Function and structure of the eye muscles in myasthenia gravis: novel methods to aid in diagnosis and understanding of pathophysiology

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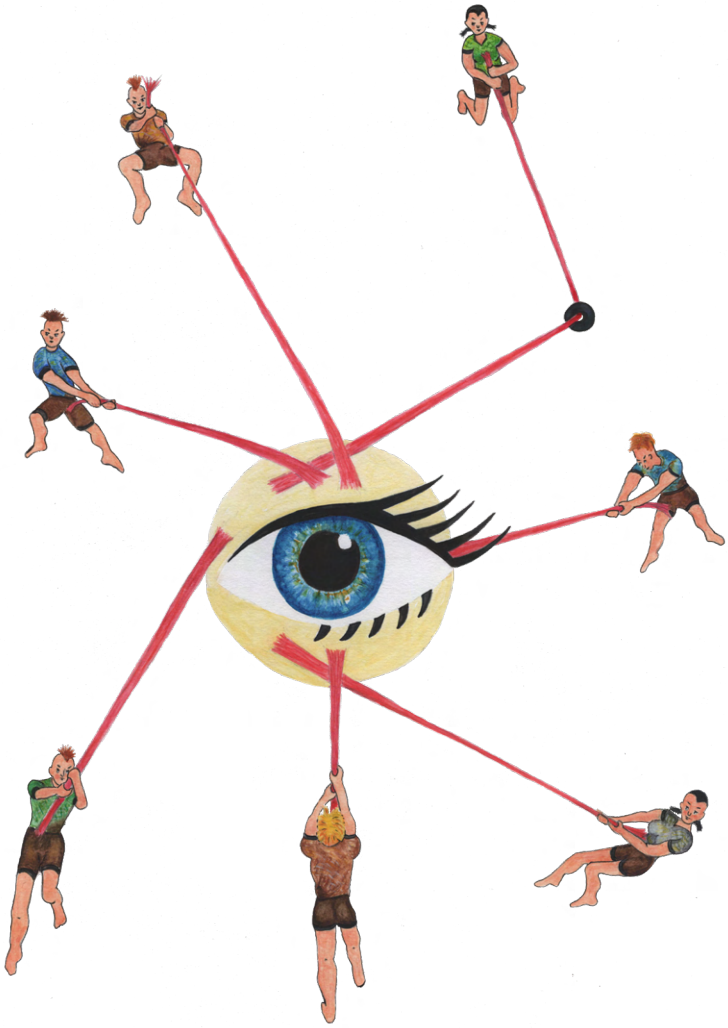

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General introduction

Myasthenia gravis (MG) is a muscle disease characterized by fluctuating and fatigable muscle weakness. Ocular and bulbar involvement is most common, where ocular symptoms, like double vision (diplopia) and drooping eye lids (ptosis), are experienced during the course of the disease.[1] In the generalized subtype of the disease all skeletal muscles can be involved including neck, arms, legs and respiratory muscles.
In this introductory chapter, MG will first be explained in more detail. Then, the challenges in diagnosis, therapy and outcome measure development of the clinical phenotype ocular MG are introduced, followed by three advanced methods that are studied to aid in these challenges: neurophysiological assessments, orthoptic measures and MR-imaging. The chapter ends with the overall aim of the thesis.

PART I – MYASTHENIA GRAVIS

Background and clinical course

MG is caused by auto-antibodies targeting proteins at the neuromuscular junction, including the acetylcholine receptor (AChR), muscle-specific tyrosine kinase (MuSK) or LRP4.[2,3] These antibodies can block neuromuscular signaling directly, block formation of a healthy neuromuscular structure and/or lead to the destruction of muscle membranes at the neuromuscular junction via complement activation (figure 1).
The prevalence of the disease is between 1 and 3.5 per 10.000 people.[4] The age of onset has a peak at an early age around 30 years and at a later age of around 50 years. The early peak is mainly caused by an early onset in young females, as is also common for other auto-immune diseases.[5] MG can or cannot be associated with a thymoma, a tumor of the thymus. In patients with thymoma either MG-related proteins are expressed, causing an auto-immune response, or dysregulation of the thymus results in the survival of auto-immune T-cells. No cure is available for MG, however with immunosuppressive treatments, thymectomy and medicine directly improving neuromuscular transmission muscle weakness and fatigue can be improved in many patients.[6,7]
In 10-15% of MG patients refractory symptoms occur, or the side effects of treatment do not outweigh the treatment effects.[8] The refractory symptoms can include ocular refractory ophthalmoplegia.[9] In the past few years, new medicine targeting complement, the FcRn receptor, or B-cell antigens have been developed, which is promising for these patient groups.[7]

Diagnosis

Diagnosis of MG consists of several steps (Figure 2). The first step involves serum analysis, where the auto-antibodies against AChR, MuSK or LRP4 can be detected. The presence of these antibodies form a definitive diagnosis of MG. If no antibodies are found, diagnosis can

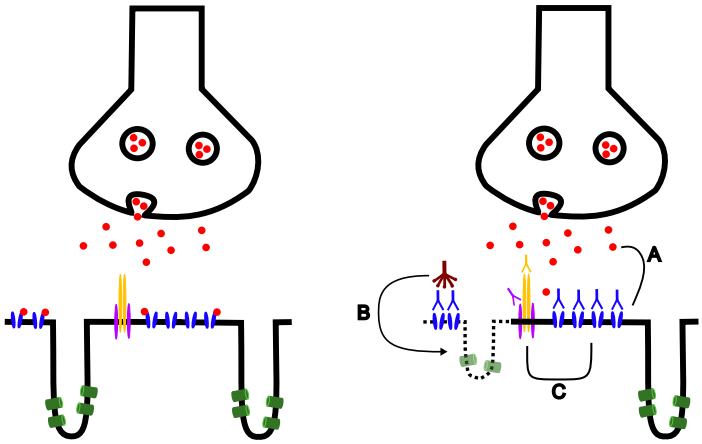

Figure 1. *Schematic of the neuromuscular junction. Left: The situation in a healthy control, where upon release of acetylcholine (AChR, in red) into the synaptic cleft, the AChR binds to the AChR-receptor (in blue) causing sodium influx eventually leading to activation of the voltage-gated sodium channels (in green) causing a fiber contraction. In purple and yellow the MuSK/LRP4 complex is shown which is responsible for AChR-receptor clustering.*
On the right the different disease mechanisms are shown in MG: ***A****. AChR-antibodies (blue) block the AChR receptors, and thereby neuromuscular signaling directly.* ***B.*** *Via complement activation (brown) and membrane-attack-complexes AChR-antibodies can lead to destruction of neuromuscular folds.* ***C.*** *MuSK (purple) and LRP4 (yellow) antibodies lead to neuromuscular failure due to blockage of healthy AChR clustering.*

be made definitive with additional electrophysiological tests. When a decrement is observed with repetitive nerve stimulation [RNS] or jitter is observed on single-fiber EMG (SF-EMG) the diagnosis is also definite. If these tests fail, diagnosis can be made probable with clinical improvement during additional clinical tests like the acetylcholinesterase inhibitor trial (see diagnostic flowchart in figure 2) or the ice-pack test. [2,3,10] If no definitive diagnosis can be made with serum testing and neurophysiological testing, but there is a clinical improvement after administration of a acetylcholinesterase inhibitor or there is a very clear ocular subtype with asymmetry and fluctuations in diplopia and ptosis, a probable diagnosis of seronegative MG can be made.

MG subtypes

MG patients are often divided into subgroups based on their clinical phenotype.[4] Ocular MG patients have sole involvement of the eye muscles with diplopia and drooping eye lids.[11] Bulbar MG patients have sole involvement of the muscles of the mouth and throat, affecting speech and swallowing. In generalized MG other muscles are also involved, like neck, arm, legs and respiratory muscles. There is an association between clinical phenotype and the

different antibodies. In AChR antibody positive MG the disease often starts ocular. MuSK antibody positive MG patients tend to have a more bulbar phenotype. Many patients start with either an ocular or bulbar phenotype and progress to a more generalized phenotype. In 85% of patients generalization occurs during the course of their disease. However, some patients remain purely ocular or bulbar. For example, in 90% of patients who have had the ocular form for more than 2 years no generalization will occur.[11]

The purely ocular subtype of MG is known to have specific challenges. In purely ocular MG half of patients are seronegative, while in a cross-section of all MG patients only 5% is seronegative.[12] Also, other diagnostic tests fail due to anatomical constraints in testing the eye muscles. Ocular MG is currently often excluded from clinical trials into the development of new therapies, because improvement of ocular symptoms is harder to measure than improvement of generalized muscle weakness. Additionally, the ocular symptoms can be refractory to therapy, leaving patients with invalidating ocular symptoms. Due to the number of challenges in ocular MG, this thesis will focus on this subtype.

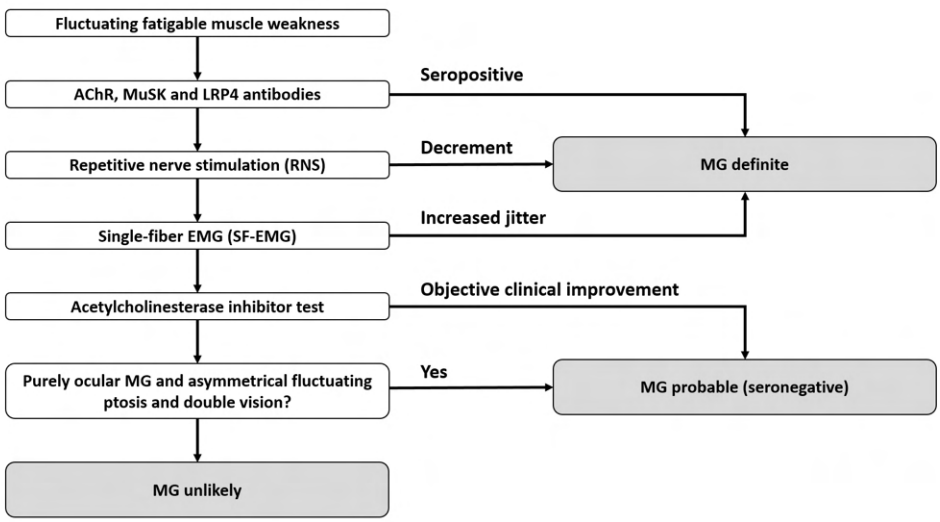

***Figure 2**. Diagnostic algorithm for myasthenia gravis. Clinical pattern evaluation, antibody testing and neurophysiological testing are central elements in the diagnostic workup. Adapted from Gilhus NE et al. Myasthenia gravis. Nat Rev Dis Prim. 2019;5(1):30.*

PART II – CHALLENGES IN OCULAR MG

To understand the difficulties of diagnosing ocular MG and the challenges in studying ocular function and pathology, understanding of the ocular anatomy is important. The extra-ocular muscles, or the eye muscles, located behind the eye in the bony orbit, move the eye in all directions of gaze. The lateral rectus muscle abducts the eye and the medial rectus muscle adducts the eye. When the eye is in abduction the superior rectus muscle moves the eye up and the inferior rectus muscle moves the eye down. When the eye is in adduction the inferior oblique muscle moves the eye up and the superior oblique muscle moves the eye down (figure 3). When any of these muscles is weak it can result in double vision as experienced by the patient. The levator palpebrae superioris muscle, which is located above the superior rectus muscle, is responsible for lifting the upper eye lid.

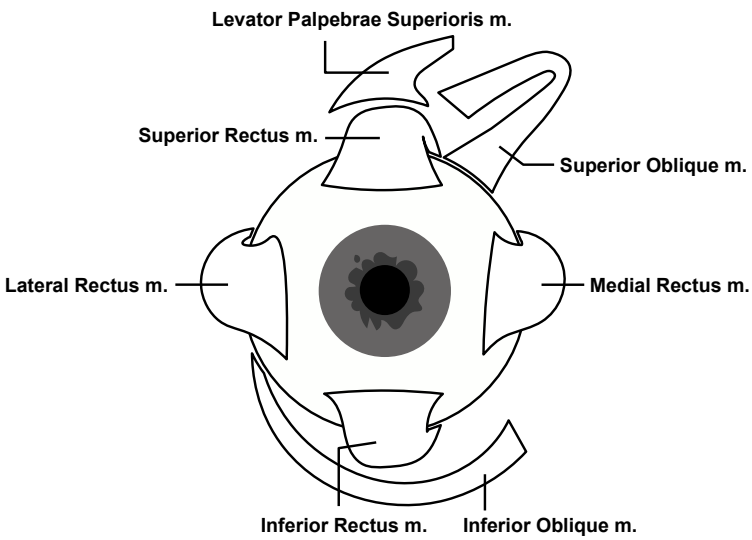

***Figure 3**. Anatomy of the right eye with the six eye muscles and the levator palpebrae superioris muscle. The four recti muscles, the lateral, medial, superior and inferior rectus, and the two oblique muscles, the inferior and the superior oblique, move the eye in all directions. The levator palpebrae superior muscles is responsible for lifting the upper eyelid.*

Diagnostic challenge in seronegative myasthenia gravis

There is a diagnostic challenge in seronegative MG patients. Around 50% of ocular MG patients are seronegative. Moreover, the sensitivity of RNS is low in this ocular subgroup of MG. In figure 4 the amount of undiagnosed patients in the world are depicted after RNS for

the generalized and ocular subgroups. While SF-EMG has a higher sensitivity, it requires a specifically trained neurophysiologist to perform the measurement and is thus operator-dependent. In addition, these neurophysiological tests of the eye muscles are not directly possible due to anatomical constraints; the eye muscles and their innervating nerves are located behind the eye in the bony orbit. The ice-pack test and the acetylcholinesterase inhibitor test, however useful, are not fully objective and require symptoms to be present at that current moment. Therefore, additional diagnostics are needed for patients with seronegative disease, who are still regularly misdiagnosed or diagnosed after a considerable delay.[4]

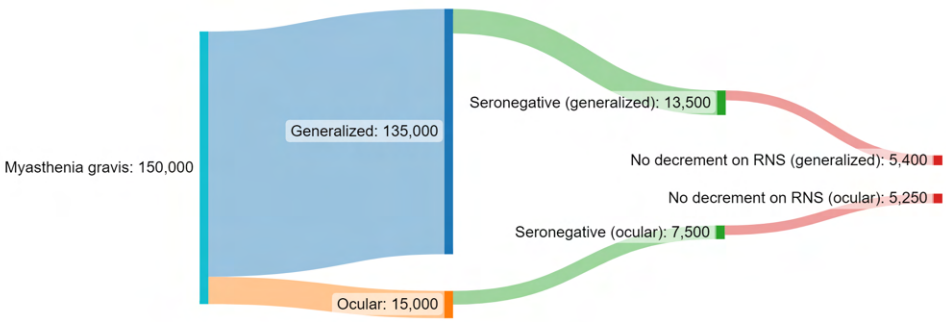

***Figure 4**. Diagram depicting the amount of ocular MG patients in the world that remain undiagnosed in generalized MG as compared to ocular MG after antibody testing and repetitive nerve stimulation. 10% of MG patients remain purely ocular, of which 50% of patients are seronegative.*

Therapeutic challenge in refractory ocular myasthenia gravis

There is a therapeutic challenge in the MG patients who struggle with refractory disease, including those with refractory ophthalmoplegia.[9] Little is known about the pathophysiology of refractory ocular ophthalmoplegia in MG. From some case reports [13–16] and a case control study[17] into untreated ocular MG patients, atrophy and fat replacement of the eye muscles have been observed. Fat replacement is known to occur when skeletal muscles are damaged. Also in MuSK and AChR MG, muscle volume decrease and fat replacement of other muscles, like the bulbar muscles responsible for chewing, swallowing and speech, are observed.[18–21] Atrophy and damage of muscles could be an explanation why refractory disease can develop in MG, because severely atrophic and damaged muscles might not respond to therapy. As such, studying to what extent atrophy occurs in the eye muscles, as well as the influence of treatment on this, could aid in pathophysiological understanding of refractory ophthalmoplegia.

Lack of outcome measures in ocular myasthenia gravis

Several outcome measures are used to follow the severity of MG-related muscle weakness over time: Patient reported outcome measures like the *MG activities of daily living* (MG-ADL)[22] and quantitative muscle tests evaluating the severity of MG-related muscle weakness like the *quantitative myasthenia gravis score* (QMG).[23,24] For ocular MG patients, however, outcome measures specific for the ocular symptoms are not yet developed or used in the clinic. In recent clinical trials on new treatments[7], the ocular subtype of MG was usually excluded, probably also because the degree of ocular weakness has been difficult to quantify so far.[25,26] Patient reported outcome measures for ocular MG, like the *ocular myasthenia gravis rating scale* (OMGRate)[27] are being developed and evaluated, however more objective scales for the severity of ocular MG, like the QMG is for generalized MG, are missing.

PART III – POTENTIAL AIDS IN DIAGNOSTIC, THERAPEUTIC AND OUTCOME MEASURE CHALLENGES IN OCULAR MG

To overcome the challenges outlined above, methods are needed that can directly measure structure or function of the extra-ocular muscles. Three of these are outlined below: a new neurophysiological test using repetitive ocular vestibular evoked myogenic potentials (RoVEMP), orthoptic measurements and quantitative MRI. The RoVEMP test and orthoptic tests measure eye muscle function, and MRI is used to study eye muscle structure. If there are distinct differences in structure and function between diseases and between MG and healthy controls these methods could be used to aid in diagnosis. Structural and functional changes during the disease course could help in the pathophysiological understanding of refractory ophthalmoplegia, for example if the dysfunctional muscles are severely atrophic. And both distinct structural and functional measures that change during the disease course could function as outcome measures for clinical trials.

Repetitive ocular vestibular myogenic potentials

RNS of peripheral nerves is used to measure the pathologic decremental response of compound muscle action potentials (CMAPs) in MG. Due to depletion of acetylcholine at the neuromuscular junction after repetitive stimulation and partial neuromuscular blockage in MG, the excitation threshold of a part of the fibers is not reached resulting in a decrement in CMAPs.[28] As discussed above, RNS of the eye muscles is not directly possible due to anatomical constraints. However, via stimulation of the vestibular organ, ocular vestibular evoked myogenic potentials (oVEMP) can be elicited. The oVEMP reflex is generated by stimulation of the vestibular organ via vibration applied to the skull or sound to the ear.[29] Using electrodes under the eye, this reflex can be measured for the inferior oblique muscle,

which is located close to the electrodes in up-gaze.[30] Repetitive stimulation (RoVEMP) is in this way considered as a variant of RNS for the eye muscles and indeed a decrement is observed in MG patients (figure 5). [31,32] The diagnostic use of the RoVEMP test is established on a group level. However due to technical difficulties, like a low signal-to-noise ratio and blinking artefacts, the usefulness of the RoVEMP test for an individual patient and for follow-up is unknown. The test-retest reliability should be studied first. I hypothesize that with a good test-retest reliability the RoVEMP test could be useful in diagnostics, as an outcome measure and could help in understanding neuromuscular failure in the eye muscles.

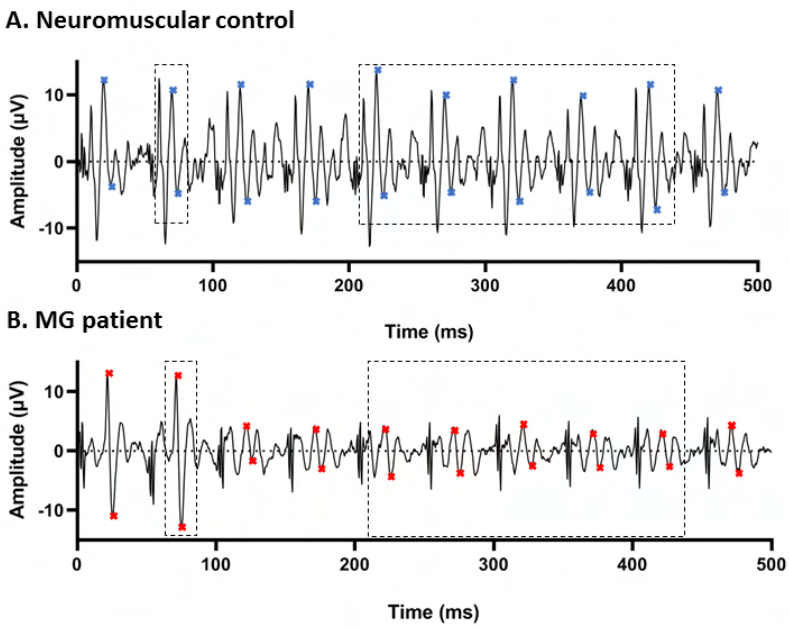

Figure 5. *Example RoVEMP signal in a myasthenia gravis (MG) patient (B) and a neuromuscular control. The signal from the MG patient clearly shows a decremental response after repetitive stimulation. From De Meel et al. Repetitive ocular vestibular evoked myogenic potentials in myasthenia gravis. Neurology. 2020;94(16):E1693-E1701.*

Orthoptic measurements

Orthoptists are the experts in diagnosing and treating defects in eye movement and problems with how the eyes work together. Several orthoptic tests enable measurements of the maximal movement of the eyes and relative deviations between the gazes of the eyes in all directions. These tests can objectively measure eye muscle function and the severity of double vision. A synoptophore can for example be used to measure the maximal movement

of the eye, or duction angles, in all eight gaze directions (up, down, abduction, adduction and the four diagonal gazes).[33,34] The Hess-chart is a test to measure the relative difference in gaze direction between two eyes. Using glasses with a red filter for one eye and a green filter for the other eye, and a screen with red LEDs and a green laser pointer, the difference in gaze direction between the eyes can be objectified.[35] (figure 6) These tests are currently not adapted for the measurement of MG-related fatigability of eye muscles. Since orthoptic measures are direct measures of eye muscle function, I hypothesize that these test when adapted for MG are promising in diagnostics, as an outcome measure and can help to understand the pathophysiology of diplopia in MG.

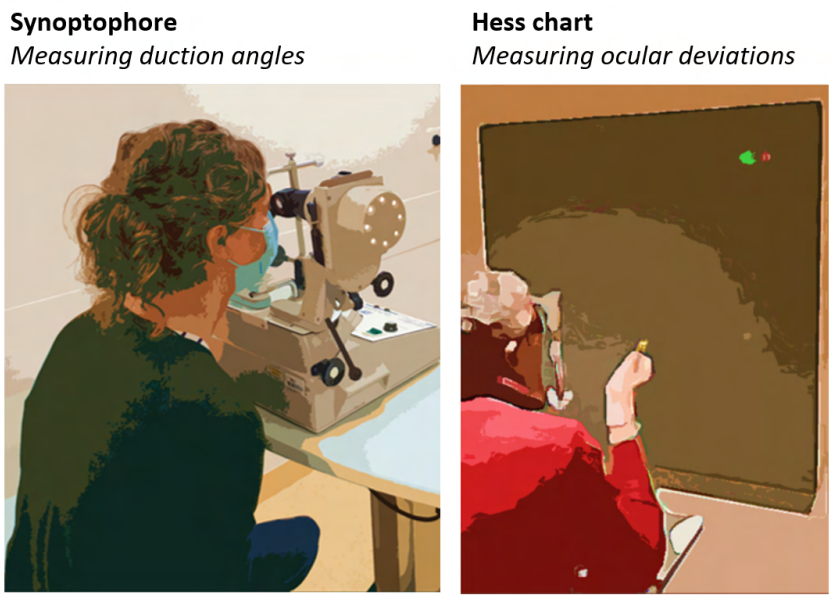

***Figure 6**. Example orthoptic measurements. On the left a synoptophore that is used for duction angle measurements. Duction angles are measured per eye individually and are a quantification of the angle an eye can move into all 8 primary directions of gaze. On the right a Hess chart that is used to measure the deviation between two eyes by using glasses with a red/green filter, red dots on a screen and a green laser pointer. Adapted from Keene KR et al. Diagnosing myasthenia gravis using orthoptic measurements: Assessing extra-ocular muscle fatigability.*

Quantitative MRI in neuromuscular disease

Magnetic resonance imaging (MRI) is a medical imaging technique that uses a strong magnetic field and radio waves to visualize tissues in the body. Quantitative MRI can then assess certain tissue properties. In research into neuromuscular disease, quantitative MRI

is widely used as a biomarker for disease progression and disease activity.[36] Physical disability gets worse (disease progression) when disease activity causes more and more damage to muscle fibers. For example in dystrophic muscular disease, like Duchenne muscular dystrophy, Becker muscular dystrophy and facioscapulohumeral muscular dystrophy, muscles are progressive replaced by fat and fibrosis due to muscle damage. The fat fraction of these muscles as measured with MRI correlates with strength measures in many diseases[37], and can even predict clinical milestones in Duchenne muscular dystrophy[38]. Fat fraction is therefore a marker of disease progression and is used as surrogate outcome measure in clinical trials developing new therapies.[39] Using MR, the fat fraction of a muscle can be quantified and mapped using chemical shift based water fat separation (the Dixon method[40]). The Dixon method uses the difference in precession speed of water and fat to produce separate water and fat fraction maps (figure 7). A proton density weighted fat fraction (PDFF) can be calculated using these maps by dividing the fat signal by the water plus fat signal after correction for specific tissue properties.[37]

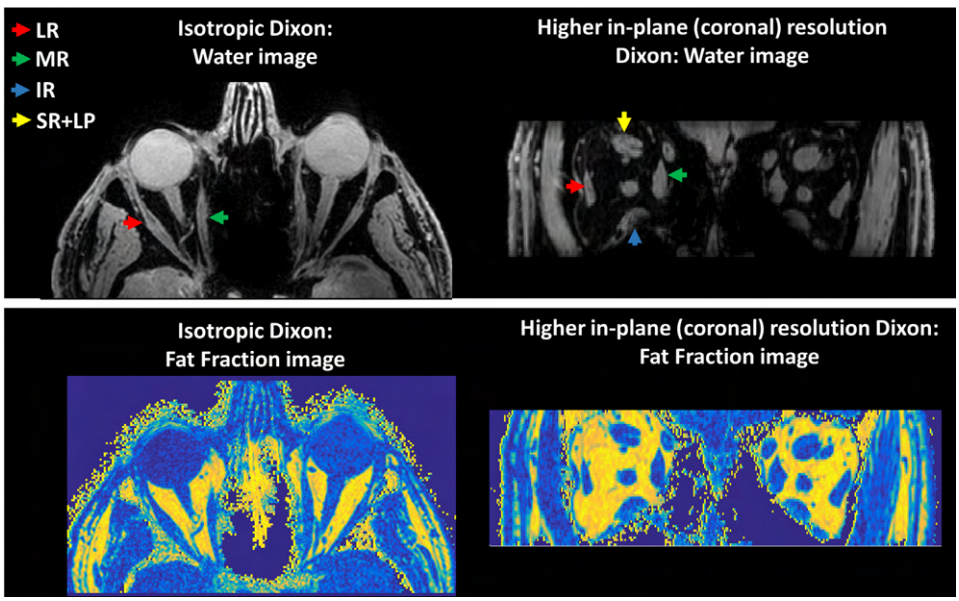

Figure 7. *Examples of water images on top and fat fraction maps below as measured with a chemical shift based water fat separation gradient echo MRI-scan. The colored arrows point out the lateral rectus muscle (LR; red), the medial rectus muscle (MR; green), the inferior rectus muscle (IR; blue) and the superior rectus and levator palpebrae muscle complex (SR+LP; yellow). Adapted from Keene KR et al. The feasibility of quantitative MRI of extra-ocular muscles in myasthenia gravis and Graves' orbitopathy. NMR Biomed 2021;34:e4407.*

The T2 relaxation time of the muscle is proposed as a measure of inflammation and edema reflecting disease activity.[41] Muscle cells mainly consist of water and the T2 of the muscle is around 30 ms. As more free water (e.g. edema) is present between and around the muscle cells the T2 of the tissue increases. Therefore, it is a very sensitive marker of inflammation, however it lacks specificity because it also increases in the presence of membrane leakiness and necrosis.[42] Measuring the T2 of only this water or muscle compartment is challenging, because in neuromuscular disease inside the voxels a combination of fat and water tissue is present. The muscle can be partly replaced by fat due to the disease process. In the presence of this fat, the signal inside a voxel originates from both the water from the muscle cells and the fat. Because fat also has a longer T2 relaxation time than muscle, this biases the T2 measurements towards a longer T2. The signal that is originating from the intramuscular fat should be accounted for in the used models by separating the signal in its two-components: the water signals and the fat signal.[43]

Quantitative MRI of the eye muscles

Applying quantitative MRI of the eye muscles is particularly challenging as compared to the skeletal muscles. Given the large amount of movement of the eye muscles, MR-measurements and MR-images are very prone to movement artefacts. The eye muscles are also very small with diameters of approximately half a centimeter and a length of approximately four centimeter. Therefore a high resolution is needed to image and measure the eye muscles without contamination from the surrounding orbital fat. In MRI the higher the resolution and the smaller the voxels, the lower the signal to noise ratio. Also due to the close proximity of air from the sinuses and bone from the orbit, the inhomogeneity of the main magnetic field (B0) is a challenge to overcome for quantitative measurements in this anatomical area.[44,45]. Recent studies have shown that the increased signal to noise ratio in an MRI with a magnetic field strength of 7 Tesla, combined with a cued instructed blinking to reduce eye-motion artefacts and localized shimming to minimize B0 artefacts, makes it possible to do high resolution images of the eyes (figure 7).[45,46] Since these challenges are overcome, I aimed to combine high resolution orbital MRI and quantitative MRI of muscles to study the structure of the eye muscles of MG patients as compared to healthy controls and other ocular diseases. These structural differences could aid patients in diagnostics, as outcome measure in clinical trials and to understand why ocular symptoms are resistant to therapy in some MG patients.

PART IV - AIMS AND OUTLINE OF THIS THESIS

I aimed to develop novel methods to improve the clinical care of ocular MG patients. First, I aimed to aid in the difficult diagnosis of ocular seronegative MG using the RoVEMP test, orthoptic measurements and quantitative MRI as diagnostic tools. Moreover, I aimed to gain a better understanding of EOM involvement in MG to better understand the pathogenesis of refractory ophthalmoplegia in MG. Lastly, I aimed to directly measure eye muscle function and structure in ocular MG to use as an outcome measure in clinical trials (figure 8).

An extensive literature review was performed into clinical and imaging clues to the diagnosis and follow-up of all diseases with ptosis and ophthalmoparesis (**chapter 2**). With the mentioned techniques I measured the EOM as directly as possible in MG patients. I examined the test-retest reliability of the RoVEMP test, a test that directly measures CMAP decrement of the EOM (**chapter 3**). I examined the EOM functionally by assessing maximal duction angles and deviations as proxies for muscles strength (**chapter 4**). Lastly, I examined structural changes of the EOM using quantitative MRI (**chapter 7**) after developing a method to qualitatively measure muscle inflammation (**chapter 5**) and after assessing the feasibility of doing quantitative MRI of the EOM on a 7 Tesla MR-scanner (**chapter 6**).

Methods

Challenges	 **Ocular myasthenia gravis**	**RoVEMP test** *Neurophysiological measure of eye muscle fatigability*	**Orthoptic measures** *Functional measures of eye muscle weakness and fatigability*	**Quantitative MRI** *Structural quantitative measures of eye muscles*
	Diagnostic *Half of patients is seronegative; need for diagnostic tests*	*Is the test-retest reliability sufficient for diagnosis?*	*Can orthoptic measures be adapted to measure MG-related fatigability to aid diagnostics?*	*Are there differences in eye muscle volume, T2 and fat fraction between MG, healthy controls and other diseases?*
	Understanding refractory ophthalmoplegia *Needed for developing effective therapies*	*Can neuromuscular transmission failure be measured in the eye muscles?*	*Do eye muscles have a fatigable muscle weakness as is present in skeletal muscles?*	*Are refractory ocular symptoms in MG caused by eye muscle atrophy and fat replacement?*
	Ocular outcome measures *Need for objective outcome measures of eye muscle weakness*	*Does decrement correlate with severity of ocular symptoms?*	*Can orthoptic measures aid as an objective outcome measure for eye muscle involvement in ocular MG?*	*Can volume, fat fraction and T2 of the eye muscles serve as an outcome measure in clinical trials?*

Figure 8. *Summary of the three clinical challenges in ocular myasthenia gravis, the three introduced advanced methods that might aid these challenges and the hypotheses.*