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Differential Involvement of Orbital Fat and Extraocular Muscles in Graves' Ophthalmopathy

Wilmar M. Wiersinga[a] Noortje I. Regensburg[b] Maarten P. Mourits[b]

[a]Department of Endocrinology and Metabolism, and [b]Orbital Center, Department of Ophthalmology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Key Words
Graves' ophthalmopathy · Volumetry · Orbital fat · Extraocular muscles · Determinants

Abstract
Graves' ophthalmopathy (GO) is characterized by swelling of orbital fat and extraocular muscles, but little attention has been given to differential involvement of fat and muscles. Advancements in imaging allow rather accurate measurements of orbital bony cavity volume (OV), fat volume (FV) and muscle volume (MV), and are the topics of this review. Ratios of FV/OV and MV/OV neutralize gender differences. In adult Caucasian controls, mean values ± SD of FV/OV are 0.56 ± 0.11 and of MV/OV are 0.15 ± 0.02. FV increases substantially and MV decreases slightly with advancing age, requiring age-specific reference ranges. In 95 consecutive untreated Caucasian GO patients, both FV and MV were within normal limits in 25%, increased FV but normal MV was present in 5%, normal FV but increased MV was detected in 61%, and both increased FV and MV was evident in 9%. Increased FV was associated with more proptosis and longer GO duration. Increased MV was associated with older age, more severe GO (more proptosis and diplopia, worse eye muscle ductions), higher TBII and current smoking. At the cellular and molecular level differential involvement of fat and muscles might be related to differences between fibroblast phenotypes and cytokine profiles in each compartment, to different orbital T cell subsets during the course of the disease and to peroxisome proliferator activator receptor-γ polymorphisms and modulation of 11β-hydroxysteroid dehydrogenase-1. Enlarged muscles are apparently a rather early phenomenon in GO, whereas increases in fat mass occur relatively late. Why a minor subset of GO patients presents with an increase of only fat remains poorly understood.

Introduction

Graves' ophthalmopathy (GO) is characterized by swelling of extraocular muscles and orbital fat [1]. Orbital imaging with CT or MRI allows evaluation of muscle and fat swelling, and most GO patients seem to have both an increased muscle volume (MV) and an increased fat volume (FV) [2]. Early studies in the 1980s, however, already reported some isolated cases of GO patients in whom only the retrobulbar fat compartment is increased without muscle enlargement [3–5] (fig. 1). This is intriguing because the finding suggests different phenotypes of GO with pos-

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Wilmar M. Wiersinga
Department of Endocrinology and Metabolism, Academic Medical Center
Meibergdreef 9
NL–1105 AZ Amsterdam (The Netherlands)
E-Mail w.m.wiersinga@amc.uva.nl

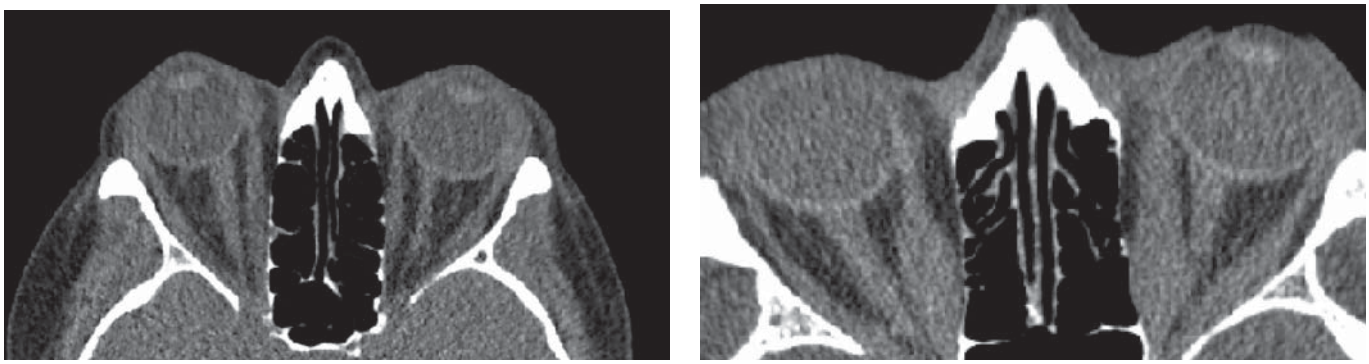

Fig. 1. Orbital CT scans of 2 patients with GO. The left panel depicts a CT scan in which only FV is increased, whereas in the right panel only MV is increased.

sible differences in pathogenesis. So far, the differential involvement of orbital fat and extraocular muscles in GO has scarcely been explored in a systematic manner. Technological advancements in 3D imaging of the orbit and computed volumetry now enables more accurate quantitative measurement of orbital fat and muscle volumes. In this review we evaluate the prevalence of increased orbital FV and increased extraocular MV in untreated GO patients, as judged from reference values of orbital volumes obtained in controls. We also try to link fat or muscle enlargement to clinical and biochemical parameters, and discuss possible cellular and molecular mechanisms for differential involvement of orbital fat and muscles.

Orbital Volumetry – Technique

Early volume measurements were based on 2D images of orbital CT scans, suffering from subjective visual interpretation and poor measurement of dimensions. Calculated orbital volumes are significantly greater using axial CT scans as compared to coronal CT scans [6]. The introduction of thin slices and high spatial resolution of more advanced CT scanners made it possible to perform more precise measurements, reducing the error rate [7]. Since CT can distinguish between various tissues due to different tissue densities on the basis of x-ray absorption, tissue segmentation on CT scans can be performed by computer-assisted selection of all pixels with particular densities to include the preferred tissue. Regensburg et al. [8] used both region-growing and manual segmentation simultaneously in axial, coronal and sagittal images of orbital CT scans with control of the 3D reconstruction, applying commercially available software. Orbital soft tissue CT numbers were set at –200 to +100 Hounsfield units (HU) for bony orbital volume, –200 to –30 HU for fat tissue and –30 to +100 HU for muscle tissue. Borders of the orbital aditus were determined by the frontal bone, frontozygomatic suture, inferior orbital rim and the anterior orbital crest. The entrance of the optic nerve in the orbital canal, the fossa pterygopalatina and the orbital fissures were used for the cut-off point of the orbital end. Extraorbital fat is erased by deleting fat tissue in the skin of the eyelids and fat in the superior and inferior orbital fissures. The method was validated using a phantom of butter and chicken wing muscles as equivalents for orbital fat and extraocular muscles, respectively. Intraobserver and interobserver variation of orbital volumes was in the order of 1–4%. Orbital volumetry can also be done on MRI, with T2-weighted scans and T1-weighted fat suppression scans [7, 9, 10]. 3D image sets offer better results than 2D MRI [11, 12]. Using high resolution scans and computer-assisted programs both CT and MRI provide rather accurate and comparable orbital volume measurements.

Orbital Volumetry – Reference Values

Applying the technique described by Regensburg et al. [8] we assessed orbital volumes in adult Caucasian control subjects in order to obtain reference values. Subjects (52 men and 55 women, aged 20–80 years) were stratified per decade in six age groups, according to gender [13]. Orbital volumes in men were greater than in women, but the sex difference disappeared when fat and muscle volumes were related to orbital bony cavity volumes (OV): the mean ratios of FV/OV (0.56) and MV/OV (0.15) were the same in men and women (table 1). Thus, on average

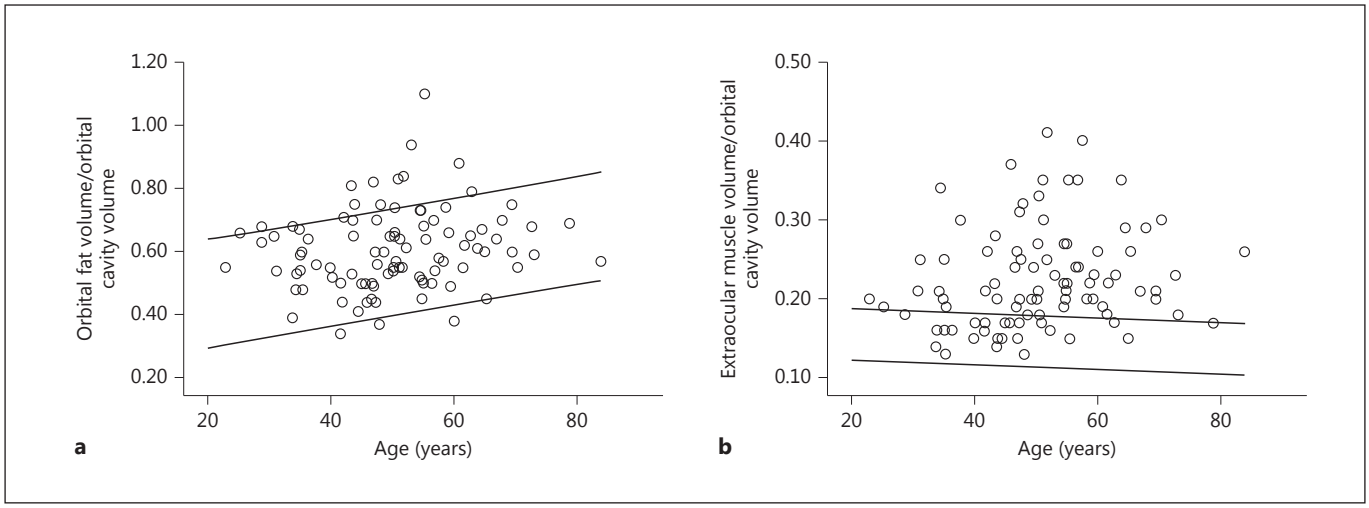

Fig. 2. Scatter plots of orbital FV (expressed as FV/OV; **a**) and orbital MV (expressed as MV/OV; **b**) of 95 consecutive patients with untreated GO. Continuous lines indicate percentiles 2.5 and 97.5 of age-specific reference values [21].

56% of the orbital volume consists of fat, and 15% of extraocular muscles. Whereas orbital bony cavity volume was not age-dependent, we observed an increasing FV and FV/OV with advancing age in both genders: FV/OV increased gradually from 0.49 in the 3rd decade to 0.68 in the 8th decade. Muscle volume did not change as a function of age in men, but slightly decreased in women (mean MV/OV ratio in men was 0.15, and in women 0.16 in the 3rd decade and 0.15 in the 8th decade). In constructing reference values, the use of FV/OV and MV/OV neutralized gender differences and to a certain extent also the rather wide interindividual variation observed in orbital volumes (OV range 20.7–34.5 cm^3, FV range 9.2–25.7 cm^3, MV range 2.47–5.42 cm^3). Reference values, however, had to be age specific. Reference intervals of FV/OV and MV/OV depicting the 2.5 and 97.5 percentile boundaries as a function of age are depicted in figure 2. The observed orbital volumes agree reasonably well with those reported in other papers, small differences are likely explained by differences in methodology [6, 14–16]. Orbital volumes in Japanese people (but not in Chinese) are somewhat lower than in Caucasians, indicating ethnic differences [17, 18]. Most studies report gender differences, with greater volumes in men compared to women. The effect of age has received little attention. The rapid growth of the orbit comes presumably to an end by 11 years in females and by 15 years in males [10, 17]. A recent publication reports that inferior periocular soft tissue anterior to the anteroposterior globe axis increases with age,

Table 1. Orbital fat volume (FV), orbital extraocular muscle volume (MV), orbital bony cavity volume (OV) and their ratios in 160 normal Caucasian orbits

	Male orbits (n = 72)	Female orbits (n = 88)	p value
FV, cm^3	16.2±3.4	14.0±2.9	<0.001
MV, cm^3	4.2±0.5	3.7±0.5	<0.001
OV, cm^3	28.9±2.4	24.9±2.2	<0.001
FV/OV	0.56±0.11	0.56±0.10	NS
MV/OV	0.15±0.02	0.15±0.02	NS

Data are given as mean ± SD [13]. NS = Not significant.

predominantly due to fat expansion [19]. Volumetry of the supraorbital area shows an age-related increase of fat volumes, whereas muscle volume decreases with age in healthy female controls [20]. These findings provide supporting evidence that orbital fat expansion occurs with advancing age. Taken together, determinants of orbital volumes include sex, age and ethnicity.

Orbital Volumetry in GO

Orbital volumetry – using the method described by Regensburg et al. [8] – was done in 95 consecutive patients with untreated GO, in whom thyroid function al-

Table 2. Characteristics of 95 consecutive untreated GO patients according to volume measurements

	Group 1 (n = 24) FV + MV ≤P97.5	Group 2 (n = 5) FV only >P97.5	Group 3 (n = 58) MV only >P97.5	Group 4 (n = 8) FV + MV >P97.5	p
Age, years	45 (37–51)	44 (27–50)	53 (47–59)	52 (44–59)	0.02
FV/OV	0.56 (0.50–0.63)	0.75 (0.67–0.79)	0.57 (0.50–0.65)	0.83 (0.80–0.93)	0.00
MV/OV	0.16 (0.15–0.17)	0.17 (0.14–0.19)	0.24 (0.21–0.28)	0.25 (0.22–0.33)	0.00
Lid aperture, mm	12 (10–13)	12 (12–14)	12 (11–14)	14 (12–15)	NS
Proptosis, mm	20 (17–21)	24 (23–26)	22 (20–23)	24 (22–27)	NS
Diplopia[1]	0 (0–0)	0 (0–0)	1 (0–2)	0 (0–1)	0.00
Elevation	45 (38–53)	34 (30–52)	38 (26–45)	43 (25–46)	0.03
Clinical activity score	2 (1–3)	1 (1–2)	2 (1–3)	2 (1–4)	NS
TBII, U/l	4.4 (1.9–9.2)	2.7 (1.0–3.9)	9.9 (2.8–22.6)	5.5 (0.5–22.1)	0.02

Data are median values with interquartile range in parentheses [21]. p values for group differences are by Kruskal-Wallis test. NS = Not significant.

[1] Score 0 = no diplopia, 1 = intermittent diplopia, 2 = inconstant diplopia, 3 = constant diplopia.

ready had been restored [21]. Orbital FV and MV were considered to be enlarged if the FV/OV and MV/OV was above the 97.5 percentile of age-specific reference intervals, respectively. The results are as follows: 25% had normal FV and MV, 5% had increased FV but normal MV, 61% had normal FV but increased MV, and 9% had increased FV and MV (table 2). The high proportion of GO patients with normal FV and MV was unexpected. These patients had all been diagnosed with Graves' disease (positive TSH binding inhibitory immunoglobulins; TBII) and GO (by experienced ophthalmologists and endocrinologists at combined thyroid-eye clinics), whereas alternative diagnoses had been ruled out by CT scan. Thus, there can be little doubt about the correctness of the diagnosis of GO. It cannot be excluded, however, that in this group there might already have been some increase in FV and/or MV which were too small to exceed the upper normal limit of the reference interval. Especially, single muscle enlargement may not result in increased total muscle volume. The finding of GO patients with only increased FV supports the notion that different phenotypes may exist [22]. There has been one previous study in which the prevalence of increased FV and/or MV (defined as exceeding 2SD above the mean of normal controls, adjusted for gender but not for age) in GO patients is reported [2]. The study comprised 62 GO patients, a mixture of 17 untreated and 22 treated hyperthyroid patients with GO and 23 euthyroid GO patients. The prevalence of normal FV and MV was 13%, of increased FV but normal MV 8%, of normal FV but increased MV 32%, and of both increased FV and MV 47%. There is a sharp contrast between this study and our study in the prevalence of increased FV (55 vs. 14%), whereas the prevalence of increased MV is comparable (79 vs. 70%). The discrepancy may be explained by the more homogeneous group of GO patients in our study, involving consecutive untreated GO patients in whom hyperthyroidism had already been treated. In addition, patients with early disease, and therefore possibly less MV and FV increase, are referred nowadays more often than in the past. If one accepts this line of reasoning, the increase of FV would be a late rather than an early phenomenon in the natural history of GO (see also below).

During our volumetric studies we clearly saw a difference in the structure of orbital fat between controls and GO patients. Therefore, we formally studied orbital fat and muscle densities [23]. In the control subjects fat densities were not age dependent, but muscle densities decreased with advancing age (fig. 3); densities were not related to FV or MV. In the GO patients fat densities (–70.3 ± 4.9 HU) were different compared to controls (–79.1 ± 5.4 HU, $p < 0.001$), indicating a shift towards densities closer to that of water (HU = 0). Muscle densities did not differ between GO and controls (+24.0 ± 5.8 and +22.9 ± 6.5 HU, not significant; fig. 3). Fat densities exceeding the upper normal limit were associated with smaller FV and greater MV. Changes in fat density in GO are unlikely to be caused by compression of fat (although the clinical experience of orbital surgeons is that large masses of fat bulge forward upon incision of the periorbit). Other explanations are absorption of fat (which, also provoked by increased retrobulbar pressure due to enlarged MV,

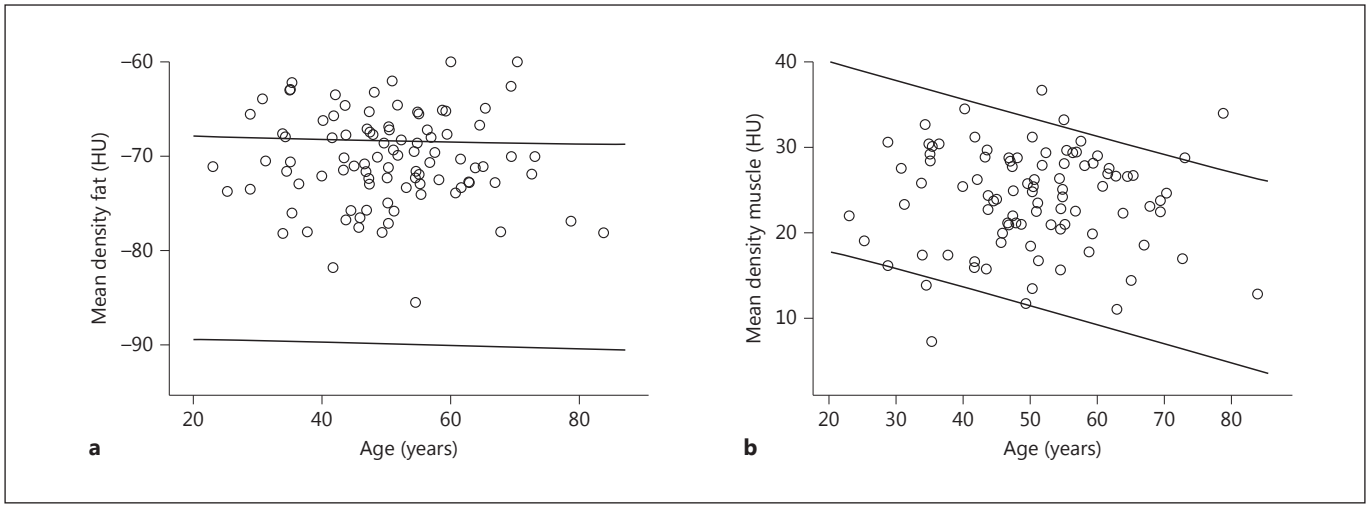

Fig. 3. Scatter plots of mean CT densities (expressed as HU) of orbital fat (**a**) and extraocular muscles (**b**) of 95 consecutive patients with untreated GO. Continuous lines indicate percentiles 2.5 and 97.5 of age-specific reference values [23].

would preserve the connective tissue strands with higher x-ray absorption), or tissue edema (in view of the shift in densities towards HU of 0). Fat and muscle densities in GO were directly related to each other and higher muscle densities were related to higher MV (and lower FV). High muscle density in GO is unlikely to be due to edema (in which case one would expect the density to be lower than in controls), but might be caused by muscle fibrosis or muscle fiber degeneration. Hypoxia may also play a role as extraocular muscles are extremely sensitive to hypoxia in view of their large amount of mitochondria and high oxygen need [24]. A previous study noted an inverse relation between duration of GO and rectus muscle density [25]. It is clear that more and especially longitudinal studies are required to clarify the causes and significance of density changes in GO.

Table 3. Associations of orbital fat and muscle volume with clinical and biochemical parameters in patients with untreated GO

Associations with increased orbital fat volume
1. Longer duration of GO
2. More proptosis
Associations with increased muscle volume
1. Higher age
2. More proptosis
3. More diplopia, worse eye muscle ductions
4. Higher TSH receptor antibodies (TBII)
5. Current smoking

Clinical Associations with Orbital Fat and Muscle Volumes in GO

In our consecutive series of 95 untreated GO patients we compared clinical characteristics of patients with an FV/OV >P97.5 and ≤P97.5; MV/OV did not differ between both groups [21]. Increased FV was associated with more proptosis (24 vs. 21 mm, $p < 0.00$) and less diplopia; TBII was not significantly different (3 vs. 7 U/l, not significant). In contrast, comparing patients with an MV/OV ratio >P97.5 and ≤P97.5 (FV/OV ratio being similar in both groups), patients with increased MV were older (52 vs. 45 years, $p = 0.00$), had more diplopia and worse eye muscle ductions ($p = 0.00$) and higher TBII (9.7 vs. 4.2 mU/l, $p = 0.01$). The observed association of increased FV with a greater degree of exophthalmos is in line with previous reports in patients with isolated FV increase [3–5]. The observed associations with MV indicate more severe GO when MV is increased, in accordance with the notion that GO is more severe in patients with high TBII and older age [26–28] (table 3). Smoking behavior might be involved as well. Smoking is a risk factor for GO, and smokers have more severe GO than nonsmokers [29]. Hyaluronan secretion and adipogenesis markedly increase in cultured orbital fibroblasts (OF) ex-

Table 4. Putative cellular and molecular mechanisms related to differential involvement of orbital fat and muscles in GO

1. Phenotypic differences in fibroblasts from orbital fat and extraocular muscles
2. Changes in orbital T cell subset over time
3. Differences in cytokine profiles between orbital fat and extraocular muscles
4. Single nucleotide polymorphisms in the PPARγ gene
5. Cytokine modulation of 11β-hydroxysteroid dehydrogenase-1 activity in fat

posed to cigarette smoke, in a dose-dependent manner [30]. We found higher MV in current smokers versus never and ex-smokers (MV/OV 0.24 vs. 0.20, p = 0.03), but no difference in FV (FV/OV 0.62 vs. 0.57, not significant) [31]. The proportion of current smokers increased from 17 to 30 and 39% in the lowest, middle and highest MV/OV tertiles, respectively, suggesting a dose-dependent relationship (this was not observed in FV/OV tertiles). Szucs-Farkas et al. [15], however, reports an increased connective tissue volume (but not MV) in smokers. The discrepancy might be related to the smaller sample size (n = 32) and less accurate volume measurements (using thick axial MRI images and manual outlining of compartments) in their study. Lastly, we observed a relationship between duration of GO and orbital volumes. FV was greater at a duration >1 year compared to a duration <1 year (FV/OV 0.65 vs. 0.55, p = 0.004), whereas MV was similar (MV/OV 0.22 vs. 0.21, not significant) [32]. Although further longitudinal studies are required, the data suggest that the increase of FV is a rather late event in the natural history of GO. This view is supported by in vitro experiments on adipogenesis: cultured undifferentiated OF require prolonged exposure to particular cytokines like IL-1β and IL-6 in order to differentiate into mature adipocytes [33].

Cell Biology and Orbital Fat and Muscle Volumes in GO

Considerable progress has been made in unraveling the cellular and molecular mechanisms leading to fat and muscle swelling in GO [34]. It has become clear that OF are the primary target cells of the autoimmune attack [33]. Some differences have been noted between OF and cytokine profiles derived from retrobulbar fat and extraocular muscles (table 4), which are now discussed.

Firstly, OF derived from the interstitium between the muscle cells (perimysium) uniformly express the cell surface glycoprotein Thy-1: they are all Thy-1$^+$. OF from the fat compartment can be either Thy-1$^+$ or Thy-1$^-$. Only Thy-1$^-$ OF have the capacity to differentiate into mature adipocytes, associated with increased TSH receptor expression [35]. Both Thy-1$^+$ and Thy-1$^-$ OF produce IL-6 after stimulation with IL-1β or activation of the CD40 pathway, but Thy-1$^+$ OF produce higher levels of prostanoids and display higher CD40 levels than Thy-1$^-$ OF [35, 36]. PGE2 and CD40 ligand (also known as CD154) provoke production of proinflammatory cytokines (like IL-6) and secretion of glycosaminoglycans by OF [36, 37]. Adipogenesis is thus restricted to the fat compartment, whereas the inflammatory reaction might be more severe in the muscle compartment. More severe inflammation is expected in early rather than late stages of GO, compatible with the suggestion that increases in MV occur earlier than increases in FV.

Secondly, in the early stages of GO the predominant T cells are Th1 (related to cell-mediated immunity), whereas in the later stages they are Th2 (related to humoral immunity) dominant [38]. Thus, the role of Graves' IgG would be more prominent in later stages when TSH receptor expression markedly increases after differentiation of a subset of OF into mature adipocytes. The data again suggest increased FV to be a relatively late phenomenon, which is in agreement with our clinical findings.

Also, cytokine profiles seem to differ between orbital fat and muscles in GO, although studies on this issue are few. Th1-like cytokines predominate in muscle, whereas Th1- and Th2-derived cytokines are present in fat, albeit with a wide interindividual variation [39].

Furthermore, peroxisome proliferator activator receptor-γ (PPARγ) is involved in orbital adipogenesis. PPARγ is elevated in orbital fat from GO patients relative to controls [40], and exposure of OF to PPARγ agonists (like rosiglitazone) stimulates expression of PPARγ, TSH receptor and differentiation into adipocytes [41]. Treatment with pioglitazone has been associated with exacerbation of GO [42] and an increase in proptosis of about 2 mm in diabetic patients [43]. Proptosis is 2 mm lower in GO patients who are carriers of the single nucleotide polymorphism Pro12Ala in the PPARγ gene [44].

Finally, 11β-Hydroxysteroid dehydrogenase-1 activity of OF from orbital fat is greater in GO patients than in controls, due to induction by proinflammatory cytokines (notably TNFα, IL-6). The enzyme increases local cortisol bioavailability, thereby enhancing adipogenesis [45].

The available data on cell biology support the notion that an increase of MV is more prominent in early stages of GO, whereas an increase of FV occurs later. Remodeling of muscle and fat tissue may, however, start simultaneously but proceed at a different rate. Why a small minority of GO patients present with enlargement of only fat mass remains incompletely understood. It should be realized that most of our knowledge on OF is based on fibroblasts residing in the orbital fat, which are easy to obtain and culture; OF derived from muscles are difficult to harvest and have scarcely been studied.

Disclosure Statement

The authors have no conflicts of interest to disclose.

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