Graves' disease

Graves' disease (GD) is a relatively common autoimmune disease (approximately 2% prevalence) (1) where the body develops autoantibodies – Graves' antibodies – to the thyroid stimulating hormone receptor (TSH-R), which cause the receptor to be constantly stimulated. Secondary to the constant stimulation of the TSH-R by Graves' antibodies, which are not inhibited by the normal feedback mechanism, there may be uncontrolled secretion of T3 and T4, leading to clinical hyperthyroidism. The thyroid gland itself is often enlarged due to the growth-promoting activity of Graves' antibodies. The most common extrathyroidal association of GD is the ocular disturbance known as Graves' ophthalmopathy (GO) (2), the topic of this review. Other extrathyroidal manifestations are proximal myopathy, acropachy, pretibial myxedema, vitiligo, and eyelash loss (3). GD has a long and variable natural history which makes efficacy of any therapy difficult to evaluate without a control group (4) and makes studies containing control groups particularly valuable (5, 6).

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GO may be associated with other thyroid pathology including Hashimoto's thyroiditis and thyroid cancer (7). The manifestations of GO are diverse and include protrusion of the eye within the orbit (proptosis), upper eyelid retraction, diplopia, and pressure effects including optic nerve compression (8). Diplopia is caused by extraocular muscle involvement, which may include one or more of acute and chronic inflammation, active muscle contraction (9), and fibrotic changes. The spectrum of severity of GO can...
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vary from mild ocular discomfort through to diplopia, to
abnormal appearance and to sudden visual loss.

GO has been one of the more controversial diseases in
modern endocrinology, without a clear consensus regard-
ing pathogenesis, classification, diagnosis, and treatment.
During the early stages of GO, a variety of inflammatory
cells (macrophages, T cells, mast cells, and occasional
plasma cells) infiltrate the orbital connective tissue, adi-
pose tissue, and muscle (10). Several inflammatory cy-
tokines have been associated with the evolution of the or-
bital tissue changes in GO (11). These include interferon
gamma (IFN-g), interleukin-1 (IL-1), and transforming
growth factor-beta (TGF-b) (12) and tumor necrosis factor
(TNF) (13). Growth factors such as insulin-like growth fac-
tor-I (IGF-I) (14, 15) and platelet-derived growth factor (12)
have also been implicated.

Diagnosis and treatment

There is much unmentioned and unrecognized selection
bias in many studies on GO. Patients seen first by oph-
thalmologists can be expected to have different clinical
and laboratory characteristics to those first seen by en-
docrinologists. These differences have not been studied.

It is generally accepted that clinically obvious ophthal-
mopathy develops in 20 to 40% of patients with GD (16).
In some populations orbital imaging shows abnormalities
in virtually all patients with GD. Magnetic resonance imag-
ing spectroscopy measures more sensitive parameters,
e.g., the concentration of chondroitin sulphate proteogly-
can in the retrobulbar tissue as a marker for the activity of
GO (17).

The onset of GO in relation to GD is variable. GO ap-
ppears prior to GD in approximately 20% of cases, 40%
during GD onset and 40% after the occurrence of hyper-
thyroidism (18).

To classify the severity of GO, and effectiveness of
 treatment, there are two main grading systems. The first is
the clinical grading NOSPECS, developed and used
mostly by endocrinologists with little or no acceptance by
ophthalmology (19). Mourits (an ophthalmologist special-
izing in orbital disease) developed the Clinical Activity
Score (CAS), which places greater emphasis on inflam-
atory changes, giving a range from 0 to 10 to grade the
activity of GO (20).

The clinical presentation of GO can be subdivided into
predominantly congestive ophthalmopathy and predomi-

nantly ocular myopathy. Predominantly congestive oph-
thalmopathy accounts for about 30% of all GO cases (21)
and is characterized by an inflammatory infiltrate seen
predominately in the orbital connective tissues and orbital
fat with relative sparing of the extraocular muscles (EOM).
In the ocular myopathy variant, inflammation, swelling,
and dysfunction of the EOM are the major presenting is-
sues, with patients usually complaining of painless
diplopia (21). Muscle involvement can rarely present as
acute orbital myositis. While up to 10% of GO patients
develop isolated ocular myopathy, most have a combina-
tion of the myopathy and congestive subtypes (21).

Euthyroid GO

Patients with visual symptoms and no known or sus-
ppected biochemical or immunologic thyroid disease typi-
cally present to an ophthalmologist. GO is confirmed by
the clinical picture (proptosis, diplopia, lid retraction, et

cetera).

Euthyroid patients with GO often have multiple thyroid
immunologic abnormalities (22). The commonest labora-
tory abnormality is the presence of TSHR Abs, and if neg-
ative, the diagnosis is unlikely. This is not the antibody
usually assayed by laboratories when thyroid antibodies
are requested, and should be requested in addition to the
standard antibodies (and is routinely available).

The tests known as the TSHR Ab assay and the TSH
binding inhibitory immunoglobulin assay (TBII) are the
same. There are two components of this test: the thyroid
stimulating antibodies (TSAb) (aka thyroid stimulating Ig
or TSI) and the thyroid blocking antibodies (TBAb) (23). A
negative TSHR Ab result in a case strongly suspected of
being GD should be discussed with the laboratory and
additional antibody tests may need to be performed (see
the antigenic target, below). The particular feature of the
TSHR Ab may dictate the detail of the clinical presenta-
tion (24).

Imaging parameters of GO are fairly well defined. The
commonest radiologic abnormality is tendon-sparing en-
largement of all the muscles (25). This is different from the
clinical situation – it is involvement of medial and inferior
rectus, which causes most symptoms (reading). It may be
that medial and inferior rectus are more likely to be in-
volved, or it may be that involvement of these muscles
causes more symptoms than involvement of other mus-
cles and drives the patient to seek treatment.
Specialized orbital clinics have expertise in ultrasound diagnosis and follow-up. There is a small literature on situations that simulate GO both clinically and radiologically (26). If ordering computed tomography in a patient with diplopia who may have GO/GD, be cognizant of the iodine in contrast and the potential for subsequent disturbed thyroid biochemistry; contrast is better avoided in cases of suspect GD/GO.

The usual diplopia pattern in symptomatic patients is esotropia ± vertical diplopia. The esotropia is due to involvement of the medial recti. Compression of the abducens nerve at the apex of the orbit by swollen muscles may also be a factor in the frequently seen esotropia (27). Exotropia is so uncommon in GO that one should suspect myasthenia gravis as the cause. Vertical diplopia is due to differential involvement of the eight cyclovertical muscles, the most clinically significant usually being the inferior rectus.

Treatment

There are three main treatments of GO: glucocorticosteroids (GCs), orbital irradiation, and surgery (for strabismic, oculoplastic, and orbital complications of GO). There is continuing uncertainty about the place of each of these treatments – when and how to use them – and we need more studies with control groups.

Steroids are used in a number of different ways in GO. With optic nerve compression there is no doubt that high dose (either oral or pulsed) steroids are sight-saving and allow deferment of orbital decompression to a convenient time. High dose steroids usually have an ameliorating effect on GO while they are being used but it is not known if their effect lasts beyond the period of treatment. Bartalena et al (28) found that adding 3 months of oral steroids to thyroid radioablation had a potent positive effect on the outcome that lasted long beyond the duration of therapy. This arm - 3 months of oral steroids independent of other therapy - probably deserves independent evaluation as a treatment that may improve on the natural history in GO. There is the odd patient whose symptoms are markedly improved by low dose steroid (say, 5 mgm a day). One study assessed the effect of oral prednisone combined with cyclosporine versus prednisone alone. The patients who received combination therapy had a greater reduction in proptosis and diplopia and fewer relapses on ceasing treatment (29).

Although orbital radiotherapy is a well established and widely accepted treatment of GO, one prospective, randomized, double-blind, placebo-controlled study of the sort of patients for whom radiotherapy is typically recommended could not demonstrate a beneficial effect (5, 30). Another study using radiotherapy with sham control demonstrated a strabismus surgery-sparing effect in 25% of patients (6), with no change in proptosis or eyelid swelling. Others have shown little or slight effect on strabismus (31, 32). Other studies have shown that any effect may be independent of the actual dose used (33). Many prominent thyroidologists debate the therapeutic nihilism of these studies and still promote the use of orbital irradiation (34).

The oculoplastics specialist is central to the ongoing management of many GO patients. Operations to improve appearance, comfort, and function in these patients include surgeries for upper and lower lid retraction (35, 36), tarsorrhaphy to lessen exposure and the staring appearance, and orbital surgeries for proptosis and for optic nerve compression. Guanethidine eye drops are useful for some patients with upper lid retraction (37).

Orbital decompression surgery for proptosis may cause diplopia de novo (38). The incidence of diplopia is uncertain – reports vary from as low as an 18% incidence of new diplopia in a series undertaken for cosmetic reasons (39), to as high as a 52% worsening of diplopia (40), without any apparent explanation for these differing incidences.

Botulinum toxin appears to be effective in treating some of the manifestations of GO, being effective in the treatment of diplopia (41), upper lid retraction (36, 42), and (anecdotally) optic neuropathy (43). There is some evidence that in the early stages, active muscle contraction, not fibrosis, limits ocular rotations and that this suggests why botulinum toxin injections may help in some cases (27).

Studies into somatostatin analogues such as octreotide as a new treatment for GO look promising. One study saw improvements in 7 out of 12 patients when treated with 0.1 mg of octreotide, three times per day for 3 months, compared to no improvement or worsening of GO in the 8-patient control group (44). A longer acting analogue, lanreotide, has been under investigation, in an attempt to reduce the number of injections required (45).

Plasmapheresis has also been investigated as a therapy for GO, but the literature is inconclusive (46, 47). Until a randomized and controlled study is reported, the useful-
ness of intravenous immunoglobulins is in doubt. Specific TNF treatment has been trialled with some success (13). Prolonged GO can be associated with an increased incidence of glaucoma and these patients should be under periodic review by an ophthalmologist (48).

The course of GO is unpredictable. Though hyperthyroidism typically responds to antithyroid treatment, GO may persist or develop further. Some studies have suggested that thyroid radioablation has a worsening effect on GO (28); following this seminal article, there was much apprehension about using radioablation in patients with any orbitopathy without 3 months of concurrent steroids, a supplementary treatment of some morbidity. The ophthalmologist may be asked for guidance by the endocrinologist who is considering radioablation for a toxic patient. Our advice is as follows:

1. If there are no unusual contraindications to using steroids as in the Bartalena et al article, do so.
2. Some of the worsening documented in the Bartalena et al article was trivial, e.g., increased caruncular edema.
3. Approximately 1/3 of GO is seen prior to, coincident with, and following GD; more of the latter category appears post-radioablation and it is possible that the increased incidence of worsening GO seen in the Bartalena et al article may be a statistical artifact (at least in part).
4. If radioablation is used without steroids, the ophthalmologist can usually manage any subsequent GO.

GO can actively progress for a period of several months to several years (49). It may be quiescent for years then seem to become active again. Consequently, corrective squint surgery for diplopia or oculoplastic surgery for eyelid or orbital pathology is usually deferred until stability has been demonstrated so that changes postsurgery are kept to a minimum (16). Some anecdotal reports suggest that this traditional deferral of surgery until stability has been demonstrated may not be necessary, at least for the treatment of diplopia (3).

**Predisposition**

There is controversy as to the extent of genetic influences in GD, although the general consensus is that GD is a disorder of multifactorial etiology with a polygenic mode of inheritance. Weak or no links have been demonstrated to specific candidate predisposition genes such as Ig allotypes or T-cell receptor polymorphisms, to HLA-DR3 (50), P1 blood group (51), or CTLA-4 (52). Links have been demonstrated to HLA-B8 (53).

Autoimmune diseases in general are more prevalent in women. While approximately 85% of those with GD will be women (54), of those who already have GD, there appears to be no correlation between sex and frequency of overt ophthalmopathy (55). However, it does appear as though the severity of the disease is more pronounced in older men (10). Other autoimmune diseases are seen with greater frequency in patients with GD, e.g., myasthenia gravis. This can be a difficult diagnosis to appreciate in a patient who already has diplopia from GO (56, 57).

Smoking is strongly associated with GO (58) and patients are very unlikely to quit smoking, even after strong advice from an ophthalmologist (59).

**The antigenic target**

The most popular theory to explain the association of GO with autoimmune thyroid disease is immunologic cross-reactivity of autoantibodies and/or sensitized T-lymphocytes against antigen(s) shared between the thyroid and the orbit. There have been several proposed antigenic targets for GO:

1. Mitochondrial succinate dehydrogenase flavoprotein subunit (SDHFp, a.k.a. 64 kDa protein) (60-63)
2. Thyroid peroxidase (TPO) (64)
3. Thyroglobulin (Tg) (16, 65, 66)
4. A fusion protein, G2s (67, 68)
5. TSH receptor (24, 69-75)
6. Yersinia enterocolitica (24, 76).

Proposed mechanisms for the EOM cell damage are cytotoxic T-cell mediated apoptosis, antibody-dependent cell-mediated cytotoxicity, and cytokine-mediated cytotoxicity (77). Complement-fixing EOM cell cytotoxic autoantibodies are not seen in patients with GO.

In euthyroid patients with GO, 28.6% were positive for TSH binding inhibitory immunoglobulins (TBI) and 82.9% were positive for thyroid stimulating antibodies (TSAb). Of those with GD and GO, 100% were positive for both TBI and TSAb (78).

**Localization of the antigen**

Controversy exists between which type of cell(s) are the primary sites for the TSH-R in GO. The main candidate cells are orbital fibroblasts, adipocytes, and muscle cells. Supporting the theory that the fibroblast is the target tis-
sue is that much of the orbital swelling is due to high levels of glycosaminoglycans (GAGs), produced by fibroblasts (79). mRNA transcripts of the TSH-R have also been shown to be expressed in the orbital fibroblast (80).

TSH-R antibodies from the sera of Graves’ patients can stimulate fibroblast collagen synthesis in vitro (81). TSH-R protein expression has been found in fibroblasts from several anatomic locations other than the orbit, which suggests other factors (either cell or antigen) are involved in GO manifestations. No currently identified abnormal antigen expressed on fibroblasts results in increased binding of antibodies in GO or enhanced response to TSH (82). A study where extraocular fibroblasts were exposed to recombinant TSH in vitro showed no stimulus of protein synthesis, cAMP, or GAG production, even at high concentrations (83).

It has been shown that the TSH-R mRNA transcript and protein are expressed in adipose tissue (84); however, like fibroblasts, expression is not exclusive to the orbital area (85). More recently, research on interscapular brown adipose tissue (BAT) suggests that TSH stimulates cAMP, but also BAT-specific proteins, which indicates that the TSH-R might be multifunctional, with cell specific properties (86).

Extraocular muscle (EOM) cells are the other obvious target, as EOM enlarges in GO (8). Muscle enlargement has been identified in patients with no other clinical signs of GO, indicating such changes are early manifestations of the disease (2, 87). Dysfunction of the muscles restricts eye movement, and their bulk can cause pressure on the optic nerve in the restricted orbital apex. Antibody-dependent, cell-mediated cytotoxicity against orbital myocytes has been confirmed by in vitro data (77). Most lymphocytic infiltration occurs in or around the EOM (88), and analysis of EOM-infiltrating T cells in GO has shown that they are thyroid-reactive (89). Moreover, antibodies from patients with GO have the ability to stimulate EOM growth in vitro, while not stimulating growth in other skeletal muscle (90). It has also been shown that IgGs bind to porcine eye muscle membranes and increase the growth of myoblast culture (90); however, the same group did not find a correlation between myoblast growth stimulation and thyrotropin receptor (TSH-R) antibody levels. From the biological viewpoint, one study has found 64% of patients with clinically overt GO had sera that reacted with human eye muscle membranes, which did not react with non-ocular skeletal muscle, liver, or fat (91). When sera from patients with GO were tested for antibody dependent cell-mediated cytotoxicity (ADCC), the tests were positive in 10 of 20 patients against EOM cells, but only in 2 of 25 against orbital fibroblasts (92).

EOM also differs from normal muscle by having a greatly increased blood flow, increased cell cycle, and unique innervation and spindle structure (93, 94). EOM fibers also differ from other skeletal muscle with respect to their gene expression profile (95), myosin isotypes, and enzyme profiles (96). Previous studies conducted at our laboratory have shown that the thyroid target of GD, the TSH receptor (TSH-R), is expressed in EOM and thyroid but not in other skeletal muscles at an RNA level (75, 97), and at a protein level (98).

Animal models of Graves’ disease and Graves’ ophthalmopathy

Very early animal studies have shown that injections of thyroid or pituitary extracts or TSH induces exophthalmos and an increase in retro-orbital tissue (99-103). While there is no reliable animal model for GO at this point in time, there are some recent animal models generated that do show several GD, and some GO characteristics. Using genetic immunization, Costagliola et al reported anti-TSH-R antibodies in 57 of 59 immunized mice, and EOM of the hyperthyroid animals displayed histologic changes typical of GO, such as edema, fibrosis, and cellular infiltration when compared to the control mice (70). More recently, Yamada et al demonstrated another mouse model using a similar technique of immunization with TSHR cDNA and other cDNAs of interest (104).

Summary

There is an incomplete understanding of the mechanisms of Graves’ ophthalmopathy, and a lack of effective or complication-free treatment or preventative measures. While not a life threatening disease, GO has the potential to seriously impair a patient’s quality of life, with concerns regarding cosmetic appearance and physical discomfort. In its most extreme form GO may lead to visual loss. Identification of the target autoantigen and tissue localization of the antigen may lead to more specific immunotherapies for GO.

Eye muscle is a tissue frequently evaluated in research due to strong evidence that it is a primary target of damage (105). There are some important differences between eye and other skeletal muscles, not least of which is the expression of the TSH-R mRNA (75, 97).
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Various researchers consider that due to the TSH-R being the immunologic target in Graves’ disease itself, this protein is also a possible candidate autoantigen in GO, if expression can be shown to be restricted to the orbit and thyroid. However, which orbital tissue type(s) are involved or the sequence of their involvement during the immunopathogenesis is in dispute. Once this issue is answered, it will be possible to determine the downstream biochemical pathways by which GO occurs, and consequently find new drug targets.

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REFERENCES


59. Wiersinga WM, Smit T, van der Gaag R, Mourits M, Kornfled SP. Myasthenia gravis in conjunction with Graves’ dis-
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57. Kusuhara T, Nakajima M, Imamura A. Ocular myasthenia
gravis associated with euthyroid ophthalmopathy. Muscle
58. Prummel MF, Wiersinga WM. Smoking and risk of Graves’
disease [comment]. JAMA 1993; 269: 479-82.
59. Karadimas P, Bouzas EA, Mastorakos G. Advice against
smoking is not effective in patients with Graves’ ophthal-
60. Kubota S, Gunji K, Ackrell BA, et al. The 64-kilodalton eye
muscle protein is the flavoprotein subunit of mitochondrial
succinate dehydrogenase: the corresponding serum anti-
bodies are good markers of an immune-mediated damage
to the eye muscle in patients with Graves’ hyperthyroidism.
61. Zhang ZG, Wall JR, Bernard NF. Tissue distribution and
quantitation of a gene expressing a 64-kDa antigen associ-
ated with thyroid-associated ophthalmopathy. Clin Immunol
Immunopathol 1996; 80: 236-44.
62. Ross PV, Koenig RJ, Arscott P, et al. Tissue specificity and
serologic reactivity of an autoantigen associated with au-
toimmune thyroid disease. J Clin Endocrinol Metab 1993;
77: 433-8.
63. Gunji K, Skolnick C, Bednarzuk T, et al. Eye muscle antib-
obodies in patients with ocular myasthenia gravis: possible
mechanism for eye muscle inflammation in acetylcholine-
receptor antibody-negative patients. Clin Immunol
Immunopathol 1998; 87: 276-81.
64. Khooh DH, Ho SC, Seah LL, et al. The combination of ab-
sent thyroid peroxidase antibodies and high thyroid-stimu-
lating immunoglobulin levels in Graves’ disease identifies a
group at markedly increased risk of ophthalmopathy. Thy-
65. Kuroki T, Ruf J, Whelan L, Miller A, Wall J R. Antithyroglobu-
lin monoclonal and autoantibodies cross-react with an or-
bital connective tissue membrane antigen: a possible
mechanism for the association of ophthalmopathy with au-
62: 361-70.
Use of monoclonal antibodies to investigate a possible role
of thyroglobulin in the pathogenesis of Graves’ ophthal-
tion of the novel thyroid and eye muscle shared protein G2s:
autoantibodies against G2s are closely associated with ophthal-
mopathy in patients with Graves’ hyperthyroidism. J Clin
Endocrinol Metab 2000; 85: 1641-7.
68. De Bellis A, Bizzarro A, Conte M, et al. Relationship be-
tween longitudinal behaviour of some markers of eye au-
toimmunity and changes in ocular findings in patients with
Graves’ ophthalmopathy receiving corticosteroid therapy.
69. Gerdina MN, van der Meer J W, Broenink M, Bakker O,
Wiersinga WM, Prummel MF. Association of thyrotropin re-
ceptor antibodies with the clinical features of Graves’ oph-
70. Costagliola S, Many MC, Denef JF, Pohlenz J, Refetoff S,
Vassart G. Genetic immunization of outbred mice with thy-
rotropin receptor cDNA provides a model of Graves’ dis-
M. Muscle autoantigens in thyroid associated ophthalmop-
athy: the limits of molecular genetics. J Endocrinol In-
72. Kosugi S, Sugawa H, Mori T. Epitope analysis of the thy-
73. Cundiff J G, Kaitthamaiah S, Seetharamaiah GS, Baker J R,
Jr., Prabhakar BS. Studies using recombinant fragments of
human TSH receptor reveal apparent diversity in the bind-
ing specificities of antibodies that block TSH binding to its
receptor or stimulate thyroid hormone production. J Clin
Endocrinol Metab 2001; 86: 4254-60.
74. Feliciello A, Porcellini A, Ciullo I, Bonavolonta G, Avvedi-
mento EV, Feni G. Expression of thyrotropin-receptor mR-
NA in healthy and Graves’ disease retro-orbital tissue [com-
75. Major BJ, Cures A, Frauman AG. The full length and splice
variant thyrotropin receptor is expressed exclusively in
skeletal muscle of extracellular origin: a link to the patho-
genesis of Graves’ ophthalmopathy. Biochem Biophys Res
76. Luo G, Fan J L, Seetharamaiah GS, et al. Immunization of
mice with Yersinia enterocolitica leads to the induction of
antithyrotropin receptor antibodies. J Immunol 1993;
151: 922-8.
Immunologically mediated cytotoxicity against human eye
muscle cells in Graves’ ophthalmopathy. J Clin Endocrinol
Metab 1986; 63: 316-22.
78. Kazuo K, Fujikado T, Ohmi G, Hosohata J, Tano Y. Value of
thyroid stimulating antibody in the diagnosis of thyroid as-
associated ophthalmopathy of euthyroid patients. Br J Oph-
79. Bahn RS. The fibroblast is the target cell in the connective
tissue manifestations of Graves’ disease. Proc Allergy
80. Menguia M, Lukes YG, Nagy EV, et al. TSH receptor gene
expression in retrocular fibroblasts. J Endocrinol Invest
81. Franklin JA, Graves’ ophthalmopathy and the TSH recep-
82. Weetman AP. Thyroid-associated eye disease: pathophys-
83. Paschke R, Metcalfe A, Alcalde L, Vassart G, Weetman A,
Ludgate M. Presence of nonfunctional thyrotropin receptor
variant transcripts in retroocular and other tissues [com-


98. Kloprogge SJ, Kapsa R, Frauman AG. The extraocular muscle thyroid stimulating hormone receptor (TSHR) and its relevance to Graves' ophthalmopathy. Proceedings of the Endocrine Society of Australia; Adelaide, Australia; 2002.


101. Dobyns BM. Studies on exophthalmos produced by thyrotropic hormone II. Changes induced in various tissues and organs (including the orbit) by thyrotropic hormone and their relationship to exophthalmos. Surg Gynecol Obstetrics 1946; 82: 609-10.


