ns</td>
<td>33-69</td>
</tr>
<tr>
<td>2003</td>
<td>Detorakis</td>
<td>MRI 2.3</td>
<td>3</td>
<td>b</td>
<td>5</td>
<td>ns</td>
<td>37-62</td>
</tr>
<tr>
<td>2005</td>
<td>Szucs</td>
<td>MRI 3</td>
<td>3</td>
<td>a</td>
<td>40</td>
<td>ns</td>
<td>23-72</td>
</tr>
<tr>
<td>2010</td>
<td>Regensburg</td>
<td>CT 1.3</td>
<td>1</td>
<td>b</td>
<td>160</td>
<td>cauc</td>
<td>20-80^</td>
</tr>
</tbody>
</table>

*Method of measuring, 1= region growing, 2= pen outlining, 3= screen outlining, ns= not stated
cauc= Caucasian, select control: a= considered normal, b= disorder fellow eye/orbit (no orbital disease), c= healthy, ^10 orbits / decade
3. Orbital volumetry in GO

Volumetry in GO can be applied to assess the relative contributions of fat and muscle volumes to the overall increase of orbital soft tissue volumes. Although muscle volume increase in GO is the most striking phenomenon in GO, an increase in orbital fat volume can also be assessed.\textsuperscript{29,32,45} Forbes et al. measured orbital fat and muscle volumes on CT-scans in the orbit of 22 healthy subjects and 72 Graves’ patients. They found increased volumes in GO patients when compared with controls.\textsuperscript{46} In 49% of their patients there was an increase in both muscles and fat volume, in 39% of patients an increase of only muscle volume and in 11% an increase of only fat was observed. Although few volumetric studies focussed on the orbital fat from CT scans, the available data suggested that not all GO patients have enlarged eye muscles; a subset of patients may exist with just fat enlargement.\textsuperscript{31,32,47} What are the determinants of this increase in fat volume? To evaluate the possible increase in fat volume, the ratio found had to be corrected for age, since we showed that the orbital fat volume increased with age. In doing so, we showed that the majority of GO patients in our group had no increase in fat volume. Only a limited number (5.2%) had only fat volume increase and a few (8.4%) had muscle- and fat volume increase. The number of patients, who had only muscle volume increase, was large (61.1%) and there was also a substantial group with no increase of fat or muscle volume (25.3%). This assessment is not in line with the finding of Zonneveld et al., who reported (in 40 orbits) 2% no increased fat or muscle volume, 27.5% increased fat volume only, 20% only muscle volume increase and 47% increased fat and muscle volume.\textsuperscript{29} Forbes et al. had the same percentage for the group of muscle and fat volume increase and no group without any soft tissue volume increase. But, as we stated before, age was not taken into account. We concluded that in our patient group, absolute fat volume increase (e.g. exceeding the borders of the 95% confidence interval) was limited. However, one could have a substantial increase of FV within the 95% confidence interval. Is there a difference between the group with fat volume increase vs. no fat volume increase? Yes,

<table>
<thead>
<tr>
<th>gender</th>
<th>FV mean cm$^3$</th>
<th>range cm$^3$</th>
<th>MV mean cm$^3$</th>
<th>range cm$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>♂/♀</td>
<td>♂/♀</td>
<td>♂/♀</td>
<td>♂/♀</td>
<td>♂/♀</td>
</tr>
<tr>
<td>12/9</td>
<td>10.10/ 11.19</td>
<td>8.22-12.20/ 8.56-14.0</td>
<td>4.69/4.79</td>
<td>3.07-6.20</td>
</tr>
<tr>
<td>17/23</td>
<td>9.5/10.6</td>
<td>7.1- 11.9/ 7.7-14.3</td>
<td>1.5/2.2</td>
<td>1.1- 1.9/ 1.2-3.2</td>
</tr>
<tr>
<td>11/10</td>
<td>21.59</td>
<td>18.72- 24.46</td>
<td>2.4</td>
<td>2.3- 2.8</td>
</tr>
<tr>
<td>ns</td>
<td>9.0</td>
<td>6.6 - 11.4</td>
<td>8.8</td>
<td>7.4-10.2</td>
</tr>
<tr>
<td>7/6</td>
<td>19.97</td>
<td>15.92-24.02</td>
<td>2.98</td>
<td>2.51-3.54</td>
</tr>
<tr>
<td>2/3</td>
<td>15.7</td>
<td>13.4-20.4</td>
<td>2.42</td>
<td>1.89-2.96</td>
</tr>
<tr>
<td>15f/ 5</td>
<td>15.95</td>
<td>14.27-18.40</td>
<td>3.32</td>
<td>2.83-3.64</td>
</tr>
<tr>
<td>88/ 72</td>
<td>14.0/16.2</td>
<td>11.9-17.2/13.8-18.7</td>
<td>3.7/4.2</td>
<td>3.86-3.77/4.01-4.04</td>
</tr>
</tbody>
</table>
we did find differences. The patients in the group with fat volume increase had more proptosis. In our patient group there were more patients with only muscle volume increase than expected. A possible explanation could be that in patients with proptosis, without striking muscle enlargement visible on a CT scan, the muscles are stretched. These stretched muscles appear to have a normal, not enlarged, volume visually, but calculating the volume proves otherwise. The subgroup with only muscle volume increase was older and had more proptosis, more impaired ductions, more diplopia and higher TBII than the groups without muscle volume increase. This is in line with reports indicating that GO runs a worse course in elderly patients.  

The duration of GO is a factor that might influence the fat- and/or muscle volume. If we divided our patient group according to the duration of GO in less than 1 year and more than 1 year, the latter group had significant higher fat volumes (table 2, p=0.004). Thus it is possible that fat volume increases with longer duration of GO. Here the finding of Hiromatsu et al. is most interesting. He observed that in orbital soft tissues from patients with GO, TH1-like cytokines were predominantly found in eye muscle tissue and related to the eye muscle enlargement and possibly had a role in the development of the eye muscle component in GO. In orbital fat tissue, however, there were predominantly TH2-like cytokines negatively related to orbital fat volume. Therefore, TH2-like cytokines, anti-inflammatory cytokines, may play a protective role in the chronic stage of GO. Studies of Bahn et al. indicated that TH1-like cytokines were predominant in the early stages of GO, whereas the profile of TH2-like cytokines dominated in the late stages of GO. Our results apparently are in agreement with the notion that fat volume enlargement is a rather late phenomenon in the course of GO.

In our study, we could not confirm that FV is frequently increased in GO, but looking at the 3D reconstructions of the orbital fat in GO, one can clearly see a difference in the structure of the orbital fat. In search for an explanation of this difference, we measured the mean CT density of orbital fat and muscles. A higher density would suggests that not only the volume, but also the structure of the orbital tissue undergoes changes in GO. The CT density of orbital fat and/or muscles is sometimes mentioned in the literature, but no systematic calculation was found.  

It is generally accepted that in the course of GO the orbital fat is infiltrated by T-lymphocytes and that, through the influence of cytokines and other factors, glycosaminoglycans (GAG’s) are produced that attract water. Our finding that the mean fat density in GO patients is significantly higher than in controls (p<0.000) supports this. However an unexpected finding was that the higher the density the smaller was the fat volume. The question arises whether the subgroup of GO patients with fat densities P>97.5 differed clinically from fat densities P<97.5. 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. 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. This would imply a similar cellular infiltration or water absorption in fat and muscle tissues in GO. Given the used HU 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. Insufficient 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. 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

### Table 2.

<table>
<thead>
<tr>
<th></th>
<th>&lt;1 year</th>
<th>&gt;1 year</th>
<th>M-W p=</th>
</tr>
</thead>
<tbody>
<tr>
<td>FV/OV</td>
<td>0.55</td>
<td>0.65</td>
<td>0.004</td>
</tr>
<tr>
<td>MV/OV</td>
<td>0.21</td>
<td>0.22</td>
<td>0.194</td>
</tr>
<tr>
<td>age(years)</td>
<td>52</td>
<td>50</td>
<td>0.087</td>
</tr>
<tr>
<td>lidaperture (mm)</td>
<td>12</td>
<td>12</td>
<td>0.522</td>
</tr>
<tr>
<td>proptosis^</td>
<td>21</td>
<td>22</td>
<td>0.030</td>
</tr>
<tr>
<td>abductie (degree)</td>
<td>46</td>
<td>46</td>
<td>0.869</td>
</tr>
<tr>
<td>adductie (degree)</td>
<td>46</td>
<td>46</td>
<td>0.806</td>
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<tr>
<td>elevatie (degree)</td>
<td>42</td>
<td>40</td>
<td>0.903</td>
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<tr>
<td>depressie (degree)</td>
<td>58</td>
<td>60</td>
<td>0.083</td>
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<tr>
<td>diplopia*</td>
<td>0</td>
<td>1</td>
<td>0.721</td>
</tr>
<tr>
<td>CAS</td>
<td>2</td>
<td>2</td>
<td>0.185</td>
</tr>
<tr>
<td>TSH (mu/L)</td>
<td>2.20</td>
<td>0.61</td>
<td>0.258</td>
</tr>
<tr>
<td>FT4 (pmol/L)</td>
<td>15.2</td>
<td>16.0</td>
<td>0.183</td>
</tr>
<tr>
<td>Anti TPO (kU/L)</td>
<td>160</td>
<td>100</td>
<td>0.417</td>
</tr>
<tr>
<td>TBII (U/L)</td>
<td>7.60</td>
<td>5.20</td>
<td>0.762</td>
</tr>
</tbody>
</table>

M-W= Mann-Whitney non parametric test, p<0.05 is considered significant, FV/OV= ratio fat volume/orbital volume, MV/OV= ratio muscle volume/orbital volume, ^measured with Hertel exophthalmometer, * 0= no diplopia, 1= intermittent diplopia, 2= constant diplopia, CAS= clinical activity score, TSH= thyroid stimulating hormone, FT4= free thyroxin 4, Anti TPO= thyroid per oxidase antibodies, TBII= thyroid binding inhibitory immunoglobulin.
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. The resulting orbital hypoxia may influence negatively adipogenesis by inhibiting triglyceride accumulation in adipose tissue. In contrast to fat density, we found no significant change of the mean muscle density in GO patients. 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. We also assessed that with increasing muscle volume the mean muscle density increased as well. As the muscle volume in GO patients is increased in as much as 70%, the question arises whether this increase is due to the presence of more muscle fibres or swelling of the muscle fibres. If muscle fibres 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, arguments are in favour of more fibres. A relative increase of mean muscle density in GO patients may be caused by muscle fibrosis or muscle fibre 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. The limitation of our present studies is that we calculated volumes and densities on a given point in time and we could not establish whether these patients were at the beginning of their disease or already in the steady state. Future longitudinal studies will hopefully clarify this issue. We plan to calculate the soft tissue volumes and densities of the same group after 1 and 3 years with follow up clinical evaluation to see if we can detect temporal changes in the soft tissues of the orbit in GO.

Smoking is an environmental factor that has a deleterious effect on the course of GO. Several papers in the literature point that in smokers the disease is more severe, more active and more resistant to therapy. Is the influence of smoking directed against fat or muscle? In the literature there are reports of experiments in vitro with cultured human fibroblasts exposed to tobacco smoke. The results of these experiments show an increased differentiation of fibroblasts into adipocytes, but this was only seen in laboratory experiments. Szucs-Farkas et al. were the first to calculate orbital connective tissue and muscles from MRI scans of GO patients and reported that the connective tissue volume in smokers was increased. However, the method used by Szucs-Farkas, outlining on axial MRI images is less precise than our method, due to the thickness of the MRI slices and the inclusion of other materials in the calculations. For instance, if there is edema, outlining will include this edema in the results, whereas with region growing in CT scans only the pixels within the fat range are taken into account. Moreover, their numbers were very small, they had only 16 current smokers, 6 never- and 8 ex-smokers. In our group we had 41 patients who never smoked, 23 ex- smokers and 27 current smokers and comparing them we found the muscle volume in current smokers to be
larger than in never- and ex smokers (p=0.034). There was no statistically significant increase in fat volume between these groups. 

Why would it be important to know the changes in orbital fat and muscle volumes? 

Volumetry has been proposed as an independent outcome measure in trials assessing the efficacy of treatment in GO. The assumption is that effective treatment will be associated with a decrease in orbital soft tissue volumes. Although this assumption has a histologic substrate it could also be the natural course. In recent literature, orbital decompression seems to give rise to an increase of EOM volume postoperatively. This could be due to a rarely observed activation of the GO that was previously described with subsequent increase in muscle volume. It is more likely that the surgical fenestration of the tough periorbit allows the muscles to expand and restore better blood circulation with an increase in volume as a result. In future studies we will calculate the volumes after decompression in our patient group and see if we can confirm these observations. 

Volumetry is important, because knowing the sequence of events in orbital soft tissue volumes may give us a better understanding of the pathogenesis and understanding differential behaviour of muscle and fat could lead to a novel tissue targeted treatment.
Reference List


