THYROID ASSOCIATED OPHTHALMOPATHY:
TREATMENT FOR HYPERTHYROIDISM AND
EVALUATION OF METHODS FOR MEASURING
SACCADIC EYE MOVEMENTS

Frank Träisk

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To the memory of Anja Luhtasela
ABSTRACT

Thyroid associated ophthalmopathy (TAO) is the orbital manifestation of autoimmune thyroid disease that clinically affects about 30-50 % of patients with Graves’ disease (GD). The clinical features of TAO are in most patients mild and transient. In some cases though, a severe orbital immune reaction develops, which may lead to permanent exophthalmos and double vision. Optic neuropathy in TAO is rare and denotes very severe and sight-threatening disease.

The objectives of the herein presented studies include first, the evaluation of techniques for the measurement of the velocity of saccades in normal subjects and patients with TAO and second, the assessment of the potential effect of treatment with anti-thyroid drugs or radioactive iodine for Graves’ hyperthyroidism on worsening or development of TAO.

Objective assessment of TAO and the detection of a potential subclinical extraocular muscle involvement in TAO may be difficult. Previous studies from our eye-movement laboratory have given way to optimism about using saccade velocity measurements in the detection of early and subclinical TAO. The magnetic scleral search coil (MSC) system has long been considered as a “gold standard” method for measuring saccadic velocity in the laboratory milieu. A modern development of the infrared reflection (IR) method was considered as a more suitable method for eye-tracking in the clinical setting. The technique had though until now not undergone evaluations for saccadic velocity measurements.

In the present studies saccadic eye movements were recorded in healthy subjects and patients with TAO with both the MSC and IR methods. The data were analysed regarding the characteristic amplitude-velocity relationship of the saccades (the main sequence). The results showed that the IR method generated saccadic velocities that were higher compared to the MSC method in healthy subjects. Intra individual as well as inter individual variability of the main sequence was shown with both methods, but was more pronounced with the IR recordings. No significant differences were shown for the main sequence relationship between patients with TAO and healthy controls with either of the recording systems.

The primary treatment for most patients with Graves’ hyperthyroidism is either radioactive iodine or anti-thyroid drugs. The results from the here presented study showed that the patients who were randomized to treatment with anti-thyroid drugs had a significantly lower risk for development or worsening of TAO than those randomized to treatment with radiiodine. Deterioration of the eye disease was not found to be related to either therapy among the patients who had TAO already before treatment for hyperthyroidism, whereas patients with no clinically detectable eye involvement from the start showed a significantly higher proportion of de novo development of TAO in the radiiodine treatment group. Smoking was confirmed as a risk factor for TAO. In the subgroup of smokers, the mode of treatment for hyperthyroidism did not significantly influence the outcome of worsening or development of TAO.

Keywords: Graves’ disease, thyroid associated ophthalmopathy, saccade, main sequence, magnetic scleral search coil, infrared reflection, radiiodine, anti-thyroid drugs, smoking.


## LIST OF ABBREVIATIONS

<table>
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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>ATD</td>
<td>Anti-thyroid drug</td>
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<td>C</td>
<td>Main sequence constant</td>
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<td>CAS</td>
<td>Clinical activity score</td>
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<tr>
<td>CT</td>
<td>Computerized tomographic scanning</td>
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<td>CTLA-4</td>
<td>Cytotoxic T-Lymphocyte Antigen 4</td>
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<td>CXCL-10</td>
<td>Chemokine (C-X-C motif) ligand 10</td>
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<td>EOG</td>
<td>Electro-oculogram</td>
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<td>EUGOGO</td>
<td>The European Group on Graves’ Orbitopathy</td>
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<td>GAG</td>
<td>Glycosaminoglycans</td>
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<td>GD</td>
<td>Graves’ disease</td>
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<td>HLA</td>
<td>Human leukocyte antigen</td>
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<td>IGF 1</td>
<td>Insulin-like growth factor 1</td>
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<td>IL</td>
<td>Interleukin</td>
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<td>IR</td>
<td>Infrared-like growth factor 1</td>
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<td>MBq</td>
<td>Mega Bequerel</td>
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<td>MRF</td>
<td>Midbrain reticular formation</td>
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<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<td>MSC</td>
<td>Magnetic scleral search coil</td>
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<td>NOSPECS</td>
<td>N – No signs or symptoms, O – Only signs, S – Soft tissue involvement, P – Proptosis, E – Extraocular muscle involvement, C – Corneal involvement, S – Sight loss</td>
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<tr>
<td>PPAR</td>
<td>Peroxisome proliferator-activated receptor</td>
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<td>PPRF</td>
<td>Paramedian pontine reticular formation</td>
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<td>PV</td>
<td>Peak velocity</td>
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<td>RANTES</td>
<td>Regulated upon Activation, Normal T-cell Expressed and Secreted</td>
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<td>RCT</td>
<td>Randomized controlled trial</td>
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<td>RI</td>
<td>Radioiodine</td>
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<td>SNP</td>
<td>Single nucleotide polymorphism</td>
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<td>TS</td>
<td>Thyroid surgery</td>
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<td>TAO</td>
<td>Thyroid associated ophthalmopathy</td>
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<td>TBII</td>
<td>Thyrotropin binding inhibiting immunoglobulin</td>
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<td>TPO</td>
<td>Thyroperoxidase</td>
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<td>TRAb</td>
<td>Thyrotropin receptor antibody</td>
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<td>TSAb</td>
<td>Thyrotropin receptor stimulating antibody</td>
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<td>TSH</td>
<td>Thyrotropin</td>
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<td>TSI</td>
<td>Thyroid stimulating immunoglobulin</td>
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<td>T3</td>
<td>Triiodothyronine</td>
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<td>T4</td>
<td>Thyroxine</td>
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<td>$V_{\text{MAX}}$</td>
<td>Maximum velocity of the main sequence</td>
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<td>VOG</td>
<td>Video-oculography</td>
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<tr>
<td>W/D</td>
<td>Worsening or development</td>
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1. INTRODUCTION

1.1. GRAVES’ DISEASE

Graves’ disease (GD) owes its name to Robert James Graves, an Irish physician who described a case of goiter with exophthalmos in 1835 [Graves 1835]. In Germany, Doctor Carl Adolph von Basedow independently described the disease in 1840, which explains why the disease is sometimes referred to as von Basedow’s disease [von Basedow 1840].

GD affects approximately 0.5 % of the population and has been reported to be responsible for 50-80 % of all cases of hyperthyroidism [Brent 2008]. The female predominance of GD is between 5:1 and 10:1 and although patients at any age can be affected, there is a noticeable peak incidence between the ages of 40 to 60 years [Brent 2008]. The incidence of Graves’ disease in Stockholm County in Sweden was recently reported to be 25 patients per 100000/year [Abraham-Nordling et al. 2008].

The clinical characteristics of GD include hyperthyroidism, goitre and the three extrathyroidal manifestations; thyroid associated ophthalmopathy (TAO), pretibial dermopathy and acropathy. The two latter signs occur in less than five and one percent of the patients with GD respectively [Orgiazzi 2000], whereas clinically apparent TAO is observed in about 30-50 % of the patients [Brent 2008]. Hyperthyroidism in GD is explained by an autoimmune production of TSH receptor antibodies, which bind to the TSH receptor on the follicular cells of the thyroid gland and stimulate a chronic excess production of thyroid hormones. Symptoms of hyperthyroidism include e.g. weight loss, tachycardia, tremor, arrhythmia, muscle weakness, anxiety, fatigue and insomnia. Stimulation of the thyroid may cause enlargement of the gland, but clinical goitre is not present in all patients with GD [Brent 2008].

A small number of patients with GD have no detectable levels of serum TSH receptor antibodies, but still display a typical diffuse thyroid radionuclide uptake on scintigraphy, clinical thyrotoxicosis, heredity for Graves’ disease and/or typical eye signs of Graves’ disease. In hyperthyroid GD, the prevalence of detectable serum TSH receptor antibodies (TRAb) is about 70-100 % [Orgiazzi 2000]. In antibody-negative GD thyroid stimulation may be an effect of exclusively intrathyroidal production of TRAb or explained by laboratory assay insensitivity or misdiagnosis [Weetman 2000]. TAO has in earlier reports been found in patients with normal levels of thyroid hormones in 6-21 % of the cases. This number has though been reduced considerably with novel and more sensitive laboratory assays [Khoo et al. 2000].

Although the underlying cause for GD is not known, both inheritance and environmental factors are likely to play pathogenetic roles [Prummel et al. 2004]. In one report, thirty percent of the patients with GD had a positive family history (in any relative) for hyperthyroidism [Manji et al. 2006]. The concordance rate for GD in monozygotic twins has been reported to be approximately 35 % [Brix et al. 2001] and there is emerging evidence about the existence of both immune-modulating and thyroid
specific susceptibility genes in autoimmune thyroid disease [Jacobson and Tomer 2007]. Smoking has been shown to increase the risk for GD, although to a lesser extent than the risk for TAO [Weetman 2000]. GD may also be associated with other factors, e.g. stressful life events [e.g. Winsa et al. 1991].

Graves’ hyperthyroidism is generally treated with anti-thyroid drugs, radioactive iodine or thyroid surgery (chapter 1.4.3.).

1.2. THYROID ASSOCIATED OPHTHALMOPATHY (TAO)

1.2.1. Clinical aspects

TAO has almost exclusively been observed in patients with GD, even though a few cases have been found in association with other thyroid disorders, e.g. Hashimoto thyroiditis [Bartalena et al. 2000]. In a large cohort study from Olmsted County in Minnesota the incidence of TAO was 16 women and three men per 100000 population/year [Bartley 1994]. The peak ages for occurrence of TAO in that study was 40-44 and 60-64 years for women and slightly higher for men (age range for both genders was 8-88 years).

TAO occurs in about 30-50 % of unselected patients with GD [Brent 2008], but with imaging techniques orbital changes consistent with TAO have been observed also in patients with no clinical signs of the eye disease [Forbes et al. 1986, Villadolid et al. 1995, Lennerstrand et al. 2007]. Changes in saccadic eye motility has been observed not only in patients with clinical signs of TAO, but also in those with GD and no apparent eye involvement [Schworm et al. 2002]. Hence, the number of patients with either clinical or subclinical manifestations of TAO may be higher than the generally reported numbers.

The clinical features and symptoms of TAO depend on the activity and severity of the disease. Activity denotes the presence of periocular inflammatory signs and a potential for change of severity of the eye disease. Severity, on the other hand, represents the functional and cosmetic deficits associated with the disease [Dickinson and Perros 2001]. Rundle’s curve is often used to describe the typical course of TAO [Rundle 1957] (here a modified version):

![Rundle's curve](image)

[Wiersinga 1995 Copyright 1995, The Endocrine Society]

Clinically, three phases of TAO may be identified; the active progressive, the active regressing and the inactive phase [Dickinson and Perros 2001]. The initial phase of deterioration may be acute or insidious and may last for several months and is characterized by increasing signs of inflammation and swelling of the orbital tissues.
After the initial deterioration, the active phase subsides and a plateau phase is reached. Eventually, a slow decrease of the inflammatory activity takes place, which may last a year or more [Perros and Kendall-Taylor 1998]. As shown in “Rundle’s curve”, the course of TAO activity is paralleled by the severity curve. In severe cases of TAO, eye changes may sustain even though the orbital immune reaction has subsided (burned out phase) and is explained by the development of fibrosis and increased adipose tissue in the orbit [Kendall-Taylor 2007]. Clinically, this may e.g. be observed as residual permanent exophthalmos and dysfunction of the extra ocular muscles. Permanent loss of vision may be the consequence of optic neuropathy or scarring of the cornea.

The reported proportions of patients with various symptoms and different noticeable signs of TAO depend naturally on patient selection. In one study, the most common symptoms of TAO were pain and discomfort (30 %), lacrimation and photophobia (15-20 %), diplopia (17 %) and blurred vision (7 %) [Bartley 1994]. Nearly all patients with TAO have upper eyelid retraction at some stage [Bartley et al. 1996], which may give rise to a staring appearance, grittiness, tearing and pain in the eyes. Since eyelid retraction may be a sign of augmented adrenergic stimulation in patients without clinically obvious inflammation in the orbital tissues, it is sometimes not included in the eye signs that constitute TAO. Persisting upper eyelid retraction is generally the result of either scarring between the lacrimal gland fascia and the eyelid levator muscle or associated with restriction of the inferior rectus muscle motility [Dickinson 2007].

The periocular inflammation that typifies the active phase of TAO is present in 34-72 % of the patients and is recognized as e.g. redness and oedema of the conjunctiva and eyelids [Kendler et al.1993, Bartley et al.1996]. The activity of TAO may be difficult to assess clinically in a uniform manner. Colour photographs for comparisons may be useful [Dickinson and Perros 2001] and according to Mourits et al. [1997] proper assessment of TAO activity requires two visits with an interval of one month.

Some patients with TAO develop exophthalmos (proptosis). The fascial sheet that stabilizes the position of the eye bulb (anterior orbital septum) normally holds back the orbital contents from bulging forward. In exophthalmos, the eyes are pushed forwards because of the increased volume of the orbital tissues. In patients with tight septal fascia, exophthalmos may not develop, but rather, a rise in the orbital pressure can evolve, which in turn may lead to optic nerve compression [Dickinson 2007]. Exophthalmos is clinically measured by a Hertel exophthalmometer, i.e. the distance between the lateral orbital margin and the corneal apex. These measurements may be associated with an inter-observer variation, at least when multiple observers are involved [Musch et al.1985]. Moreover, racial differences in the habitual distance between the corneal apex and bony margin of the orbit have been reported [De Juan et al.1980] as well as variability related to age and gender [Mourits et al. 2004]. To diminish the errors of measurement, exophthalmos should ideally be assessed by the same observer at each occasion using the same instrument. The prevalence of exophthalmos in patients with TAO depends on its definition. In a frequently quoted study, exophthalmos was defined as Hertel exophthalmometry readings of 20 millimetres or more, and herein the prevalence among patients with TAO was 62 % [Bartley et al. 1996]. Exophthalmos or upper eyelid retraction may result in exposure keratopathy and, in worst cases, sight-threatening corneal ulceration and scarring. Diplopia in TAO may be intermittent or permanent, present in only extreme directions of gaze or in the primary position [Dickinson and Perros 2001]. The restriction of eye
motility is a consequence of failure to relax the extraocular muscles and/or an effect of swelling and fibrosis in the orbital tissues. In clinical practice, ocular motility is assessed by mere observation or by quantitative measurement of the binocular field of single vision, prism cover tests or by the range of ductions (uniconular field of fixation; UFOF) [Dickinson and Perros 2001, Steel et al. 1995, Mourits et al. 1994]. The latter has shown to have repeatability within eight degrees for single muscle measurement and is hence useful in patient follow-up [Haggerty et al. 2005]. In the study by Bartley et al.[1996] restrictive extraocular myopathy was present in 43% of the patients with TAO.

Optic neuropathy is a serious complication, which has been reported to affect six percent of the patients with TAO [Bartley et al. 1996]. The optic nerve may be affected by either compression of the nerve at the orbital apex or by exophthalmos-associated stretching of the nerve. The signs of optic neuropathy can be more or less prominent, but most commonly include optic disc swelling, impairment of colour vision and radiological evidence of apical optic nerve compression [McKeag et al. 2007]. Patients with diabetes and TAO have been found to be at particular risk for developing optic neuropathy [Kalmann and Mourits 1999].

Imaging techniques are sometimes used in TAO. Orbital CT and MRI scanning are generally used for differential diagnostic purposes, especially in asymmetrical orbital involvements and in patients who might have compression of the optic nerves [Pitz 2007]. Also, echography of the extraocular muscles has by some authors been found to be valuable for diagnosis and follow up of TAO [e.g. Erickson et al. 1995].

During the active phases of TAO, anti-inflammatory treatments may reduce symptoms and hamper or prevent the progression of severe TAO. Oral or intravenous corticosteroids are most commonly used, but also orbital irradiation may be considered, in particular for patients with impairment of ocular motility [Bartalena et al. 2008 a,b]. In the late phases of TAO anti-inflammatory therapies are of no benefit and restoration by means of surgical intervention (orbital decompression, extraocular muscle or eyelid surgery) may be necessary [Bartalena et al. 2000].

GD is known to affect the patients’ quality of life, particularly if TAO is present [Gerding et al. 1997, Estcourt et al. 2008]. In a comparison with other diseases, TAO has been found to be associated with a worse quality of life than e.g. diabetes, emphysema or heart failure [Gerding 1997]. Despite appropriate management, one third of the patients with TAO are dissatisfied with their eye appearances, according to a long-term follow up study [Bartley et al. 1994]. The change of the facial expression has been found to lead to the sense of an altered identity and, by consequence, a psychosocial burden of stigmatization and social withdrawal [Estcourt et al. 2008]. Even a clinically mild eye disease may cause a severe social, psychological and economical burden [Bartalena et al. 2000].

1.2.2. Classification of TAO

The orbital inflammatory process in TAO generally gives rise to multiple clinical ophthalmological signs. Therefore, it has been considered practical to classify the disease by means of clinical scoring systems. The NOSPECS classification has been
widely used for characterizing TAO (N – No signs or symptoms, O – Only signs, S – Soft tissue involvement, P – Proptosis, E – Extraocular muscle involvement, C – Corneal involvement, S – Sight loss) and has over the years undergone several revisions [Werner 1969, Werner 1977, Van Dyk 1981]. Although, NOSPECS regards most of the features of TAO, precise descriptions of them are lacking [Dickinson and Perros 2001]. The system has also shortcomings for the assessment of TAO activity [Gorman 1998]. 

The clinical activity score (CAS), which was introduced by Mourits et al. [1989] has been used for the evaluation of clinical changes of TAO during the active phases of the disease. In the first version, seven signs of orbital inflammation and three changes in TAO severity were scored as either present or absent. In a later version, only seven activity signs were included (see below). Despite its many advantages, the lack of grading may be considered as a shortcoming for CAS (the scoring changes only when the signs and symptoms appear or disappear in a binary mode).

Recently, the EUGOGO group suggested that the signs and symptoms described in the following table should be included in the routine clinical practice in specialist multidisciplinary clinics and for clinical trials [Wiersinga et al. 2006, Bartalena et al. 2008 a,b].

(a) Activity measures based on the classical features of inflammation: clinical activity score (CAS). The final score (maximum 7) is the sum of all items present

- Spontaneous retrobulbar pain
- Pain on attempted up- or down gaze
- Redness of the eyelids
- Redness of the conjunctiva
- Swelling of the eyelids
- Inflammation of the caruncle and/or plica
- Conjunctival oedema

(b) Severity measures

- Lid aperture
- Swelling of the eyelids (absent, mild, moderate, severe)
- Redness of the eyelids (absent, present)
- Redness of the conjunctiva (absent, mild, moderate, severe)
- Conjunctival oedema (absent, present)
- Inflammation of the caruncle or plica (absent, present)

- Exophthalmos
- Subjective diplopia score (0, no diplopia; 1, intermittent, i.e. diplopia in primary position of gaze, when tired or when first awakening; 2, inconstant, i.e. diplopia at extremes of gaze; 3, constant, i.e. continuous diplopia in primary or reading position)
- Eye muscle involvement (ductions in degrees)
- Corneal involvement (absent/punctate keratopathy/ulcer)
- Optic nerve involvement

  - Best corrected visual acuity
  - Colour vision
  - Visual fields (to be included only if optic nerve compression is suspected)
  - Optic disc
  - Relative afferent papillary defect (absent/present)
Here, the recommended cut off point for “active TAO” was three or more activity points out of seven possible. Sight-threatening TAO was defined by optic nerve involvement and/or corneal breakdown. TAO was denoted moderate to severe if the eye disease had sufficient impact on daily life to justify the risks of immune suppression or surgical intervention. According to the authors, this usually is associated with at least one of the following signs: Lid retraction of two millimetres or more, moderate or severe soft tissue involvement, exophthalmos of three millimetres or more for race and gender and inconstant or constant diplopia. Signs and symptoms of lesser severity were labelled as mild TAO [Bartalena 2008 a,b].

1.2.3. Pathogenesis

In TAO the orbital tissues are infiltrated by inflammatory cells and undergo fat expansion and hyaluronan accumulation as a response to a postulated autoimmune reaction [Gianoukakis and Smith 2008]. The interstitial cells, primarily the orbital fibroblasts, rather than the extraocular muscle cells are considered to be primary targets of the autoimmune attack [Khoo and Bahn 2007]. The observed immune responses against eye muscle antigens have been thought to be epiphenomenons of eye muscle fibre damage [Mizokami et al. 2004].

Organ specificity of the immune reactions in GD cannot be easily explained. The orbital fibroblast population display specific phenotypic characteristics that distinguish them from those of other tissues. Also, the action of cytokines (e.g. IL 1, IL 6) may be different in the orbits compared to other tissues. In orbital tissues, cytokines have been found to promote both adipogenesis and fibrosis in TAO [Orgiazzi 2007, Gianoukakis and Smith 2008]. Single nucleotide polymorphism (SNP) has been found for different genes that encode for regulators of the T-lymphocyte activation and proliferation (e.g. CTLA-4 and PTPN-22) in patients with GD, but their role in TAO is uncertain. Also, some HLA loci (DRB1, DQB1 and DQA1) have been found to be associated with GD and might be involved in the auto-antigen presentation in GD and TAO [Gianoukakis and Smith 2008].

In the search for antigens involved in the orbital immune process, the TSH and IGF-1 receptors have achieved particular recognition. The TSH receptor has been suggested to be mostly involved in adipogenesis and the IGF-1 receptor in the recruitment and activation of T-cells as well as the production of GAG in the orbits of patients with TAO [Khoo and Bahn 2007].

In Graves’ hyperthyroidism, the TSH receptors in the thyroid gland are stimulated by auto-antibodies to an excess production of thyroid hormones. TSH receptors are also present in the orbital tissues, but their pathogenetic role here is less apparent. The clinical observation that TAO is related to high levels of serum TRAb has suggested a role for the receptor in the orbital disease process [e.g. Eckstein et al. 2006, Gerding et al.2000]. Other tissues express the TSH receptor as well, but in the orbital tissues of patients with TAO, mRNA levels of the TSH receptor have been found to be higher than in patients without TAO [Khoo and Bahn 2007]. Furthermore, the expression of the TSH receptor have been found to be increased during the active phase of TAO as opposed to the late inactive phase [Wakelkamp et al. 2003]. There are reports that suggest that TSH receptor ligation by TRAbs leads to increased adipogenesis and this would hence play a role in the fat expansion in the orbit. Stimulation of the PPAR-gamma receptor (which takes part in the regulation of adipogenesis) has been found to
promote TSH receptor expression and influence the recruitment and differentiation of orbital preadipocytes, which are subpopulations of fibroblasts [Valyasevi et al. 2002, Zhang et al. 2006]. Specimens of orbital adipose tissue from TAO patients have shown to overexpress both adipocyte associated genes e.g. PPAR gamma as well as the TSH receptor [Khoo and Bahn 2007]. The PPAR-gamma agonist rosiglitazone and pioglitazone, which is used in the treatment of diabetes, has also clinically been observed to cause enlargement of the orbital tissues [Starkey et al. 2003, Dorkhan et al. 2006].

The assumption that the IGF-1 receptor also is involved in the pathogenesis of TAO is based on the observations that antibodies against the IGF-1 receptor are present in the serum of patients with TAO and that there is increased expression of the receptor in the orbital tissues from patients with TAO. Stimulation of the IGF-1 receptor on the orbital fibroblasts increases the expression of IL 16 and RANTES, which both act in T cell migration and activation. Moreover, the receptor activation increases the production of GAG and display adipogenetic effects [Khoo and Bahn 2007].

There is, to date no robust animal model for TAO, although progress in this field has been reported [Ludgate and Baker 2004].
1.3. THE SACCADES

1.3.1. General aspect as regards TAO

There are few solid and readily available ways to objectively quantify both the activity and severity of TAO in clinical practice [Gorman 1998]. Reproducibility and standardisation of the measurements is challenging and if only the measures that are of more objective nature (e.g. exophthalmos, width of the lid fissure and eye motility) would be taken into account, this would render difficulties in the evaluation of disease activity [Dickinson and Perros 2001].

Recordings of rapid eye movements, the saccades, have been studied as potential measures for extraocular muscle involvement and in the detection of early disease (chapter 1.3.4.). The results, however, have been rather unconvincing and the available measuring techniques have so far not met the requisites for measurements in clinical practice [Dickinson and Perros 2001, Schworm et al. 2000, 2002].

1.3.2. The saccades – basic features and neural control

The ability to move the eyes is the basis for both stabilizing gaze and for shifting the directions of gaze. The ocular motor system can be divided into different categories, depending on the purpose of the movement and the neuronal processes that characterize them [Leigh and Zee, 1999a]; visual fixation, smooth pursuit, vergence, vestibular eye movements, optokinetic eye movements, quick phase of nystagmus and saccades.

The saccades bring the image of interest onto the fovea by rapid redirections of the eyes. The velocity of these eye movements is very high. According to the literature peak velocity of a saccade can exceed 500 degrees/s if the amplitude is large [Leigh and Zee, 1999b].

The saccade begins with a rapid start (in computerized detection often defined as velocity exceeding 20-30 degrees/s) and an acceleration phase which terminates at the peak velocity. After this, the deceleration phase begins, which is quite similar in shape to the acceleration phase. The amplitude (position; left graph) and velocity (right graph) of a saccade [Leigh and Zee 1999b] is illustrated in the following figure (time on the x-axis):

In recordings of saccades, several parameters can be evaluated, e.g. the latency, amplitude, duration and velocity of the movements. As shown in the previous
figure, the peak velocity (PV), i.e. the highest point of the saccade velocity curve, occurs in the approximate middle of the saccade. This peak velocity has been found to be closely linked to the saccadic amplitude in a mathematical relationship called the main sequence [Bahill et al. 1975, Boghen et al. 1974]. The peak velocity increases with amplitude in a linear fashion, but only up to about 20 degrees. For larger amplitudes the amplitude-peak velocity relationship saturates and approaches an asymptotic maximum value of peak velocity, called the maximum velocity ($V_{\text{MAX}}$). The slope of the main sequence curve depends on the value of the constant ($C$) and both parameters can be calculated from the equation

$$PV = V_{\text{MAX}} \left(1 - e^{-\text{amplitude}/C}\right).$$

The numerical value of $C$ is calculated as 63% of $V_{\text{MAX}}$. The correlation between the main sequence constant $C$ and maximum velocity $V_{\text{MAX}}$ are described in the following graphs. In the left graph all three curves have the same value for $C (=8)$ and in the right graph the curves share the same value for $V_{\text{MAX}} (=500 \text{ degs/sec})$.

The duration and velocity of the saccadic eye movement cannot be voluntarily controlled, which makes saccadic dynamics interesting for characterization of diseases that affect the ocular motor system. However, the saccadic velocity can be affected by other factors. For instance, several authors have reported changes in the saccadic velocity profile by fatigue; e.g. changes in the postsaccadic glissadic drift, occurrence of overlapping saccades and slowing of the saccadic velocity [Bahill and Stark 1975, Fuchs and Binder 1983, Schmidt et al.1979, van Opstal and van Gisbergen 1987]. The saccadic velocity may also be different depending on the eye movement task. Slower saccades have been registered when the saccades were centrifugal instead of centripetal [Pelisson and Prablanc 1988]; when made to auditory targets or remembered locations in complete darkness [van Gelder et al. 1997] or when the targets are presented at a low frequency [Lueck et al. 1991]. Increasing age may influence the saccadic velocity, although this has not been consistently shown in all studies [e.g. Abel et al. 1983, Wilson et al. 1993, Moschner and Baloh 1994, Munoz et al. 1998, Huaman and Sharpe 1993]. Gender has not been found to influence the velocity of the saccades [Wilson et al. 1993].
The neural control of the saccade is complex. According to a simplified scheme, the descending pathway of the rapid eye movement leads signals from the cortex to the superior colliculus, either directly or via the basal ganglia, and further pass them on to gaze centres in the reticular formation of the brain stem [Leigh and Zee 1999b]. The cerebellum plays an important role in the adaptational control of the saccades [Optican and Robinson 1980]. Since saccades are normally combined with head movements there is a close connection between the saccadic and vestibular neural systems [Troost 1981].

The pulse and the step of the saccade is generally used to describe the neurological basis of the eye movement [Leigh and Zee, 1999b]. The pulse is the excitatory brainstem activity that via the ocular motor nerves induces a sudden contraction in the extra ocular muscles that overcomes the orbital elastic forces that drag the eyes into a central primary position. Concurrent inhibitory signals to the antagonist muscles are elicited, which enables the release of the counteracting forces. When the end point of the movement, i.e. the final amplitude has been reached, a certain degree of neural activity persists, which hinders the eye to bounce back into the mid position. This part of the saccade is called the step.

The neuronal command signals responsible for saccades are elicited in the gaze centres of the brain stem; within the rostral interstitial nucleus of the midbrain reticular formation (MRF) for vertical movements and paramedian pontine reticular formation (PPRF) for horizontal movements. Two types of neurones are critical for generating the saccade in the brainstem; the burst cells (excitatory and inhibitory) and the omnipause cells. The excitatory burst neurons are silent between saccades and start firing at a high frequency a few milliseconds prior to the saccade. Simultaneously, the inhibitory burst neurons silence all antagonist muscle activity. Omnipause cells discharge during fixation and in the saccadic system they work through inhibition of the burst cells. A pulse can not be elicited unless the constant activity of the omnipause neurons cease. For this, the omnipause cells themselves need to be inhibited by higher-level centres, e.g. the superior colliculus and the cerebellum [Catz and Thier 2007].

The so called neural integrator system has been suggested to contribute to the saccadic step, which is the tonic activity in an ocular motor nerve, which serves to keep the eye in an eccentric position. A copy of the eye velocity signal, which is sent to the neural integrator generates a tonic command that codes for the eye position [Catz and Thier 2007]. A simplified scheme of the neural control system with illustrations of the neural activity and interconnections of the brainstem saccadic neurons is shown below:

![Diagram of neural control system](image)

P=Omnipause neurons
B= Excitatory burst neurons
NI= Neural integrator
OMN= Ocular muscle neuron
E= Eye

(Leigh and Zee, 1999b).
1.3.3. Methods for recording saccades

The most frequently used techniques for recording eye movements are, to our knowledge the electro-oculogram (EOG), video-oculography (VOG), the infrared reflection (IR) and the magnetic scleral search coil (MSC).

An optimal eye movement recording system need to meet several requirements [Ygge, 1999b]:
1. High sensitivity, i.e. accurate detection of small changes of the eye position.
2. The ability to record at a high frequency to catch fast eye movements, such as saccades and nystagmus.
3. Linearity over wide amplitudes.
4. Insensitivity to extraneous bio-electrical signals such as interference from activity in the facial muscles.
5. Insensitivity to ambient illumination.
6. It should not restrict the visual field.
7. Recording of at least six channels simultaneously, i.e. horizontal, vertical and torsional movements from the two eyes.
8. Easy application for routine clinical evaluations and acceptance by patients.
9. It should be possible to subtract the head movements from the combined eye and head movement signals by means of a head movement recording system.

The different devices exhibit different advantages and shortcomings.

The electro-oculogram (EOG) records eye movements by utilizing the fluctuating polarity in the facial plane when the eye (being a dipole) rotates within the orbit. Electrodes that are placed around the eyes record the changing polarity. The amplitude range of the EOG system is not limited, although linearity decreases for amplitudes beyond 30 degrees from the primary position. Horizontal and vertical eye movements can be recorded, but vertical recordings are easily affected by the co-contraction of facial muscles that produce artefacts, e.g. by the lid muscles. The system resolution is low and ambient light and external electrical interference increase recording noise. In video-oculography (VOG) a miniature video camera, which is mounted in a head mask, records the anterior eye with the pupil and pigmented iris. The changed position of the pupil is processed in a computer system and interpreted as eye movements. Spatial resolution is high and also torsional eye movements can be recorded. The recording range is vertically and horizontally approximately +/- 25 degrees. The main drawback of the system has long been its low sampling frequency. New systems, which exhibit markedly improved capacities, have recently been developed. The infrared reflection and Scleral search coil systems as well as the recent developments of VOG display particular advantages in the recordings of high velocity eye movements, since their sampling frequencies can be high.

The infrared reflection system (IR)

In the IR system infrared light (radiation) is transmitted from illuminators and reflected against the eyes. The cornea absorbs the light while the white sclera reflects the light. The eye position signal derives from the reflected light that is sampled by photo detectors. The fluctuation of the reflected light, which is caused by changes of the visible ocular surface area during the eye movements, is translated into eye movements. The signals, which basically are photocurrent subtractions, are conducted into the
soundcard of a computer and finally presented by a software program as eye movements [Ober 1994].

The IR recording goggles (Orbit XY-1000, IOTA AB, Sweden)

IR devices may differ according to what landmarks of the eyes are traced, how illumination is projected, the way the eyes are viewed and how selectivity of directions is achieved. The IR system has since the introduction of the technique in the early 1950s been continuously modified and improved. Previous systems have used small areas for illumination and detection and have been associated with circumstantial set-up procedures, which consequently have limited their use in the clinical settings. The IR eye tracking system (Orbit XY-1000, IOTA AB, Sweden), which was used in our studies, was designed to trace the corneoscleral limbus, the corneal bulge and the pupil. In this system, illumination is projected with a wide angle and the photo detectors receive the reflected IR light from a three-dimensional surface (in contrast to small defined areas of illumination and photo detection from indirect two-dimensional representations of the eyes). This wide-angle system requires a minimum of set-up time; a manual adjustment of the goggles for the inter-pupillary distance, a computerized separation of horizontal and vertical eye movements and equalization of the photo detectors to ambient illumination. According to the manufacturer, the goggles and eyes should be in a stable position and lighting conditions should not change during the initial system calibration, which is performed separately for every subject before the actual recording. The light injector is in this procedure adjusted to compensate for sensor-eye displacements as well as to ambient light. Separation of the x-horizontal and y-vertical trajectories is performed in a so called timesharing mode. When tracing the horizontal position, a pulse of infrared light is emitted from vertically placed diodes. The reflected light is subsequently recorded by 90 degrees horizontally placed photo detectors. The next pulse of light that emits 350 microseconds afterwards from horizontally placed diodes is detected by vertically positioned photo detectors, which generates the vertical eye position. In an optimal recording with this device, the landmarks generating the eye position signal would be the corneoscleral limbus, corneal bulge and pupil. However in a wide-angle illumination and photo detection system, the signals are not merely generated by positions of the ocular structures, but also the eye lids. The lids cover parts of the corneoscleral limbus and cornea and the three-dimensional differences of the reflecting surface, particularly the lid-eye surface distance may affect the desired signal. Furthermore, the lids may drift due to eye-eyelid friction, which may introduce artefacts. According to manufacturer’s specifications the sensitivity of the system is sufficiently high to be influenced even by the cardiac pulse of the vessels surrounding
the eyes. Being an advantage for saccadic signal detection, this sensitivity may undesirably lead to the system picking up artefacts, especially if the headband is strapped too tight.

Even though an initial system calibration for ambient light is performed, fluctuations of surrounding light can affect the reflected infrared signal. Therefore, according to the manufacturer, lighting conditions should be kept stable during the recordings, which preferably should be conducted in a half dimmed room.

Another drawback of the system is the aperture of the goggles, which limits the angular recording range to a horizontal +/- 20 degrees and a vertical +/- 15 degrees. This makes analysis of large saccades impossible and has consequences for the fitting of the main sequence amplitude-peak velocity plot as the bending of the curve generally starts at high amplitudes.

Maximum measurement frequency is high. A digital filtering of the modulated 2450 Hz light yields a maximum output frequency of 1000 Hz. Spatial resolution is 0.01 degrees under optimal conditions. Maximum non-linearity is +/- 10 % over +/- 15 degrees horizontally and +/- 10 degrees vertically.

**The magnetic scleral search coil system (MSC)**

The MSC system was first introduced in the early 1960s [Robinson 1963] and has since been considered as the gold standard for eye movement recordings [Eggert 2007]. The device has undergone considerable improvement mainly during the 60s-80s [e.g. Collewijn et al. 1975] and the current systems demonstrate low noise, good linearity (better than 0.25 % for an output signal equivalent to the sine of the coil deviation) as well as high temporal and spatial resolution (down to one min. of arc with a high temporal sampling frequency). Also, the angular range of movement is large and not limited by boundaries in the visual field.

The system picks up the eye position (i.e. coil position) through a thin copper wire that is moulded in a soft contact annulus with an inner radius approximately equal to the corneal radius. The coil is placed on the eye surface and moves together with the eyes. During the recording, the patient is placed inside perpendicular magnetic fields. As the magnetic field oppositely changes intensity a voltage is induced in the coil, which is proportional to the position of the coil inside the field.

*The coil of the magnetic scleral search coil system*

Despite the technical advantages of the system, the device is, to our knowledge rarely used in the clinics, mainly because of the coil-associated discomfort for the patient. In
one study, the maximum discomfort from using the coils was rated as 3.0 ± 0.3 on a scale between no (1) and extreme discomfort (5) [Irving et al. 2003]. The drying and deformation of the cornea may lead to temporal reduction of vision, which according to the manufacturer limits the wearing time to 30 minutes. Recently, a new coil-eye lid protection device has been introduced, which allows recording intervals of up to two hours with considerably reduced discomfort [Sprenger et al. 2008].

1.3.4. Saccadic velocity in TAO

Most diseases that affect the saccadic dynamics make the eye movements slower. Examples of diseases that have been found to slow down saccades are dementia, Parkinson’s disease, Huntington’s disease, progressive supranuclear palsy, spinocerebellar ataxia, lipid storage disease, paraneoplastic syndromes and internuclear ophthalmoplegia [Leigh and Zee 1999b].

The results from saccadic eye motility studies on patients with TAO have been quite unpersuasive [Dickinson and Perros 2001]. The reports are scarce and have included few patients. Mauri et al. [1984] looked at recordings of horizontal saccades in 25 patients with the intention to detect early stages of TAO (IR method). They found that conventional saccadic parameters were usually normal, albeit muscle fatigue was seen more frequently in patients with TAO (16 patients) than in patients with GD with no TAO (9 patients). Feldon and Unsöld [1982] recorded the peak velocity (PV) in 13 patients with mild, moderate and severe TAO, using an IR recording system. They found that the PV was reduced in patients with more severe TAO, particularly in the amplitude range of 6-15 degrees; and that orbital decompression was associated with improvements of PV. Later, in a report by Feldon et al. [1990] it was shown that patients with optic neuropathy, large extraocular muscle volumes (on CT scans) and limited ocular motility also had lower saccadic PV; particularly for amplitudes of 14 degrees (49 subjects, IR technique). Wouters et al. [1998] found that that saccades from 9 out of 12 patients with TAO (without overt dysmotility) were less conjugate and had a lower maximum main sequence velocity than those made by control subjects (MSC recordings). Restrictive fibrosis was suggested to explain these findings.

In a systematic evaluation of multiple saccade parameters associated with early TAO, no clinically relevant changes were identified by Schworm et al [2000] (MSC recordings; ten patients with early TAO and ten control subjects). Despite significant differences in some of the evaluated 15 dynamic parameters (particularly for large amplitude saccades) patients and controls could not be differentiated by velocity measures because of large standard deviations. They suggested that the differences between their results and those from earlier studies might be explained by inclusions of patients with fibrotic disease in the preceding studies. Also, a central adaptation of the saccade-generator may have compensated for the eye muscle involvement and led to normalization of the saccadic velocities in their study group.

In a later study by Schworm et al. [2002] patients with different degrees and phases of TAO were analysed separately. Here it was shown that all patient groups (late longstanding, ten patients; early severe, ten patients; early mild disease, ten patients; dysthyroid disease without signs of ophthalmopathy, six patients) exposed a different vertical eye motility pattern than normal subjects (n=10). The normal volunteers displayed higher velocities in the downward direction whereas the opposite relationship (upward faster than downward) was found in all the thyroid patient groups. The authors
suggested that the observed changes in the vertical saccadic eye movements might be explained by early, subclinical eye muscle involvement in GD. In that study, as in previous reports, patients with severe and longstanding restrictive disease had slower saccades than control subjects. The relative discomfort from using the MSC coils was considered a drawback in further clinical studies and therefore it was suggested that a non-invasive system should be evaluated for the ensuing experiments.

In many of the earlier studies, the experiments have been performed with horizontal and not vertical saccadic eye movements, which may be a problem since vertical eye movements are preferably affected in TAO [Wouters et al. 1998]. Also, patients with early active and late fibrotic stages of disease have not always been separated. This may be important, since neuronal adaptation may “normalize” the saccadic eye velocity in the early inflammatory and contractile phases of the eye disease [Optican and Robinson 1980, Simonsz and Kommerell 1989, Wouters et al. 1998, Schworm et al. 2000].
1.4. RISK FACTORS FOR TAO

1.4.1. General aspects

The risk for developing TAO may be innate or related to environmental factors. Susceptibility genes have been identified for TAO [Orgiazzi 2007], but only a small number of patients with severe TAO have been observed to have a positive family history for thyroid eye disease. Also, age and gender have been found to affect the development of TAO [Perros et al. 1993, Kendler et al. 1993, Benker et al. 1999, Manji et al. 2006]. The female preponderance is not as strong for TAO as it has been found to be for GD. Most patients with TAO are women, but men have been observed to develop more severe TAO. Older patients (more than 50-60 years) have been shown to be at higher risk for more severe TAO.

Cigarette smoking has a rather indisputable status as a risk factor for development of TAO [Bartalena et al. 2008 a,b], but also risk factors that are related to hyperthyroidism and its treatment may be associated with development of TAO, e.g. high serum levels of TRAb and T3, pre-existing TAO before treatment for hyperthyroidism, post-treatment hypothyroidism and radioiodine treatment.

Cigarette smoking

There is strong evidence that smoking is associated with an increased risk for TAO [Vestergaard 2002, Thornton et al. 2007] and particularly for influencing development of severe eye disease [e.g. Shine et al. 1990, Prummel and Wiersinga 1993, Winsa et al. 1993]. In a recent review, where 15 case-control and cohort studies met the criteria for scrutiny, current smokers were found to have a significantly higher risk for TAO compared to non-smokers or never smokers [Thornton et al. 2007]. The number of cigarettes consumed daily has also been found to play an independent role for development of proptosis and diplopia in patients with TAO [Pfeilschifter and Ziegler 1996]. Moreover, the outcome of treatment with steroids and radiotherapy for active TAO has been reported to be negatively affected by smoking [Bartalena et al. 1998b]. Since the increased risk for TAO has predominantly been associated with current smoking habits, as opposed to ex-smoking or ever-smoking, many authors have emphasized the importance of giving up smoking to prevent the development of the eye disease [Thornton et al. 2007, Wiersinga 2007, Krassas and Wiersinga 2006]. The suggested mechanism by which smoking is thought to potentiate TAO are: proliferation of fibroblast as a response to superoxide radicals; proliferation and stimulation of fibroblasts to produce GAG as a response to hypoxia; increased expression of HLA class II by orbital fibroblasts by nicotine and tar in the presence of IFN-gamma; increased GAG production as a response to circulating chemicals from cigarette smoke and adipogenesis in the orbit synergistic with that of IL-1 [Orgiazzi 2007, Cawood et al. 2007].

TRAb

Mounting data support a relationship between TAO and serum levels and prevalence of TRAb. Khoo et al. [1999] found that high levels of serum TSI correlated to an increased risk for TAO, particularly in the absence of detectable levels of TPOAb. Gerding et al. [2000] observed that the activity of TAO correlated with the levels of TSH receptor antibodies and in a retrospective analysis of patients with TAO. Eckstein
et al. [2004] also showed that severity and activity of TAO corresponded with the prevalence of TRAb. Later, the same group reported from a follow-up of 159 patients with TAO that patients with a mild course and a severe course of TAO differed at all time points for the prevalence and levels of TBII [Eckstein et al. 2006]. Here, TBII was found to have a prognostic value for TAO in half of the patients, except for the first time point.

1.4.2. Treatment for hyperthyroidism

In a consensus statement about the management of TAO, the EUGOGO group has made four major conclusions regarding the management of hyperthyroidism in relationship to TAO [Bartalena et al. 2008 a,b]:

1. Euthyroidism should be restored promptly and maintained stably in all patients with TAO.
2. Frequent monitoring of thyroid status (every 4-6 weeks) is imperative in the initial phases of treatment when changes in thyroid status are expected.
3. a. Patients with active TAO given radioiodine should be offered prophylactic steroid cover (commencing with 0.3-0.5 mg of prednisone /kg bw per day orally 1-3 days after radioiodine and tapering the dose until withdrawal about 3 months later).
   b. Shorter periods of glucocorticoid therapy (1-2 months) may be equally protective.
4. Patients with inactive TAO can safely receive radioiodine without steroid cover, as long as hypothyroidism is avoided, particularly if other risk factors for TAO progression, such as smoking, are absent.

There has been merging evidence that the achievement and maintenance of stable euthyroidism is important in the management and prevention of TAO. Prummel et al. [1989] found that amelioration of TAO followed the achievement of euthyroidism in patients who by the time of referral were dysthyroid, whereas patients who were euthyroid had no change. Later, the same authors observed more severe TAO in the patients in whom treatment for hyperthyroidism did not result in euthyroidism [Prummel et al. 1990]. A similar relationship between abnormal levels of thyroid hormones and severe TAO was confirmed in a large retrospective study by Kim et al. [2004]. According to some reports, albeit not all [e.g. Sisson et al. 2008], high levels of pre-treatment thyroid hormones has shown to increase the risk for TAO. Tallstedt et al. [1992] found that a high pre-treatment level of serum T3 (>=5 nmol/L) was associated with an increased risk of TAO regardless of the chosen treatment for hyperthyroidism. The level of serum free T4 was also found in a multicentre cohort study to constitute an independent predictor for TAO [Manji et al. 2006]. It is not known if severe hyperthyroidism has a pathogenetic influence by itself, or simply reflects a more severe immunological disturbance in GD.

Post-treatment hypothyroidism and unstable euthyroidism has also been observed to associate with the occurrence and severity of TAO. It has been proposed that the high levels of serum TSH levels during the hypothyroid state may increase the thyroid cell antigen presentation [Volpé et al.1986].
Hamilton et al. [1967] found in a large retrospective analysis that TAO developed more frequently in those patients that had become myxedematous after treatment, than those who remained euthyroid. Others have found similar and timely associations between post-treatment hypothyroidism and progression periods of TAO [Almquist and Algvere 1972, Sjöberg et al. 1989, Karlsson et al. 1989]. Tallstedt et al. [1994] retrospectively analysed the charts of 492 patients treated with radioiodine and found that early administration of thyroxine reduced the occurrence of TAO. In that study, 18% of the patients who received thyroxine substitution therapy by the time of post-treatment hypothyroidism had experienced deterioration or development of TAO compared to 11% of the patients who received thyroxine as a rule at two weeks after initiation of therapy to avoid hypothyroidism. In a post hoc analysis of the RCT by Tallstedt et al. [1992] where thyroxine had not been introduced until the time of radioiodine induced hypothyroidism, an association could not be confirmed between TAO and hypothyroidism [Törring et al. 1996]. Rather, more severe disease or varying T3 or free T4 levels after treatment were observed as a common denominator for development of TAO. A similar association had been observed previously by Karlsson et al. [1989] between the development of TAO and rapid shifts in the levels of thyroid hormones during a short period of time.

1.4.3. Anti-thyroid drugs; Radioiodine; Thyroid surgery

Anti-thyroid drugs (ATD), radioiodine (RI) or thyroid surgery (TS) are the three generally used treatments for Graves’ disease. The choice of primary treatment for Graves’ hyperthyroidism is known to vary between different parts of the world [Glinser et al. 1987, Wartofsky et al. 1991, Weetman and Wiersinga 1998, Vaidya 2008]. While the preferred alternative in Europe and Asia often is medical therapy, radioiodine is more favoured in the USA. On the level of the patients, the decision regarding treatment is generally guided by the age and preference of the patient, the severity of hyperthyroidism, size of the thyroid gland, local practice and resources. If the patient has TAO or smokes may also play a role [Berg et al. 1999]. In terms of quality-of-life outcome, no differences have been found between the three treatment modalities in a long-term follow-up [Abraham-Nordling et al. 2005].

In cases of relapse of GD many physicians recommend ablative therapy with RI or TS [Weetman and Wiersinga 1998]. Ablative therapy may also be of benefit to patients with GD and concomitant TAO, since the depletion of the thyroid antigens and intrathyroidal autoreactive T lymphocytes might hamper the autoimmune reactions associated with the development of TAO [e.g. DeGroot 1997, Marcocci et al. 1998, Bartalena et al. 2004 and 2008c]. The levels of TRAb have shown to normalize faster in patients that undergo total instead of a subtotal thyroidectomy, which supports the idea that the antibody production is thyroid dependent and in consequence, total ablation of the thyroid in severe TAO might hasten remission of the eye disease [Winsa et al. 1995]. Total thyroidectomy has in a recent review also been suggested as the preferred surgical approach in patients with TAO [Stålberg et al. 2008]. RI treatment might also be considered for thyroid ablation in patients with TAO according to some authors, since the observed treatment-related risk for exacerbation of the eye disease may be hindered by parallel treatment with corticosteroids [Bartalena et al. 2004]. Recently, the combination of total thyroidectomy and RI treatment in association with corticosteroid treatment has been reported to be favourable for progression of TAO.
compared to total thyroidectomy alone [Menconi et al. 2007]. According to Eckstein et al. [2007] the choice to perform thyroid ablation would be possible to make as early as 6-12 months after the introduction of ATD therapy. In that study remission was found to be less likely if, by then, the levels of TBII were high and there was evidence of a severe course of TAO.

The clinical signs of TAO develop or worsen in less than half of the patients with GD after the start of treatment for hyperthyroidism and it may be difficult to determine whether this is a result of the natural course of the disease or a consequence of the chosen treatment. Therefore, the question regarding treatment associated risks for TAO is preferably sorted out in randomized trials. The RCTs comparing the therapeutic options for GD that hitherto have been published [Tallstedt et al. 1992, Bartalena et al. 1998a] have pointed to a negative effect by RI treatment for development or deterioration of TAO.

**Anti-thyroid drugs**

According to the general Swedish recommendations [Hallengren 2008] anti-thyroid drug treatment for GD (methimazole, tiamazole, propylthiouracil) is a primary treatment option for patients younger than 20 years and older than 50 years, as well as for patients aged 20-50 with a moderate disease activity and a small or moderate sized goitre. Anti thyroid drugs block the enzyme thyroperoxidase in the thyroid follicular cells and hereby inhibits the production of thyroid hormones. Propylthiouracil also inhibits the peripheral conversion of T4 to T3. ATD treatment can be given in fixed or titrated doses, with or without the replacement of thyroxine and for different treatment time periods (generally 12-18 months [Cooper 2005]). ATD treatment very seldom leads to permanent hypothyroidism, which is an advantage compared to treatment with RI and TS. The disadvantages of ATD include a rather high risk for relapse after discontinuation of therapy (about 50 % [Cooper 2005]) and side effects, e.g. rash, joint pain, liver inflammation and agranulocytosis; the latter being an uncommon (0.1-0.3 %), but feared adverse effect of the drugs [Brent 2008]. Male gender, age under 40 years and a very active thyroid disease has been associated with a higher risk for failure to achieve remission with ATD treatment [Allahabadia et al. 2000]. Also, patients with high levels of serum TBII or severely active TAO have been found to be at particular risk for relapse of GD after one year of treatment with ATD [Eckstein et al. 2007]. Repeated recurrences after treatment with ATD have been proposed to have a potential detrimental effect on the long term risk for development of TAO [Perros and Kendall-Taylor 1998]. Accordingly, ATD treatment might be inferior to RI and TS in patients with TAO [Bartalena et al. 2000, Bartalena et al. 2004]. On the other hand, ATD treatment has in general been found to be associated with a relatively low risk of worsening or development of TAO and as such, not been thought to influence the overall risk for the orbital disease [Wiersinga 2007]. The figures for worsening and development of TAO after ATD treatment have been reported to be 3 % [Bartalena et al. 1998a] and 12 % [Tallstedt et al. 1992] respectively in the two RCTs that have evaluated the risk for TAO in ATD compared to RI treatment. In non-randomized studies the incidence levels have been similar [e.g. Barbosa et al. 1972, Sridama and DeGroot 1989].

ATDs have also been suggested to have protective effects against the development of TAO. Serum TSH-receptor antibodies and other immunologically active factors, e.g.
intracellular adhesion molecule 1, CXCL-10, soluble IL-2 and IL-6 receptors have been shown to decrease with ATD treatment [Cooper 2005]. ATDs have also been found to induce apoptosis of intrathyroidal lymphocytes and a decrease in HLA class II expression. Moreover, circulating suppressor T cells have been found to increase, whereas helper T cells, natural killer cells and activated intrathyroidal T cells have decreased. It is not clear whether these effects are related to the ATD induced suppression of the thyroid functions or if they are independent immunological effects of the drug [Cooper 2005].

**Radioiodine**

Radioiodine treatment (RI) is generally recommended for adult patients as an initial treatment option and for patients who do not go into remission after initial treatment with ATDs [Brent 2008, Hallengren 2008]. In Sweden, RI treatment has been recommended especially for middle-aged and elderly patients with GD [Berg et al. 1999], whereas in the USA it has been used more frequently also in younger patients [Wartofsky et al.1991].

The radioisotope I-131, which is given on a one-time dose basis, accumulates in the thyroid gland and exerts a destructive effect on the gland. In most patients, permanent hypothyroidism develops within the first two years after treatment and virtually all patients treated with RI will end up with life-long substitution therapy with thyroxine [Berg et al. 1999]. The role RI treatment plays in the development and exacerbation of TAO has been much debated and it has generally been difficult to draw conclusions from the published reports. For instance, most reports are retrospective; patients have been enrolled with different degrees of TAO and varying duration of hyperthyroidism and ophthalmopathy; more than one treatment for hyperthyroidism and different dose regimens for iodine-131 has been used and there has been a lack of standardized descriptions of TAO. Acharya et al. [2008] recently undertook a systematic review of randomized controlled trials that dealt with the RI treatment related risk for TAO. Their conclusion was that RI treatment significantly increases the risk for TAO compared with ATD (RR 4.23 with CI 2.04-8.77). The reported rough estimate of the risk of worsening of pre-existing TAO or developing of new TAO was 20 % after RI treatment (severe TAO in 7 %) and 5 % after ATD therapy. The risk for development of severe TAO after RI treatment was also found to be higher compared with ATD therapy (RR 4.35 with CI 1.28-14.73). A weakness in this analysis was the limited number of available trials, heterogeneity in the descriptions of TAO and that most studies excluded patients with severe TAO [Bahn 2008]. Until now, only two randomized controlled trials have compared RI and ATD for the worsening or development of TAO [Tallstedt et al. 1992, Bartalena et al.1998a]. In the study by Tallstedt et al. [1992] development or worsening of TAO occurred after treatment with RI, ATD and TS in 33 % (13 out of 39 patients), 10 % (4 out of 38 patients) and 16 % (6 out of 37 patients) respectively in the group of patients who by inclusion were 35-55 years. These patients had no previous history of treatment for hyperthyroidism. Severe TAO was one of the exclusion criteria and the time for follow-up was as least 24 months. The study has met some criticism regarding confounding factors [Gorman 1995]. In the RI treatment group there were more smokers and hypothyroidism was allowed to develop before L-thyroxine substitution was introduced.
after RI treatment. In most institutions at that time, a slight hypothyroidism was allowed to develop before thyroxine was added. In the study by Bartalena et al. [1998a] it was found that patients treated with RI had a deterioration or development of TAO in 15 % (23 out of 150 patients) of the patients, compared to 3 % (4 out of 148 patients) in the ATD group within six months after treatment. In the RI treatment group worsening of pre-existing TAO occurred in 24 % and de novo development in 8 % of the patients. The total time for follow-up was 18 months and only patients with no or mild eye disease were included. Seventy percent of the patients had by enrolment previously been treated with ATD and all patients received a three months course of methimazol prior to the randomized treatment. In that trial post-radioiodine short phase hypothyroidism occurred in 25 % of the patients. The results from non-randomized studies have been partly contradictory. In a recent observational study of 72 patients [Perros et al. 2005] it was shown that radioiodine treatment did not lead to deterioration of TAO in patients who showed signs of minimally active TAO in a stable phase of the disease. Here, hypothyroidism was avoided by early administration of thyroxine and follow-up was one year. Sisson et al. [2008] found that manifestations of the clinical activity score (CAS) was non-substantial in the 76 patients who were followed after RI treatment. Patients with positive CAS points were insignificantly fewer at one year follow-up compared to baseline. However, thirty patients (39 %) had developed a two millimetre or more proptosis at one year of follow-up. During the same time period eye motility impairment had developed in 13 patients, but had disappeared in ten patients. In earlier reports, the reported ranges of progression and development of TAO after radioiodine treatment has been larger in retrospective studies (3-53 %) than in prospective studies (15-37 %) [Bonnema et al. 2002]. The occurrence of development and/or exacerbation of TAO after RI treatment in some of these reports are listed below (if development and worsening was determined separately, this is labelled as D and W respectively):

<table>
<thead>
<tr>
<th>Study</th>
<th>TAO</th>
<th>Patients (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietlein et al. 1999</td>
<td>D 4 %, W 15 %</td>
<td>85</td>
</tr>
<tr>
<td>Abe et al. 1998</td>
<td>10 %</td>
<td>67</td>
</tr>
<tr>
<td>Kung et al. 1994</td>
<td>D 27 %, W 17 %</td>
<td>114</td>
</tr>
<tr>
<td>Tallstedt et al. 1994</td>
<td>15 %</td>
<td>492</td>
</tr>
<tr>
<td>Fernandez-Sanchez et al. 1993</td>
<td>25 %</td>
<td>25</td>
</tr>
<tr>
<td>Barth et al. 1991</td>
<td>D 17 %, W 33 %</td>
<td>89</td>
</tr>
<tr>
<td>DeGroot et al. 1990</td>
<td>10 %</td>
<td>175</td>
</tr>
<tr>
<td>Vestergaard and Laurberg 1989</td>
<td>D 4 %, W 23 %</td>
<td>50</td>
</tr>
<tr>
<td>Sridama and DeGroot 1989</td>
<td>D 5 %, W 23%</td>
<td>241</td>
</tr>
<tr>
<td>Barbosa et al. 1972</td>
<td>14 %</td>
<td>72</td>
</tr>
<tr>
<td>Kriss et al. 1967</td>
<td>33 %</td>
<td>24</td>
</tr>
<tr>
<td>Hamilton et al. 1967</td>
<td>D 5 %, W 16 %</td>
<td>136</td>
</tr>
</tbody>
</table>

A proposed trigger for TAO after RI therapy is the radiation-induced damage of thyrocytes and the subsequent release of antigens that are thought to be shared by the thyroid and the orbital tissues. This antigen exposure to the immune system may lead to the production of auto-antibodies and activation of T-cells found in the peripheral
blood, which are thought to be involved in the pathogenesis of TAO [e.g. Bonnema et al. 2002, Teng et al.1990].

After the treatment with RI, a rise in the level of serum TSH receptor antibodies has been observed [Törring et al. 1996]. This elevation of serum TRAbs has been shown to be altered by ATD treatment and accordingly it has been hypothesised that ATDs administered parallel to RI treatment might decrease the RI-treatment-related risk for TAO. Gamstedt et al. [1986] followed patients who received RI therapy alone or RI with concomitant ATDs (methimazol two months before and three months after) and found that the commonly observed transient rise in TSH receptor autoantibodies after iodine-131 therapy did not occur in the group who received the combination therapy. In neither of the groups was however TAO confirmed. Andrade et al. [2004] randomized patients with Graves’ disease into treatment with radioiodine alone or radioiodine with pre-treatment with ATD (methimazol). They also found that methimazol attenuated the iodine-131 induced rise in serum TRAb levels, but in neither of the groups clinical ophthalmopathy developed. Kung et al. [1994] randomized 114 patients with newly diagnosed Graves’ disease into RI treatment alone or RI with a subsequent twelve months course of ATD (methimazol) treatment (follow-up time of two years). The two groups did not differ as regards the development or worsening of TAO (23% and 24% in the respective groups). In total, worsening of pre-existing TAO occurred in 17% and de novo TAO developed in 27% of the patients.

It has been proposed that patients who are at particular risk for worsening or development of TAO should be offered a concomitant course of oral corticosteroids together with RI treatment [e.g. Bartalena et al. 2008 a,b]. In a randomized trial, [Bartalena et al. 1989] patients with no TAO or mild and stable TAO received either RI treatment alone or RI with concomitant treatment with Prednisolone (0,4-0,5 mg/kg bw. for one month with gradual tapering of the dose over three months). The results showed that 56% of the patients who received RI alone and had pre-existing TAO got worse during a follow-up time of 18 months. On the other hand, improvement of pre-existing TAO occurred in 52% of the patients who received parallel treatment with corticosteroids after RI. In this group no patient had worsening of TAO. In a later and larger RCT from the same group [Bartalena et al. 1998a] the protective effect of steroid treatment was confirmed. RI treatment alone was associated with deterioration or new development of TAO in 15% of the patients, but no patient in the group where steroids were given together with RI got worse.

The possible benefit of steroids along with RI treatment has also been evaluated by others. In Gamstedt et al. [1991] patients who were treated with RI received parallel treatment with either placebo or betamethasone. One patient in each group developed TAO during the one year follow-up. In Karlsson et al. [2006] 22% of the patients with pre-existing TAO who received RI and prophylactic treatment with Prednisolone (initial dose of 20 mg and a tapering period of approximately five months) experienced transient worsening of eye symptoms during a follow-up of 6-12 months. In the study by Dederichs et al. [2006] clinically transient mild TAO developed in 3% of the patients with no pre-existing TAO during one year after RI treatment, which was followed by Prednisone 0,4-0,5 mg /kg bw every second day for five weeks.
**Thyroid surgery**

Thyroid surgery (TS) is rarely the primary option for treatment for Graves’ hyperthyroidism, unless the patients (especially younger individuals) have highly toxic and/or large goitres [Brent 2008]. TS has also been recommended for patients with severe eye signs and hyperthyroidism that does not respond sufficiently to ATD therapy [Stålberg et al. 2008]; for those with complications of ATDs or who refuse treatment with radioiodine and for pregnant women requiring high doses of ATDs [Brent 2008]. The disadvantages of TS are, besides the need for post-treatment thyroxin substitution, the risks related to the surgical procedure, e.g. paralysis of the vocal cords and hypocalcaemia [Stålberg et al. 2008].

The reported figures for worsening or development of TAO after TS are generally relatively low [Sridama and DeGroot 1989, Miccoli et al. 1996, Fernandez-Sanchez et al. 1993, Abe et al. 1998, Marcocci et al. 1999]. In the previously quoted study by Tallstedt et al. [1992] development or worsening of TAO after subtotal TS occurred in 11 % of the patients aged 20-35 years and in 16 % of the patients aged 35-55 years. Subtotal thyroidectomy has been advocated as standard procedure in many centres to avoid post-surgical hypothyroidism and for safety reasons. Despite some reports that total thyroidectomy would be associated with a better outcome for TAO compared to the subtotal procedure [Winsa et al.1995, Miccoli et al. 1996], this has not been confirmed in other case-control and randomized trials [Marcocci et al. 1999, Järhult et al. 2005].
AIM OF THE PRESENT STUDIES

- To compare the MSC and IR methods for eye movement recordings in healthy subjects as regards the main sequence relationship between the peak velocity and amplitude of the saccade.

- To assess the inter individual and intra individual variability of the main sequence relationships in recordings of saccadic eye movements with the MSC and IR recording techniques.

- To compare healthy control subjects and patients with TAO as regards the main sequence relationships of the saccades from recordings with the MSC and IR recording techniques.

- To compare radioiodine and anti-thyroid drug treatment for the worsening or development of TAO.
SUBJECTS

Paper I and II

Ten healthy volunteers (seven men and three women) participated in the experiments. Their ages ranged between 31 and 49 years. Eight of the ten subject’s recordings were accepted for further analysis. Two were rejected because of noise. Three of the subjects (age 34, 35 and 49; all male) performed additional four eye-tracking sessions at different occasions with a minimum time interval of 24 hours.

Paper III

Fourteen patients with active TAO and 14 healthy control subjects were enrolled for recordings of saccadic eye movements with two different eye-tracking systems. The patients with TAO were enrolled from the out-patient clinic at St. Erik Eye Hospital. All 14 patients were female, age 21-65 years (mean age 48, SD 13 years). Inclusion was possible if the time from the thyroid diagnosis did not exceed 18 months and the patients had signs of active TAO. The enrolled patients’ mean duration of thyroid disease was seven months (SD 4). By the time of the experiments, ten patients were on treatment with anti-thyroid drugs and five patients had been given radioiodine treatment (one patient received both treatments). All patients, except one, were euthyroid, three were smokers and in all patients but two, serum TSH receptor antibodies was present by the time of the experiment. The characteristics of the patients’ eye disease are summarized in the following table:

<table>
<thead>
<tr>
<th>Subject</th>
<th>Anti-inflammatory treatment</th>
<th>Retraction</th>
<th>CAS</th>
<th>Proptosis</th>
<th>Adduction R/L</th>
<th>Abduction R/L</th>
<th>Up R/L</th>
<th>Down R/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>No</td>
<td>Right+Left</td>
<td>5</td>
<td>19-19/98</td>
<td>45/50</td>
<td>50/45</td>
<td>45/45</td>
<td>55/55</td>
</tr>
<tr>
<td>II</td>
<td>Rx</td>
<td>Left</td>
<td>3</td>
<td>22-22/95</td>
<td>55/55</td>
<td>45/45</td>
<td>35/35</td>
<td>60/60</td>
</tr>
<tr>
<td>III</td>
<td>Rx</td>
<td>Left</td>
<td>2</td>
<td>14-14/92</td>
<td>55/55</td>
<td>70/65</td>
<td>45/40</td>
<td>65/65</td>
</tr>
<tr>
<td>IV</td>
<td>No</td>
<td>Right+Left</td>
<td>6</td>
<td>20-17/102</td>
<td>50/45</td>
<td>50/50</td>
<td>25/45</td>
<td>45/50</td>
</tr>
<tr>
<td>V</td>
<td>OR + OP</td>
<td>No</td>
<td>4</td>
<td>23-23/98</td>
<td>45/40</td>
<td>45/45</td>
<td>30/30</td>
<td>45/45</td>
</tr>
<tr>
<td>VI</td>
<td>No</td>
<td>No</td>
<td>4</td>
<td>20-21/97</td>
<td>55/45</td>
<td>45/55</td>
<td>45/35</td>
<td>60/60</td>
</tr>
<tr>
<td>VII</td>
<td>No</td>
<td>No</td>
<td>3</td>
<td>20-20/94</td>
<td>55/50</td>
<td>60/55</td>
<td>45/40</td>
<td>65/65</td>
</tr>
<tr>
<td>VIII</td>
<td>OP</td>
<td>Right+Left</td>
<td>4</td>
<td>18-19/93</td>
<td>50/55</td>
<td>55/60</td>
<td>35/35</td>
<td>70/70</td>
</tr>
<tr>
<td>IX</td>
<td>No</td>
<td>No</td>
<td>5</td>
<td>19-19/99</td>
<td>50/40</td>
<td>50/50</td>
<td>40/35</td>
<td>55/55</td>
</tr>
<tr>
<td>X</td>
<td>No</td>
<td>Right+Left</td>
<td>4</td>
<td>19-19/97</td>
<td>55/60</td>
<td>65/55</td>
<td>35/35</td>
<td>60/60</td>
</tr>
<tr>
<td>XI</td>
<td>Rx</td>
<td>No</td>
<td>3</td>
<td>17-18/99</td>
<td>50/45</td>
<td>55/60</td>
<td>45/45</td>
<td>55/55</td>
</tr>
<tr>
<td>XII</td>
<td>No</td>
<td>Right+Left</td>
<td>3</td>
<td>20-22/97</td>
<td>45/45</td>
<td>55/50</td>
<td>40/35</td>
<td>55/55</td>
</tr>
<tr>
<td>XIII</td>
<td>No</td>
<td>Right</td>
<td>3</td>
<td>19-17/90</td>
<td>55/55</td>
<td>55/60</td>
<td>45/45</td>
<td>70/70</td>
</tr>
<tr>
<td>XIV</td>
<td>No</td>
<td>No</td>
<td>4</td>
<td>19-19/97</td>
<td>55/55</td>
<td>55/55</td>
<td>40/40</td>
<td>60/60</td>
</tr>
</tbody>
</table>

OR= Orbital irradiation, OP=Oral prednisolone, Rx= Rofecoxib.
CAS= Clinical activity score [Mourits et al. 1989].

Eye movement directions represent the uniocular range of fixation (degrees) in four directions for the right (R) and left (L) eyes.
The control subjects were members of the laboratory staff, family and friends; seven were male and seven female. Their ages were 29-63 years (mean age 44, SD 11) and no individual had a history of known disorder affecting the thyroid or ocular motility.

**Paper IV**

Patients with non-treated Graves’ disease, who were seen at the participating endocrinology or oncology clinics were enrolled in the trial if they met the inclusion criteria and accepted to participate. The criteria for inclusion and exclusions were:

**Inclusion:**
- Age 35-69 years
- Symptomatic Graves’ hyperthyroidism
- Confirmation of the diagnosis by serum TSH (≤ 0.1 mIU/L) and elevated levels of T3 and/or free T4
- Thyroid uptake of iodine-131 and radionuclide scans of the thyroid compatible with Graves’ disease, i.e. an even radionuclide distribution
- The activity of an orally administered dose of iodine-131 (as calculated for the individual patient to give an absorbed radiation dose of 120 Gray) should not exceed 600 MBq, enabling the therapy to be given on an out-patient basis (see equation in the methods section)

**Exclusion:**
- Previous history of treatment with anti-thyroid drugs, iodine-131 or thyroid surgery
- Patients with severe TAO, requiring treatment with corticosteroids by the time of inclusion
- Incipient toxic crisis
- Coronary heart disease
- Pregnancy
- Breast-feeding or planned pregnancy within the following two years

Randomization into two treatment arms; radioiodine (group I) and anti-thyroid drugs (group M) was made in blocks over time within the four centres (stratified randomization). The enrolment started in 1996 and ended in 2003, which was earlier than estimated due to an insufficient rate of inclusion. The power estimates were based on the results from the previous trial by Tallstedt et al. [1992]. A comparison (α=0.05, two-tailed test) of the binomial proportions between two groups of 300 patients in each group would have yielded more than 90 % probability (power) to detect a true difference of 0.10. Now, with the same specifications and the number of observations obtained by the termination of the study, the power was at least 70 %.

The following scheme shows the number of patients in the study who were randomized, excluded and included respectively:

\[ G=Gothenburg \]
\[ L= Lund \]
\[ M= Malmoe \]
\[ S= Stockholm \]
The number of enrolled patients was 333. Twenty patients could not be included in the final study group: One patient because of incorrect diagnosis (Hashimoto thyroiditis); 17 had no ophthalmological assessment at randomization and two had no follow-up visits. The excluded patients had an average age of 50.1 years, the male/female ratio was 5/15 and five patients out of 18 were smokers (two missing data). Thus, the final study group comprised of 313 patients: 150 patients in the medical therapy group (group M) and 163 patients in the radioiodine treatment group (group I):

**Characteristics of the patients before start of treatment**

<table>
<thead>
<tr>
<th></th>
<th>Group I (n=163)</th>
<th>Group M (n=150)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>51 (SD 8)</td>
<td>50 (SD 8)</td>
</tr>
<tr>
<td>Age range (years)</td>
<td>36-68</td>
<td>35-69</td>
</tr>
<tr>
<td>Female gender (patients)</td>
<td>141 (87 %)</td>
<td>137 (91 %)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>65 (SD 12)/160*</td>
<td>67 (SD 13)/145*</td>
</tr>
<tr>
<td>Previous smoking (patients)</td>
<td>52 (32 %)</td>
<td>39 (27 %)/146*</td>
</tr>
<tr>
<td>Current smoking (patients)</td>
<td>59 (36 %)</td>
<td>62 (42 %)/148*</td>
</tr>
<tr>
<td>Cigarettes/day (number)</td>
<td>13 (SD 10)</td>
<td>12 (SD 7)</td>
</tr>
<tr>
<td>No TRAb (patients)</td>
<td>10 (6 %)</td>
<td>11 (7 %)</td>
</tr>
<tr>
<td>Estimated pretreatment duration of hyperthyroidism (months)</td>
<td>5 (SD 4)/162*</td>
<td>6 (SD 5)/148*</td>
</tr>
<tr>
<td>(U24) (percent)</td>
<td>64 (SD 11)/162*</td>
<td>62 (SD 11)</td>
</tr>
<tr>
<td>Thyroid volume &lt;30 g (patients)</td>
<td>29 (18 %)</td>
<td>35 (24 %)</td>
</tr>
<tr>
<td>Thyroid volume 30-39 g (patients)</td>
<td>69 (42 %)</td>
<td>53 (36 %)</td>
</tr>
<tr>
<td>Thyroid volume &gt;40 g (patients)</td>
<td>65 (40 %)</td>
<td>58 (39 %)</td>
</tr>
<tr>
<td>TAO – present (patients)</td>
<td>22 (13 %)</td>
<td>19 (13 %)</td>
</tr>
<tr>
<td>Only eyelid retraction – present (patients)</td>
<td>31 (19 %)</td>
<td>28 (19 %)</td>
</tr>
<tr>
<td>TAO and retraction – absent (patients)</td>
<td>110 (67 %)</td>
<td>103 (69 %)</td>
</tr>
<tr>
<td>Proptosis right eye (mm)</td>
<td>16 (SD 2)</td>
<td>16 (SD 2)</td>
</tr>
<tr>
<td>Proptosis left eye (mm)</td>
<td>16 (SD 2)</td>
<td>16 (SD 2)</td>
</tr>
</tbody>
</table>

**Patients with TAO before start of treatment**

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>22</td>
<td>19</td>
</tr>
<tr>
<td>Proptosis right eye (mm)</td>
<td>18 (SD 2)</td>
<td>18 (SD 2)</td>
</tr>
<tr>
<td>Proptosis left eye (mm)</td>
<td>17 (SD 2)</td>
<td>17 (SD 3)</td>
</tr>
<tr>
<td>TAO activity index (see appendix I)</td>
<td>2.3 (SD 1.7)</td>
<td>2.0 (SD 0.0)</td>
</tr>
<tr>
<td>Clearly impaired eye motility (patients)</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Eyelid retraction (patients)</td>
<td>14</td>
<td>8</td>
</tr>
</tbody>
</table>

The cumulative drop-out (LOCF) from the ophthalmological follow-up was in group I and group M as follows; at one year three and one percent; at two years six and three percent and at three years ten and nine percent respectively. At four years (i.e. post-protocol for ophthalmological follow-up) 20 % of the patients in both groups were still followed by ophthalmologists.
METHODS

Paper I, II and III

Paradigm:
The eye movement recordings (Paper I, II, III) were performed in a darkened room at a 150 cm distance from the stimulus, which was presented on a back-projected translucent screen. The stimulus consisted of a horizontally and vertically jumping red square target subtending a visual angle of 23 arc minutes. A vertical and horizontal 15 degrees’ three point calibration was performed at the beginning of each recording. Horizontal recordings included 68 non-randomised stimulus jumps of increasing amplitude of 5 up to 40 degs. (5, 10, 20, 30 and 40 degs.) at every 1500 msec. to the left or right. The vertical paradigm consisted of 73 stimuli jumps in the vertical direction in the midline with amplitudes of 5 up to 30 degs. (5, 10, 20 and 30 degs.). A separate calibration for adjustment of the goggle position was also performed before the IR recordings in order to avoid horizontal-vertical crosstalk.

Recordings of the saccadic eye movements and data processing:
All recording sessions were identical (Paper I, II, III). The IR recordings (Orbit XY-1000, IOTA AB, Sweden) were performed first and followed directly by the MSC recordings (Skalar Medical, the Netherlands). The order of the recordings was chosen to avoid blurring of vision by the MSC coils before the IR registration. Simultaneous recordings were not possible owing to the risk of interference between the two techniques.

The four channel recordings (right and left eyes, horizontal and vertical eye movements) were processed using the JR program, which is a computer software program for automatic detection of rapid eye movements [Ygge et al. 1999a]. Grossly abnormal data and blinks were removed and the obtained ASCII data of the saccades were transferred into an analysis program for calculation of the main sequence parameters. A multivariate analysis of variance for repeated measures with the procedure GLM – general linear model was performed with the SPSS statistical package, ver. 10. The statistical analysis focused on the asymptotic maximum peak velocity (\(V_{\text{MAX}}\)) and constant (C) of the main sequence curve that was evaluated by fitting recorded data with the exponential function defined by Peak Velocity = \(V_{\text{MAX}}(1 - e^{-\text{amplitude}/C})\).

Intra individual and inter individual variability was evaluated using the coefficient of variability (Paper I, II). For the relationship of between-subject and within-subject variations in the three subjects who performed repeated measurements, also the intraclass coefficient of variation was calculated (paper II). The effect of different lighting conditions on the IR recordings was assessed by measuring basic recording noise on an eye prosthesis. The temperature and air humidity was recorded in the immediate proximity of the goggle cards of the IR system while wearing the goggles (paper II).
Paper IV

Treatment and follow of Graves’ hyperthyroidism:
To the patients who were randomized to medical therapy, Methimazol was given at a dose of 15 mg twice daily. The drug was replaced by 150 mg of Propylthiouracil three times daily if the patients experienced minor adverse reactions. The anti-thyroid drugs were discontinued after 18 months with an additional month of L-thyroxine substitution of 100 μg daily, which thereafter was discontinued. Radioiodine treatment was given with the intention to give one dose of radioactive iodine aiming for an estimated absorbed radiation dose in the thyroid gland of 120 Gy. The administered activity was calculated using the following formula [Berg et al. 1996]:

\[
\text{Activity (MBq)} = \left(23.4 \times \text{thyroid mass (g)} \times 120 \text{ (Gy)}\right) / \left(\text{estimated uptake (0 h; \%)} \times \text{effective half-life (days)}\right)
\]

The mass of the thyroid was assessed by means of thyroid scintigraphy and by palpation. Reference models of a thyroid gland were made to help in the assessment (30, 40, 50 and 60 ml). The half-life of iodine-131 and the estimated thyroid uptake at zero hours were calculated from the initial 24 hour thyroid iodine uptake and a new uptake test four to nine days later, i.e. the same day the radioiodine therapy was given. Beta-blockers were used as pre-treatment in group I and for symptomatic relief also in group M. The same regimen for L-thyroxine substitution was employed in both groups:
- At 2 weeks: Initiation of a 50 μg daily dose of L-thyroxine
- At 4 weeks: Increase of the dose to L-thyroxine 100 μg daily
- At 6 weeks: Adjustment of the L-thyroxine dose to normalize the levels of serum T3 and free T4 and to bring TSH to <0.4 mIU/L. A slightly elevated serum free T4 was accepted up to 20 % above the upper normal limit.

Treatment for hyperthyroidism was monitored by clinical assessments and laboratory evaluations of the serum T3, free T4 and TSH levels at the following time points: At six and ten weeks and at six, nine, 12, 15, 18, 24, 36 and 48 months in both groups and in group I also at four months. Serum TRAb and TPOAb (optional) were analyzed at randomization, six, 12, 18, 24, 36 and 48 months in both groups. The methods for the laboratory analyses were different in the different study centres and in some centres they also changed during the time of follow-up.

An ophthalmological assessment was also performed at each appointment with the endocrinologist or oncologist. If at any time TAO developed or deteriorated, the patients were referred to the ophthalmologist for additional eye examinations.

Ophthalmological follow-up:
The ophthalmologists performed the baseline eye examination within the first two weeks after enrolment. Thereafter, the patients were assessed regarding TAO at three, 12, 24, 36 months and also later (or in-between) if referred by the thyrodiologist. In patients with active TAO, eye examinations were performed at least every six weeks until the condition had markedly improved. In each centre, the majority of patients were followed by the same ophthalmologist throughout the study. The ophthalmological protocol included assessments of visual acuity, proptosis, eyelid retraction, eyelid swelling, chemosis, conjunctival redness, impairment of the eye movements, corneal ulceration and sight loss. Also, the patients’ own assessments of eye symptoms were documented. Eye lid retraction only was not classified as TAO.
The primary endpoint in the study was the difference in the proportion of patients with worsening or development of TAO in the two treatment groups (intention-to-treat). At each eye examination it was determined if TAO was present; and in those cases better, worse or unchanged.

Validation of the events referred to as worsening or development (W/D), improvement and no change of TAO during the time of follow-up compared to the baseline ophthalmological assessments, was performed using modified criteria from the trial by Bartalena et al. [1998a]. For the criteria-based event W/D or improvement of TAO, two of the following four criteria were required: 1) Change in exophthalmometry readings of two millimetres or more; 2) improvement or deterioration of the patient’s eye movements between the four scoring levels (no impairment; clearly impaired; diplopia in the primary position; fixation of the globe), 3) changes of visual acuity caused by optic neuropathy and 4) changes in two of the three TAO activity measures chemosis, eyelid oedema and conjunctival redness. The patients who did not meet the criteria of improvement or W/D of TAO were referred to as having no change of TAO.

Statistical analysis:
The primary endpoint hypothesis was tested by means of a Chi-square test. The influence of possible prognostic factors on the time to TAO was investigated with Cox regression analysis. The development within the various subgroups was illustrated by means of Kaplan Meier technique. P-values < 0.05 were considered significant. The Medlog software system (Information Analysis Corp., Medlog Systems 2008-2, USA) was used for storage of data and statistical analysis.
RESULTS

Paper I

The mean of the horizontal and vertical $V_{\text{MAX}}$ for the saccades recorded with the IR method was 830 degs./sec and with the MSC system 460 degs./sec. The mean value for the constant C measured with the IR technique was 19.0 and for the MSC technique 8.9. In general, the IR method showed a 31% higher peak velocity than the MSC method for 20 degrees saccades (540 degs./sec compared to 412 degs./sec) and a 47% higher peak velocity for 30 degrees saccades (653 degs./sec compared to 444 degs./sec). There was a significant difference between the main sequence relationship in the recordings from the two systems ($p=0.047$):

There were no significant differences between the main sequence relationships for abducting and adducting saccades with either the IR or the MSC systems.

The inter individual variability between the eight subjects was generally higher in the IR compared with the MSC recordings; here presented as the coefficient of variability for $V_{\text{MAX}}$ (CV):
Paper II

Analysis of the within subject variability in the three subjects performing repeated tasks at five occasions revealed that the IR system yielded larger variability for $V_{\text{MAX}}$ compared with the MSC system ($p=0.044$). The following table shows the $V_{\text{MAX}}$ (degs./sec) and C of the main sequence; mean, standard deviation (Sd) and calculated coefficient of variability (CV) from the five different recording sessions performed on three different individuals with the IR and MSC techniques:

<table>
<thead>
<tr>
<th>Subject</th>
<th>IR $V_{\text{MAX}}$</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Sd</th>
<th>Mean</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>548</td>
<td>862</td>
<td>714</td>
<td>650</td>
<td>505</td>
<td>142</td>
<td>656</td>
<td>0.216</td>
<td></td>
</tr>
<tr>
<td></td>
<td>348</td>
<td>364</td>
<td>412</td>
<td>269</td>
<td>363</td>
<td>52</td>
<td>351</td>
<td>0.145</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.6</td>
<td>10.7</td>
<td>7.0</td>
<td>12.5</td>
<td>14.0</td>
<td>2.6</td>
<td>10.9</td>
<td>0.238</td>
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</tr>
<tr>
<td></td>
<td>5.4</td>
<td>6.4</td>
<td>8.2</td>
<td>6.5</td>
<td>1.4</td>
<td>6.2</td>
<td>0.231</td>
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<td></td>
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<tr>
<td></td>
<td>547</td>
<td>714</td>
<td>575</td>
<td>573</td>
<td>473</td>
<td>87</td>
<td>576</td>
<td>0.152</td>
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<tr>
<td></td>
<td>513</td>
<td>446</td>
<td>396</td>
<td>397</td>
<td>398</td>
<td>51</td>
<td>430</td>
<td>0.119</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.4</td>
<td>13.5</td>
<td>9.6</td>
<td>9.2</td>
<td>10.7</td>
<td>1.7</td>
<td>10.7</td>
<td>0.161</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.9</td>
<td>8.0</td>
<td>9.7</td>
<td>7.9</td>
<td>7.5</td>
<td>1.4</td>
<td>7.8</td>
<td>0.177</td>
<td></td>
</tr>
<tr>
<td></td>
<td>440</td>
<td>446</td>
<td>355</td>
<td>323</td>
<td>311</td>
<td>64</td>
<td>375</td>
<td>0.171</td>
<td></td>
</tr>
<tr>
<td></td>
<td>304</td>
<td>285</td>
<td>314</td>
<td>356</td>
<td>384</td>
<td>40</td>
<td>328</td>
<td>0.123</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.3</td>
<td>8.1</td>
<td>5.3</td>
<td>5.9</td>
<td>7.4</td>
<td>1.3</td>
<td>7.0</td>
<td>0.191</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.2</td>
<td>5.7</td>
<td>7.9</td>
<td>7.4</td>
<td>8.3</td>
<td>1.4</td>
<td>6.9</td>
<td>0.201</td>
<td></td>
</tr>
</tbody>
</table>

The intraclass coefficients of variation ($R_{\text{icc}}$) for the IR and MSC systems was calculated for both $V_{\text{MAX}}$ and C. $R_{\text{icc}}$ for $V_{\text{MAX}}$ was 0.64 with the IR method and 0.52 with the MSC method. $R_{\text{icc}}$ for C was 0.51 with the IR method and 0.12 with the MSC method. In other words, for the IR recordings 36 % of the variance for $V_{\text{MAX}}$ and 49 % of the variance for C resulted from intra individual variability. The same data for the MSC recordings regarding $V_{\text{MAX}}$ and C were 48 % and 88 % respectively.

At close analysis of single saccades, an individual tendency for performing saccades with an oscillating saccadic amplitude and velocity were revealed. These unexpected saccadic amplitude and velocity profiles were only observed in the IR recordings and not with the MSC system.

The following left graph is an example of unevenness of the velocity profile of a 20 degrees single saccade recorded with the IR system. The right graph describes a
saccade of the same amplitude performed by the same subject but recorded with the MSC method (note the different scales of the y-axis).

Filtering of the IR recordings’ raw data reduced the peak velocity, but did not reduce variability in more than one subject. The degree of velocity reduction did not seem to correlate with the level of basic noise in the recordings.

Different lighting conditions changed the basic level of noise. Temperature and air humidity within the IR goggles changed within the first five and two minutes respectively (rough limits) after starting to wear the goggles, but was fairly stable during the subsequent 30 minutes of the recordings.

**Paper III**

The multivariate statistical analysis for repeated measures of the main sequence did not reveal significant differences between the patients with TAO and healthy control subjects (p=0.235). This was showed also when separating the results for the two recording techniques (p=0.254).
There were also no significant differences when the two groups were compared for up
versus down movements (p=0.863) with either of the MSC and IR methods.
Upwards velocity was subtracted from the downwards velocity (delta; down minus up)
and the following graph illustrates the main sequence subtraction results.

The maximum amplitude of the vertical saccade paradigm was limited by the aperture
of the IR goggles and consequently smaller in this study than in the one by Schworm et
al. [2002]. Since this might have influenced the outcome additional experiments were
performed in healthy subjects. Herein, it was shown that the main sequence curve when
the maximum amplitude of the vertical saccades was 40 degrees instead of 30 degrees
(MSC recordings) did not change the up versus down relationship of the main sequence
curve.
In the original experiments the head position on the chin rest was not checked. In
additional experiments two healthy subjects performed saccades with a slightly gaze
down, gaze up and gaze forward position (MSC recordings). In all three head positions,
both C and V\text{MAX} were lower for the downward saccades than the upward saccades. For
both individuals the large saccades were slowest downwards in the down gaze position.
For different amplitudes, the small (up to approximately 10-15 degrees) downwards saccades were faster than the small upwards saccades, whereas the large downwards saccades were slower than the large upward saccades.

**Paper IV**

In the group of patients who were randomized to treatment with radioiodine, worsening or development (W/D) of TAO was found to occur significantly more frequently (63 patients; 38.7 %) than in the medical treatment group (32 patients; 21.3 %; p<0.001). W/D of TAO occurred during the first year after start of therapy in most of the patients (78 % in group I and 75 % in group M) and in only a few patients this took place after two years of follow-up (6 % in group I and 3 % in group M).

Besides treatment, smoking was also found to be a risk factor for W/D of TAO. In a Cox regression analysis both the choice of therapy and smoking habits were shown to significantly influence the risk of W/D of TAO. Also the combination of smoking and treatment with iodine-131 significantly influenced the risk for TAO:

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Odds ratio (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Therapy (iodine-131 vs medical therapy)</td>
<td>4.05 (1.95 to 8.43)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Set criteria</td>
<td>7.72 (2.31-25.75)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Current smoking (yes vs no)</td>
<td>5.20 (2.35 to 11.53)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Set criteria</td>
<td>9.80 (2.75-34.90)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Iodine-131 therapy in smokers</td>
<td>0.29 (0.11 to 0.74)</td>
<td>0.010</td>
</tr>
<tr>
<td>(smokers treated with iodine-131 vs all others)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Set criteria</td>
<td>0.14 (0.04-0.60)</td>
<td>0.007</td>
</tr>
<tr>
<td>Serum free T4 (pmol/L)</td>
<td>1.01 (1.00 to 1.03)</td>
<td>0.012</td>
</tr>
<tr>
<td>Set criteria</td>
<td>1.03 (1.01-1.04)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

In the following Kaplan-Meier plots (inverted) the relationship between treatment, smoking and W/D of TAO are shown. Significant differences were found in all Mantel-Haenszel comparisons, except in the one where the groups I and M were compared only in smokers:
Radioiodine vs ATD treatment

Smokers vs non-smokers

Radioiodine vs ATD treatment in non-smokers
Radioiodine and ATD treatment in smokers

Of the 41 patients (13%) who presented with clinical signs of TAO already at the first visit, 19 patients (ten in group I and nine in group M) had worsening of ophthalmopathy at some point during follow up. Approximately half of the patients had improvement of the eye disease, some got better before deterioration and only two patients had no change:

The number of patients with more severe eye disease among the patients with pre-existing TAO was similar between the two treatment groups throughout the study. In those patients, the number of individuals with an increased proptosis of three millimetres or more (as compared to measurements at randomization) and/or a deterioration of eye motility was three patients in both treatment groups at three months; three and four at 12 months respectively; three in both groups at 24 months and five patients in both groups at 36 months (missing data treated with last observation carried forward). In that subgroup, steroid treatment was given to three patients in group M and four in group I (one patient also received retrobulbar irradiation).

Development of de novo TAO was more common in group I (53 patients) than in group M (23 patients). The patients with development of TAO in group I also had more severe ophthalmopathy (defined as an increase in proptosis of three millimetres or more (as compared to measurements at randomization) and/or a deterioration of eye motility) at different time points after start of therapy:
In the group of patients with no TAO at the start of therapy, 52 % in group I and 70 % in group M were smokers. The corresponding numbers of smokers in the whole study group were 36 % and 42 % in group I and M respectively.

In group I and group M steroid treatment and/or orbital radiotherapy to treat TAO were required in nine and three percent of the patients respectively.

When the set criteria were used for evaluation of the change in TAO, W/D of TAO was also found to occur more often in group I (40 patients; 25 %) than in group M (16 patients; 11 %; p=0.002). The Cox regression results and the Mantel- Haenszel comparisons between different groups as shown on the pages 35-37 showed comparable results. Patients with TAO at randomization had the following change during the time of follow up according to the set criteria: Worsening of TAO in five and four patients, improvement in seven and zero patients and no change in ten and fifteen patients in group I and group M respectively.

Twenty-nine (19 %) of the patients in group M had adverse effects of the ATDs. These were:

- 13 % dermatological events including allergy
- 4 % joint pain
- 3 % change of taste
- 1 % rise in liver enzymes
- 1 % (one patient) long term fever and suspected impending agranulocytosis.
- 1 % non-specified

Therapy was changed into radioiodine in twelve patients because of adverse effects at a median time of three months (SD 4.3 months) after start of therapy.

In both treatment groups, 19 % of the patients had a recorded serum TSH level exceeding 4.5 mIU/L at any point during follow-up. During the first year, this occurred in 12 % and 14 % in group I and group M respectively. Preceding the deterioration of TAO, 10 % of the patients in group I and 16 % in group M had a recorded serum TSH of more than 4.5 mIU/L (6 % of the patients in both groups at less than six months prior to deterioration).
GD relapsed in 22.0% of the patients in group M and in 1.2% in group I (not including re-treatment for persisting hyperthyroidism). Re-treatment with RI in the previously medically treated patients was followed by W/D of TAO in only one patient.
DISCUSSION

Paper I-II

In Paper I it was shown that the IR recording system gave rise to generally higher velocity data, compared to the MSC system. The MSC recordings have in a previous report been found to give rise to lower velocity data than the VOG system [van der Geest and Frens 2000]. It might be, that the MSC system yields velocity data that are too low compared to the true saccadic velocity of the eyes; possibly owing to the mechanical load on the eyeball or increased friction related to the coil. It has also been suggested that the presence of the coils on the surface of the eyes might change the oculomotor command signals that drive the saccadic eye movements [Frens and van der Geest 2002]. There is at least a theoretical possibly that sliding of the coil on the surface of the eyes affects the MSC recordings [Houben et al. 2006], even though this was not observed in a more recent comparison between the MSC system and a videobased device [Schmitt et al. 2007]. That group also found no differences between a video-based recording system and MSC recordings in the same individuals in recordings of horizontal saccades. These new reports indicate that the MSC method generates reliable data. In consequence, it is likely that the true saccadic velocity of the eyes in our study has been overestimated by the IR system. Houben et al. [2006] suggested that the higher level of signal noise in the VOG system may have generated a tendency of higher peak velocities for the video than for coil signals. This is a probable contributing explanation also for the higher IR peak velocities in our study. Indeed, we found that filtering of the IR recordings gave rise to lower velocity data. Filtering of the IR recordings did however not smear out the variability within subjects. This might have been expected if the main reason for variability were basic noise. We believe that artefacts associated with the IR system also have had an essential influence on our recordings. If the positional signal is altered by e.g. concurrent lid movements the velocity curve might show steep velocity shifts, abnormal profiles and falsely high peak velocities. This was observed within many of the IR recorded saccades and has most likely contributed to variability, both between subjects and within subjects. Moreover, large number of artefacts in a recording might necessitate the elimination of a considerable proportion of the saccades from further analysis. Too small numbers of useful peak velocity data in a recording makes fitting of the main sequence curve inferior, which in turn means less reliable results and increased overall variability.

Furthermore, the inferiority of the IR system regarding spatial resolution and linearity for large amplitudes is a setback. Reliable peak velocities for large amplitude saccades are obviously important for proper fitting of the exponential curve, which normally starts to saturate at amplitudes exceeding approximately 20 degrees.

As mentioned, the detailed analysis of the saccadic velocity profiles of the IR recordings uncovered the partly individual existence of saccades that showed irregular patterns of intrasaccadic amplitude gain and velocity. If this observed oscillating amplitude and velocity pattern also reflects physiological intra-saccadic unevenness is not known, but is most likely to have principally been caused by artefacts, probably related to the changes in the reflecting surface of the lids and eyes. However, if assumed to represent physiological oscillations, the MSC recording technique may not have detected these short lasting alterations due to intrinsic filtering. According to Bahill and Stark [1975] fatigue changes the neurological control signal strategy of the
saccade and causes e.g. overlapping saccades, which show monocular patterns of amplitude and velocity variations that resemble the oscillations found in our study. An improved calibration procedure and a standardized hour-of-the-day recording session might, at least in theory, yield less variability.

The IR system that was used in this study (Orbit XY-1000, IOTA AB, Sweden) is seemingly less reliable than the MSC technique in the measurements of the main sequence relationship of saccadic eye movements. The disadvantage of the MSC technique still lies in its invasiveness, albeit novel coil designs associated with less discomfort have recently been introduced. The current developments of video-based eye tracking systems holds promise for a probably superior alternative to the IR reflection system for future research as well as for clinical use. In recent reports these have shown good agreement with the gold standard MSC method for recordings of the velocity of saccades [Houben et al. 2006, Schmitt et al. 2007].

**Paper III**

The results from earlier saccadic eye motility studies in patients with TAO have been somewhat contradictory (chapter 1.3.4.). Saccadic dynamics may have been more influenced in severe and late fibrotic TAO [e.g. Feldon and Unsöld 1992, Feldon et al. 1990, Schworm et al. 2002], whereas patients with early and mild disease have shown minor or no changes [e.g. Mauri et al. 1984, Schworm et al. 2000, 2002]. In one study, the patients displayed signs of active disease and no overt extraocular muscle involvement (duration of TAO not presented), but still exhibited changes in saccadic dynamics [Wouters et al. 1998]. The presence of eye muscle damage in early TAO is supported by findings of antibodies against eye-muscle antigens in patients with TAO of less than twelve months duration [Gopinath et al. 2006]. Hypothetically, this early eye muscle involvement might give rise to alterations of the saccadic velocity in patients with TAO. The results presented by Schworm et al. in 2002 (from the same laboratory where the current experiments were undertaken) generated an idea that patients with different stages of TAO, and even patients without clinically obvious eye disease, might be characterized by saccadic velocity recordings. In that study, it was shown that the patient groups with GD, with or without TAO had a different vertical eye velocity pattern than normal subjects when the MSC technique was used. Some of the patients with GD and no TAO were found to have impairment of the ocular motility by tests of binocular single vision and monocular ductions [Rydberg et al. 2003]. As suggested by Schworm et al. (2002), for the ensuing eye velocity experiments a non-invasive technique needed to be evaluated. For this purpose we chose the IR method (Orbit XY-1000, IOTA AB, Sweden). In our current studies we were, however not able to reproduce the results by Schworm et al. [2002] with either the MSC or the IR methods. The reasons for this is not known, but e.g. different examiner, subjects, range of recorded amplitudes and the use of a bite-board in this study instead of a head rest in the previous study, as well as the limited number of patients and controls in both the current and the previous studies, may have played a role. The innate variability of the data may probably also set back the likelihood of finding significant differences between the groups.

The observed lack of differences between patients and normal subjects may also have physiological explanations. The previously postulated adaptational capacity of the central neural saccadic generator [Kommerell et al.1976; Optican and Robinson 1980]
may have compensated for the early eye muscle changes in TAO. Tian et al. [2003] showed that patients with TAO generally have a higher eye muscle tension and their hypothesis was that increasing tension is an adaptational mechanism for compensating for eye muscle restriction. As suggested by Schworm et al. [2000] the earlier studies may have found changes in saccadic dynamics because of inclusion of patients with late fibrotic disease. In that quoted study where only patients with early TAO were included no clinically relevant differences were found in saccadic dynamics. Also in the current study, patients with early TAO had been selected.

The workings and limitations of the IR system may make the technique less preferable for saccadic motility assessments in patients with TAO. The IR system tracks the eye position by reflections from the ocular surface. The ocular surface is normally changed in TAO and may, at least in theory, also cause amendment of the recorded data; the lids may be retracted and swollen, there is generally dryness of the ocular surface, the distance between the bulb and the goggles may change because of exophthalmos, etc. Moreover, the poorer reliability and larger variability of the recordings is an important setback. Large amplitudes cannot be recorded with the IR system owing to the limited aperture of the goggles. Hence, the velocity of saccades with large amplitudes that may be affected more in TAO would not be possible to detect [Schworm et al. 2000]. The restriction of the amplitude range is also a drawback for fitting of the main sequence curve. On the other hand, the effect of a possible maladjustment of the coil on the surface of eyes that are affected by TAO it is not known.

The IR method for eye tracking (Orbit XY-1000, IOTA AB, Sweden) is currently not considered for future saccadic velocity measurements in patients with TAO. Novel non-invasive devices may be better candidates for this purpose. However, our inability to reproduce the results by Schworm et al. [2002] also with the MSC system diminishes somewhat the scope for using saccadic velocity measurements for early detection and quantitative characterization of mild and early TAO. Perhaps, the measurement of saccades in the directions of single eye muscle actions as proposed in the UFOF assessment [Haggerty et al. 2005], may prove to be better than our paradigm where only two vertical and two horizontal directions were included.

**Paper IV**

This trial is, to our knowledge, the third prospective, randomized study where radiiodine (RI) and anti-thyroid drug treatment (ATD) has been compared for worsening or development (W/D) of TAO. In the earlier RCT that was conducted in a similar population [Tallstedt et al. 1992] more patients were smokers in the radiiodine than in the medical treatment group and L-thyroxine was given only when hypothyroidism evolved after RI treatment. In the current study, these potential confounding factors were controlled for. In all three RCTs [Tallstedt et al.1992, Bartalena et al. 1998a] including the current one, RI treatment was found to be associated with a higher risk of W/D of TAO compared to treatment with ATD. Some, but not all, non-randomized prospective and retrospective studies have pointed to such a relationship, but the inferiority regarding controls, the risk of selection bias, etc, have rendered conclusions difficult on the basis of these reports (chapter 1.4.3.). Thyroid surgery was not considered as a third treatment arm in this current study, since the results in Tallstedt et al. [1992] could not demonstrate significantly higher occurrence of W/D of TAO in that treatment group compared to the ATD group. Besides,
including a third treatment arm would have rendered further difficulties in reaching the required number of patients for sufficient power of the study. This decision was also reasonable, considering that thyroid surgery is a rare option for primary treatment of GD [Wartofsky et al. 1991].

The limitations of this trial should be considered. First, our final study group did not meet the requisite of the trial, but according to power estimates, the study still had a power of about at least 70%. Second, the interpretation of our results are confined to patients who e.g. by the time of diagnosis are 35-69 years of age (general recommendations for radioiodine treatment in most centres in Sweden by the time the trial was designed) and do not exhibit signs of TAO that require corticosteroid therapy. After the termination of the trial, twenty patients needed to be excluded from the final study group, mainly because of insufficient ophthalmological follow up. The age, gender and smoking habits of these patients did however not differ greatly from the final study group. A further limitation is the non-blinded study-design.

The primary endpoint in this study was worsening or development (W/D) of TAO. This event was not outlined by means of a pre-set score (even though a comprehensive eye assessment protocol was completed at each visit), but rather, ophthalmologists well familiar with the eye disease determined this on the basis of clinical observations. Today, recommendations exist regarding the use of evaluated scoring systems in the clinical and scientific setting [Bartalena et al. 2008 a,b]. In the midst of the 1990s, when this trial was planned, no general consensus was available and the ophthalmological protocol that was used in this trial was a combination of some of the measures that were recommended in the CAS and in the NOSPECS classification systems [Mourits et al. 1989, Werner 1977]. The weighed combined activity and severity points (Total points) were used to indicate the course of the eye disease. Total points were calculated as follows (see appendix, paper IV): Activity-index + (3 x “Hertel increase”) + (4 x “Impaired eye movement”) + (5 x “Corneal involvement) + (6 x “Visual impairment”)

After closure of the trial, we were able to conclude that the ophthalmological assessment “Total points” at the time of the primary outcome event changed at a mean of three points in the radioiodine and four points in the ATD treatment groups respectively. Consequently, bias in favour of ATD treatment did not seem likely. The following graphs show the distribution of the Total points (appendix paper IV) before and after the deterioration of TAO in the groups of patients with W/D of TAO:
In order to assess the clinical relevance of the observed change of TAO we evaluated the results according to set criteria. We chose criteria for change of TAO, which had been used in a previous randomized trial where radioiodine and medical therapy was compared for worsening or development of TAO [Bartalena et al. 1998a]. In the current study, lid width and some of the features of the clinical activity score had not been recorded, which explains why minor modifications of the criteria were necessary. Our results confirmed the significant differences between worsening or development of TAO related to the randomized treatment for hyperthyroidism as well as for smoking. In some patients, TAO is short-lasting and for those, the deterioration of TAO might be of a minor concern. We also calculated the treatment-related risk for W/D of TAO only in patients who had a more sustained deterioration (total points of a higher value than that at baseline at two or more occasions plus those with deterioration at only one occasion but who received corticosteroid treatment), and still found significantly higher occurrence of W/D of TAO in the patients randomized to RI treatment (chi-square test, p<0.05). These results suggest that the employed primary outcome measure of our study was relevant for comparisons between the two treatment groups. Additionally, exophthalmos and impaired ocular motility, which are innately less likely to have been biased, were also found to be more common in the radioiodine group. Also, the use of corticosteroids was more frequent among the patients who received treatment with RI.

It has been shown that patients who receive RI treatment may benefit from concomitant treatment with corticosteroids in order to avoid W/D of TAO [Bartalena et al. 1989, 1998a]. The use of corticosteroids may however not be necessary in patients with mildly active TAO who undergo RI treatment, since deterioration has not been observed in such patients [Perros et al. 2005] or has been considered to be transient and non-severe [Sisson et al. 2008]. In our study, 14 patients out of 22 in the RI group who exhibited TAO at first visit had very mild disease (defined as total points ≤2). Six of these patients were observed to have worsening of TAO; of which four cases had an increase of proptosis of three millimetres or more and/or an impairment of ocular motility at some point during follow-up. This subgroup of patients is comparatively small and conclusions should be made with caution. However, on the basis of these results it cannot be excluded that patients with minor eye changes also run a risk of clinically important worsening of TAO after RI that is given as primary treatment for Graves’ hyperthyroidism. An interesting finding in our study was that the risk of worsening of pre-existing TAO was as frequent in the ATD group as in the RI group. A large proportion of the patients who did not have TAO at the start of the trial experienced de novo development of TAO after RI treatment. This implies that other risk factors than pre-existing TAO also might be important to consider in the decision making regarding immune-modulating prophylaxis. The large number of smokers among the patients with de novo development of TAO suggests that smoking habits may be important. The role of pre-treatment levels of thyroid hormones and anti-thyroid antibodies could regrettably not be determined from this study at this point, since laboratory methodology was different in the different centres (see methods section).

In Bartalena et al. [1998a] the risk of de novo development of TAO after RI treatment was generally found to be small. In that study, seventy percent of the patients had been treated with ATD before referral to the clinic that run the study and may consequently have entered the trial at a later phase of GD and of the natural course of TAO. Pre-
existing TAO in the current study and in Tallstedt et al. [1992] was 13%, whereas in Bartalena et al. [1998a] it was 50%. In the latter study, the sum of the patients who had development of TAO at any time point (i.e. before and after study enrolment) was 52% and 51% respectively in the RI and ATD groups. In the current study the corresponding figures were 46% (group I) and 28% (group M) respectively. The possible impact of this later referral to an orbital centre in the Italian study may need to be taken into account. Also, the number of smokers was higher in the study by Bartalena et al. [1998a] (56%; RI and ATD groups together) than in the current study (39%) and the one by Tallstedt et al. [1992] (41%).

The tertiary nature of the centre that hosted the study by Perros et al. [2005] may possibly also have influenced their results. In that study 69% of the patients had a history of moderate to severe TAO and almost all patients (69 out of 72) were already on treatment with ATD (median duration of hyperthyroidism 18 months). It might be hypothesized that, in their study group and that of Bartalena et al. [1998a] some of the patients’ ophthalmopathy were already in a slow “decelerating” phase (see Rundle’s curve), whereas the patients in our current study were in an early phase of GD and feasibly immunologically more “accelerating” phase of TAO. Our results regarding the risk associated to the presence and absence of pre-treatment TAO for W/D of TAO, apply only to patients with newly diagnosed GD and no previous history of treatment for GD. Newly diagnosed patients were also randomized in Kung et al. [1994] and also herein, more patients developed de novo ophthalmopathy (27%) than exacerbation of pre-existing TAO (17%) after RI therapy. These results underscore the need to be clinically sensitive for development of TAO in RI treated patients also in the ones without pre-existing TAO when treatment is provided in the early stages of GD.

In summary, the results from this study showed that when therapy was given to newly diagnosed patients for Graves’ hyperthyroidism, the risk of W/D of TAO was as follows (moderately severe TAO is here defined as an increased proptosis of three millimeters or more and/or ocular motility impairment):

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Any degree of W/D of TAO during a four year follow-up</th>
<th>Moderately severe after one year</th>
<th>Moderately severe after three years</th>
</tr>
</thead>
<tbody>
<tr>
<td>RI no TAO</td>
<td>38%</td>
<td>15%</td>
<td>11%</td>
</tr>
<tr>
<td>ATD no TAO</td>
<td>18%</td>
<td>5%</td>
<td>2%</td>
</tr>
<tr>
<td>RI or ATD + TAO</td>
<td>45-47%</td>
<td>16-18%</td>
<td>24%</td>
</tr>
</tbody>
</table>

It should be emphasized that the allocation of patients into having or not having TAO at diagnosis is arbitrary and merely based on clinical estimates. Also, the total number of patients with pre-treatment TAO was only 41 out of 313 individuals in this study.

Recurrency of TAO has been pointed out to be a possible risk factor for TAO [Perros and Kendall-Taylor 1998, Bartalena et al. 2000]. Owing to the long duration of our study, the patients who were classified as having W/D of TAO might have experienced
this event after treatment for relapse. However, W/D of TAO occurred in the majority of cases before re-treatment and hence, the observed deteriorations of TAO should be related to the randomized treatment, if to any treatment at all.

Cigarette smoking is a known risk factor for TAO [Thornton et al. 2007] and was confirmed as such in the current trial. Avoiding smoking is hardly controversial from any medical point of view. However, there is to date no firm evidence that patients with TAO who stop smoking have a better outcome than those who are not able to do so. Patients who smoked and received treatment with ATD did not have a significantly different outcome for W/D of TAO than the smokers who were treated with RI. Hence, the treatment-related risk factor does not seem to be as strong in smokers as in non-smokers. Nonetheless, since smokers who received radioiodine had the overall highest risk of TAO, one might argue that radioiodine should be avoided in smokers or be given together with immune-suppressive prophylaxis. Smoking has also been proposed as an indicator for considering steroid prophylaxis together with RI treatment by the EUGOGO group [2008 a,b].
CONCLUDING REMARKS

- The IR recording system generated generally higher $V_{\text{MAX}}$ and C values than the MSC system for the main sequence relationship between amplitude and peak velocity of the saccadic eye movements. Inter subject variability was larger with the IR technique.

- Intra individual variability for $V_{\text{MAX}}$ was found in both the IR and MSC recordings, but was more pronounced in recordings with the IR system.

- Main sequence analysis of saccadic eye movements did not show significant differences between normal control subjects and patients with TAO in this experimental setting with either the IR or the MSC recording systems. Innate shortcomings of both techniques should be considered in studies of saccadic eye movements in normal subjects and in patients with TAO.

- Radioiodine treatment for Graves’ hyperthyroidism was found to increase the risk for worsening or development of TAO when compared to anti-thyroid drug therapy. In the decision-making regarding treatment for hyperthyroidism in patients with Graves’ disease, this should be considered.

- The occurrence of de novo development of TAO was higher among the patients who were randomized to radioiodine treatment than among those randomized to medical treatment for Graves’ hyperthyroidism. On the other hand, worsening of TAO among the patients who had ophthalmopathy already at the start of therapy, occurred at similar rates in both treatment groups.

- Smokers had the highest risk for worsening or development of TAO irrespective of treatment modality. This risk factor should be paid attention to in the management of patients with Graves’ disease.
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