Statins for Graves' orbitopathy (STAGO): a phase 2, open-label, adaptive, single centre, randomised clinical trial

Giulia Lanzolla, Elena Sabini*, Marenza Leo, Francesca Menconi, Roberto Rocchi, Angela Sframeli, Paolo Piaggi, Marco Nardi, Claudio Marcocci, Michele Marinò*

Summary

Background A protective action of statins on development of Graves' orbitopathy suggests that statins might be used for treatment of the disease. We aimed to assess the efficacy of the addition of a statin, atorvastatin, to intravenous glucocorticoids (ivGCs) on Graves' orbitopathy outcomes in patients with hypercholesterolaemia.

Methods We did a randomised, open-label, phase 2, adaptive, clinical trial at a single, tertiary, referral hospital in Pisa, Italy. Patients with moderate-to-severe, active Graves' orbitopathy, with a low-density lipoprotein cholesterol concentration between 2·97 and 4·88 mmol/L were eligible for inclusion. Patients were randomly assigned (1:1) in 11 blocks of eight, using a computer-based system, to the ST group or the NST group. The ST group received ivGCs (methylprednisolone 500 mg once a week for 6 weeks followed by 250 mg once a week for an additional six weeks) for 12 weeks and oral atorvastatin (20 mg once a day) for 24 weeks. The NST group only received the ivGC regimen. Patients were unmasked to group allocation; however, the ophthalmological investigator was masked to randomisation. The primary endpoint was the Graves' orbitopathy outcome (composite evaluation of exophthalmos, clinical activity score, eyelid aperture, and diplopia) at 24 weeks in the modified intention-to-treat (ITT) population (patients who attended the week 12 visit). Patients were considered responders when at least two of the following criteria were fulfilled in the most affected eye, without worsening in any of the same measures in both eyes: (1) reduction in exophthalmos of 2 mm or more, with no increase by 2 mm or more in the other eye; (2) reduction of clinical activity score by two or more points; (3) reduction in eyelid aperture by 2 mm or more, with no increase by 2 mm or more in the other eye; and (4) disappearance or improvement (change from constant to inconstant, intermittent, or absent, or from inconstant to intermittent or absent) of diplopia, and (5) improvement in visual acuity by 0·2 decimals or more. The trial is registered with EUDRACT, 2018-001317-33, and ClinicalTrials.gov, NCT03110848.

Findings Between June 1, 2020, and Nov 30, 2020, 119 patients were screened for inclusion, of whom 88 (74%) patients were enrolled and randomly assigned to one of the two treatment groups (44 [50%] to the ST group and 44 [50%] to the NST group). Eight (9%) patients did not attend the 12 week visit; 80 (91%) patients (18 [23%] men and 62 [78%] women) were included in the modified ITT population (41 [51%] in the ST group and 39 [49%] in the NST group]. The proportion of Graves' orbitopathy composite evaluation responders at 24 weeks was higher in the ST group (21 [51%] of 41 patients) than the NST group (11 [28%] of 39 patients; attributable risk 0·23 [95% CI 0·02–0·44]; p=0·042). 26 adverse events occurred in 21 (24%) of 88 patients in the safety population. One (2%) of 44 patients in each group required treatment discontinuation, with no serious adverse events and no difference between groups.

Interpretation Addition of oral atorvastatin to an ivGC regimen improved Graves' orbitopathy outcomes in patients with moderate-to-severe, active eye disease who were hypercholesterolaemic. Future phase 3 studies, which could potentially recruit patients regardless of low-density lipoprotein cholesterol concentration, are required to confirm this association.

Funding Associazione Allievi Endocrinologia Pisana.

Lancet Diabetes Endocrinol 2021; 9: 733–42

Published Online September 27, 2021 https://doi.org/10.1016/S2213-8587(21)00238-2

See Comment page 726

*Authors contributed equally

Department of Clinical and Experimental Medicine, Endocrinology Units (G Lanzolla MD, E Sabini MD, M Leo MD, F Menconi, MD, R Rocchi MD, Prof C Marcocci MD, Prof M Marinò MD), **Department of Surgical, Medical and Molecular Pathology, Ophthalmopathy Unit I** (A Sframeli MD, Prof M Nardi MD), **Department of Information Engineering** (P Piaggi PhD), **University of Pisa and University Hospital of Pisa, Pisa, Italy**

Correspondence to Prof Michele Marinò, Department of Clinical and Experimental Medicine, Endocrinology Unit II, University of Pisa and University Hospital of Pisa, 56124 Pisa, Italy **michele.marino@med.unipi.it**

Introduction

Graves' orbitopathy is an autoimmune extrathyroidal manifestation of Graves' disease[1,2] due to autoantigens expressed by orbital fibroblasts and thyroid cells.[3] High dose intravenous glucocorticoids (ivGCs) are the first line treatment for moderate-to-severe, active Graves' orbitopathy, as recommended by the 2016 guidelines of the European Thyroid Association and the European Group On Graves' Orbitopathy (ETA/EUGOGO).[4] ivGCs result in a beneficial effect in 35–80% of patients.[5] The addition of other medications might improve the benefits of ivGCs and reduce dosage, thereby minimising side-effects. Since 2015, a number of studies have focused on the role of cholesterol and cholesterol-lowering medications.[6,7] Two large cross-sectional investigations showed a reduced risk of Graves' orbitopathy in patients with Graves' disease who were receiving statins.[8,9] Another cross-sectional investigation in patients with Graves' disease showed a correlation between the presence of Graves' orbitopathy and total and low-density lipoprotein

Research in context

Evidence before this study

We searched PubMed for studies published between Jan 1, 1980, and Jan 1, 2020, using the search terms "Graves' orbitopathy", "Graves' ophthalmopathy", "thyroid eye disease", "thyroid associated ophthalmopathy", "thyroid ophthalmopathy", "endocrine ophthalmopathy", "cholesterol", "low-density lipoprotein", "lipids", and "statins". Only English language articles were included. A large retrospective, cross-sectional investigation reported a reduced risk of Graves' orbitopathy in patients with Graves' hyperthyroidism who received statins. Moreover, a cross-sectional investigation done in consecutive patients with Graves' hyperthyroidism showed a correlation between the presence of Graves' orbitopathy and both total cholesterol and low-density lipoprotein cholesterol and a correlation between the Graves' orbitopathy clinical activity score and cholesterol. The association between low-density lipoprotein cholesterol and Graves' orbitopathy was confirmed by a retrospective study. The 2016 European Thyroid Association and European Group on Graves' Orbitopathy guidelines recommended intravenous glucocorticoids as the first line treatment for moderate-to-severe, active Graves' orbitopathy. However, the proportion of responders to this treatment is variable and a discrete percentage of patients do not respond to treatment satisfactorily, which prompts alternative or additional treatments.

Added value of this study

To our knowledge, our study is the first that investigates the efficacy and the safety of a statin (atorvastatin) added to glucocorticoid treatment in patients with moderate-to-severe, active Graves' orbitopathy. Our findings show a beneficial effect of atorvastatin on Graves' orbitopathy outcome and quality of life in patients who are hypercholesterolaemic and are receiving intravenous glucocorticoids.

Implications of all the available evidence

To ameliorate the response to the first-line treatment for moderate-to-severe, active Graves' orbitopathy, the available evidence supports the use of a combination treatment with medications acting at the different steps of the pathogenesis of the eye disease. Our study offers an additional tool for disease management. Future studies should aim to investigate the molecular mechanisms underlying our findings in detail and expand the clinical research by doing a phase III clinical trial, possibly involving patients with Graves' orbitopathy regardless of their cholesterol concentrations.

cholesterol.[10] Although Graves' orbitopathy severity did not correlate with high cholesterol, the Graves' orbitopathy clinical activity score was higher in patients with high cholesterol and correlated with total and low-density lipoprotein cholesterol concentration.[10] The association between low-density lipoprotein cholesterol and Graves' orbitopathy is supported by the findings of a retrospective investigation published in 2018.[11]

See Online for appendix

These observations[6–11] might have implications in disease management. Not only might statins protect patients with Graves' disease from developing Graves' orbitopathy, they might be useful in the treatment of Graves' orbitopathy when present. We aimed to investigate the effects of the addition of a statin to an ivGC regimen in patients with Graves' orbitopathy.

Methods

Study design and participants

We did a phase 2, open-label, adaptive, single-centre, randomised, clinical trial to evaluate the effects of the addition of atorvastatin to an ivGCs regimen in patients who were hypercholesterolaemic with moderate-to-severe, active Graves' orbitopathy. The study was done at a single tertiary referral centre: the University Hospital of Pisa, Pisa, Italy.

For the **Clinical Trial Facilitation Group recommendations** see https://www.hma.eu/fileadmin/dateien/Human_Medicines/01-About_HMA/Working_Groups/CTFG/2014_09_HMA_CTFG_Contraception.pdf?fbclid=IwAR3AY5Ha0ESDyqIBeUaYI9VTFWmx9bbt8NZ-80N-5ME6pkBb1UHvFsTwqlQ

Men and women (18–75 years) with Graves' disease—diagnosed based on hyperthyroidism associated with anti-thyrotropic hormone (TSH) receptor autoantibodies (TRAbs)—and moderate-to-severe, active Graves' orbitopathy—defined as presence of at least one of the following criteria associated with clinical activity score of three out of seven or more in the most affected eye: exophthalmos of 2 mm or more compared with normal for sex and race (appendix p 2); inconstant to constant diplopia; and lid retraction of 2 mm or more were eligible for inclusion. Additionally, eligible patients had to have a fasting low-density lipoprotein cholesterol of 2·97–4·88 mmol/L and a triglyceride concentration less than 4·51 mmol/L, based on European Society of Cardiology and European Society of Hypertension guidelines.[12] Patients who did not have more than one of the additional cardiovascular risk factor (diabetes, hypertension, smoking, familial history of cardiovascular events, obesity [BMI ≥30 kg/m²]), which combined with high low-density lipoprotein cholesterol concentration makes statin treatment mandatory, were included in the study; [12,13] as were individuals with alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatine phosphokinase (CPK) no more than three-times the upper normal range. Participants were asked to use contraceptive methods with a failure rate of less than 1% a year; those who complied were eligible for inclusion: women of childbearing potential (not in menopause or started menopause <2 years before enrolment) were asked to use these methods for six months after the last dose of the study drug and men sexually active with women of childbearing age were asked to use these methods for 7·5 months after the last dose of the study drug in accordance with the Clinical Trial Facilitation Group recommendations.

Patients with optic neuropathy, as defined by the 2016 ETA/EUGOGO guidelines;[4] those receiving

treatment with glucocorticoids or any immunosuppressive medication in the 3 months preceding recruitment; patients who used selenium in the 3 months preceding recruitment; individuals who received previous surgical treatment for Graves' orbitopathy; and participants with contraindications to ivGCs and statins (appendix p 2) were not eligible for inclusion. Pregnant or lactating women; patients with liver diseases; and those who were receiving medications that increase statin and ivGC toxicity, or interfere or interact with statins and ivGCs—especially Cytochrome P450 Family 3 Subfamily A Member 4 (CYP3A4) inhibitors or inductors (appendix p 2) were also not eligible for inclusion. Patients with relevant malignancy of any type, but that was not in remission or had been diagnosed in the 5 years before recruitment); those with a recent (≤1 year) history of alcoholism or drug misuse; and those with mental illness preventing comprehensive informed consent were excluded from the trial. Additionally, patients with a low-density lipoprotein cholesterol concentration more than 4·88 mmol/L were excluded because these concentrations render statin treatment mandatory, thereby preventing randomisation.[12,13]

Written, signed informed consent including compliance with requirements and restrictions listed in the consent form was required for the patients to be recruited into the trial. The study was approved by the local Ethic Committee (Comitato Etico Area Vasta Nord-Ovest; approval number 15893_MARINO') and done in accordance with the International Conference on Harmonisation Good Clinical Practice guidelines and the principles of the Declaration of Helsinki.

Randomisation and masking

Patients were recruited consecutively over a 6 month period and randomly assigned (1:1) in 11 randomisation blocks of eight patients to receive methylprednisolone and atorvastatin (ST group) or methylprednisolone alone (NST group; the control group). Randomisation was done by the quality control system of University Hospital of Pisa (the sponsor), who received anonymous identification numbers of enrolled patients and, using PASS (2021), randomly assigned patients to one of the two groups. Assignment outcomes were sent to the principal investigator. No one in the study team had access to the randomisation procedure. The patients and the study team were unmasked, but the ophthalmological investigator was masked to group assignment.

Procedures

Starting at baseline (day 1), intravenous methylprednisolone was administered, according to a previously described protocol:[4,14] 500 mg once a week for 6 weeks followed by 250 mg once a week for another 6 weeks (cumulative dose 4·5 g; appendix p 8). Patients in the ST group also received 20 mg oral atorvastatin once a day for 24 weeks. Patients in both groups were given omeprazole 20 mg once a day for the first 12 weeks of the study alongside the intravenous methylprednisolone regimen. Omeprazole was administered to counter side-effects of the ivGC regimen.

An ophthalmological evaluation was done at baseline, 12 weeks, and 24 weeks. The evaluation included exophthalmometry (Hertel exophthalmometer), eyelid aperture, assessment of diplopia (Gorman score),[4] ocular ductions, corneal status, fundi, visual acuity (Snellen chart), and clinical activity score.[15] Patients were seen by the same ophthalmologist who was masked to treatment group assignment.

Blood tests were done at baseline, 12 weeks, and 24 weeks. The blood tests assessed free thyroxine (FT4) and triiodothyronine (FT3) concentrations using chemiluminescence immunoassays (Vitros Immunodiagnostics, Raritan, NJ, USA); TSH concentrations using immunochemiluminometric assay (Immulite 2000, Siemens Healthcare, Gwynedd, UK); TRAb concentrations using enzyme-linked immunoassay (ElisaRSR TRAb 3rd Generation, Cardiff, UK); and total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides concentrations using enzymatic-colorimetric assays (Roche, Mannheim, Germany). Additionally, AST, ALT, CPK, and fasting blood glucose concentrations were measured at baseline and at safety visits using enzymatic-colorimetric assays (Roche).

Quality of life (QoL) was evaluated using the Graves' orbitopathy QoL questionnaire (GO-QoL).[16] Patients completed the questionnaire at baseline, 12 weeks, and 24 weeks. The questionnaire consisted of two subscales: visual functioning (eight questions about potentially decreased visual acuity, diplopia, or both), and appearance (eight questions about psychosocial functioning attributable to changes in appearance). Questions were scored as severely limited (one point), a little limited (two points), or not limited at all (three points). The two scores (8–24 points) were summed into a total score (16–48 points). A higher score meant a better QoL. The total score and the two subscales were compared.

Outcomes

The primary objective was the overall Graves' orbitopathy outcome at 24 weeks based on a composite evaluation of exophthalmos, clinical activity score, eyelid aperture, diplopia, and visual acuity assessed in the modified intention-to-treat (ITT) population. Patients were considered responders when at least two of the following criteria were fulfilled in the most affected eye, without worsening in any of the same measures in both eyes: 1) reduction in exophthalmos of 2 mm or more, with no increase by 2 mm or more in the other eye; 2) reduction of clinical activity score by two or more points; 3) reduction in eyelid aperture by 2 mm or more, with no increase by 2 mm or more in the other eye; 4) disappearance or improvement (change from constant to inconstant, intermittent, or absent, or from inconstant

to intermittent or absent) of diplopia; and 5) improvement in visual acuity by 0·2 decimals or more.

Secondary objectives were overall outcome of Graves' orbitopathy at 12 weeks, change in QoL, and Graves' orbitopathy relapse at 24 weeks. The secondary outcomes were also assessed in the modified ITT population. Graves' orbitopathy relapse at 24 weeks was defined as change in two of the following measures compared with week 12: worsening in clinical activity score ≥2 points; worsening in exophthalmos by 2 mm or more; worsening in eyelid aperture by 2 mm or more; worsening in diplopia (appearance or change in degree); and worsening in visual acuity by 0·2 decimals or more.

In post-hoc analyses we evaluated single eye features, FT4, and TRAbs at 12 and 24 weeks. Additionally, we evaluated the outcome of the single eye features at 12 and 24 weeks using the same criteria as the composite outcome analysis.

Safety visits were done at baseline and at week 2, 4, 6, 8, 10, 12, 15, 24, and 28 (appendix p 8). Adverse events were reported for all patients who were randomly assigned to one of the treatment groups and were documented and coded according to the standardised medical dictionary for regulatory affairs,[17] as recommended by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use.

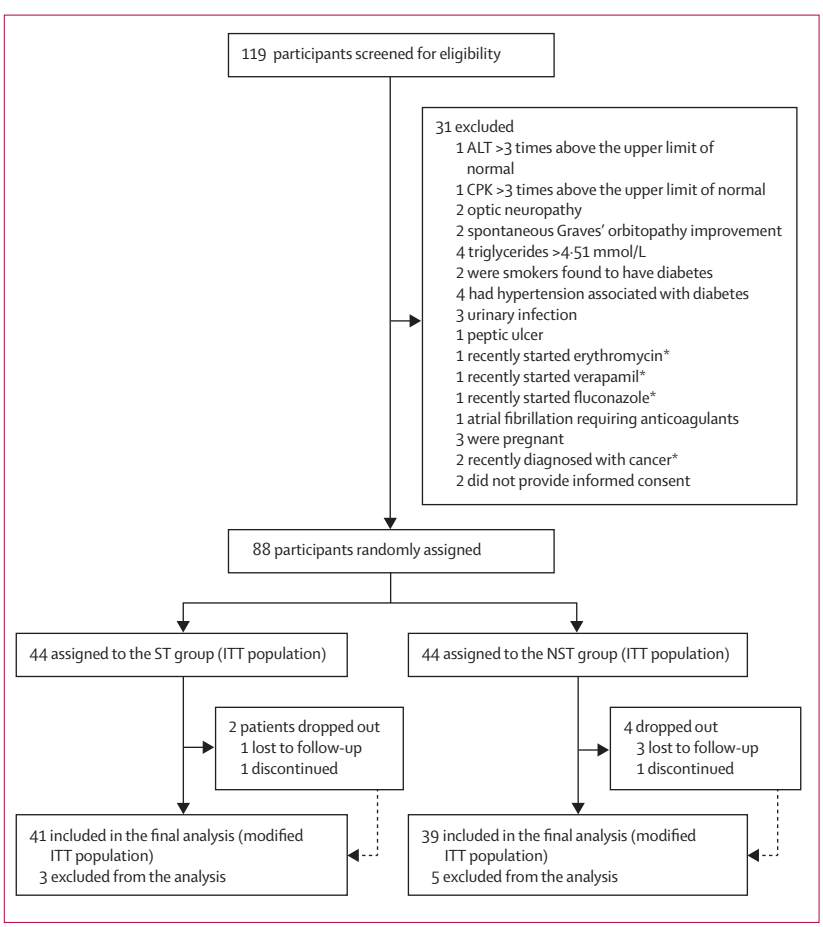

Figure 1: **Trial profile**
*Within the 6 weeks preceding the baseline visit.

Statistical analysis

Previous studies have shown that a cumulative ivGCs dose of 4·5 g over 12 weeks resulted in an improvement in Graves' orbitopathy in about 65% of patients,[5] and we assumed that atorvastatin would increase this percentage to 91% (target difference), on the basis of a 40% protective effect of statins reported by Stein and colleagues;[8] therefore, we estimated that 88 patients were sufficient for statistical significance with $p<0·05$ by Yates χ^2 test for the primary outcome in the final analysis, with a statistical power of 0·8, considering a 10% dropout. The expected result was that 36 (90%) of 40 patients in the ST group would be responders and 26 (65%) of 40 patients in the NST group would be responders, with a drop-out rate of four (9%) patients per group. The target attributable risk was calculated to be 0·25 (95% CI 0·07–0·42; χ^2 5·81; $p=0·015$). Per protocol, an efficacy interim analysis was planned when 44 (50%) of patients completed the 24 week visit, which was done by the sponsor. The aim of the interim analysis was to assess whether overall Graves' orbitopathy outcome at 24 weeks was significantly superior ($p<0·05$) in the ST group. No other changes were planned on the basis of the interim analysis, including final sample size, drug dosage, and procedures. Sample size for interim analysis was arbitrary, as imposed by the sponsor and the Ethics Committee. No p values adjustments were planned and reported p values were nominal. Interim analysis was disclosed 3 months before completion of the study. Thus, in accordance with the sponsor, we decided to complete the study as planned. The results of interim analysis were in line with those obtained in the final analysis, even though not statistically significant (appendix p 3). No bias adjustments based on interim analysis were made. Because there was a single primary endpoint, no multiplicity adjustments were made. There were no major amendments to the original protocol.

To avoid errors the following database handling procedures were employed: allowed character checks, batch totals, missing records check, cardinality check, digits check, consistency check, control totals, cross-system consistency check, data type check, hash totals, limit check, logic check, presence check, range check, spelling and grammar check, and uniqueness check.

Continuous variables are presented as mean (SD) or median (IQR). Continuous variables were standardised to determine their effect on overall Graves' orbitopathy outcome at 24 weeks. Categorical variables were converted into continuous variables for comparisons, by converting each category into a progressive number starting from zero.

Continuous variables were compared using ANOVA with Bonferroni's correction or Mann-Whitney U test.

Mean changes from baseline were analysed using a restricted maximum likelihood-based repeated measures approach in combination with the Newton Raphson Algorithm. Analyses included the fixed categorical effects of treatment, visit, and treatment-by-visit interaction and the continuous fixed covariates of baseline score and baseline score-by-visit interaction. A first-order auto-regressive covariance structure was used to model the within-patient errors. The Satterthwaite approximation was used to estimate denominator degrees of freedom. Significance tests were based on least-squares means using a two-sided α of 0·05 (two-sided 95% CI). Analyses were implemented using SPSS (version 21.0). The primary treatment comparisons were the contrast between treatments at each visit using the Šídák's multiple comparisons test. Categorical data were compared using two-tailed Fisher's exact test, which allows a more conservative approach compared with Yates χ^2 test. Point estimates represent attributable risk followed by 95% Wilson's CI. There was no allowance for multiplicity for secondary outcomes.

Data from patients who did not attend any of the follow-up visits were not included in the analysis. The ITT population included patients who attended all follow-up visits or attended the 12 week, but not the 24 week visit. Data from the patients who attended the 12 week visit but not the 24 week visit were kept in the analysis based on last observation carried forward as a conservative assumption. Data from patients who withdrew from study treatments or were not compliant but did attend follow-up visits were kept in the analysis. There was no external monitoring. The study was monitored by the sponsor. The study is registered with EUDRACT, 2018-001317-33, and ClinicalTrials.gov, NCT03110848.

Role of the funding source

The funder had no role in study design, data collection, analysis and interpretation, and writing of the report.

	ST group (n=41)	NST group (n=39)
Gender		
Men	9 (22%)	9 (23%)
Women	32 (78%)	30 (77%)
Age	55·3 (9·2)	52·4 (11·2)
Smoking habits		
Non-smoker	21 (51%)	14 (36%)
Former smoker	11 (27%)	11 (28%)
Current smoker	9 (22%)	14 (36%)
Mean BMI, kg/m² (SD)	25·4 (3·3)	26·1 (2·9)
Thyroid treatment		
Methimazole	23 (56%)	17 (44%)
Levothyroxine after radioiodine	7 (17%)	9 (23%)
Levothyroxine after thyroidectomy	11 (27%)	13 (33%)
Time since radioiodine, months	14·5 (10·2–20·2)	11·0 (8·0–15·0)
Free thyroxine (ng/dL, reference range: 0·7–1·7)	1 (0·8–1·2)	1·1 (0·9–1·4)
Thyroid stimulating hormone (mU/L, reference range: 0·4–4)	1·5 (0·2–3·5)	1 (0·3–1·9)
Thyroid stimulating hormone receptor autoantibodies (IU/L; cut-off <1·5)	5·4 (2·1–10·2)	3·7 (2–15·8)
Graves' orbitopathy duration, months	12·5 (7·7–24)	15·5 (9·8–24)
Previous Graves' orbitopathy treatment		
None	34 (83%)	33 (85%)
Low cumulative dose (1·3 g) oral prednisone	7 (17%)	6 (15%)
Time since oral prednisone treatment, months	9·5 (7·2–17·2)	9·0 (6·0–13·0)
Mean low-density lipoprotein cholesterol concentration (mmol/L)	3·89 (0·58)	3·73 (0·54)
Mean exophthalmometry in the most affected eye (mm)	22·6 (2·9)	23 (2·9)
Mean clinical activity score	4·3 (1)	4·1 (1·1)
Mean eyelid aperture (mm)	12·7 (2·5)	12·6 (2·2)
Diplopia		
Absent	11 (27%)	12 (31%)
Intermittent	5 (12%)	8 (21%)
Inconstant	13 (32%)	13 (33%)
Constant	12 (29%)	6 (15%)
Mean best corrected visual acuity in the most affected eye (decimals)	0·9 (0·05)	0·9 (0·05)
Mean Graves' orbitopathy quality of life score	32 (5·5)	30·7 (5·3)

Data are mean (SD), median (IQR) or n (%).

Table 1: **Baseline characteristics of the modified intention-to-treat population**

Results

Between June 1, 2020, and Nov 30, 2020, 119 patients were screened. 31 (26%) patients were not eligible for inclusion (figure 1). 88 (74%) patients (23 [26%] men and 65 [74%] women) with a mean age of 53·9 years (SD 10) were recruited and randomly assigned to the ST group (44 [50%] patients) or the NST group (44 [50%] patients; figure 1). Demographic and clinical variables of ITT population at baseline are reported in (table 1). All patients received thyroid treatment (table 1). All patients maintained FT4 and TSH within normal range, with no significant differences between the groups (table 1; appendix p 4). Minimal changes in thyroid medication dosage were necessary in 24 patients (13 [30%] in the ST group and 11 [25%] in the NST group), with no cases of overt hypothyroidism or thyrotoxicosis and no effects on study outcomes (data not shown). Median dose of methimazole was 5·0 mg (IQR 2·5–10·0) in 40 (50%) patients who were receiving hyperthyroidism treatment. No patients had previously received immunosuppressive treatments or orbital irradiation for Graves' orbitopathy. Across the two groups, 13 (16%) patients had received low-dose prednisone no longer than 3 months before enrolment (table 1).

Three (6%) of 44 patients in the ST group and five (11%) of 44 patients in the NST group were lost to follow-up before the 12 week visit (all informed consent withdrawal) and not included in the analysis (figure 1). One (2%) patient in the ST group and three (6%) patients in NST group were lost to follow-up between the 12 week and 24 week visits (they kept informed consent, but were not able to attend the 24 week visit for personal reasons), and were kept in the analysis according to last observation

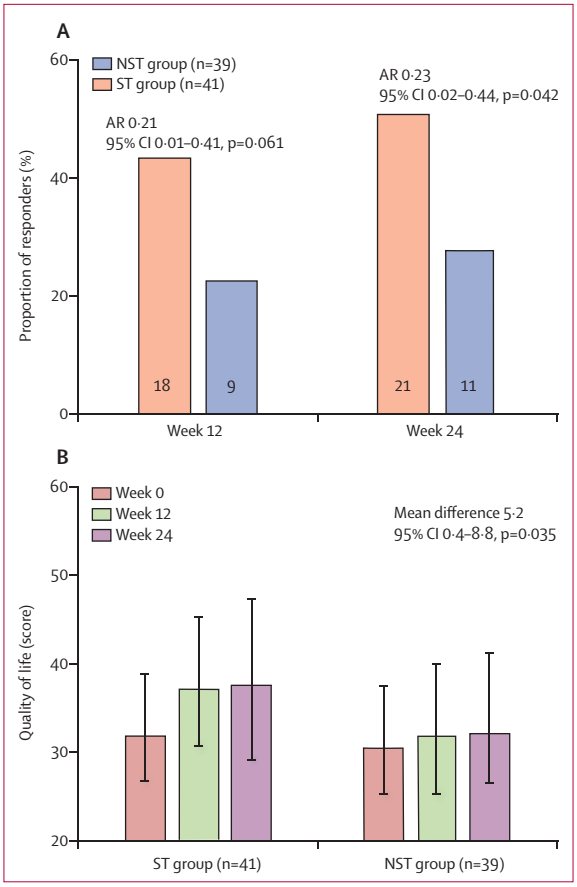

Figure 2: **Graves' orbitopathy outcome at 12 weeks and 24 weeks (A) and change in quality of life score throughout the study (B) in the modified intention-to-treat population**
AR=Attributable risk.

	ST group (n=41)	NST group (n=39)	Attributable risk (95% CI; p value)	Mean difference (95% CI; p value)
Overall response at 24 weeks	21/41 (51%)	11/39 (28%)	0·23 (0·02 to 0·44 p=0·042)	..
Overall response at 12 weeks	18/41 (44%)	9/39 (23%)	0·21 (0·01 to 0·41; p=0·061)	..
Quality of life across the follow-up period	NA	NA	..	5·2 points (0·4 to 8·8; p=0·035)
Relapse	0/41	6/39 (15%)	–0·15 (–0·27 to –0·04; p=0·011)	..

Data are n/N (%). NA=not applicable.

Table 2: **Primary and secondary study endpoints in the modified intention-to-treat population**

carried forward. These patients were contacted by telephone for safety analysis up to 28 weeks. One (2%) patient in ST group stopped atorvastatin at 12 weeks because of myositis, and one (2%) patient in the NST group stopped ivGCs treatment because of gastritis. Both patients were kept in the analysis because they attended all visits. There were no other patients who discontinued the medications or needed dose reductions. Overall, six (7%) of 80 patients were imputed (two [4%] of 41 in the ST group and four [10%] of 39 patients in the NST group) and last observation carried forward was used. A sensitivity analysis for primary outcome following the removal of imputations supported our findings (appendix p 5). Overall, the modified ITT population included 41 (51%) patients in the ST group and 39 (49%) patients in the NST group, with a dropout of 10·2% after random assignment.

Low-density lipoprotein cholesterol concentration decreased in the ST group but not in the NST group; there was a statistically significant difference between groups (mean difference –0·4 mmol/L [95% CI –0·7 to –0·1]; p=0·015; appendix p 9). A post-hoc analyses showed a statistically significant difference between the low density lipoprotein cholesterol in the ST group and the NST group at 12 weeks (mean difference –0·7 mmol/L [–1·2 to –0·2]; p=0·0031) and 24 weeks (mean difference –0·5 mmol/l [–0·9 to –0·1]; p=0·0051). Using a composite evaluation, the proportion of Graves' orbitopathy responders at 24 weeks (primary outcome) was significantly higher in the ST group than in the NST group (figure 2A; table 2). A non-statistically significant higher proportion of responders at 12 weeks (secondary outcome) was observed in the ST group (figure 2A; table 2).

The total Graves' orbitopathy-QoL score (secondary outcome) increased in the ST group but not in the NST group, with a statistically significant difference between the groups (figure 2b; table 2; appendix p 6). Post-hoc analyses showed a statistically significant difference between the ST group and the NST group at 24 weeks (mean difference 6·4 points [95% CI 0·6–12·1]; p=0·031), and a non-statistically significant difference at 12 weeks (mean difference 5·2 points [0·3–5·6]; p=0·063; appendix p 6). Concerning the two subscales of Graves' orbitopathy QoL, there was a statistically significant difference between groups in functioning subscale at 12 weeks (mean difference 3·3 points [0·1–6·5]; p=0·043) and 24 weeks (mean difference 4 points [0·8–7·2]; p=0·014), whereas the appearance subscale was not statistically significantly different between the two groups (appendix p 6).

No one in the ST group had Graves' orbitopathy relapse at 24 weeks (secondary outcome; table 2), whereas six (15%) of 39 patients relapsed in the NST group (table 2).

In addition to study endpoints, we evaluated outcome of single eye features and behaviour of FT4 and TRAbs (appendix p 4). There was a significant amelioration of clinical activity score (mean difference –1 point [95% CI –1 to –1]; p<0·0001) and diplopia (mean difference –1·5 classes, [95% CI –1 to –1]; p=0·035) in the ITT population regardless of statin treatment over the course of the study (data not shown). Exophthalmos, eyelid width, and visual acuity in the ITT population regardless of statin treatment did not change significantly over the course of the study (data not shown). There was no difference between groups for all the eye features over the 24 week study (appendix p 4). Single eye features in 24 week responders versus non-responders are reported in the

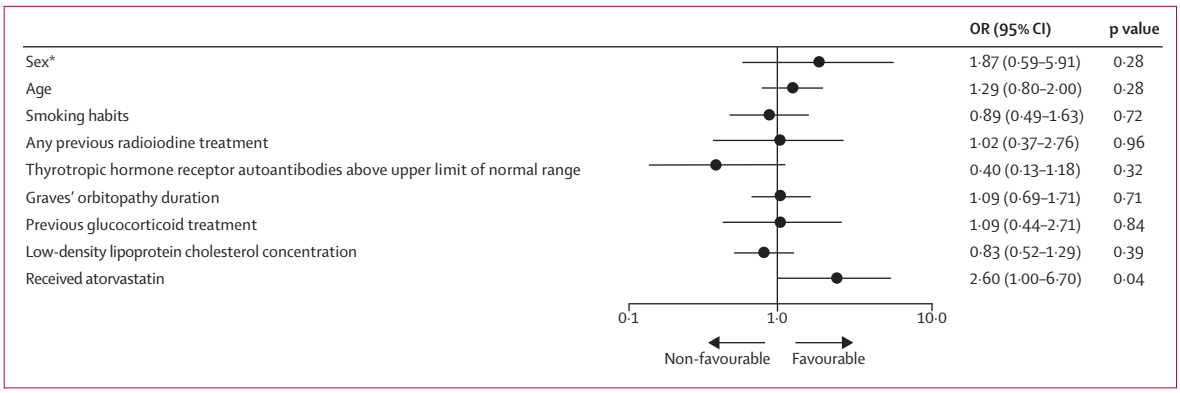

Figure 3: **Effect of various determinants on 24 week outcome of Graves' orbitopathy in the modified intention-to-treat population**
Sex is female versus male.

appendix (p 7). Outcome of eye features were not statistically significantly different between the two groups at 12 weeks and 24 weeks (appendix p 10). The number of patients with constant diplopia was higher, although not statistically, in the ST group (table 1). To estimate the effect of diplopia on overall Graves' orbitopathy outcome at 24 weeks, we removed diplopia from the analysis. The results showed a non-significantly different better outcome in the ST group (14 [34%] of 41 patients) than in the NST group (seven [18%] of 39 patients; AR 0·16 [95% CI –0·03 to 0·35]; p=0·13; data not shown).

FT4 did not change across follow-up and was not significantly different between the ST group and the NST group (appendix p 4). TRAbs did not change during the study, with no significant differences between the groups, which also held true when patients treated with radioiodine were excluded (appendix p 4).

Gender, age, smoking, radioiodine, TRAbs, Graves' orbitopathy duration, prednisone treatment, and low-density lipoprotein cholesterol concentration at baseline did not affect Graves' orbitopathy outcome at 24 weeks (figure 3), nor did they affect secondary endpoints (data not shown). The apparent positive effect of female gender and negative effect of TRAbs were not statistically significant (figure 3). The only variable that significantly affected overall Graves' orbitopathy outcome at 24 weeks was atorvastatin (figure 3). To determine if the effect of atorvastatin reflected lowering of cholesterol, we compared responders and non-responders within the ST group, but low-density lipoprotein cholesterol concentration did not significantly differ between them (appendix p 11).

All 88 randomly assigned patients were included in the safety population. These patients were visited or contacted by telephone for up to 28 weeks. 26 adverse events occurred in 21 (24%) of 88 patients, with no serious or unexpected events and no difference between the groups (table 3). One (2%) patient in the ST group stopped atorvastatin because of myositis. One (2%) patient in the NST group stopped ivGC because of gastritis.

	ST group (n=44)	NST group (n=44)
Total number of adverse events	12 (27%)	14 (32%)
Cardiac disorders		
Palpitations	1 (2%)	2 (5%)
Vascular disorders		
Hot flush	1 (2%)	1 (2%)
Face swelling	1 (2%)	0
Hypertension	1 (2%)	1 (2%)
Gastrointestinal disorders		
Nausea	0	0
Dyspepsia	0	1 (2%)
Gastritis	0	1 (2%)
Abdominal discomfort	1 (2%)	0
Infections		
Cystitis	2 (5%)	1 (2%)
Herpes simplex	0	1 (2%)
Oral fungal infection	1 (2%)	0
Metabolism and nutrition disorders		
Hyperglycaemia	1 (2%)	1 (2%)
Weight increase	0	1 (2%)
Biochemistry investigations		
Increase in serum liver enzyme concentrations	0	1 (2%)
Musculoskeletal and connective tissue disorders		
Myositis	1 (2%)	0
Psychiatric disorders		
Sleeping disorders	1 (2%)	1 (2%)
Depressive mood	0	1 (2%)
Skin and subcutaneous tissue disorders		
Eczema	1 (2%)	0
Rush	0	1 (2%)

Data are n (%).

Table 3: **Adverse events in the safety population**

Discussion

Advances in our understanding of Graves' orbitopathy pathogenesis have changed the management of the disease, especially through the introduction of novel

treatment procedures.[4] Our study stemmed from the association between Graves' orbitopathy, statins, and cholesterol.[6,7] In addition to the protective role of statins on the development of Graves' orbitopathy in Graves' disease and the association between Graves' orbitopathy and high cholesterol,[6–11] low-density lipoprotein cholesterol was found to be a predictor of response to treatment.[18] Moreover, in patients with Graves' orbitopathy-related strabismus, individuals who used statins had less need for decompressive surgery compared with those who did not use statins,[19] suggesting that statins might be a therapeutic tool in the treatment of Graves' orbitopathy. Thus, we designed this clinical trial, which aimed to investigate the effects of atorvastatin on Graves' orbitopathy outcome in patients with moderate-to-severe, active Graves' orbitopathy who were receiving ivGCs.

We choose atorvastatin because it is the most commonly used lipid-lowering medication,[12,13] and previous studies showed that had the strongest protective effect on Graves' orbitopathy development.[6–9] The most common atorvastatin starting dose is 10 mg per day; however, this dose is known to not be effective in all patients. Therefore, to have a correct stratification a dose of 20 mg per day was chosen, which previous studies have shown to result in low-density lipoprotein cholesterol reduction in about 95% of patients.[12,13]

Our results suggest that addition of atorvastatin to an ivGCs regimen improves the outcome of moderate-to-severe, active Graves' orbitopathy. The overall response of Graves' orbitopathy to ivGCs at 24 weeks was significantly better in patients given atorvastatin compared with those not given the statin. QoL also improved in patients given atorvastatin, with a statistically significant difference compared with patients not given atorvastatin. None of the patients given atorvastatin had Graves' orbitopathy relapse at 24 weeks, compared with six patients who were not given atorvastatin. No major adverse events related to atorvastatin were observed.

Despite the better overall outcome, no differences between groups were observed concerning single eye features, although there was a trend to a better outcome of clinical activity score and diplopia in the atorvastatin group. Because a non-statistically significant, higher proportion of patients had constant diplopia at baseline in the ST group, paralleled by a non-statistically significant, slightly higher median diplopia and slightly worse ocular ductions, it can be presumed that in the composite evaluation a change in diplopia contributed to the better overall Graves' orbitopathy outcome. However, when we excluded diplopia from the analysis, a trend towards a better response in atorvastatin group was confirmed, although not statistically significant.

The apparent discrepancy between overall outcome and outcome of single eye features is not surprising. The composite score includes all eye features simultaneously, as recommended by the ETA/EUGOGO guidelines.[4] A difference in overall outcome might not be paralleled by a difference in single eye features outcomes, and only the combination of single eye features might result in a statistically different overall outcome. The composite overall Graves' orbitopathy outcome was chosen as the primary endpoint because a change in single eye features might not reflect a true modification of Graves' orbitopathy and because they can be affected by a number of unrelated factors. By contrast, the composite evaluation offers a more realistic picture. In confirmation of the ETA/EUGOGO guidelines,[4] expert opinion recommends the use of a composite score.[20]

The effects of statins might be related to their cholesterol lowering mechanism, pleiotropic actions, interaction with methylprednisolone.[6,7] Hypercholesterolaemia is known to exert systemic proinflammatory actions.[6,7,21,22] The mechanisms include oxidative stress and release of proinflammatory cytokines, some of which are involved in Graves' orbitopathy.[3] Furthermore, hypercholesterolaemia elicits activation of Toll-Like Receptor 4-Myeloid Differentiation Factor 2 (TLR4–MD2), resulting in tissue inflammation.[23] In a study, published in 2021, we found a non-significant overexpression of the *TLR4* gene (+1·58-times increase) and a non-significant down-regulation of low-density lipoprotein receptor gene (–1·64-times decrease) in orbital fibroblasts of patients with Graves' orbitopathy compared with orbital fibroblasts from patients without Graves' orbitopathy,[7,24] which supports our findings from this study of an association between cholesterol and Graves' orbitopathy.[10,11]

However, in previous studies, non-statin lipid-lowering medications did not affect development of Graves' orbitopathy in Graves' disease,[8,9] suggesting that mechanisms other than cholesterol-lowering might be responsible.[6,7] Our study suggests that low-density lipoprotein cholesterol did not differ between Graves' orbitopathy responders and non-responders within the ST group. Thus, the effects of atorvastatin might be related to its pleiotropic actions rather than to lowering of cholesterol. Statins block the mevalonate pathway, which, through reduction of protein prenylation, promotes apoptosis, autophagy, and unfolded protein response stress.[25] By affecting these pathways, statins might influence innate immunity and regulate inflammatory cell balance. In addition, in-vitro studies and experimental models of autoimmune diseases showed that statins have direct immunoregulatory effects by inducing tolerogenic dendritic cells, a specialised subset of dendritic cells promoting immune tolerance and counteracting autoimmune responses.[26] Statins might also counteract orbital changes through additional mechanisms that influence tissue remodelling because they inhibit adipogenesis in preadipocytes and fibroblasts by reducing expression of adipogenic genes.[27] A study published in 2020 showed that statins have anti-fibrotic activity in Graves' orbitopathy orbital fibroblasts, through inhibition

of myofibroblast differentiation induced by tumour growth factor-β.[28] Another possibility relates to the pharmacological interaction of atorvastatin with methylprednisolone. Atorvastatin is metabolised by CYP3A4 and might act as a CYP3A4 inhibitor, thereby affecting the pharmacokinetic properties of glucocorticoids. Thus, the combined therapy might increase the effects of methylprednisolone. These alternative mechanisms suggest that the effects of atorvastatin might be cholesterol independent. To our knowledge, no data are available on the effects of statins on Graves' orbitopathy outcome and risk of Graves' orbitopathy development in patients with Graves' disease regardless of low-density lipoprotein cholesterol levels, for which new studies are needed.

The response to ivGCs in the NST group was lower than the ones reported in two previous studies with the same regimen.[14,29] In the previous studies, patients had more severe Graves' orbitopathy of shorter duration, and outcome was evaluated according to different criteria (change in single eye features rather than the composite score we used), which might explain the discrepancy. However, the response to ivGCs in the NST group was similar to that reported in the EUGOGO dose-finding study,[30] in which patients were given a similar treatment regimen. Furthermore, the response rate in the ST group was similar to that in patients given a higher cumulative dose of methylprednisolone (7·47 g *vs* 4·5 g used in our study) reported in the same EUGOGO dose-finding study,[30] supporting the conclusion that atorvastatin increases Graves' orbitopathy response to treatment.

A possible limitation of our trial is that it was not masked to patients, which might have affected Graves' orbitopathy QoL. A double-blind design would have been preferable, but this was not possible due to internal rules of our Ethics Committee, according to which the study drug and placebo must be provided by the same pharmaceutical company, which requires active participation of a company. Despite several attempts, no companies manufacturing statins agreed to participate. However, because ophthalmological examination was masked, our findings on 24 week outcomes of Graves' orbitopathy can be considered reliable.

Another potential limitation is that the same ophthalmologist examined all patients. Although this approach offers the advantage of avoiding interobserver variations, intraobserver variations are still possible, which could have been prevented by a multiple-observer approach.

No stratification was done concerning smoking, but smoking did not differ between the study groups and had no effects on study outcomes. Additionally, some patients with constant diplopia could have taken advantage of a higher methylprednisolone dose, in accordance with ETA/EUGOGO guidelines.[4] However, this would have prevented a correct stratification and increased the risk of adverse events. Our findings and conclusions are restricted to a relatively short follow-up period. Whether the beneficial effects of atorvastatin hold true long term remains to be established.

In conclusion, atorvastatin addition to an ivGCs regimen improves the response of Graves' orbitopathy in patients with Graves' disease who are hypercholesterolaemic. No differences in the number or severity of adverse events was observed between the study groups, suggesting that atorvastatin does not increase treatment-related risks. A phase 3 clinical trial, regardless of low-density lipoprotein concentration, with a longer follow-up is needed to confirm our findings and introduce statins into clinical practice.

Contributors

MM, GL, ES, ML, and AS conceived and designed the study. MM was the principal investigator of the clinical trial. GL and MM coordinated the trial, collected the raw data, and reviewed adverse events and drug-related side-effects. GL, MM, and PP verified the underlying data, analysed all findings, and take responsibility for the integrity and the accuracy of the data analysis. ES, ML, and AS set up the database. GL entered the data. GL, FM, RR, CM, and MM screened and enrolled the participants, obtained informed consent from the participants, provided patient care and documentation of study data. MN did the ophthalmological evaluation. GL and MM wrote the manuscript. All authors contributed to data interpretation, revised, and approved the manuscript. All authors had access to the data. MM takes final responsibility for the decision of submitting the study for publication.

Declaration of interest

We declare no competing interests.

Data sharing

Upon request the corresponding author, deidentified participants' data will be available to others after publication, as will the study protocol and the statistical analysis plan.

Acknowledgments

The study was funded in part by a donation from Associazione Allievi Endocrinologia Pisana, Pisa, Italy.

References

1 Piantanida E, Tanda ML, Lai A, Sassi L, Bartalena L. Prevalence and natural history of Graves' orbitopathy in the XXI century. *J Endocrinol Invest* 2013; **36:** 444–49.
2 Bartalena L, Fatourechi V. Extrathyroidal manifestations of Graves' disease: a 2014 update. *J Endocrinol Invest* 2014; **37:** 691–700.
3 Bahn RS. Current insights into the pathogenesis of Graves' ophthalmopathy. *Horm Metab Res* 2015; **47:** 773–78.
4 Bartalena L, Baldeschi L, Boboridis K, et al. The 2016 European Thyroid Association/European Group on Graves' Orbitopathy guidelines for the management of Graves' orbitopathy. *Eur Thyroid J* 2016; **5:** 9–26.
5 Zang S, Ponto KA, Kahaly GJ. Clinical review: intravenous glucocorticoids for Graves' orbitopathy: efficacy and morbidity. *J Clin Endocrinol Metab* 2011; **96:** 320–32.
6 Lanzolla G, Vannucchi G, Ionni I, et al. Cholesterol serum levels and use of statins in Graves' Orbitopathy: a new starting point for the therapy. *Front Endocrinol* 2020; **22:** 933.
7 Marinò M, Lanzolla G, Marcocci C. Statins: a new hope on the horizon of Graves' orbitopathy? *J Clin Endocrinol Metab* 2021; **106:** e2819–21.
8 Stein JD, Childers D, Gupta S, et al. Risk factors for developing thyroid-associated ophthalmopathy among individuals with Graves disease. *JAMA Ophthalmol* 2015; **133:** 290–96.
9 Nilsson A, Tsoumani K, Planck T. Statins decrease the risk of orbitopathy in newly diagnosed patients with Graves disease. *J Clin Endocrinol Metab* 2021; **106:** 1325–32.
10 Sabini E, Mazzi B, Profilo MA, et al. High serum cholesterol is a novel risk factor for Graves' orbitopathy: results of a cross-sectional study. *Thyroid* 2018; **28:** 386–94.

11 Lanzolla G, Sabini E, Profilo MA et al. Relationship between serum cholesterol and Graves' orbitopathy (GO): a confirmatory study. *J Endocrinol Invest* 2018; **41:** 1417–23.

12 Piepoli MF, Hoes AW, Brotons C. Main messages for primary care from the 2016 European Guidelines on cardiovascular disease prevention in clinical practice. *Eur J Gen Pract* 2018; **24:** 51–56.

13 Stone NJ, Robinson JG, Lichtenstein AH, et al. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol* 2014; **63:** 2889–34.

14 Kahaly GJ, Pitz S, Hommel G, Dittmar M. Randomized, single blind trial of intravenous versus oral steroid monotherapy in Graves' orbitopathy. *J Clin Endocrinol Metab* 2005; **90:** 5234–40.

15 Mourits MP, Prummel MF, Wiersinga WM, Koornneef L. Clinical activity score as a guide in the management of patients with Graves' ophthalmopathy. *Clin Endocrinol* 1997; **47:** 9–14.

16 Terwee CB, Gerding MN, Dekker FW, Prummel MF, Wiersinga WM. Development of a disease specific quality of life questionnaire for patients with Graves' ophthalmopathy: the GO-QOL. *Br J Ophthalmol* 1998; **82:** 773–79.

17 Riedl M, Kolbe E, Kampmann E, Krämer I, Kahaly GJ. Prospectively recorded and MedDRA-coded safety data of intravenous methylprednisolone therapy in Graves' orbitopathy. *J Endocrinol Invest* 2015; **38:** 177–82.

18 Naselli A, Moretti D, Regalbuto C, et al. Evidence that baseline levels of low-density lipoproteins cholesterol affect the clinical response of Graves' ophthalmopathy to parenteral corticosteroids. *Front Endocrinol* 2020; **11:** 609895.

19 Reynolds AL, Del Monte MA, Archer SM. The effect of oral statin therapy on strabismus in patients with thyroid eye disease. *J AAPOS* 2018; **22:** 340–43.

20 Bartalena L, Wiersinga WM. Proposal for standardization of primary and secondary outcomes in patients with active, moderate-to-severe Graves' orbitopathy. *Eur Thyroid J* 2020; **9** (Suppl 1)**:** 3–16.

21 Ridker PM. Rosuvastatin in the primary prevention of cardiovascular disease among patients with low levels of low-density lipoprotein cholesterol and elevated high-sensitivity C-reactive protein: rationale and design of the JUPITER trial. *Circulation* 2003; **108:** 2292–97.

22 Verma A, Lavie CJ, Milani R V. C-Reactive protein: how has JUPITER impacted clinical practice? *Ochsner J* 2009; **9:** 204–10.

23 Tall AR, Yvan-Charvet L. Cholesterol, inflammation, and innate immunity. *Nat Rev Immunol* 2015; **15:** 104–116.

24 Rotondo Dottore G, Bucci I, Lanzolla G, et al. Genetic Profiling of orbital fibroblasts from patients with Graves' ORBITOPATHY. *J Clin Endocrinol Metab* 2021; **106:** e2176–90.

25 Ghavami S, Yeganeh B, Stelmack GL, et al. Apoptosis, autophagy and ER stress in mevalonate cascade inhibition-induced cell death of human atrial fibroblasts. *Cell Death Dis* 2012; **3:** e330.

26 Li H, Wang C-C, Zhang M, et al. Statin-modified dendritic cells regulate humoral immunity in experimental autoimmune myasthenia gravis. *Mol Cell Neurosci* 2015; **68:** 284–292.

27 Shahida B, Johnson PS, Jain R, et al. Simvastatin downregulates adipogenesis in 3T3-L1 preadipocytes and orbital fibroblasts from Graves' ophthalmopathy patients. *Endocr Connect* 2019; **8:** 1230–39.

28 Wei Y-H, Liao S-L, Wang S-H. Simvastatin and rock inhibitor Y-27632 inhibit myofibroblast differentiation of Graves' ophthalmopathy-derived orbital fibroblasts via rhoa-mediated ERK and p38 signaling pathways. *Front Endocrinol* 2020; **11:** 607968.

29 Aktaran S, Akarsu E, Erbağci I, Araz M, Okumuş S, Kartal M. Comparison of intravenous methylprednisolone therapy vs. oral methylprednisolone therapy in patients with Graves' ophthalmopathy. *Int J Clin Pract* 2007; **61:** 45–51.

30 Bartalena L, Krassas GE, Wiersinga W, et al. Efficacy and safety of three different cumulative doses of intravenous methylprednisolone for moderate to severe and active Graves' orbitopathy. *J Clin Endocrinol Metab* 2012; **97:** 4454–63.